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# Octet<sup>®</sup> BLI Discovery

User Guide 13

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# About Octet<sup>®</sup> Systems

Octet<sup>®</sup> systems enable real-time quantitation or kinetic characterization of biomolecular interactions. Each system includes:

- Octet<sup>®</sup> instrument
- Computer
- Hardware accessories
- Octet<sup>®</sup> Software Modules–Octet<sup>®</sup> BLI Discovery, and Octet<sup>®</sup> Analysis Studio. For more details on the Octet<sup>®</sup> Analysis Studio software, see the User Guide.

### Table 1-1: Octet<sup>®</sup> System Software Functions

Octet <sup>®</sup> Software	Functions
Octet <sup>®</sup> BLI Discovery	<ul> <li>Define quantitation or kinetic experiments and save them for future use.</li> <li>Define custom assays.</li> </ul>
<mark>[<u></u>]</mark>	<ul><li>Run experiments and acquire binding data.</li><li>View and save binding data.</li></ul>
Octet <sup>®</sup> Analysis Studio	<ul> <li>Analyze binding data and view analysis results.</li> <li>Export or copy analysis results.</li> <li>Generate reports of quantitation or kinetic results.</li> </ul>

For information on preparing samples for quantitation or kinetics experiments, please see the appropriate Octet<sup>®</sup> biosensor product instructions.

# Conventions and Symbols Used in This Guide

**NOTICE:** Presents pertinent details on a topic. For example, general information, tips or alternate options.

IMPORTANT: Indicates the assay or procedure will not work if the guidelines provided are not properly followed.



**WARNING & CAUTION:** Informs the user that specific actions could cause irreversible consequences or damage. To prevent hazards, the manual should be read before operating the equipment

# Octet<sup>®</sup> Systems Safety Information

### Getting Started

### All users must read the following safety information.



**WARNING:** Do not operate the Octet<sup>®</sup> system in any other way than described in the user manual. Failure to comply may expose you to hazards that can lead to personal injury and may cause damage to the equipment.



**WARNING:** Octet <sup>®</sup>systems should only be installed, relocated, and/or moved by trained Sartorius personnel. To obtain more information, please contact Sartorius Technical Support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel installing, relocating and/or moving an Octet<sup>®</sup> system.

For more information on and safety precautions for the supplied computer and computer equipment, please refer to the manufacturer's documentation supplied with the computer packaging.

## Product Labeling Definitions

Table 1-2: Label Definitions

Symbol	Definition
CE	The system complies with applicable European directives.
	The system complies with the requirements for electromagnetic compliance (EMC) in Austra- lia and New Zealand.
FC	The electromagnetic interference from this system is under limits approved by the Federal Communications Commission (United States).
	This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if device is used in a domestic environment. South Korea
X	Electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. lease contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	High voltage; potential electrical shock hazard.
$\underline{\land}$	Keep hands clear of moving parts.

# Consignes de securite des systemes Octet<sup>®</sup>

### Avant de commencer

Tous les utilisateurs sont tenus de lire impérativement les consignes de sécurité suivantes.



**WARNING:** N'utilisez pas le système Octet<sup>®</sup> pour un usage autre que celui décrit dans le manuel utilisateur. Le non-respect de cette consigne peut vous exposer à des risques susceptibles d'occasionner des blessures et d'endommager votre équipement.



**WARNING:** Seul le personnel qualifié de Sartorius est habilité à installer, déménager et/ou transférer les systèmes Octet<sup>®</sup>. Pour plus d'informations, veuillez contacter l'assistance technique de Sartorius. Le non-respect de ces consignes pourra conduire à l'annulation de votre contrat de garantie ou d'assistance. Sartorius décline toute responsabilité en cas de blessures ou de dommages consécutifs à une installation, un déménagement et/ou transfert d'un système Octet<sup>®</sup> effectués par du personnel non qualifié.

Pour plus d'informations sur les mesures de sécurité concernant l'ordinateur et l'équipement informatique fournis, veuillez consulter la documentation du fabricant jointe à l'emballage du produit.

## Définitions de l'étiquetage des produits

Table 1-3: Label Definitions

Symbole	Définition
CE	Ce système est conforme aux directives européennes en vigueur.
	Ce système répond aux exigences relatives à la compatibilité électromagnétique (CEM) en vigueur en Australie et en Nouvelle-Zélande.
FC	Les interférences électromagnétiques émises par ce système se situent dans les limites approuvées par la Federal Communications Commission (Commission fédérale des commu- nications) américaine.
	Cet appareil a été testé pour sa conformité pour une utilisation dans un environnement de laboratoire. Des interférences radio peuvent survenir si l'appareil est utilisé dans un environnement domestique. South Korea
	Les équipements électriques et électroniques ne doivent pas être jetés comme des déchets municipaux non triés ; ils doivent faire l'objet d'une collecte sélective. Pour toute information concernant le démantèlement de vos équipements, veuillez contacter un représentant agréé.
	Haute tension : risque potentiel de choc électrique.
$\underline{\mathbb{A}}$	Ne touchez pas les pièces mobiles.

# Sicherheitshinweise für Octet-Systeme

### Erste Schritte

Die folgenden Sicherheitshinweise sind von jedem Benutzer zu lesen.



**WARNING:** Bedienen Sie das Octet-Systeme nur wie im Benutzerhandbuch beschrieben. Eine Missachtung kann Sie Gefahren aussetzen, die zu Personen- und Sachschäden führen können.



**WARNING:** Octet-Systeme sollten nur durch geschultes Personal von Sartorius installiert, umgelagert und/oder bewegt werden. Für weitere Informationen wenden Sie sich bitte an den technischen Support von Sartorius. Durch Nichtbeachtung dieser Hinweise werden alle bestehenden Gewährleistungen oder Dienstleistungsvereinbarungen nichtig. Sartorius übernimmt keine Verantwortung für Personen- oder Sachschäden, die infolge der Installation, Umlagerung und/oder Bewegung eines Octet<sup>®</sup>-Systems durch ungeschultes Personal entstehen.

Weitere Informationen und Sicherheitsmaßnahmen für den im Lieferumfang enthaltenen Computer samt Computerzubehör finden Sie in der Herstellerdokumentation, die mit der Computerverpackung geliefert wurde.

### Definitionen der Produktkennzeichnungen

Table 1-4: Label Definitions

Symbol	Definition
CE	Das System erfüllt die geltenden europäischen Richtlinien.
	Das System erfüllt die Anforderungen für elektromagnetische Verträglichkeit (EMV) in Aus- tralien und Neuseeland.
FC	Die elektromagnetische Störausstrahlung dieses Systems unterschreitet die von der Federal Communications Commission (Vereinigte Staaten) genehmigten Grenzwerte.
	Dieses Gerät wurde auf Konformität für die Verwendung in einer Laborumgebung getestet. Funkstörungen können auftreten, wenn das Gerät in einer häuslichen Umgebung verwendet wird. South Korea
	Elektrische und elektronische Geräte dürfen nicht mit dem gewöhnlichen, unsortierten Hausmüll entsorgt werden, sondern sind getrennt zu entsorgen. Informationen zur Stilllegung der Geräte erhalten Sie von einem autorisierten Vertreter des Herstellers.
	Hochspannung; Stromschlaggefahr.
$\underline{\mathbb{A}}$	Hände von beweglichen Teilen fernhalten.

# Sartorius Technical Support

You can contact Sartorius technical support at:

Sartorius BioAnalytical Instruments, Inc

47661 Fremont Boulevard

Fremont, CA 94538

USA Tel: +1-650-322-1360 Fax: +1-650-322-1370 E-mail: octetsupport@sartorius.com

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# User Safety Guidelines and Warnings



**WARNING:** Do not block, push objects into, or allow dust to accumulate in the air vents. Do not store an Octet<sup>®</sup> system in a low airflow environment, such as a closed cabinet, while in operation. Restricting the airflow can damage the instrument or cause a fire.



**WARNING:** Connect the power cord between the product and a grounded AC outlet. Power connectors and power strips vary among countries. Using incompatible cables or improperly connecting cables to a power strip or electrical outlet may damage the equipment or cause a fire.



**WARNING:** Use only certified power cord sets having at least 16 AWG/3G (3 x 0.75mm2) cable with power plug and connector rated 250 V, 10 A.



**WARNING:** If the Octet<sup>®</sup> system is not used as specified, injury to the user and/or damage to the instrument may result.



**WARNING:** Keep the area around the sample door clear and unobstructed.

**NOTICE:** Do not position the Octet<sup>®</sup> instrument in a way that makes it difficult to disconnect the power.

**NOTICE:** Octet<sup>®</sup> system and software installation should be performed by Sartorius personnel only.

# Directives et mises en garde relatives à la sécurité des utilisateurs



**WARNING:** N'obtusrez pas les ouïes d'aération, n'y insérez pas d'objets et ne laissez pas la poussière s'accumuler à l'intérieur. N'utilisez pas le système Octet<sup>®</sup> dans un environnement mal ventilé (armoire fermée). Limiter la ventilation peut endommager l'instrument ou provoquer un incendie.



**WARNING:** À l'aide du cordon secteur, branchez le produit à une prise CC reliée à la terre. Les connecteurs d'alimentation et les blocs multiprises peuvent varier selon les pays. L'utilisation de câbles incompatibles ou le mauvais branchement des câbles à un bloc multiprise ou à une prise électrique peut endommager l'équipement ou provoguer un incendie.



**WARNING:** N'utilisez que des cordons secteur certifiés munis d'au moins un câble 16 AWG/3G (3 x 0,75 mm2) avec prise électrique et connecteur de 250 V, 10 A.



**WARNING:** Le non-respect des consignes d'utilisation du systme Octet<sup>®</sup> peut occasionner des blessures à l'utilisateur et/ou endommager l'instrument.



WARNING: Veillez à laisser la porte du compartiment échantillons accessible et dégagée.

*NOTICE:* Ne placez pas l'instrument Octet<sup>®</sup> de manière à rendre difficile la dèconnexion de l'alimentation.

**NOTICE:** Seul le personnel de Sartorius est habilité à procéder à l'installation du système et du logiciel Octet<sup>®</sup>.

# Sicherheitsrichtlinien und Hinweise für den Benutzer



**WARNING:** Blockieren Sie niemals die Lüftungsöffnungen, stecken Sie keine Gegenstände in sie und lassen Sie keinen Staub in sie eintreten. Lagern Sie ein Octet-System während des Betriebs niemals in Umgebungen mit geringem Luftstrom, wie z. B. einem geschlossenen Schrank. Ein eingeschränkter Luftstrom kann zu Schäden am Gerät führen oder einen Brand verursachen.



**WARNING:** Schließen Sie das Netzkabel des Geräts an eine geerdete Wechselstrom-Steckdose an. Netzstecker und Steckerleisten unterscheiden sich von Land zu Land. Die Verwendung inkompatibler Kabel oder die unsachgemäße Verbindung von Kabeln mit einer Steckerleiste oder Steckdose kann zu Schäden am Gerät führen oder einen Brand verursachen.



**WARNING:** Verwenden Sie ausschließlich zugelassene Netzanschlusskabel mit mindestens 16 AWG/3G (3 x 0,75 mm2) und Netzstecker sowie einen Anschluss mit 250 V, 10 A.



**WARNING:** Ein nicht bestimmungsgemäßer Gebrauch des Octet-Systems kann zu Verletzungen des Benutzers und/oder Schäden am Gerät führen.



WARNING: Halten Sie den Bereich um die Probenklappe frei.

**NOTICE:** Positionieren Sie das Octet-Instrument nicht so, dass es schwierig ist, die Stromversorgung zu unterbrechen.

**NOTICE:** Die Installation des Octet-Systems und der dazugehörigen Software sollte ausschließlich durch Personal von Sartorius erfolgen.

# Installing Octet<sup>®</sup> BLI Discovery Software

**NOTICE:** Octet<sup>®</sup> BLI Discovery and Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software require a compatible version of the Octet<sup>®</sup> GxP Server module. The software automatically checks the version of the Octet<sup>®</sup> GxP Server module in use and will display a message if it is incompatible. Contact your administrator to install the correct version of the Octet<sup>®</sup> GxP Server module if this happens.

- 1. Insert the software CD into your CD drive. If software was provided on a USB thumb drive, plug the thumb drive into the USB port on the computer.
  - If the Autoplay dialog box appears, open the CD or USB thumb drive to view files.
  - If the Autoplay dialog box does not appear, navigate to the installation drive using Windows Explorer.
- 2. CD and USB thumb drives are typically found under the D:\ or E:\ drive.

📙   🛃 🚽 OctetBLIDiscovery			_	
File Home Share View				~ 🕐
$\leftrightarrow \rightarrow \checkmark \uparrow$ E:\Installation Files\Sartor	ius\OctetBLIDiscovery	ب 5	Search OctetBLIDiscovery	
This PC	Name	^	Date modified	Туре
3D Objects	🚭 Setup		8/8/2022 2:09 PM	Application
E. Desktop				
🗄 Documents				
🕂 Downloads				
b Music				
Pictures				
📑 Videos				
🏪 Local Disk (C:)				
🖸 DVD Drive (E:) Octet Installer 13.0				
🛖 Share (\\VBoxSvr) (S:)				
🛖 VM_Shared_Folder (\\VBoxSvr) (Z:)				
🔿 Network				
	/ <			>
1 item				

Figure 2-1: Location of Installation Program

3. Double-click **Setup.exe** in Installation Files\Sartorius\OctetBLIDiscovery. The installation wizard appears.

Cotet® BLI Discovery 13.0	Setup — 🗌 X
SVIPCTEVS	Welcome to Octet® BLI Discovery 13.0 Setup
	Setup will guide you through the installation of Octet® BLI Discovery 13.0.
	It is recommended that you close all other applications before starting Setup. This will make it possible to update relevant system files without having to reboot your computer.
	Click Next to continue.
	Next > Cancel

Figure 2-2: Software Setup Wizard

4. Click **Next** to display the Choose Install Location dialog box.

	_		×
Choose Install Location Choose the folder in which to install Octet® BLI Discovery 13.0.			Œ(
Setup will install Octet® BLI Discovery 13.0 in the following folder. T folder, dick Browse and select another folder. Click Next to continue	io install in a	different	
Destination Folder <u>C:\Program Files (x86)\Sartorius\OctetBLIDiscovery13</u>	Brov	vse	]
Space required: 206. 1 MB Space available: 78.7 GB			
Sartorius - www.sartorius.com	lext >	Cano	el

Figure 2-3: Choose Install Location Dialog Box

5. Click **Next** to accept this path location.

The Choose Start Menu Folder dialog box appears.

🖽 Octet® BLI Discovery 13.0 Setup	-	×
Choose Start Menu Folder Choose a Start Menu folder for the Octet® BL	I Discovery 13.0 shortcuts.	Œ
Select the Start Menu folder in which you woul can also enter a name to create a new folder.	d like to create the program's shortcuts.	You
Octet		
Accessibility		^
Accessories Administrative Tools		
Galil		
Greenshot		
Maintenance Microsoft Office Tools		
Oracle VM VirtualBox Guest Additions		
System Tools	ð	
Visual Studio 2019		¥
Sartorius - www.sartorius.com		
	< Back Install C	ancel

Figure 2-4: Choose Start Menu Folder Dialog Box

### 6. Click Install.

The installation wizard	takes a few	second	s to install.
🖽 Octet® BLI Discovery 13.0 Setup		-	$\Box$ $\times$
Installing Please wait while Octet® BLI Discovery 13.0 i	is being installed.		Œ
Extract: OctetBLIDiscovery.pdb			
Extract: OctetBLIDiscovery.local Extract: OctetBLIDiscovery.VisualElementsM Extract: AcquisitionEngine.dll Extract: MultiWellPlate.dll Extract: DataSecurity.dll 100%	1anifest.xml		^
Extract: PEGRP32E.DLL Extract: DMC32.dll Extract: Dmcbus32.dll Extract: Dmcser32.dll Extract: OctetBLIDiscovery.pdb	\$		*
Sartorius - www.sartorius.com	< Back	Next >	Cancel

Figure 2-5: Installation Progress

The installation wizard displays the "Completing the Octet BLI Discovery Setup Wizard' dialog box.

<b>ธงาร</b> บารง	Completing Octet® BLI Discovery 13.0 Setup
	Octet® BLI Discovery 13.0 has been installed on your computer.
	Click Finish to close Setup.
	< Back Finish Cancel

Figure 2-6: Completing the Setup

7. Click **Finish** to complete the installation.

If you are installing the 21 CFR Part 11 version of the software you will also need to install and setup the GXP Server. Go to "Installation of the Octet<sup>®</sup> GxP Server Module" on page 569 for those instructions.

# Starting the Octet<sup>®</sup> System and Octet<sup>®</sup> BLI Discovery Software

To start the system and software:

- 1. Turn on the computer.
- 2. Use the power switch located on the external electrical box to turn on the system

**NOTICE:** The instrument requires a minimum one-hour warm-up time. Sartorius also recommends leaving the instrument on for a minimum of eight hours prior to using it for the first time.

3. Launch the Octet<sup>®</sup> BLI Discovery software by double-clicking on the Octet<sup>®</sup> BLI Discovery desktop icon

NOTICE: If you have the CFR11 version, your icon will indicate that, see Figure 2-7.



Figure 2-7: Desktop Icon

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, users are required to log in and start a user session before the software will launch. Please refer to "Starting a User Session" on page 74 for more information.

## Software Overview

After the software is launched, the Octet<sup>®</sup> BLI Discovery software **Main Screen**. Screen components along with the default windows appear are shown in Figure 2-8.



Figure 2-8: Main Screen

### Main Menu and Toolbar

The Main Menu and Toolbar are located in the upper left of the Main Screen (Figure 2-9).



Figure 2-9: Main Menu and Toolbar

**NOTICE:** The Security menu is only available in the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software.

### File Menu

The **File** menu (Figure 2-10) allows users to open and save method files, view experiments, print files and set system and software options.

A method file (.fmf) contains sample plate configuration, sample plate table information, sensor assignments, and assay step information that allow the Octet<sup>®</sup> instrument and software to run an experiment. A read-only copy of the method file will automatically be saved in the experiment folder when the run is started. When the run is complete, the data in the experiment folder can be reviewed.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.



#### Figure 2-10: File Menu

#### Table 2-1: File Menu Commands (Sheet 1 of 2)

Menu Command	Toolbar Button	Function
Open Method File	<del>1</del>	Opens an experiment method file (.fmf).
Close Method File	N/A	Closes the active experiment method file but does not save changes.
Save Method File	đ	Saves the active experiment method file (.fmf).
Save All Method Files	Ċ <b>t</b>	Saves all open method files (.fmf).
Save Method File As	N/A	Save the active experiment method file as a new file without overwriting the original method file.
Open Experiment	N/A	Opens an experiment folder.
Save Experiment	N/A	Saves the active experiment.
Print	N/A	Opens the <b>Print</b> dialog box to print a file.
Print Preview	N/A	Opens a print preview window of a method or assay definition file.
Print Setup	N/A	Opens the <b>Print Setup</b> dialog box to print a file.
File History	N/A	Displays a list of previously opened files.

#### Table 2-1: File Menu Commands (Sheet 2 of 2)

Menu Command	Toolbar Button	Function
Options	N/A	Opens the <b>Options</b> dialog box. Please refer to "Octet <sup>®</sup> BLI Discovery Options" on page 23 for more information on changing system and soft- ware options.
Exit	N/A	Closes the software.

### View Menu

The **View** menu allows users to show or hide the **Toolbar** and status windows. A check mark next to the menu item indicates the option is currently shown.

<ul> <li>Toolbar</li> <li>Status Bar</li> <li>Instrument Status</li> </ul>	Vie	w Experiment Instrumen
<ul> <li>Status Bar</li> <li>Instrument Status</li> </ul>	$\checkmark$	Toolbar
Instrument Status	$\checkmark$	Status Bar
	$\checkmark$	Instrument Status

#### Figure 2-11: View Menu

Table 2-2: View Menu Commands

Menu Command	Function
Toolbar	Shows or hides the <b>Toolbar</b> .
Status Bar	Shows or hides the <b>Status bar</b> .
Instrument Status	Displays the <b>Instrument Status</b> window.

### Experiment Menu

The **Experiment** menu provides access to the **Experiment Wizard**, assay and experiment options as well as experiment templates.



Figure 2-12: Experiment Menu

#### Table 2-3: Experiment Menu Commands

Menu Command	Toolbar Button	Function
New Experiment Wizard		Opens the <b>Experiment Wizard</b> .
Edit Assay Parameters	N/A	Opens the <b>Edit Assay Parameters</b> dialog box to define a new assay, edit an existing assay, or remove an assay from the quantitation application. See "Managing Assay Parameter Settings" on page 245 for more information.
Edit Sensor Types	N/A	Opens the <b>Sensor Types</b> dialog box to view current biosensor types, add new biosensor types and remove biosensor types. See "Managing Biosen- sor Types" on page 30 for more information.
Set Plate Temperature	N/A	Opens the <b>Temperature Setting</b> dialog box that displays the current sam- ple plate temperature and allows users to change the current temperature setting of the instrument. See "Setting the Plate Temperature" on page 24 for more information. To set the default temperature, see "Defining a New Default Sample Plate Temperature" on page 25.
Templates	N/A	Allows users to select from a set of predefined quantitation or kinetics method templates.
Skip Step	N/A	Skips the step in the method that is currently executing (kinetics experi- ments only).
Stop	$\otimes$	Stops the experiment. Data from the active biosensor is not saved, but all data prior to the active biosensor will be available.

### Instrument Menu

The **Instrument** menu provides direct control of the Octet<sup>®</sup> instrument.



Figure 2-13: Instrument Menu

 Table 2-4: Instrument Menu Commands (Sheet 1 of 2)

Menu Command	Toolbar Button	Function
Reset	N/A	Resets the instrument and the log in the <b>Instrument Status window</b> .
Stop Shaker	N/A	Stops the sample plate shaker.

Menu Command	Toolbar Button	Function
Present Stage	▲	Presents the instrument stage that houses the biosensor tray, sample and reagent plates (Octet <sup>®</sup> RH16, and Octet <sup>®</sup> QK384 only).

### Table 2-4: Instrument Menu Commands (Continued) (Sheet 2 of 2)

### Security Menu

The **Security** menu is only available in the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software. For complete details on menu options, please refer to "Accessing Compliance Features" on page 76.

Sec	urity Window Help
	Verify Document View Audit Trail
	Change Project Change Password Server Administration
	Lock Application Logoff

Figure 2-14: Security Menu

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### Window Menu

The Window menu provides options for the open windows in the Main Screen.

All open windows are listed at the bottom of the menu, and a check mark indicates the window that is currently active. To view another window, select it from the list.

Wir	Idow Help	
	New Window	
	Cascade	
	Tile	
	Arrange Icons	
	1 Experiment Wizard	
	2 Instrument Status	Open Windows
	3 Basic Quantitation Experiment - Example Quantitation Method file.fmf	
	4 Basic Kinetics Experiment - BH081908-4 ADAM titration_LABUSER081908_ExpMethod.fmf	
$\checkmark$	5 Basic Quantitation Experiment - LABUSER090811_ExpMethod.fmf	

Figure 2-15: Window Menu

#### Table 2-5: Window Menu Commands

Menu Command	Function
New Window	Opens a new Runtime Binding Chart window.
Cascade	Organizes all windows in a cascade.
Tile	Tiles all windows vertically.
Arrange Icons	Arranges the minimized window icons in a row at the bottom of the screen.
Open Windows	Lists the windows currently open.

### Help Menu

The **Help** menu provides access to software and instrument support information.

#### <u>H</u>elp

Octet BLI Discovery User Guide	F1
Sartorius Web Site	
Octet Support Link	
About Octet BLI Discovery	

Figure 2-16: Help Menu

Table 2-6: Help Menu Commands (Sheet 1 of 2)

Menu Command	Toolbar Button	Function
BLI Discovery User Guide	N/A	Opens the online Octet <sup>®</sup> BLI Discovery Software User Guide.

#### Table 2-6: Help Menu Commands (Sheet 2 of 2)

Menu Command	Toolbar Button	Function	
Sartorius Web Site	N/A	Opens a web browser and displays the Sartorius web page (www.sarto-rius.com).	
Octet <sup>®</sup> Support	N/A	Opens a web browser and displays the Octet <sup>®</sup> support landing page.	
About Octet <sup>®</sup> BLI Discovery	0	Displays software, user and instrument information.	

**NOTICE:** Clicking on the Sartorius logo in the upper right corner of the **Main Screen** also displays the **About Octet**<sup>®</sup> **BLI Discovery** window.

### Status Bar

The **Status Bar** is located at the bottom of the **Main Screen** and displays current instrument and experiment status and plate temperature.

Instrument: Ready

Experiment: Not Started 🛷 Plate temp: 30 °C 🖽

#### Figure 2-17: Status Bar

In the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, the **Status Bar** also displays the User and Project name entered at login.

Instrument: Ready Experiment: Not Started 🖉 Plate temp: 35 °C 🖺 Project: Antigen:Antibody screen 👗 User: JBlack (John Black) 🔬

Figure 2-18: Status Bar with User and Project Names

### Instrument Status Window

The Instrument Status window displays a log of all instrument activity.



Figure 2-19: Instrument Status Window

Select the **Auto Scroll to bottom** check box to auto-scroll the log to display the most current events. Click **Save to File** to save a copy of the instrument log.

### NOTICES:

If a problem occurs during operation of the instrument, save a copy of the system log to assist our technical support staff in diagnosing the issue.

The instrument log automatically resets when Octet <sup>®</sup>BLI Discovery software is closed.

## Experiment Wizard

The **Experiment Wizard** guides users through the complete set up of an experiment. Using the wizard is described in detail in the Quantitation and Kinetics experiment chapters.

🖄 Experiment Wizard	
Choose an option to start	Available Templates for - Octet R8  Blank Experiment Protein A G or L biosensors_8CH_96W fmf Protein A G or L biosensors_high sensitivity_8CH_96W fmf
New Kinetics Experiment <ul> <li>Basic Kinetics</li> <li>Epitope Binning</li> </ul>	
Recent Methods	$\rightarrow$

Figure 2-20: Experiment Wizard

# Octet<sup>®</sup> BLI Discovery Options

Acquisition options allow users to set system and data preferences for quantitation and kinetic data acquisition. To view these options (Figure 2-21), click **File > Options** from the **Main Menu**.

puona		
Data Files		
Quantitation data repository:	C:\data	
Kinetics data repository:	C:\data	
	Use old 5.	.0 file format for FRD file 🔽 Use extended sample types
Simulation	the application	is configured using the properties of the selected
Octet RH96 Octe	t R2 00	ctet RED96e Octet K2
Octet RH16 Octe	tR4 ○00	ctet RED96
Octet QK384 Octe	±R8 ◯00	ctet QKe
Startup		Sensor Options - New Experiments
Temperature: 30	≜ °c	Replace sensors in tray after use
Veb Server Port: 8080	Connect as: DESKTOP- DESKTOP- 192.168.13	0EHTC34 0EHTC34 6.128
Refresh (s): 10		
Refresh (s): 10	http://DESK	TOP-0EHTC34:8080
Refresh (s): 10	http://DESK	(TOP-0EHTC34:8080
Refresh (s):         10           Automation           TCP-IP           Port:         20000	http://DESK	(TOP-0EHTC34:8080
Refresh (s):         10         Image: Constraint of the second se	http://DESK	(TOP-0EHTC34:8080
Refresh (s): 10 🖨 Automation TCP-IP Port: 20000 💠 Serial (RS232) Port: Communications Port (COM1	Localhost	CTOP-0EHTC34:8080

Figure 2-21: Options Dialog Box

 Table 2-7: User Options (Sheet 1 of 2)

Item	Description
Data Files	
Quantitation data repository	The default location where quantitation data files (.frd) are saved. Click. <b></b> ( <b>Browse</b> ) to select a different folder.
	<b>NOTICE:</b> Sartorius recommends that the data be saved to the local machine first, then transferred to a network drive if needed.
Kinetics data repository	The default location where kinetics data files (.frd) are saved. Click <b></b> ( <b>Browse</b> ) to select a different folder.
	<b>NOTICE:</b> Sartorius recommends that the data be saved to the local machine first, then transferred to a network drive if needed.

#### **Table 2-7:** User Options (Sheet 2 of 2)

ltem	Description
Use old 5.0 file format for FRD files	Select this option to save data in the earlier Octet <sup>®</sup> RED software 5.0 format.
	<b>NOTICE:</b> Saving data in the old file format produces larger files and may result in slower data analysis.
Use extended sample types	Select this option to extend the sample types available in the right-click menu of the <b>Sample Plate Map</b> and <b>Sample Plate Table</b> to include negative and positive controls.
Startup Temperature	User-defined default startup plate temperature. This temperature is used as the default setting for all experiments.
	<b>NOTICE:</b> This changes the startup plate temperature only, not the current plate temperature. The software must be restarted after entering the new value for the new setting to take affect.
Data Options	
Significant digits	Specifies the number of significant digits the software uses for Molecular Weight, Concentration and Dilution values during data analysis.
	NOTICE: Use Six decimal places for the Protein A assay.
Simulation	If the workstation is not connected to an instrument, this option enables users to create and save an experiment to a method file (.fmf) using the properties of the selected instrument type.
Web Server	Selecting this option enables remote monitoring of the experiment using a web browser. See "Designing Experiments Remotely" on page 26 for more information.
Automation	Allows users to select the appropriate connection for automation interfaces used with Octet <sup>®</sup> RH16 and Octet <sup>®</sup> QK384 systems only. For more information, please refer to Appendix A, Using Octet <sup>®</sup> RH16, Octet <sup>®</sup> RH96 and Octet <sup>®</sup> QK384 Systems with an Automation Interface on page 545.
Default Sample Plate	Select the default sample plate format to use when creating a new method file. Applies to instruments that support 96-well and 384-well plates.
Sensor Options - New Experiments	Select the default behavior for a new method created with the Experiment Wizard.

# Setting the Plate Temperature

Plate temperature range depends on the type of instrument. Please refer to the specific instrument specifications. A factory-set default plate temperature of 30 °C is used as a system startup plate temperature and the experiment default temperature. This default value can be customized by the user. In addition, the plate temperature setting can be changed for individual experiments when needed. The current plate temperature displays in the Status bar at the bottom of the Main Screen.

### Changing the Plate Temperature for Individual Experiments

To set the plate temperature to a value different than the default setting for a specific experiment:

- 1. From the Main Menu, click Experiment > Set Plate Temperature.
- 2. Select the desired temperature in the Set temperature to field (Figure 2-22) and, then click OK.



Figure 2-22: Temperature Setting

3. Allow e the sample plate to equilibrate to the new temperature before beginning an experiment. For experiments set to 25 or 30 °C, allow approximately 10 minutes for a plate at room temperature. For experiments set to 15 °C, allow approximately 20 minutes. If the temperature is increased to 30 °C from a previous run at 15 °C, then 20 minutes should be sufficient time for the plate to equilibrate.

**NOTICE:** If the Octet<sup>®</sup> BLI Discovery software is closed, the plate temperature will reset to the default startup value specified in the Options dialog box when the software is relaunched.

### Defining a New Default Sample Plate Temperature

To define a new default temperature that will be used at startup and as the default plate temperature for all experiments:

1. From the **Main Menu**, click **File > Options**.

2. In the **Options** dialog box (Figure 2-23), select a new temperature in the **Startup** box and click **OK**. The plate temperature will then adjust to the new value, and this setting will be used as the new default startup temperature whenever the software is launched.

Quantitation data repo	sitory: C·\data	C:\data		
	o. add	C:\data		
Kinetics data reposito	ry: C:\data			
	Use old 5.	Use old 5.0 file format for FRD file Use extended sample types		
Simulation				
If no instrument is cor	nnected, the application i	is configured using the p	roperties of the selected	
◯ Octet HTX	Octet QKe	Octet K2	Octet R2	
Octet QK384	Octet RED96	Octet QK	Octet R4	
Octet RED384	◯ Octet RED96e	Octet RED	Octet R8	
Startup		Sensor Options - Nev	w Experiments	
Temperature: 25 C		Replace sensors in tray after use		
Data Options		Default Sample Plate		
Significant digits:	4	96 wells	◯ 384 wells	
Web Server				

Figure 2-23: Setting the Default Startup Temperature in the Options Dialog Box

3. Allow the sample plate to equilibrate to the new temperature before beginning an experiment. For experiments set to 25 or 30 °C, allow approximately 10 minutes for a plate at room temperature. For experiments set to 15 °C, allow approximately 20 minutes for plate at room temperature. If the temperature is increased to 30 °C from a previous run at 15 °C, then allow 20 minutes for the plate to equilibrate.

**IMPORTANT:** For the new default temperature value to take affect, you must restart the software.

# Designing Experiments Remotely

You can install the BLI Discovery software on another computer, say a laptop computer in another room, and design the experiment on that laptop.

To do this, perform the following steps:

- 1. 1nstall the BLI Discovery on any Windows 10 PC following the steps described earlier in this chapter.
- 2. Start the BLI Discovery software.
- 3. On the main screen, click File > Options to open the Options dialog box.
- 4. In the Simulation group box, select the target instrument type for the experiment.
- 5. Click **OK** to accept the settings.
- 6. Use BLI Discovery to design an experiment using the instructions in this guide.
- 7. After designing the experiment, save the experiment method file to a USB device or a file server connected to the Octet<sup>®</sup> PC.
8. Start BLI Discovery software and load the experiment method file. Mount the appropriate sensor trays and sample plates and start the assay.

**NOTICE:** If you are using the CFR version of the software, the remote computer must be able to connect to the GxP Server. CFR and non-CFR method files are not interchangeable.

## Monitoring Experiments Remotely

If the Octet<sup>®</sup> system computer is connected to a local network, experiment progress can be monitored remotely from any networked computer, smartphone or mobile device using any web browser. In addition, instrument log files and previously run experiments can also be accessed remotely for review.

- 1. From the **Main Menu**, click **File** > **Options**.
- In the Options dialog box (Figure 2-25), select the Web Server check box. Adjust the Port and Refresh settings and change the Connect as IP address if needed. The default Refresh rate of 10 will refresh the experiment view in the web browser every 10 seconds. Click OK.

**NOTICE:** Sartorius recommends using the **Port** and **Connect as** (IP address) settings shown as default in the **Web Server** box, as they are unique to your Octet<sup>®</sup> system.

Options	×	
Data Files Quantitation data repository: C:\Temp Kinetics data repository: C:\Temp		
Simulation If no instrument is connected, the applicat	tion is configured using the properties of the selected	
Octet HTX Octet QKe Octet QK384 Octet RED9 Octet RED384 Octet RED9	Octet K2         Octet R2           5         Octet QK         Octet R4           6e         Octet RED         Octet R8	
Temperature: 30 C	Sensor Options - New Experiments	
Data Options Significant digits:	Default Sample Plate <ul> <li>96 wells</li> <li>384 wells</li> </ul>	
✓ Web Server         Connect as:           Port:         8080          DESKTOP-0EHTC34           DESKTOP-0EHTC34         192.168.136.128           http://DESKTOP-0EHTC34:8080         http://DESKTOP-0EHTC34:8080		
Automation		

Figure 2-24: Selecting the Web Server in the Options Dialog Box

3. Click **File** > **Options** to access the **Options** dialog box again. A **Web Server URL** will now be listed under the **Connect as** box (Figure 2-25). Record this URL as it will be needed to access the experiment remotely.



Figure 2-25: Web Server URL

- 4. Start the experiment in the Octet<sup>®</sup> BLI Discovery software as you normally would.
- 5. Open a web browser on a remote computer or device that is on the same network as the Octet<sup>®</sup> system.

**NOTICE:** The remote computer or device must be on the same network as the Octet<sup>®</sup> system, or connected to the network the instrument is on via VPN.

6. Enter the **Web Server URL** in the browser window or click the **Web Server URL** link in the **Options** dialog box. The experiment in progress appears(Figure 2-26).



Figure 2-26: View of Quantitation Experiment (top) and Kinetics Experiment (bottom) via Web Browser

- 7. In the browser window, you can:
  - Click the experiment name to view experiment details.
  - · Click Log File to display a log of current instrument activity.
  - Click **Kinetics Data Repository** or **Quantitation Data Repository** to open and view previously run experiments.

## Managing Biosensor Types

The Octet<sup>®</sup> BLI Discovery software includes a default list of all the types of biosensors available for quantitation or kinetic analysis. The available biosensor types appears in the Sensor Assignment tab. Users can add custom biosensors as needed.

### Viewing Available Biosensor Types

To view the available types of biosensors, from the Main Menu, click Experiment > Edit Sensor Types.

Sensor Types Quantitation Sensors Kinetics Sensors Anti-Human IaG F SA (Streptavidin) Add. Add.. Anti-Mouse IgG Fv SAX (High Precision Streptavidin) SAX2 (High Precision Streptavidin 2.0 Anti-GST Delete AHC (Anti-hlgG Fc Capture) FAB2G (Anti-Fab 2nd generation) HIS2 (Anti-HIS) Anti-GST Anti-CHO HCP FAB2G (Anti-Fab 2nd generation) Protein A HIS1K (Anti-Penta-HIS) Protein G APS (Aminopropylsilane) Protein L AR2G (Amine Reactive 2nd Gen) GlyS (Sialic Acid) SSA (Super Streptavidin) GlyM (Mannose) AMC (Anti-mlgG Fc Capture) SA (Streptavidin) Ni-NTA SAX (High Precision Streptavidin) Custom SAX2 (High Precision Streptavidin) Residual Protein A HIS1K (Anti-Penta-HIS) Ni-NTÀ Close

The Sensor Types window appears (Figure 2-27).

Figure 2-27: Sensor Types Dialog Box



## Adding a Biosensor Type

To add a biosensor type:

- 1. From the Main Menu, click Experiment > Edit Sensor Types.
- 2. In the **Sensor Types** window (Figure 2-28), click **Add** next to the **Quantitation Sensors** or **Kinetic Sensors** box (depending on the type of biosensor that will be added).
- 3. In the Add Sensor dialog box, enter a name for the biosensor type and click OK.

Quantitation Sensors				Kinetic	s Sensors			
Anti-Human IgG Fc		Add		SA (S	treptavidin)	<b>a 1 1 1</b>	Add	
Anti-Mouse IgG FV Anti-GST		Dele	te	SAX (	(Anti-hIaG Ec (	Streptavidir Capture)	Delete	=
Anti-Human Fab-CH1 (F	AB)	Dele	œ	Anti-	GST		Delete	-
Anti-FLAG (FLG)				Anti-ł	Human Fab-CH	1 (FAB)		
Anti-HIS (HIS2)	Add Sen	sor				×		
Protein A								
Protein G	Name:				OK	d Gen		
Protein L SA (Streptavidin)				1	UK			
SAX (High Precision St	Ľ.				Cancel			
Residual Protein A						_		
Anti-Penta-HIS			_	Prote	in A			
Custom				riote		_		

Figure 2-28: Adding a Biosensor Type

## Removing a Biosensor Type

To remove a biosensor type, select the biosensor name in the **Quantitation Sensors** or **Kinetic Sensors** box and click **Delete**.

**NOTICE:** The default software biosensor types cannot be deleted. Only the biosensor types that users add to the system can be deleted.

### Chapter 3:

# Octet<sup>®</sup> System Specifications and Site Requirements

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**NOTICE:** The names of the following instruments have changed.

- Octet<sup>®</sup> HTX is now the Octet<sup>®</sup> RH96
- Octet<sup>®</sup> RED384 is now the Octet<sup>®</sup> RH16

## Getting Started

#### All users must read the following safety information.



**WARNING:** Do not operate the Octet<sup>®</sup> system in any other way than described in the user manual. Failure to comply may expose you to hazards that can lead to personal injury and may cause damage to the equipment.



**WARNING:** Octet<sup>®</sup> systems should only be installed, relocated, and/or moved by trained Sartorius personnel. To obtain more information, please contact Sartorius Technical Support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel installing, relocating and/or moving an Octet<sup>®</sup> system.

For more information on and safety precautions for the supplied computer and computer equipment, please refer to the manufacturer's documentation supplied with the computer packaging.

## Product Labeling Definitions

#### Table 3-1: Label Definitions

Symbol	Definition
CE	The system complies with applicable European directives.
	The system complies with the requirements for electromagnetic compliance (EMC) in Austra- lia and New Zealand.
FC	The electromagnetic interference from this system is under limits approved by the Federal Communications Commission (United States).
X	Electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. lease contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	High voltage; potential electrical shock hazard.
$\underline{\mathbb{A}}$	Keep hands clear of moving parts.

## Octet<sup>®</sup> RED96 System Specifications and Site Requirements



Figure 3-1: OCTET-RED96 Model Instrument–Door Closed (Left) or Open (Right)

**IMPORTANT:** Using 96-well half-area plates on the Octet<sup>®</sup> RED96 system will result in non-optimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

### System Specifications

Table 3-2. OCTET-RED96 Mode	System Specifications (Sheet 1 of 2)
TUDICO Z. OCTET RED/OT TOUC	System Speemedicins (Sheet 1 of 2)

Item	Description
Model	OCTET-RED96
Equipment Classifications	<ul> <li>Product Classification: Class 1: Detachable power cord</li> <li>Installation/Overvoltage Category: Category II</li> <li>Pollution Degree: Degree 2</li> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>
Environmental	<ul> <li>Storage Temperature: 0 to 40 °C</li> <li>Optimum Operating Temperature: 22 ± 2 °C</li> <li>Safe Operating Temperature: 15 to 30 °C</li> <li>Humidity: Non-condensing, 10 to 80% Relative Humidity</li> <li>Indoor Use Only</li> <li>Operating Altitude: 0 to 2,000 meters</li> <li>Not for use in an environment with an explosive atmosphere</li> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>

ltem	Description	
Compliance	Nemko NRTL/C, CB Scheme	
	• CE compliance as indicated on the Instrument Identification and Safety Label.	
	<ul> <li>This device has been tested for conformity for use in a laboratory environment.</li> <li>Radio interference may occur if device is used in a domestic environment.</li> </ul>	
Capabilities	Protein quantitation	
	• Kinetic and affinity analyses ( $k_{obs}$ , $k_{a}$ , $k_{d}$ , $K_{D}$ )	
	Binding specificity and cooperativity	
	Kinetic screening of proteins, peptides, and other biomolecules	
	Kinetic analysis of small molecule and fragments	
	Recommended for analyte molecular weight of 150 Da or higher	
Sampling Format	<ul> <li>Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate</li> </ul>	
	Single sample plate capacity	
Sampling Volume	180-220 μL/well (96-well plate)	
Sample Types	Purified samples, common culture media, crude lysates	
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration an or re-racking	
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color	
Optics and Mechanics	8-channel biosensor manifold	
	Optical interferometer	
	Eight spectrometers (one dedicated spectrometer per biosensor)	
Throughput	• Up to 8 biosensors in parallel, maximum of 96 tests unattended	
	One 96-well plate and one biosensor tray at once	
Orbital Flow Capacity	Static or 100-1,500 rpm	
Sample Temperature Range	(Ambient + 4 °C)-40 °C, 1 °C increments	
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)	
Weight	63 lb (28.6 kg)	
Electrical Requirements	• Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max	
·	• Power consumption: 120 W (240 W peak)	
IMPORTANT: Use the poi	wer cords provided by Sartorius or a suitable AC cord with ratings of 60 C. 300 V. 16 AWG	

#### Table 3-2: OCTET-RED96 Model System Specifications (Sheet 2 of 2)

**IMPORTANT:** Use the power cords provided by Sartorius or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

## Octet<sup>®</sup> RH16 System Specifications and Site Requirements



Automatic sliding door

Figure 3-2: OCTET-RH16 Model – Door Closed

#### NOTICE: The Octet® RED384 is now the Octet® RH16.

**NOTICE:** In Octet<sup>®</sup> BLI Discovery software Release 8.0 or later, the Sample plate and Reagent plate are referred to as Plate 1 and Plate 2.



**WARNING:** Moving the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Sartorius service personnel. To obtain more information, please contact Sartorius technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

### Instrument Identification and Safety Labeling

Please see "Octet<sup>®</sup> Systems Safety Information" on page 2 for definitions of symbols



Figure 3-3: OCTET-RH16 Model Rear Panel Label

## System Specifications

#### Table 3-3: OCTET-RH16 Model System Specifications (Sheet 1 of 3)

Item	Description
Model	OCTET-RH16
Equipment Classifications	<ul> <li>Product Classification: Class 1: Detachable power cord</li> <li>Installation/Overvoltage Category: Category II</li> <li>Pollution Degree: Degree 2</li> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>
Environmental	<ul> <li>Storage Temperature: 0 to 40 °C</li> <li>Optimum Operating Temperature: 22 ± 2 °C</li> <li>Safe Operating Temperature: 15 to 30 °C</li> <li>Humidity: Non-condensing, 10 to 80% Relative Humidity</li> <li>Indoor Use Only</li> <li>Operating Altitude: 0 to 2,000 meters</li> <li>Not for use in an environment with an explosive atmosphere</li> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>
Compliance	<ul> <li>Nemko NRTL/C, CB Scheme</li> <li>CE compliance as indicated on the Instrument Identification and Safety Label.</li> <li>This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if device is used in a domestic environment.</li> </ul>
Capabilities	<ul> <li>Protein quantitation</li> <li>Kinetic and affinity analyses (k<sub>obs</sub>, k<sub>a</sub>, k<sub>d</sub>, K<sub>D</sub>)</li> <li>Binding specificity and cooperativity</li> <li>Kinetic screening</li> <li>kinetic analysis of small molecules</li> </ul>

#### Table 3-3: OCTET-RH16 Model System Specifications (Sheet 2 of 3)

Item	Description
Sampling Format	Required plates:
	<ul> <li>96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate</li> </ul>
	• 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209)
	<ul> <li>384-well black, tilted-bottom polypropylene (Sartorius, #18-5076 or #18- 5080), SBS standard microplate</li> </ul>
	Two plate stations
	Test volume:
	+ 180–300 $\mu$ L in a 96-well plate, non-destructive and recoverable
	+ 80–130 $\mu$ L in a 384-well plate, non-destructive and recoverable
	+ 40–100 $\mu L$ in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/ or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Automation	Up to 16 biosensors in parallel
	<ul> <li>Ability to integrate the Octet<sup>®</sup> instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling</li> </ul>
Optics and Mechanics	16-channel biosensor manifold
	Optical interferometer
	<ul> <li>Sample plate platform temperature range: from 4 °C above ambient to 40 °C</li> </ul>
	16 spectrometers (one dedicated spectrometer per biosensor)
Throughput	• Up to 16 biosensors in parallel, maximum of 384 tests unattended
	<ul> <li>Two microplates, either 96- or 384-well at once. Only one plate can be used for samples. The second plate is used for reagents.</li> </ul>
Orbital Flow Capacity	Static or 100–1,500 rpm
Sample Temperature Range	(Ambient + 4 °C)-40 °C, 1 °C increments
Dimensions	30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D)
Weight	150 lb (68 kg)
Electrical Requirements	• Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max
	<ul> <li>Power consumption: 195 W (240 W peak)</li> </ul>

 Table 3-3:
 OCTET-RH16 Model System Specifications (Sheet 3 of 3)

#### Item

Description

**IMPORTANT:** Use the power cords provided by Sartorius or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

Table 3-4: Sensor Offset and Well Volumes for OCTET-RH16 and OCTET-QK384 Models

Sensor Offset (mm)	Rec	Recommended Minimum Fill Volume (µL)			
	96-well plate (Greiner Bio-One)	384-well plate (Greiner Bio-One)	384-well tilted bottom plate (Sartorius, 384TW)		
3	200	80	40		
4	200	80	60		
5	225	100	80		
6	250	120	100		
7	300	130	100		

## Octet<sup>®</sup> QKe System Specifications and Site Requirements



Figure 3-4: OCTET-QKE Model Instrument–Door Closed (Left) or Open (Right)

### Instrument Identification and Safety Labeling

Please see "Octet<sup>®</sup> Systems Safety Information" on page 2 for definitions of symbols.



Figure 3-5: OCTET-QKE Model – Rear Panel Label

## System Specifications

#### Table 3-5: OCTET-QKE Model System Specifications (Sheet 1 of 2)

Item	Description
Model	OCTET-QKE
Equipment Classifications	<ul> <li>Product Classification: Class 1: Detachable power cord</li> <li>Installation/Overvoltage Category: Category II</li> <li>Pollution Degree: Degree 2</li> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>
Environmental	<ul> <li>Storage Temperature: 0 to 40 °C</li> <li>Optimum Operating Temperature: 22 ± 2 °C</li> <li>Safe Operating Temperature: 15 to 30 °C</li> <li>Humidity: Non-condensing, 10 to 80% Relative Humidity</li> <li>Indoor Use Only</li> <li>Operating Altitude: 0 to 2,000 meters</li> <li>Not for use in an environment with an explosive atmosphere</li> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>
Compliance	<ul> <li>Nemko NRTL/C, CB Scheme</li> <li>CE compliance as indicated on the Instrument Identification and Safety Label.</li> <li>This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if device is used in a domestic environment.</li> </ul>
Capabilities	<ul> <li>Protein quantitation</li> <li>Kinetic and affinity analyses (k<sub>obs</sub>, k<sub>a</sub>, k<sub>d</sub>, K<sub>D</sub>)</li> <li>Binding specificity and cooperativity</li> <li>Kinetic screening of proteins, peptides and other biomolecules</li> <li>Biosensor re-racking</li> <li>Recommended analyte molecular weight is 5,000 Da or higher</li> </ul>
Sampling Format	<ul> <li>Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209), SBS standard microplate</li> <li>Single sample plate capacity</li> </ul>
Sample Volume	180–220 μL/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regenera- tion and/or re-racking

#### Table 3-5: OCTET-QKE Model System Specifications (Sheet 2 of 2)

Item	Description	
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color	
Optics and Mechanics	8-channel biosensor manifold	
	Optical interferometer	
	One spectrometer (shared by eight biosensors)	
Throughput	• Up to eight biosensors in parallel, maximum of 96 tests unattended	
	One 96-well plate and one biosensor tray at once	
Orbital Flow Capacity	Static or 100–1,500 rpm	
Sample Temperature Range	(Ambient + 4 °C)-40 °C, 1 °C increments	
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)	
Weight	54 lb (24.5 kg)	
Electrical Requirements	• Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max	
	<ul> <li>Power consumption: 120 W (240 W peak)</li> </ul>	
IMPORTANT: Use the power co	rds provided by Sartorius or a suitable AC cord with ratings of 60 C 300 V 16 AWG	

**IMPORTANT:** Use the power cords provided by Sartorius or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

## Octet<sup>®</sup> QK384 System Specifications and Site Requirements



Figure 3-6: OCTET-QK384 Model – Door Closed (Left) or Open (Right)

**NOTICE:** In Octet<sup>®</sup> BLI Discovery software Release 8.0 or later, the Sample plate and Reagent plate are referred to as Plate 1 and Plate 2.



**WARNING:** Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Sartorius service personnel. To obtain more information, please contact Sartorius technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

System Specifications The following table has the Octet® QK384 System Specifications:

<b>Table 3-6:</b> OC <sup>-</sup>	TET-QK384	Model System	Specifications	(Sheet 1 of 3)
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ltem	Description		
Model	OCTET-QK384		
Equipment Classifications	Product Classification: Class 1: Detachable power cord		
	Installation/Overvoltage Category: Category II		
	Pollution Degree: Degree 2		
	<ul> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>		
Environmental	• Storage Temperature: 0 to 40 °C		
	<ul> <li>Optimum Operating Temperature: 22 ± 2 °C</li> </ul>		
	<ul> <li>Safe Operating Temperature: 15 to 30 °C</li> </ul>		
	Humidity: Non-condensing, 10 to 80% Relative Humidity		
	Indoor Use Only		
	Operating Altitude: 0 to 2,000 meters		
	Not for use in an environment with an explosive atmosphere		
	<ul> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>		
Compliance	Nemko NRTL/C		
	• CE compliance as indicated on the Instrument Identification and Safety Label.		
	<ul> <li>This device has been tested for conformity for use in a laboratory environment.</li> <li>Radio interference may occur if device is used in a domestic environment.</li> </ul>		
Capabilities	Protein quantitation		
	• Kinetic and affinity analyses ( $k_{obs}$ , $k_{a'}$ , $k_{d'}$ , $K_{D}$ )		
	Binding specificity and cooperativity		
	Kinetic screening		

#### Description Item Sampling Format Required plates: • 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate • 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209) 384-well black, tilted-bottom polypropylene microplate (Sartorius, #18-5076 or #18-5080), SBS standard microplate Two plate stations Test volume: • 180–300 μL in a 96-well plate, non-destructive and recoverable • 80-130 μL in a 384-well plate, non-destructive and recoverable • 40-100 μL in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable Sample Types Purified samples, common culture media, crude lysates **Biosensor** Type Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/ or re-racking **Biosensor Tray Type** 8 x 12 format 96-biosensor tray, green color • Up to 16 biosensors in parallel Automation • Ability to integrate the Octet<sup>®</sup> instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling 16-channel biosensor manifold **Optics and Mechanics** Optical interferometer Sample plate platform temperature range: From 4 °C above ambient to 40 °C • 2 spectrometers (one dedicated spectrometer per eight biosensors) Throughput • Up to 16 biosensors in parallel, maximum of 384 tests unattended Two microplates, either 96- or 384-well at once. Only one plate can be used for samples. The second plate is used for reagents. **Orbital Flow Capacity** Static or 100-1,500 rpm Sample Temperature (Ambient + 4 °C)-40 °C, 1 °C increments Range Dimensions 30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D) Weight 150 lb (68 kg) **Electrical Requirements** Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max

#### Table 3-6: OCTET-QK384 Model System Specifications (Sheet 2 of 3)

• Power consumption: 195 W (240 W peak)

#### Table 3-6: OCTET-QK384 Model System Specifications (Sheet 3 of 3)

Item Description
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**IMPORTANT:** Use the power cords provided by Sartorius or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

Table 3-7: Sensor Offset and Well Volumes for OCTET-RH16 and OCTET-QK384 Models

Sensor Offset (mm)	Recommended Minimum Fill Volume (μL)		
	96-well plate (Greiner Bio-One)	384-well plate (Greiner Bio-One)	384-well tilted bottom plate (Sartorius, 384TW)
3	200	80	40
4	200	80	60
5	225	100	80
6	250	120	100
7	300	130	100

## Octet<sup>®</sup> RH96 System Specifications and Site Requirements



Figure 3-7: OCTET-RH96 Model – Door Closed

#### NOTICE: The HTX is now the Octet® RH96



**WARNING:** Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Sartorius service personnel. To obtain more information, please contact Sartorius technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

### Instrument Identification and Safety Labeling

Please see "Octet<sup>®</sup> Systems Safety Information" on page 2 for definitions of symbols.



Figure 3-8: Octet-RH96 Model – Rear Panel Label

## System Specifications

#### Table 3-8: OCTET-RH96 Model System Specifications (Sheet 1 of 3)

Item	Description
Model	OCTET-RH96
Equipment Classifications	<ul> <li>Product Classification: Class 1: Detachable power cord</li> <li>Installation/Overvoltage Category: Category II</li> <li>Pollution Degree: Degree 2</li> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>
Environmental	<ul> <li>Storage Temperature: 0 to 40 °C</li> <li>Optimum Operating Temperature: 22 ± 2 °C</li> <li>Safe Operating Temperature: 15 to 30 °C</li> <li>Humidity: Non-condensing, 10 to 80% Relative Humidity</li> <li>Indoor Use Only</li> <li>Operating Altitude: 0 to 2,000 meters</li> <li>Not for use in an environment with an explosive atmosphere</li> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>
Compliance	<ul> <li>Nemko NRTL/C, CB Scheme</li> <li>CE compliance as indicated on the Instrument Identification and Safety Label.</li> <li>This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if device is used in a domestic environment.</li> </ul>
Capabilities	<ul> <li>Protein quantitation</li> <li>Kinetic and affinity analyses (k<sub>obs</sub>, k<sub>a</sub>, k<sub>d</sub>, K<sub>D</sub>)</li> <li>Binding specificity and cooperativity</li> <li>Kinetic screening</li> <li>Small molecule kinetic analysis</li> </ul>

Item	Description	
Sampling Format	Required plates:	
	<ul> <li>96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate</li> </ul>	
	• 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209)	
	<ul> <li>384-well black, tilted-bottom polypropylene (Sartorius, #18-5076 or #18- 5080), SBS standard microplate</li> </ul>	
	Two plate stations	
	Test volume:	
	• 180–300 $\mu$ L in a 96-well plate, non-destructive and recoverable	
	• 80–130 $\mu$ L in a 384-well plate, non-destructive and recoverable	
	<ul> <li>40–100 μL in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable</li> </ul>	
Sample Types	Purified samples, common culture media, crude lysates	
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/ or re-racking	
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color	
Automation	Up to 96 biosensors in parallel	
	<ul> <li>Ability to integrate the Octet<sup>®</sup> instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling</li> </ul>	
Optics and Mechanics	• 8, 16, 32, 48 and 96-channel biosensor manifold	
	Optical interferometer	
	- Sample plate platform temperature range: from 4 °C above ambient to 40 °C	
	<ul> <li>16 spectrometers (selectable: one dedicated spectrometer per biosensor, up to one dedicated spectrometer per six biosensors).</li> </ul>	
Throughput	• Up to 96 biosensors in parallel, maximum of 384 tests unattended.	
	<ul> <li>Two microplates, either 96- or 384-well at once. Either or both plates may be used for samples or reagents.</li> </ul>	
Orbital Flow Capacity	Static or 100–1,500 rpm	
Sample Temperature Range	(Ambient + 4 °C)-40 °C, 1 °C increments	
Dimensions	30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D)	
Weight	200 lb (90.7 kg)	
Electrical Requirements	• Mains: 100-120/200-240 VAC, 50/60 Hz, 5 A max	
	<ul> <li>Power consumption: 195 W (240 W peak)</li> </ul>	

#### Table 3-8: OCTET-RH96 Model System Specifications (Sheet 2 of 3)

Item

#### Table 3-8: OCTET-RH96 Model System Specifications (Sheet 3 of 3)

Description

**IMPORTANT:** Use the power cords provided by Sartorius or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

Table 3-9: Sensor Offset and Well Volumes for the Octet<sup>®</sup> RH96 System

Sensor Offset (mm)	Recommended Minimum Fill Volume (µL)		
	96-well plate (Greiner Bio-One)	384-well plate (Greiner Bio-One)	384-well tilted bottom plate (Sartorius, 384TW)
3	200	80	40
4	200	80	60
5	225	100	80
6	250	120	100
7	300	130	100

## Octet<sup>®</sup> K2 Specifications and Site Requirements

The Octet<sup>®</sup> K2 system is a benchtop instrument that should be installed on a standard, non-flammable laboratory bench with a sufficient weight capacity.

The shipping weight of the Octet<sup>®</sup> K2 system (instrument, computer, and accessories ship together) is about 180 lbs (81.6 kg), and measures 48" x 32" x 46" (121.9 cm x81.3 cm x 116.8 cm).

Contents of the system as shipped include:

- The Octet<sup>®</sup> K2 instrument
- Package of 10 disposable tray liners for spent biosensors
- Software Installation CD
- Instrument Settings Backup CD
- Octet<sup>®</sup> mouse pad
- Octet<sup>®</sup> Software License agreement
- Communication cable set to connect instrument to computer workstation
- Dell computer with included Dell power cord, mouse, keyboard, and monitor connection adapters
- · Dell monitor with monitor cables and power cord
- Instrument power cord or cords dependent on end user country



Figure 3-9: OCTET-K2 Model - Door Closed (Left) or Open (Right)





**WARNING:** Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Sartorius service personnel. To obtain more information, please contact Sartorius technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

**WARNING:** Using 96-well half-area plates on the Octet<sup>®</sup> K2 system will result in non-optimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

Do not block the air inlet and outlet vents on the rear and bottom side of the instrument.

## Instrument Identification and Safety Labeling

Please see "Octet<sup>®</sup> Systems Safety Information" on page 2 for definitions of symbols.

Instrument Control	SVIFCTFA3	FB-8XXXX
AC Power	Sartorius BioAnalytical Instruments Inc. 47661 Fremont Blvd. Fremont, CA, 94538,USA	CE MARNING Cancer & Reproductive Harm
100-120/200-240V~	Model: OCTET-K2 SN: FB-8XXXX 2021-01	
50/60Hz 4A Max 2X T4AL 250V	Sartorius Lab Instruments GmbH & Co. KG 37070 Goettingen, Germany	

Figure 3-11: OCTET-K2 Model – Rear Panel Label

### System Specifications

 Table 3-10: OCTET-K2 Model System Specifications (Sheet 1 of 2)

Item	Description		
Model	OCTET-K2		
Equipment Classifications	<ul> <li>Product Classification: Class 1: Detachable power cord</li> <li>Installation/Overvoltage Category: Category II</li> <li>Pollution Degree: Degree 2</li> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>		
Environmental	<ul> <li>Storage Temperature: 0 to 40 °C</li> <li>Optimum Operating Temperature: 22 ± 2 °C</li> <li>Safe Operating Temperature: 15 to 30 °C</li> <li>Humidity: Non-condensing, 10 to 80% Relative Humidity</li> <li>Indoor Use Only</li> <li>Operating Altitude: 0 to 2,000 meters</li> <li>Not for use in an environment with an explosive atmosphere</li> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>		
Compliance	<ul> <li>Nemko NRTL/C, CB Scheme</li> <li>CE compliance as indicated on the Instrument Identification and Safety Label.</li> <li>Korea RRA/KC EMC Registration (KN11 and KN/61000-6-1:2016).</li> <li>This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if it used in a domestic environment</li> </ul>		
Capabilities	<ul> <li>Protein quantitation</li> <li>Kinetic and affinity analyses (k<sub>obs</sub>, k<sub>a</sub>, k<sub>d</sub>, K<sub>D</sub>)</li> <li>Binding specificity and cooperativity</li> <li>Kinetic analysis of proteins, peptides, and other biomolecules</li> <li>Kinetic analysis of small molecule and fragment</li> <li>Recommended analyte molecular weight of 150 Da or higher</li> </ul>		

ltem	Description	
Sampling Format	<ul> <li>Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate</li> </ul>	
	Single sample plate capacity	
Sampling Volume	180-220 μL/well (96-well plate)	
Sample Types	Purified samples, common culture media, crude lysates	
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/ or re-racking	
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color	
Optics and Mechanics	2-channel biosensor manifold     Optical interferemeter	
	<ul> <li>2 spectrometers (one dedicated spectrometer per biosensor)</li> </ul>	
Throughput	<ul> <li>Up to 2 biosensors in parallel, maximum of 96 tests unattended, subject to total assay time</li> </ul>	
	One 96-well plate and one biosensor tray at once	
Orbital Flow Capacity	Static or 400–1,500 rpm	
Sample Temperature Range	(Ambient + 4 °C)-40 °C, 1°C increments	
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)	
Weight	• 58 lb (26.3 kg)	
Electrical Requirements	• Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max	
	• Power consumption: 100 W (240 W peak)	

#### Table 3-10: OCTET-K2 Model System Specifications (Sheet 2 of 2)

#### IMPORTANT:

- Only use the power cords provided by Sartorius or a AC cord rated 60 C, 300 V, 16 AWG or better.
- Do not connect the system and computer to an electrical circuit with high intermittent power draws such as refrigerators, freezers, compressors, or vacuum pumps.
- f your site has a history of power outages, spikes, and/or drops, use an on-line uninterpreted power supply (UPS) to power the instrument and computer. Your Sartorius service representative can provide specifications for the recommended UPS system.

## Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, and Octet<sup>®</sup> R8, System Specifications and Site Requirements

The Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, and Octet<sup>®</sup> R8 systems are benchtop instruments that should be installed on a standard, non-flammable laboratory bench with a sufficient weight capacity.

The shipping weight of the Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, and Octet<sup>®</sup> R8 systems (instrument, computer, and accessories ship together) is about 180 lbs (81.6 kg), and measures 48" x 32" x 46" (121.9 cm x 81.3 cm x 116.8 cm).

Contents of the system as shipped include:

- The Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> R8 instrument
- Package of 10 disposable tray liners for spent biosensors
- Octet<sup>®</sup> R8 only: Package of 3 evaporation covers
- Software Installation CD
- Instrument Settings Backup CD
- Octet<sup>®</sup> mouse pad
- Octet<sup>®</sup> Software License agreement
- Communication cable set to connect instrument to computer workstation
- Dell computer with included Dell power cord, mouse, keyboard, and monitor connection adapters
- Dell monitor with monitor cables and power cord
- Instrument power cord or cords dependent on end user country





Figure 3-12: OCTET-R2, OCTET-R4, and OCTET-R8 Models - Door Closed



Figure 3-13: Models OCTET-R2, OCTET-R4, and OCTET-R8 Models - Rear View



**WARNING:** Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Sartorius service personnel. To obtain more information, please contact Sartorius technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the systems.

**WARNING:** Using 96-well half-area plates on the Octet<sup>®</sup> R2, Octet <sup>®</sup> R4, and Octet<sup>®</sup> R8 system will result in non-optimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

Do not block the air inlet and outlet vents on the rear and bottom side of the instrument.

### Instrument Identification and Safety Labeling

Please see "Octet<sup>®</sup> Systems Safety Information" on page 2 for definitions of symbols.



Figure 3-14: OCTET-R2, OCTET-R4, and OCTET-R8 Models – Rear Panel Labels

## System Specifications

Table 3-11: OCTET-R2, OCTET-R4, and OCTET-R8 Models System Specifications (Sheet 1 of 3)

Item	Description
Models	OCTET-R2 OCTET-R4 OCTET-R8
Equipment Classifications	<ul> <li>Product Classification: Class 1: Detachable power cord</li> <li>Installation/Overvoltage Category: Category II</li> <li>Pollution Degree: Degree 2</li> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>

Item	Description	
Environmental	<ul> <li>Storage Temperature: 0 to 40 °C</li> <li>Optimum Operating Temperature: 22 ± 2 °C</li> <li>Safe Operating Temperature: 15 to 30 °C</li> <li>Humidity: Non-condensing, 10 to 80% Relative Humidity</li> <li>Indoor Use Only</li> <li>Operating Altitude: 0 to 2,000 meters</li> <li>Not for use in an environment with an explosive atmosphere</li> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>	
Compliance	<ul> <li>Nemko NRTL/C, CB Scheme</li> <li>CE compliance as indicated on the Instrument Identification and Safety Label.</li> <li>Korea RRA/KC EMC Registration (KN11 and KN/61000-6-1:2016).</li> <li>This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if it used in a domestic environment</li> </ul>	
Capabilities	<ul> <li>Protein quantitation</li> <li>Kinetic and affinity analyses (k<sub>obs</sub>, k<sub>a</sub>, k<sub>d</sub>, K<sub>D</sub>)</li> <li>Binding specificity and cooperativity</li> <li>Kinetic analysis of proteins, peptides, and other biomolecules</li> <li>Kinetic analysis of small molecule and fragment</li> <li>Recommended analyte molecular weight of 150 Da or higher</li> </ul>	
Sampling Format	<ul> <li>Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate</li> <li>Single sample plate capacity</li> </ul>	
Sampling Volume	180-220 μL/well (96-well plate)	
Sample Types	Purified samples, common culture media, crude lysates	
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/ or re-racking	
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color	
Optics and Mechanics	<ul> <li>OCTET-R2: 2-channel biosensor manifold</li> <li>OCTET-R4: 4-channel biosensor manifold</li> <li>OCTET-R8: 8-channel biosensor manifold</li> <li>Optical interferometer</li> <li>2/4/8 spectrometers for Octet-R2, Octet-R4 and Octet-R8 (one dedicated spectrometer per biosensor)</li> </ul>	

Table 3-11: OCTET-R2, OCTET-R4, and OCTET-R8 Models System Specifications (Sheet 2 of 3)

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5	7

 Table 3-11: OCTET- R2, OCTET-R4, and OCTET-R8 Models System Specifications (Sheet 3 of 3)

Item	Description
Throughput	<ul> <li>Up to 2/4/8 biosensors in parallel, maximum of 96 tests unattended, subject to total assay time</li> </ul>
	One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Sample Temperature Range	15–40 °C, 1 °C increments
Dimensions	19.5" H x 22" W x 18.2" D (49 cm H x 56 cm W x 46 cm D)
Weight	<ul> <li>OCTET-R2: 71 lb (32.2 kg)</li> <li>OCTET-R4: 72 lb (32.7 kg)</li> <li>OCTET-R8: 76 lb (34.5 kg)</li> </ul>
Electrical Requirements	<ul> <li>Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max</li> <li>Power consumption: 100 W (240 W peak)</li> </ul>

#### IMPORTANT:

- Only use the power cords provided by Sartorius or a AC cord rated 60 C, 300 V, 16 AWG or better.
- Do not connect the system and computer to an electrical circuit with high intermittent power draws such as refrigerators, freezers, compressors, or vacuum pumps.
- f your site has a history of power outages, spikes, and/or drops, use an on-line uninterpreted power supply (UPS) to power the instrument and computer. Your Sartorius service representative can provide specifications for the recommended UPS system.

## Octet<sup>®</sup> RED96e System Specifications and Site Requirements

The Octet<sup>®</sup> RED96e system is a benchtop instrument that should be installed on a standard, non-flammable laboratory bench with a sufficient weight capacity.

The shipping weight of the Octet<sup>®</sup> RED96e system (instrument, computer, and accessories ship together) is 180 lbs (81.6 kg), and measures 48" x 32" x 46" (121.9 cm x 81.3 cm x 116.8 cm).

Contents of the system as shipped include:

- The Octet<sup>®</sup> RED96e instrument
- Package of 10 disposable tray liners for spent biosensors
- Package of 3 evaporation covers
- Software Installation CD
- Instrument Settings Backup CD
- Octet<sup>®</sup>mouse pad
- Octet<sup>®</sup>Software License agreement
- · Communication cable set to connect instrument to computer workstation
- Dell computer with included Dell power cord, mouse, keyboard, and monitor connection adapters
- Dell monitor with monitor cables and power cord
- Instrument power cord or cords dependent on end user country



Figure 3-15: OCTET-RED96E Model - Door Closed (Left) or Open (Right)



#### Figure 3-16: OCTET-RED96E Model - Rear View



**WARNING:** Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Sartorius service personnel. To obtain more information, please contact Sartorius technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

#### IMPORTANT:

Using 96-well half-area plates on the Octet<sup>®</sup> RED96e system will result in non-optimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

Do not block the air inlet and outlet vents on the rear and bottom side of the instrument.

### Instrument Identification and Safety Labeling

Please see "Octet<sup>®</sup> Systems Safety Information" on page 2 for definitions of symbols.



Figure 3-17: OCTET-RED96E Model – Rear Panel Label

## System Specifications

#### Table 3-12: OCTET-RED96E Model System Specifications (Sheet 1 of 2)

Item	Description	
Model	OCTET-RED96E	
Equipment Classifications	<ul> <li>Product Classification: Class 1: Detachable power cord</li> <li>Installation/Overvoltage Category: Category II</li> <li>Pollution Degree: Degree 2</li> <li>EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}</li> </ul>	
Environmental	<ul> <li>Storage Temperature: 0 to 40 °C</li> <li>Optimum Operating Temperature: 22 ± 2 °C.</li> <li>NOTICE: For optimal performance, the environmental temperature change should be less than 2 °C per hour.</li> </ul>	
	<ul> <li>Safe Operating Temperature: 15 to 30 °C</li> <li>Humidity: Non-condensing, 10 to 80% Relative Humidity</li> <li>Indoor Use Only</li> <li>Operating Altitude: 0 to 2,000 meters</li> <li>Not for use in an environment with an explosive atmosphere</li> <li>Mains supply voltage fluctuations of +/-10% of the nominal voltage</li> </ul>	
Compliance	<ul> <li>Nemko NRTL/C, CB Scheme</li> <li>CE compliance as indicated on the Instrument Identification and Safety Label.</li> <li>This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if device is used in a domestic environment.</li> </ul>	
Capabilities	<ul> <li>Protein quantitation</li> <li>Kinetic and affinity analyses (k<sub>obs</sub>, k<sub>a</sub>, k<sub>d</sub>, K<sub>D</sub>)</li> <li>Binding specificity and cooperativity</li> <li>Kinetic screening of proteins, peptides, and other biomolecules</li> <li>Kinetic analysis of small molecule and fragments</li> <li>Recommended for analyte molecular weight of 150 Da or higher</li> </ul>	
Sampling Format	<ul> <li>Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate</li> <li>Single sample plate capacity</li> </ul>	
Sampling Volume	180-220 μL/well (96-well plate)	
Sample Types	Purified samples, common culture media, crude lysates	
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/ or re-racking	
#### Table 3-12: OCTET-RED96E Model System Specifications (Sheet 2 of 2)

Item	Description
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Optics and Mechanics	8-channel biosensor manifold
	Optical interferometer
	Eight spectrometers (one dedicated spectrometer per biosensor)
Throughput	• Up to 8 biosensors in parallel, maximum of 96 tests unattended
	One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Sample Temperature Range	15–40 °C, 1 °C increments
Dimensions	19.5″ H x 22″ W x 18.2″ D (49 cm H x 56 cm W x 46 cm D)
Weight	72 lb (32.7 kg)
Electrical Requirements	• Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max
	<ul> <li>Power consumption: 200 W (300 W peak)</li> </ul>

#### IMPORTANT:

- Only use the power cords provided by Sartorius or a AC cord rated 60 C, 300 V, 16 AWG or better.
- Do not connect the system and computer to an electrical circuit with high intermittent power draws such as refrigerators, freezers, compressors, or vacuum pumps.
- f your site has a history of power outages, spikes, and/or drops, use an on-line uninterpreted power supply (UPS) to power the instrument and computer. Your Sartorius service representative can provide specifications for the recommended UPS system.

# Microplate Evaporation Cover

**NOTICE:** The microplate evaporation cover is used only on the Octet<sup>®</sup> RED96e and the Octet<sup>®</sup> R8 system.

- The evaporation cover was designed specifically for use with Greiner 96-well regular microplates (Part No. 655209)
- Intended to extend the length of total experiment time up to 12 hours
- · Ideal for precious samples that can be fully recovered to perform additional analyses
- Single-use only and should not be cleaned or re-used as any processing may alter its structural integrity
- The covers can withstand the standard operating temperature of the Octet<sup>®</sup> RED96e and the Octet<sup>®</sup> R8 system of 15–40°C
- They are mostly solvent resistant but should not be subjected to 100% DMSO
- All covers are individually wrapped and sold in a pack of 3

## Intended Use

Before using the evaporation cover, ensure that the push bar is installed near the sensor pickers, as shown in Figure 3-18, otherwise the biosensors will crash into the microplate evaporation cover.



Figure 3-18: Install Push Bar

For best results, immediately after preparation, place the 96-well microplate in the instrument and place the evaporation cover on it to prevent any evaporation and recover majority of the sample volume after the run. After putting the cover evaporation cover on, make sure that all four corners are pressed down onto the plate. The LED light next to the plate will be solid blue if the evaporation cover is installed properly (Figure 3-19). If the cover is not installed properly, the LED light will blink and the experiment will not be able to start.



Figure 3-19: LED is Blue When Evaporation Cover is Installed Properly

The LED status information is printed in the inside of the instrument below the home position of the sensor pickers (Figure 3-20).



Figure 3-20: LED Status Information

Start the experiment after placing the biosensor tray on the tray holder and giving the samples enough time to equilibrate to the desired temperature. During the experiment, the evaporation cover will open one column on the sample plate at a time, and enable eight biosensors to dip into the sample wells in that column (Figure 3-21). Following the column read, the panel in the evaporation cover will return to its original position. The microplate evaporation cover can extend the experiment run time to 12 hours with minimal sample evaporation so most of the samples can be recovered following the run.



Figure 3-21: Evaporation Cover Opened

# Chapter 4: 21 CFR Part 11 Compliance

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# FDA 21 CFR Part 11 Final Rule Compliance

The Octet<sup>®</sup> 21 CFR Part 11 software has features to allow users to produce electronic records that meet the requirements of the FDA 21 CFR Part 11 Final Rule. This chapter details how the features in Octet<sup>®</sup> 21 CFR Part 11 software address the requirements for compliance with the FDA 21 CFR Part 11 Final Rule according to the following guidance provided by the FDA:

Subpart A: General Provisions

- Scope
- Implementation
- Definitions

Subpart B: Electronic Records

- Controls for closed systems
- Controls for open systems
- Signature manifestations
- Signature/record linking

Subpart C: Electronic Signatures

- General requirements
- Electronic signatures and controls
- · Controls for identification codes/password

**NOTICE:** The guidance provided represents the FDA stance on this topic that was current during the development and release of this version of Octet<sup>®</sup> 21 CFR Part 11 software. For information, see http://www.fda.gov/regulatoryin-formation/guidances/ucm125067.htm

Octet<sup>®</sup> 21 CFR Part 11 software is comprised of three distinct software products: Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software and Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software and the Octet<sup>®</sup> GxP Server module. Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software is used to define quantitation, kinetic or custom assays and to run and view experiments and binding data. Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software is used to analyze binding data and view analysis results. The Octet<sup>®</sup> GxP Server software manages the user database and stores Audit Trail data.

# Overview of FDA 21 CFR Part 11 Compliance Features

# Data Integrity

The integrity of raw data is a primary design consideration of Octet<sup>®</sup> 21 CFR Part 11 software. All data acquired using Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software is time stamped and traceable to the user who initiated data acquisition. All method files, acquired data files, and analysis settings files are digitally signed to ensure data integrity. Any modification or tampering outside of the Octet<sup>®</sup> 21 CFR Part 11 software environment invalidates the digital signature. The Octet<sup>®</sup> 21 CFR Part 11 software performs integrity checks any time a method, experiment data, or analysis settings are accessed and alerts the user if unauthorized modification has occurred.

Electronic signatures can be added to an analysis workspace to prevent further modification within the Octet<sup>®</sup> 21 CFR Part 11 software-environment. An Audit Trail of all activities performed in any Octet<sup>®</sup> 21 CFR Part 11 application is stored in the Octet GxP Server database.

Data files created using Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software are strictly bound to features that support FDA 21 CFR Part 11 regulations. As a result, these files cannot be opened or modified by the non-CFR version of Octet<sup>®</sup> software to ensure the integrity of the acquired data.

## Administratively Controlled Application Access

The Octet<sup>®</sup> 21 CFR Part 11 software restricts the use of all features that can be used to acquire, modify, and analyze data, including exporting and saving the results as files. A user with no explicit privileges is considered as a Guest, and can only open and print data and method files created by the software.

The Octet<sup>®</sup> 21 CFR Part 11 software uses the Octet<sup>®</sup> GxP Server Administration software to administer user settings. The software contains the following information for each user:

- User name
- User Identifier or ID (must be unique)
- Password
- One or more of the following permissions:
  - Manage users and user settings
  - Create and edit method template
  - Build multi-dataset
  - Edit preprocess settings
  - Edit analysis settings
  - Edit annotation or display properties
  - Convert Kinetic step or step type into Quantitation
  - Edit report pages
  - Sign document
  - Set commenting requirement
  - Edit experiment info
  - Edit sensor and sample info
  - Include/exclude wells and sensors from analysis
  - Run experiment
  - Import analysis settings template to new dataset
  - Export data and Excel report
  - Review Audit Trail for any user
  - Remove Signature from document
  - · Choose repository directory when running an experiment

Octet<sup>®</sup> BLI Discovery and Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software must be linked to a Octet GxP Server module to access and enforce the features under administrative control.

## Audit Trail

Octet<sup>®</sup> 21 CFR Part 11 software automatically generates time-stamped Audit Trails that record transactions that create, delete, or modify electronic records. In each instance, the Audit Trail records the date and time of the transaction, the computer and project name, the user ID of the person who was logged on, and information on the action performed. Additional information such as old and new values are also added for some Audit Trails that log changes in method file modifications and analysis settings.

Audit trails are recorded in the database managed by the Octet<sup>®</sup> GxP Server software. Each experiment has a unique identifier and all data-specific Audit Trails are logged with the experiment identifier. Audit trails can be filtered by experiment, user, machine, project or date for viewing and printing.

Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software also has an option to require users to enter comments or notes for each Audit Trail event. This option can be enabled and disabled using the Set Commenting Requirement permission.

Users can also add comments to an Audit Trail. Once logged, the Audit Trail cannot be deleted.

# Administratively Controlled Electronic Signatures

Users who have been granted the Sign Document permission can access and electronically sign the experiment data. After the first signature is performed, the data locks out additional analysis settings modifications. A second user can counter-sign the experiment. Each electronic statement contains:

- User who signed the document
- Workstation or machine information
- Octet<sup>®</sup> GxP Server module information
- Project information
- Date and time
- Statement note

The author of the statement supplies the statement note. The electronic statement is produced when the signer agrees to the statement and Octet<sup>®</sup> 21 CFR Part 11 software verifies the User ID and password combination of the signer.

Signed statements are listed sequentially in the Sign Document dialog. The Audit Trail is logged to the Octet<sup>®</sup> GxP Server software to record the action of signing a statement.

# Automatic User Log Out (Idle Timeout)

The system administrator can set an option to have Octet<sup>®</sup> 21 CFR Part 11 software automatically log out a user if the program is idle longer than a specified time. The user is automatically logged out after the specified time period even if Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software is acquiring data from an Octet<sup>®</sup> instrument.

After data acquisition has begun, Octet<sup>®</sup> 21 CFR Part 11 software continues acquiring data until the experiment is finished and the data are saved, exported, and printed as set in Preferences, whether or not the user is logged on. If no users are logged on, data acquisition cannot be stopped manually.

### Passwords

#### Expiration

The system administrator can set an option to have user passwords expire after a set period of time. If the system administrator activates the password expiration, then users are required to change their passwords at designated intervals. When expired, users are prompted to reset their passwords on the next login.

#### Requirements

The system administrator can set the minimum number of characters passwords must contain and the level of password complexity. At a higher level of complexity, passwords need to contain at least one alpha, one numeric, and one punctuation character. After logging on, users can change their password.

#### Security

The system administrator can set the maximum number of failed login attempts. If the user tries to log in with the wrong password and reaches the set number of tries, their account locks and this action logs into the Audit Trail.

The administrator can unlock the user and reset the user password.

If a user leaves the group or company, the system administrator can inactivate the user, thereby preventing any unauthorized use of the software.

## Other Data Integrity Features

#### Overwriting Existing Files Prohibited

Existing method files cannot be overwritten using **File > Save As**. If the user attempts to save a record using the same name as a file that currently exists in the target directory, the user is notified that overwriting an existing file is prohibited, and that the file must be saved with a different name.

# Octet<sup>®</sup> 21 CFR Part 11 Software Overview

Octet<sup>®</sup> BLI Discovery and Octet<sup>®</sup> Analysis Studio software are available in an optional 21 CFR Part 11 versions that enables users in GMP and GLP laboratories to comply with 21 CFR Part 11 regulations. This version of the software includes features such as user account management, audit trails and electronic signatures. In addition, the 21 CFR Part 11 version utilizes the Octet<sup>®</sup> GxP Server module to manage the information recorded during user sessions.

This chapter explains how to use the Octet<sup>®</sup> GxP Server module, compliance features and administrative functions specific to the 21 CFR Part 11 versions of the software.

# Octet<sup>®</sup> GxP Module

**NOTICE:** Do not install the Octet<sup>®</sup> GxP Server module on the computer connected to the Octet<sup>®</sup> instrument. Install the Octet<sup>®</sup> GXP Server software on a stand alone computer in a secure area on the same network as the Octet<sup>®</sup> BLI Discovery computer. All Octet<sup>®</sup> instruments and Octet<sup>®</sup> Analysis Studio instances will authenticate users and log activity to one Octet<sup>®</sup> GXP Server instance. This simplifies account and audit trail management. This also enhances security as the Octet<sup>®</sup> users are restricted from direct access to the Octet<sup>®</sup> GXP Server files.

When Octet<sup>®</sup> BLI Discovery or Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software is launched, users are prompted to log on to the Octet<sup>®</sup> GxP Server module. This initiates a user session where all system, software and user events are recorded. During user sessions, the Octet<sup>®</sup> GxP Server module manages and stores this recorded information. User sessions are closed when the user logs out or a set period of inactivity is reached. A new user session is initiated each time a user accesses the software.

**NOTICE:** Octet<sup>®</sup> BLI Discovery and Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software require a compatible version of the Octet<sup>®</sup> GxP Server module. The software will automatically check the version of the Octet<sup>®</sup> GxP Server module in use and will display a message if it is incompatible. Please contact your administrator to install the correct version of the Octet<sup>®</sup> GxP Server module if this happens.

# Selecting a Server Location

**NOTICE:** Please contact your administrator to determine the Octet<sup>®</sup> GxP Server module host location that should be used.

When the Octet<sup>®</sup> GxP Server module host location is selected, this location is used as the default selection for the user account. It does not need to be reselected each time a new user session initiates.

Users must select the host location of the Octet<sup>®</sup> GxP Server module during the login process. The Octet<sup>®</sup> GxP Server can be run on the local host computer where Octet<sup>®</sup> BLI Discovery or Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software is installed or from a network location.

Launch Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software by double-clicking the desktop shortcut (Figure 4-1).



Figure 4-1: Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 Software Desktop Shortcut

Login	SVIECTEVS	×
Server:	localhost: 20002	
User:	~	
Password:	?	
Project:	(none) ~	
	Login Quit	

The Login dialog box appears (Figure 4-2):

Figure 4-2: Login Dialog Box

2. Click on... (Browse) to display the Octet<sup>®</sup> GxP Server dialog box (Figure 4-3).

GxP S	erver	×
er:		
20002 ÷	Find	Default
	OK	Cancel
	er: localhost Localhost 20002	Iccalhost 20002 ÷ Find OK

Figure 4-3: Octet<sup>®</sup> GxP Server Dialog Box

Choosing a remote host on same subnet—If the Octet<sup>®</sup> GxP Server module is hosted on the same subnet, deselect the Localhost check box and click Find. A list of potential Octet<sup>®</sup> GxP Server module addresses are listed (Figure 4-4). Choose the desired location from the list and click OK.



**Figure 4-4:** Octet<sup>®</sup> GxP Server Address Search Results

 Choosing a remote host on another subnet—If the Octet<sup>®</sup> GxP Server module is hosted on a different subnet, deselect the Localhost check box. Enter the IP address or fully-qualified domain name (FQDN) of the computer hosting the Octet<sup>®</sup> GxP Server module (Figure 4-5).

	GxP S	erver	×
Connection to server address:	ver: 192.168.1.78		
Port:	Localhost	Find	Default
		OK	Cancel

Figure 4-5: Manual Entry of Remote Host Address

Choosing the local host (not recommended)—If an Octet<sup>®</sup> GxP Server has been installed on the local computer and is to be used as the Octet<sup>®</sup> GxP Server module host, select the Localhost check box. Change the Port number if needed.

When the Octet<sup>®</sup> GxP Server module host location has been selected or entered, click **OK** to save changes and exit the Authentication Server dialog box. The Octet<sup>®</sup> GxP Server module location is listed as the Server in the Login box (Figure 4-6).

Login	SVIECTENS	×	:
Server:	localhost: 20002		
User:	~		
Password:		?	
Project:	(none) V		
	Login Quit		

Figure 4-6: Login Dialog Box– Octet<sup>®</sup> GxP Server Location

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Proceed to Step 3 in the next section or click Quit.

# Starting a User Session

**NOTICE:** Before starting your first user session, please contact your administrator to determine the Octet<sup>®</sup> GxP Server module host location that should be used.

1. Launch the Octet<sup>®</sup> BLI Discovery or Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software by double-clicking the respective desktop shortcut.

Login	×	
	SVIFCTF/	
Server:	localhost: 20002	
User:	~	
Password:	?	
Project:	(none) ~	
	Login Quit	

The Login dialog box appears (Figure 4-7):

#### Figure 4-7: Login Dialog Box

- 2. Confirm that the **Server** location is correct. If not, please see "Selecting a Server Location" on page 72.
- 3. From the User drop down list, select your user name (Figure 4-8).

**NOTICE:** To start an administrator session, select **Administrator** in the **User** drop down list or a user account that has been granted the Administrator privilege.

Login		×
	<b>୧୦</b> ୮୮୦୮୮୦	
Server:	localhost: 20002	
User:	DBean V	
Password:	Administrator ?	
Project:	RBrown	
	Login Quit	

Figure 4-8: User Name Selection

4. Enter your password in the **Password** text box. Click? for a password reminder if needed (Figure 4-9).

Login		×
	SVIPCTEAS	
Server:	localhost: 20002	
User:	DBean ~	
Password:	?	
Project:	~ ~ ~ ~	
ļ	Reminder: Dog Quit	



5. Select a project from the **Project** drop down list, if required (Figure 4-10).

Login		)	×
	SVIFCTFA3		
Server:	localhost: 20002		
User:	DBean v		
Password:	•••••	?	
Project:	(none) 🗸		
	(none) Antigen:Antibody Screen Cell Culture Screen Receptor:Ligand Screen		

Figure 4-10: Project Selection

6. Click Login.

Octet<sup>®</sup> BLI Discovery or Octet <sup>®</sup>Analysis Studio 21 CFR Part 11 software launches and starts the user session. During the session, the user account and project selected at login appear in the software status bar (Figure 4-11).

Experiment: Not Started 🖉 Plate temp: 35 °C 🖺 Project: Antigen:Antibody screen 🚨 User: JBlack (John Black)

#### Figure 4-11: Status Bar

#### NOTICE:

Software operation may be restricted based on your user privileges. For more information on user privileges, please contact your administrator.

User sessions are automatically locked after a period of inactivity which is set by the administrator. The Login dialog box displays and a message indicating the session has been locked displays. You can choose to log back into the session or log off at this time. User sessions do not lock during experimental data acquisition.

# Accessing Compliance Features

The 21 CFR Part 11-compliant features provided in the 21 CFR Part 11 versions of Octet<sup>®</sup> BLI Discovery and Octet<sup>®</sup> Analysis Studio software can be accessed by clicking the **Security** menu (Figure 4-12) from the software's **Main Menu**:



Figure 4-12: Security Menu

# Experiment and Method File Compliance

When using the 21 CFR Part 11 version of Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11-compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software cannot be opened, and a message displays.

# Verifying File Integrity

The integrity of method (.fmf) and data (.frd) files can be verified to ensure they were generated using 21 CFR Part 11compliant software, and have not been modified outside of Octet<sup>®</sup> software.

**NOTICE:** When verifying file integrity both method (.fmf) and data (.frd) files can be selected in Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software. The Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software automatically performs file integrity checks when opening data files.

#### 1. Click Security > Verify Document.

The Verify Digital Signature dialog box (Figure 4-13) appears:



Figure 4-13: Verify Digital Signature

2. Click... to display the Open dialog box (Figure 4-14), to browse for the desired .fmf or .frd file.

🔁 Open						×
← → ~ ↑ 📙	> This PC	→ Local Disk (C:) → data	~	ې کې Se	arch data	
Organize 🔻 Nev	v folder					?
screens2	^ N	lame	Date modified	Туре	Size	
len OneDrive		201201_ExpMethod.fmf	11/16/2020 9:25 AM	FMF File	22 KB	
This PC						
3D Objects						
E Desktop						
Documents						
🖊 Downloads						
👌 Music						
Pictures						
Videos 📔						
🟪 Local Disk (C:)						
i Network						
	~					
	File name:	Epitope Binning Experiment.fmf		Method	File (*.tmt)	~
				Ор	en Canco	el

Figure 4-14: Open Dialog Box

To change the file type available for selection, click the down arrow in the file type box to display the menu (Figure 4-15), then select the desired format.



Figure 4-15: File Type Menu

3. Select a file type, then the desired file and click **Open**.

A message in the Verify Digital Signature dialog box indicates the file compliance status: Compliant or Non-Compliant (Figure 4-16):

		Verify Digital Signature				
File:	Desktop \HT Test CFR files \Furosemide on RED96 CFR \140922_ExpMethod fmf					
		Close				
		Verify Digital Signature	×			
	File:	I:\aForetBio\Misc\Rashi\QC Kinetic Data\131122_ExpMethod.fmf was generated on a non-21 CFR Part 11 compliant system				
		Close				

Figure 4-16: File Compliant (top), File Not a CFR Document (bottom)

## Viewing the Audit Trail

The Audit Trail displays a historical log of user, system and software events recorded during user sessions. To view the Audit Trail, click **Security** > **View Audit Trail**. An example is shown in Figure 4-17.

2	Audit Trail									
From:	02/08/18		Project:	(any)		~	User:	DBea	n	~
To:	03/10/18		Experiment:	(any)		*	Machine:	MCGO	OWNPC	~
Date/1	lime	Projec	t		Machine	Action			Description	
2018/0	03/10 10:30:23	Recep	otor Ligand Scree	en	MCGOWNPC	Method parame	ters changed		C:\Users\1	
2018/0	3/10 10:25:52	Cell Ci	ulture Screen		MCGOWNPC	Method parame	ters changed		C:\Users\1	
2018/0	3/10 10:25:52	Cell Ci	ulture Screen		MCGOWNPC	Method parame	ters changed		C:\Users\1	
2018/0	3/10 10:23:35	Antige	n:Antibody Scre	en	MCGOWNPC	Method parame	ters changed		C:\Users\1	
2018/0	3/10 10:23:35	Antige	n:Antibody Scre	en	MCGOWNPC	Method parame	ters changed		C:\Users\1	
2018/0	3/10 10:16:57	Antige	n:Antibody Scre	en	MCGOWNPC	Save method fil	e		C:\Users\]	
2018/0	03/10 10:15:05	Antige	n:Antibody Scre	en	MCGOWNPC	Open method fil	e		C:\Users\]	
2018/0	03/10 10:14:54	Antige	n:Antibody Scre	en	MCGOWNPC	User login			DBean	
2018/0	03/10 10:06:35	Antige	n:Antibody Scre	en	MCGOWNPC	User logout			DBean	

Figure 4-17: Audit Trail

By default, only events associated with the currently logged in user show. Users with the Administrator or Review Audit Trail privilege can view events associated with all Users. By default, the events initially displayed in the Audit Trail are those associated with the project selected at login and the machine (computer) currently being used.

You can sort the events in the Audit Trail by clicking on any of the column headers.

You can also filter (limit) the events by selecting a particular project, Experiment, Machine and Users (Administrators only) from the corresponding drop down lists. For example, Figure 4-18 shows a drop down menu for selecting events associated with a Project.



Figure 4-18: Selecting Events by Project

You can limit your search to a specific time period by choosing the start/stop day from the calendar drop down menus (Figure 4-19).

	PI 🔊					
	Fr	om:	02/0	8/18		
4		Feb	ruary	2018		•
Sun	Mon	Tue	Wed	Thu	Fri	Sat
28	29	30	31	1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	1	2	3 i
4	5	6	7	8	9	10 ti
	0		Today	r: 03/10	/18	ti

Figure 4-19: Selecting Events in a Time Period

The Audit Trail only displays events for the entries and time period selected.

In addition to the specific project and machine selections, the following list options are also available:

- (any)-Displays events associated with all projects, experiments and/or machines for the user account. Administrators or users with the Review Audit Trail privilege can view events associated with all Users.
- (none)—Displays all project and machine events not associated with a specific project (Project list only)

# Viewing Event Details

If an action entailed a change in Method Parameters, you can view details of the change(s) by double-clicking on the individual action to display the Event Details box (Figure 4-20).

	Audit Trail						
Cell Cu	ulture Screen	~	User:	DBean	~		
(any)		~	Machine:	MCGOWNPC	~		
ne Action			scription				
WNPC Method parameters changed WNPC Method parameters changed			Users		Event Deta	ils	
			Assay	params step 11: "	Time 120 to 125		

Figure 4-20: Viewing Event Details

## Changing Projects During a User Session

During an active session, users can switch to another project in the software without having to log out.

1. Click Security > Change Projects.

A list of projects assigned to your user account shows with the current active project highlighted (Figure 4-21):



Figure 4-21: Changing Projects

2. Select the desired project from the list. The selected project becomes the active project for the user session.

## Changing Your Password

1. Click Security > Change Password to display the Change Password dialog box (Figure 4-22).

C	hange Password
Current password:	?
New password:	
Confirm new password:	
Password reminder:	
	OK Cancel

Figure 4-22: Change Password Dialog Box

- 2. Enter the Current password for your user account. Click? for a password reminder.
- 3. Enter and re-enter your **new password**. If desired, enter a Password reminder.
- 4. Click OK.

## Locking/Unlocking the Application

1. Click **Security** > **Lock Application**. The application Locked dialog box (Figure 4-23) appears and remains until you unlock it.

Application	Application Locked X						
	SUIFCITRAS						
User:	DBean (Daisy Bean)						
Password:	?						
	Unlock Logoff						

Figure 4-23: Application Locked Dialog Box

2. Enter your password and click Unlock or Logoff.

### Logging Off the Application

- 1. Click Security > Logoff.
- 2. When you see the message Are you sure you want to logoff?, click OK.

The Login dialog box (Figure 4-24) appears and is available for other Users.

Login	×
	SVIFCTFA3
Server:	localhost: 20002
User:	~
Password:	?
Project:	(none) ~
	Login Quit

Figure 4-24: Login Dialog Box

3. Quit the application as needed.

#### Chapter 5:

# Quantitation Experiments: Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, and Octet<sup>®</sup> K2 System

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# Introduction

Quantitation experiments determine the analyte concentration of a sample using a reference set of standards. After starting the Octet<sup>®</sup> system hardware and the Octet<sup>®</sup> BLI Discovery software, follow the steps (in Table 5-1) to set up and analyze a quantitation experiment.

Software		Step	See
Octet <sup>®</sup> BLI Discovery	1.	Select a quantitation experiment in the Experiment wizard or open a method file	"Starting a Quantitation Experiment" on page 84
( <u></u>		().	
	2.	Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 86
	3.	Confirm or edit the assay settings.	"Managing Assay Parameter Settings" on page 107
	4.	Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 112
	5.	Run the experiment.	"Running a Quantitation Experiment" on page 130
Octet <sup>®</sup> Analysis Studio	6.	Analyze the binding data.	Octet <sup>®</sup> Analysis Studio Software User Guide
<mark>E</mark>	7.	Generate a report.	

Table 5-1:	Setting Up	and Analyzing	a Quantitative	Experiment
------------	------------	---------------	----------------	------------

For more details on how to prepare the biosensors, see the appropriate biosensor product insert.

# Starting a Quantitation Experiment

**IMPORTANT:** Using 96-well half-area plates on the Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2 system result in non-optimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

**NOTICE:** Before starting an experiment, check the plate temperature display in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet<sup>®</sup> BLI Discovery software is closed, the plate temperature resets to the default startup value specified in the Options dialog box when the software is relaunched.

Select a method for your a quantitation experiment from the following:

- Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking File > Open Method File. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run. For more details on method files see, "Managing Experiment Method Files" on page 141.
- On the menu bar, click Experiment > Templates > Quantitation.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the using the non-compliant system cannot be opened, and cannot display a message.

## Starting an Experiment Using the Experiment Wizard

To start an experiment using the **Experiment Wizard**:

- 1. If the **Experiment Wizard** does not appear when the software is launched, click the **Experiment Wizard** toolbar button and click **Experiment > New Experiment Wizard** (**Ctrl+N**) from the **Main Menu**.
- 2. In the **Experiment Wizard**, select **New Quantitation Experiment** (see Figure 5-1, left).
- 3. Select a type of quantitation experiment (see Table 5-2 for options).

Table 5-2: Quantitation Experiment Selection

Quantitation Experiment	Description
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regenera- tion	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two- or three-step quantitation assay that enables signal amplifica- tion for higher detection sensitivity.

4. Optional: You can click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

🖄 Experiment Wizard	- • •			
Experiment Wizard      Choose an option to stat      @      Basic Quartitation     O Basic Quartitation     O Advanced Quartitation      O Advanced Quartitation      Basic Kinetics     Eptope Binning	adable Templates for - Octet R2     Bark Experiment     Arshing or Antening's biosensor _2CH_56W fmf     Arsh Perta HIS Diaton Factor Socuting, ZCH_56W fmf     Arsh Perta HIS Diaton Factor Socuting, ZCH_56W fmf     DrectDetectionImmunogencity, ZCH, 56W fmf     DrectDetectionImmunogencity, ZCH, 56W fmf     Protein A G or L. biosensor_Nigh_sensitively_ZCH_56W fmf			
Recent Methods	Essic Quantitation Experiment - Protein A G or L biosensor_2CH,96	w		
	Index de la factoria de la la construir de la construire de la construir de la construir de la construir de la construir		y well data.	

Figure 5-1: Selecting an Experiment Type in the Experiment Wizard

5. Click the  $\rightarrow$  arrow.

The **Experiment** dialog box appears (Figure 5-1, right).

# Defining the Sample Plate

Table 5-3 lists the steps to define a sample plate.

#### Table 5-3: Defining a Sample Plate

	Step	See Page
1.	Designate the samples.	86
2.	Annotate the samples (optional).	98
З.	Save the sample plate definition (optional).	104

### **Designating Samples**

Each well may be designated as a Standard, Unknown, Control or Reference. A well may remain Unassigned or be designated as Reserved by the system for Basic Quantitation with Regeneration and Advanced Quantitation experiments.

**NOTICE:** It is important to define all of the wells used in the assay. Only wells that are selected and defined using one of the sample types in Table 5-4 can be included in the assay.

#### Table 5-4: Types of Sample Wells

lcon	Description
Standard	Contains an analyte of known concentration. Data from the well is used to generate a standard curve during analysis.
Unknown	Contains an analyte of unknown concentration. The concentration of the analyte is calculated from the well data and the standard curve.
Control	<ul> <li>A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis.</li> <li>Positive Control: A control sample that contains analyte of known concentration</li> <li>Negative Control: A control sample known not to contain analyte</li> </ul>
Reference	Provides a baseline signal which serves as a reference signal for Standard, Unknown, and Con- trol. The reference signal can be subtracted during data acquisition in the Runtime Binding Chart and during data analysis.
Unassigned	Not used during the experiment.
Reserved	Used by the system during Basic Quantitation with Regeneration experiments and Advanced Quantitation multi-step experiments for Regeneration (R), Neutralization (N), Dictation (D), or Capture Antibody (C). Reserved wells are not available for use as Standards, Unknowns, Con- trols, or References.

#### **Reserved Wells**

In a Basic Quantitation with Regeneration or an Advanced Quantitation experiment, the Sample Plate Map includes gray wells. These wells are reserved by the system and specify the location of particular sample types.

Reserved samples cannot be removed from the sample plate, but you can change their column or row location. To change the location of the two reserved wells ((\*, \*, \*, \*), \*), right-click on the wells in the Sample Plate Map and select Regeneration, Neutralization, Detection, or Capture Antibody.

Table 5-5: Reserved Well Requirements (Sheet 1 of 2)

Reserved Well	Must Contain				
Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).				
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.				
Detection	Secondary antibody or precipitating substrate that is used with an enzyme-antibody conjugate to amplify the analyte signal. Sample concentrations are computed using the binding data from the detection wells.				

#### Table 5-5: Reserved Well Requirements (Sheet 2 of 2)

Reserved Well	Must Contain
Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of interest onto the biosensor.

#### Basic Quantitation with Regeneration



#### Advanced Quantitation



Figure 5-2: Default Locations for Reserved Wells in a 96-Well Sample Plate Map

## Selecting Wells in the Sample Plate Map

**NOTICE:** For the Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2 system, wells in sample plate are restricted to rows AB, CD, EF and GH. Sample wells cannot be designated in row pairs BC, DE and FG.

There are three ways to select wells in the Sample Plate Map:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 5-3, left). To select nonadjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 5-3, center).
- Click a well or draw a box around a group of wells (Figure 5-3, right).



Figure 5-3: Selecting Wells in the Sample Plate Map

**NOTICE:** Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection. Designating Standards

To designate standards:

- 1. In the **Sample Plate Map**, select the wells to define as standards.
- Click the Standard button below the Sample Plate Map (see Figure 5-3), or right-click and select Standard. The standards are marked in the plate map and the Sample Plate Table is updated.
- 3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

Plate Definition 2 Sensor Assignment 3 Review Experiment	4 Run Exp	periment					
In this step, all the information about the sample plate and its we	ls will be en	tered.					
Hirst, check the assay settings. Then highlight one or more wells	on the sam	ple plate, and i	ight-click to enter/mo	dify well da	ita.		
cquisition Rate: Standard (5.0 Hz)	Plate 1	<u>Fable (96 wells</u>					
Assay Settings	Concen	tration units:	µg/ml 🗸	Export	Import	Print	
ssay: Basic Quantitation Modify	Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	Information
Single analyte	🔵 A1			Standard		n/a	
Time (s): Shake speed (rpm):	O B1			Standard		n/a	
Juantitation: 120 400	O C1			Standard		n/a	
Plate 1 (96 wells)	O D1			Standard		n/a	
Modify	○ E1			Standard		n/a	
1 2 3 4 5 6 7 8 9 10 11 12	O F1			Standard		n/a	
A O O O O O O O O O O O O O O O O O O O	O G1			Standard		n/a	
BOOODOOOOOO				Standard		n/a	
	O AZ			Standard		n/a	
	O 62			Standard		n/a	
				Standard		n/a	
	0 52			Standard		n/a	
FORGOSOSOS	0 F2			Standard		n/a	
	G2			Standard		n/a	
	O H2			Standard		n/a	
	A3			Standard		n/a	
Standard O Control Unassigned	O B3			Standard		n/a	
	O C3			Standard		n/a	
	<u>о</u> D3			Standard		n/a	
	○ E3			Standard		n/a	
	● F3			Standard		n/a	
	0 62			Grandard		n /n	

Figure 5-4: Plate Definition Window–Designating Standards

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box appears (see Figure 5-5).

Plate 1 (96 wells)			
	Modify		
1 2 3 4 5 6 7 8 9	10 11 12		
B C C Standard			
C C C C Control			
E C C Set Well Data			
Clear Data			
	Set Well Data		×
G Copy to Clipboard	Mall Lafa and Kan	Dilation Control	
H C Extended sample lypes		Dilution Series	
Standard O Control O Una	Sample ID:	Starting value (µg/ml):	200
Unknown Reference Reference		Series operator:	
	Replicate Group:		, -
		Senes operand:	2
	Well Information:	Dilution orientation	
		Down	ŠŠ OUp
		l	
	Concentration (µg/ml):		
	Standards only		
		OK	Cancel

Figure 5-5: Sample Plate Map–Setting a Dilution Series

- 2. Select the **Dilution Series** option and enter the starting concentration value.
- 3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 5-6).



Figure 5-6: Concentration Representation in Dilution Series

#### 4. Click OK.

The Sample Plate Table appears the standard concentrations entered.

#### Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

**NOTICE:** A range of wells can be selected clicking and dragging, holding the Shift key and using the arrow keys to select sections of the plate, or holding the Ctrl key to select specific wells.

The Set Well Data dialog box appears (see Figure 5-7).

Sample Plate Sample Plate (96 wells)			
1       2       3       4       5       6       7       8       9         A       O       Standard       Unknown       O       O       0	10 11 12 000 000 Set Well Data		×
Copy to Clipboard Copy to Clipboard Extended Sample Types Outword Unknown Reference Rese	Well Information Sample ID: Replicate Group:	Dilution Series         Starting value (µg/ml):         Series operator:         Series operand:         Dilution orientation	]
	Vieli Information:	Image: Solution contraction       Image: Solution       Image: Solution </td <td></td>	

Figure 5-7: Sample Plate Map–Assigning a Standard Concentration

- 2. Select the **By value** option and enter the starting concentration value. If a range of cells was selected, all cells update with the specified value.
- 3. Click **OK**. The **Sample Plate Table** appears the standard concentrations entered.

#### Editing an Individual Standard Concentration

To enter or edit an individual standard concentration, in the Conc column of the Sample Plate Table, double-click the value and enter a new value (see Figure 5-8).

Sample Conce	Plate Table – ntration units:	µg/ml ▼	Expo	ort	Impoi	rt		
Well	Sample ID	Replicate Group	Туре	Conc	(µq/ml)	Dilution Factor	Information	
🔵 A1			Standard	1		n/a		
🔵 B1			Standard	200		n/a		
🔵 C1			Standard	100	Un	do		
🔵 D1			Standard	50				
🔵 E1			Standard	25	Cu	t		
🔵 F1			Standard	10	Co	ру		
🔵 G1			Standard	5	Pas	ste		
🔵 H1			Standard	2.5	De	lete		
🔵 A2			Standard	1				
🔵 В2			Standard	200	Sel	ect All		
🔵 C2			Standard	100	Rig	iht to left Readin	a order	
🔵 D2			Standard	50	rtig Ch	nie to iere Readin	ig order	
🔵 E2			Standard	25	Sho	ow Unicode con	trol characters	·
🔵 F2			Standard	10	Ins	ert Unicode con	trol character	+
🔵 G2			Standard	5	On	en IME		
🔵 H2			Standard	2.5	Op	CTT INTE		
🔵 A3			Standard	1	Rec	conversion		
🔵 B3			Standard	200		n/a		

Figure 5-8: Sample Plate Table–Shortcut Menu of Edit Commands

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

# Designating Unknowns

To designate unknowns in the **Sample Plate Map**, select the wells to define as unknown, right-click and select **Unknown**. The unknown wells are marked in the plate map and the sample plate table is updated (see Figure 5-9).

uisition Rate: Standard (5.0 Hz)	<ul> <li>Plate 1 Ta</li> </ul>	ble (96 wells)					
ay Settings	Concentra	ation units:	µg/ml ∨	Export	Import	Print	
ay: Basic Quantitation Mod	lify Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	Informat
Single analyte	O D2			Standard	50	n/a	
Time (s): Shake speed (rpm):	○ E2			Standard	25	n/a	
antitation: 120 400	○ F2			Standard	10	n/a	
e 1 (96 wells)	🔵 G2			Standard	5	n/a	
Mod	lify H2			Standard	2.5	n/a	
1 2 3 4 5 6 7 8 9 10 11 1	2 A3			Standard	1	n/a	
	O B3			Standard	200	n/a	
Standard	○ C3			Standard	100	n/a	
Unknown	O D3			Standard	50	n/a	
Control V	- E3			Standard	25	n/a	
Negative Control	🔵 F3			Standard	10	n/a	
Positive Control	🔵 G3			Standard	5	n/a	
Reference	🔵 НЗ			Standard	2.5	n/a	
Set Well Data	🔘 A4			Unknown	n/a		
	O 84			Unknown	n/a		
	C4			Unknown	n/a		
Copy to Clipboard	O D4			Unknown	n/a		
Standard 🛛 🧹 Extended Sample Types	🔵 E4			Unknown	n/a		
Unknown 🛑 Reference 🔿 Reserved	F4			Unknown	n/a		
	🔵 G4			Unknown	n/a		
	─ H4			Unknown	n/a		
	A5			Unknown	n/a		
	O 85			Unknown	n/a		
	C5			Unknown	n/a		

Figure 5-9: Plate Definition Window–Designate Unknown Wells

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

#### Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

- 1. In the **Sample Plate Map**, select the unknown wells (see Figure 5-9).
- 2. Right-click and select Set Well Data.

The Set Well Data dialog box appears (see Figure 5-10).

Plate 1 (96 wells)					
	Modif	fy			
1 2 3 4 5 6	7 8 9 10 11 12	2			
	000000				
BOOOC					
	Standard				
	Unknown				
	Negative Control				
	Positive Control				
	Reference				
GOOOC	Set Well Data				
	Clear Data	Set Well Data			×
Standard (		Well Information		Dilution Series	
	Copy to Clipboard	Sample ID:		Starting value:	1
	Extended Sample Types	-		o taning value.	1
		Replicate Group:		Series operator:	/ ~
				Series operand:	2
		Well Information:		Dilution orientation	
				Right	CLeft
				Down	
				88	
		Dilution Factor:	2		
		Unknowns only		0	Cancel
				01	Cancer

Figure 5-10: Sample Plate Map–Setting a Dilution Factor or a Serial Dilution

To assign a dilution factor to selected wells:

- 1. In the **Set Well Data** dialog box (see Figure 5-10), select the **By Value** option.
- 2. Enter the dilution factor value and click OK.

To assign a serial dilution to selected wells:

- 1. In the **Set Well Data** dialog box (see Figure 5-10), select the **Dilution series** option.
- 2. Enter the starting dilution, select a series operator, and enter a series operand.
- 3. Select the appropriate dilution orientation: (see Figure 5-11).



Figure 5-11: Concentration Representation in Dilution Series

4. Click OK.

The Sample Plate Table appears with the dilution factors entered.

Editing a Dilution Factor in the Sample Plate Table To edit a dilution factor in the Sample Plate Table:

- 1. In the **Set Well Data** dialog box (see Figure 5-10), double-click a cell in the **Dilution Factor** column for the desired unknown.
- 2. Enter the new value (the default dilution factor is 1).

oncent	ration units:	µg/ml ∨	Export	Import	Print		
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Fac	tor Informatic ^	
O D2			Standard	50	n/a		
) E2			Standard	25	n/a		
) F2			Standard	10	n/a		
🔵 G2			Standard	5	n/a		
🔵 Н2			Standard	2.5	n/a		
A3			Standard	1	n/a		
<b>B</b> 3			Standard	200	n/a		
C3 (			Standard	100	n/a		
D3 🔘			Standard	50	n/a		
<b>E</b> 3			Standard	25	n/a		
<b>F</b> 3			Standard	10	n/a		
🔵 G3			Standard	5	n/a		
🔵 НЗ			Standard	2.5	n/a		
A4			Unknown	n/a	2		
B4			Unknown	n/a	2 Ur	ndo	
C4			Unknown	n/a	2 Ci	ut	
D4			Unknown	n/a	2		
<b>E</b> 4			Unknown	n/a	2	- <b>F</b>	
<b>F</b> 4			Unknown	n/a	2	ste	
<b>G</b> 4			Unknown	n/a	2 De	elete	
H4			Unknown	n/a	2 Se	elect All	
A5			Unknown	n/a	2	-hade left Decilie	
B5			Unknown	n/a	2	gnt to left Keadin	g or
C5			Unknown	n/a	2 Sh	now Unicode cont	trol
0 00				,	- In	sert Unicode cont	trol

Figure 5-12: Sample Plate Table–Shortcut Menu of Edit Commands

**NOTICE**: Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

# Designating Controls or Reference Wells

Controls are samples of known concentration that are not used to generate a standard curve. A reference well contains sample matrix only, and is used to subtract non-specific binding of the sample matrix to the biosensor. During data analysis, data from reference wells can be subtracted from standards and unknowns to correct for background signal.

- To designate controls, select the control wells and click Control (below the Sample Plate Map), or right-click and select Control. Positive and Negative Control types can be assigned using right-click only if extended sample types is checked (Figure 5-13).
- To designate reference wells, select the reference wells and click the **Reference** button below the **Sample Plate Map**, or right-click the selection and choose **Reference**.

The wells are marked in the **Sample Plate Map** and the **Sample Plate Table** is updated (Figure 5-13).



Figure 5-13: Designate Controls or Reference Wells

NOTICE: Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

## Annotating Samples

You can enter annotations (notes) for multiple samples in the Sample Plate Map or enter information for an individual sample in the Sample Plate Table. For clarity, display the annotation text as the legend of the Runtime Binding Chart during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it is not available for display as a legend.

#### Annotating Wells in the Sample Plate Map

To annotate one or more wells:

- 1. In the Sample Plate Map, select the samples to annotate, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 5-14), enter the Sample ID and/or Well Information and click OK.

Sample Plate Sample Plate (96 wells)			
1     2     3     4     5     7     8     9     10     11     12       A     Image: Control contro contro control contro control contro control contro control contr			
FOC Clear Data	Set Well Data		×
Copy to Clipboard Extended Sample Types Standard Unassigned Unknown Reference Reserved	Well Information         Sample ID:       Image: Component of the system of	Dilution Series Starting value (µg/ml): Series operator: Series operand: Dilution orientation	1 / 2
	Concentration (µg/ml):	ОК	Cancel

Figure 5-14: Adding Sample Annotations from the Sample Plate Map

#### Annotating Wells in the Sample Plate Table

To annotate an individual well in the Sample Plate Table:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 5-15).

**NOTICE:** A series of Sample IDs may also be assembled in Excel and pasted into the **Sample Plate Table**.

Concent	tration units:	µg/ml ∨ Ex	port	Import	Print	
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	Information
) E3	lgG Standard		Standard	25	n/a	Sample Diluen
<b>F</b> 3	lgG Standard		Standard	10	n/a	Sample Diluen
🔵 G3	lgG Standard		Standard	5	n/a	Sample Diluen
🔵 НЗ	lgG Standard		Standard	2.5	n/a	Sample Diluen
🔵 A4	Ab1		Unknown	n/a	2	Sample Diluen
🔵 B4	Ab2		Unknown	n/a	2	Sample Diluen
🔵 C4	Ab3		Unknown	n/a	2	Sample Diluen
🔵 D4	Ab4		Unknown	n/a	2	Sample Diluen
🔵 E4	Ab5		Unknown	n/a	2	Sample Diluen
🔵 F4	Ab6		Unknown	n/a	2	Sample Diluen
🔵 G4	Ab7		Unknown	n/a	2	Sample Diluer
🔵 H4	Ab8		Unknown	n/a	2	Sample Diluen
🔵 A5	Ab1		Unknown	n/a	2	Sample Diluen
<b>B</b> 5	Ab2		Unknown	n/a	2	Sample Diluen
🔵 C5	Ab3		Unknown	n/a	2	Sample Diluen
🔵 D5	Ab4		Unknown	n/a	2	Sample Diluen
🔵 E5	Ab5		Unknown	n/a	2	Sample Diluen
🔵 F5	Ab6		Unknown	n/a	2	Sample Diluen
🔵 G5	Ab7		Unknown	n/a	2	Sample Diluer
─ H5	Ab8		Unknown	n/a	2	Sample Diluen
) A6	Ab1		Unknown	n/a	2	Sample Diluen
) B6	Ab2		Unknown	n/a	2	Sample Diluen
🔵 C6	Ab3		Unknown	n/a	2	Sample Diluer
D6 (	Ab4		Unknown	n/a	2	Sample Diluer
<b>E6</b>	Ab5		Unknown	n/a	2	Sample Diluer
<b>F6</b>	Ab6		Unknown	n/a	2	Sample Diluer
🔵 G6	Ab7		Unknown	n/a	2	Sample Diluen
─ H6	Ab8		Unknown	n/a	2	Sample Diluen
A7	hlgG		Control	10	n/a	
<b>B</b> 7	hlgG		Control	10	n/a	
🔵 C7	hlgG		Control	10	n/a	
D7 🔘	hlgG		Control	10	n/a	
🔵 E7	hlgG		Control	10	n/a	

Figure 5-15: Adding Sample Annotations in the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

### **Replicate Groups**

When samples are assigned to a Replicate Group, the software automatically calculates statistics for all samples in that group. The average binding rate, average concentration and corresponding standard deviation as well CV% are presented in the Results table for each group (see Figure 5-16).

	Sensor	Replicat	BR Avg	BR SD	BR CV	Conc. Avg	Conc. SD	Conc. CV
ĺ	Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
	Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
	Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
	Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
	Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
	Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
	Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
	Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
	Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
	Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
	Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
	Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
	Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
	Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
	Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
	Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2

Figure 5-16: Replicate Group Result Table Statistics

NOTICE: Replicate Group information can also be entered in the Results table in the software.

Assigning Replicate Groups in the Sample Plate Map

To assign Replicate Groups in the Sample Plate Map:

- 1. Select the samples to group, right-click and select **Set Well Data**.
- 2. In the Set Well Data dialog box (see Figure 5-17), enter a name in the Replicate Group box and click OK.

Set Well Data	×
Well Information	Dilution Series
Sample ID:	Starting value (µg/ml):
IgG Standard	Series operator:
Replicate Group: 200	Series operand: 2
Well Information:	Dilution orientation
Sample Diluent	Right 8888 Cleft
	Down Oup
Concentration (µg/ml): 200 Standards only	
	OK Cancel

Figure 5-17: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software only recognizes and calculates statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

**NOTICE:** When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they are treated as separate groups. Statistics for these groups calculate separately for each biosensor type.

Wells in the **Sample Plate Map** show color-coded outlines as a visual indication of which wells are in the same group (see Figure 5-18).

#### Sample Plate (96 wells)



Figure 5-18: Replicate Groups in Sample Plate Map

The Sample Plate Table update with the Replicate Group names entered (see Figure 5-19).

Conce	ntration units:	µg/ml ▼	Export	Import		
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Facto	
🔵 A1	lgG Standard	200	Standard	200	n/a	Γ
🔵 B1	lgG Standard	100	Standard	100	n/a	
🔵 C1	lgG Standard	50	Standard	50	n/a	
🔵 D1	lgG Standard	25	Standard	25	n/a	
🔵 E1	lgG Standard	10	Standard	10	n/a	
🔵 F1	lgG Standard	5	Standard	5	n/a	
🔵 G1	lgG Standard	2.5	Standard	2.5	n/a	
🔵 H1	lgG Standard	1	Standard	1	n/a	
🔵 A2	lgG Standard	200	Standard	200	n/a	
🔵 B2	lgG Standard	100	Standard	100	n/a	
🔵 C2	lgG Standard	50	Standard	50	n/a	
🔵 D2	lgG Standard	25	Standard	25	n/a	
🔵 E2	lgG Standard	10	Standard	10	n/a	
🔵 F2	lgG Standard	5	Standard	5	n/a	
🔵 G2	lgG Standard	2.5	Standard	2.5	n/a	L
🔵 H2	lgG Standard	1	Standard	1	n/a	
🔵 A3	lgG Standard	200	Standard	200	n/a	
🔵 B3	lgG Standard	100	Standard	100	n/a	
🔘 C3	lgG Standard	50	Standard	50	n/a	
🔵 D3	lgG Standard	25	Standard	25	n/a	
🔵 E3	lgG Standard	10	Standard	10	n/a	
🔵 F3	lgG Standard	5	Standard	5	n/a	
🔵 G3	lgG Standard	2.5	Standard	2.5	n/a	
🔵 НЗ	lgG Standard	1	Standard	1	n/a	
🔿 A4	Ab1	Ab1	Unknown	n/a	2	
🔵 В4	Ab2	Ab2	Unknown	n/a	2	
🔿 C4	Ab3	Ab3	Unknown	n/a	2	
🔵 D4	Ab4	Ab4	Unknown	n/a	2	
🔵 E4	Ab5	Ab5	Unknown	n/a	2	
🔵 F4	Ab6	Ab6	Unknown	n/a	2	
🔵 G4	Ab7	Ab7	Unknown	n/a	2	-

Figure 5-19: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign Replicate Groups in the Sample Plate Table:

- 1. Double-click the desired cell in the **Replicate Group** table column.
- 2. Enter a group name (see Figure 5-20).

Sample	Plate Table —				
Conce	ntration units:	μg/ml 👻	Export	Import	
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	Dilution Factor 🔺
🔵 A1	lgG Standard	200	Standard	200	n/a
🔵 B1	lgG Standard	100	Standard	100	n/a
🔵 C1	lgG Standard	50	Standard	50	n/a
🔵 D1	lgG Standard	25	Standard	25	n/a
🔵 E1	lgG Standard	10	Standard	10	n/a
🔵 F1	lgG Standard	5	Standard	5	n/a
🔵 G1	lgG Standard	2.5	Standard	2.5	n/a

Figure 5-20: Add Replicate Group from the Sample Plate Table

**NOTICE**: Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE**: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software only recognizes and calculates statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

**NOTICE:** When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they are be treated as separate groups. Statistics for these groups calculate separately for each biosensor type.

# Managing Sample Plate Definitions

NOTICE: After you define a sample plate, you can export and save the plate definition for future use.

## Exporting a Plate Definition

To export a plate definition:

1. In the Sample Plate Table (see Figure 5-21), click Export.

Sample	Plate Table —				
Concer	ntration units:	μg/ml   ▼	Export	Import	
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	Dilution Factor 🔺
🔵 A1	lgG Standard	200	Standard	200	n/a
🔵 B1	lgG Standard	100	Standard	100	n/a
🔵 C1	lgG Standard	50	Standard	50	n/a
🔵 D1	lgG Standard	25	Standard	25	n/a
🔵 E1	lgG Standard	10	Standard	10	n/a
🔵 F1	lgG Standard	5	Standard	5	n/a

Figure 5-21: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 5-22), select a folder, enter a name for the plate (.csv), and click **Save**.

🔣 Export Plate 1 Definition					×
← → × ↑ 🔒 > This PC → Local	Disk (C:) → data	~	ۍ ج	earch data	
Organize 🔻 New folder					?
OneDrive	^	Date modified	Туре	Size	
💻 This PC		No items match your search			
3D Objects					
E Desktop					
Documents					
Downloads					
Music					
Pictures					
📕 Videos					
🏪 Local Disk (C:)					
🔲 DVD Drive (D:) O					
· · · · · · · · · · · · · · · · · · ·					
File <u>n</u> ame: 96 standard plate					~
Save as type: CSV Files (*.csv)					~
∧ Hide Folders			<u>S</u> a	ave Canc	el

Figure 5-22: Export Plate Definition Window

## Importing a Plate Definition

To import a plate definition:

1. In the Sample Plate Table (see Figure 5-23), click Import.

- 8	Sample Concei	Plate Table	µg/ml ▼	Export	Import	
	Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	Dilution Factor 🔺
	🔵 A1	lgG Standard	200	Standard	200	n/a
	🔵 B1	lgG Standard	100	Standard	100	n/a
	🔵 C1	lgG Standard	50	Standard	50	n/a
	🔵 D1	lgG Standard	25	Standard	25	n/a
	🔵 E1	lgG Standard	10	Standard	10	n/a
	🔵 F1	lgG Standard	5	Standard	5	n/a

Figure 5-23: Import Button in Sample Plate Table

2. In the Import Plate Definition window (see Figure 5-24), select the plate definition (.csv), and click Open.



Figure 5-24: Import Plate Definition Window

**NOTICE:** You can also create a.csv file for import. Figure 5-25 shows the appropriate column information layout.

	А	В	С	D	E	F	G	
1	PlateWells	96						_
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information	_
3	A1	IgG Standard	200	Standard	200		Sample Diluent	
4	B1	IgG Standard	100	Standard	100		Sample Diluent	
5	C1	IgG Standard	50	Standard	50		Sample Diluent	
6	D1	IgG Standard	25	Standard	25		Sample Diluent	
7	E1	IgG Standard	10	Standard	10		Sample Diluent	
8	F1	IgG Standard	5	Standard	5		Sample Diluent	
9	G1	IgG Standard	2.5	Standard	2.5		Sample Diluent	
10	H1	IgG Standard	1	Standard	1		Sample Diluent	
11	A2	IgG Standard	200	Standard	200		Sample Diluent	-
H 4	▶ ¥ 96 s	tandard plate 🧷	1					·

Figure 5-25: Example Sample Plate File (.csv)

## Printing a Sample Plate Definition

To print a plate definition:

1. In the **Sample Plate Map** (see Figure 5-26), click **Print**.

The associated **Sample Plate Table** information prints.

Plate 1	Table (96 wel	ls) –								
Conce	ntration units:		µg/ml	$\sim$	Exp	ort	Import		Print	
Well	Sample ID	Re	eplicate (	Group	Туре	Conc	(µg/ml)	Dilu	tion Factor	Inf

Figure 5-26: Sample Plate Print Button

# Managing Assay Parameter Settings

## Modifying Assay Parameter Settings

Modify the assay parameter settings while sample plate is defined. Changes are only applied to the current experiment. To save the modified parameter settings, define a new assay. For details on creating a new assay, see "Custom Quantitation Assays" on page 142.

## Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box appears (Figure 5-27). The available settings are experiment-dependent.



Figure 5-27: Modifying Assay Parameters

## Basic Quantitation Assay Parameters

lable Assays:	Assay Parameters	
Bable Assays:         Basic Quantitation         Bable Assays:         Bable Assay:	Assay Parameters <ul> <li>Single analyte</li> <li>Multiple analyte</li> <li>Replicates per sensor type:</li> <li>1</li> </ul> <ul> <li>Ime (s):</li> <li>Shake speed (rpm):</li> <li>Quantitation:</li> <li>120</li> <li>400</li> </ul>	

Figure 5-28: Assay Parameters-Basic Quantitation Assay

Table 5-6: Basic	Quantitation Assay	Parameters
------------------	--------------------	------------

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analy- sis.
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

### Basic Quantitation with Regeneration Assay Parameters

Assay Parameters	>
Available Assays:	Assay Parameters
Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB) with regeneration     Article L-Standard ange     Arti-Human Fab-CH1 (FAB) with regeneration     Article L-Standard ange     Arti-Human Fab-CH1 (FAB) with regeneration     Article L-Standard ange     Article L-Standard ange     Article L-Standard Angel     A	Image: analyse     Image: analyse       Replicates per ternor type:     Image: analyse       Countritation:     Image: analyse       Image: analyse     Shake speed (pm):       400     Image: analyse       Regeneration:     Image: analyse       Image: analyse     Image: an
Gray indicates a built-in assay.	OK Cancel

Figure 5-29: Assay Parameters-Basic Quantitation with Regeneration

Table 5-7: Assay Parameters-Basic	c Quantitation with Regeneration
-----------------------------------	----------------------------------

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analysis.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regener- ated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

### Advanced Quantitation Assay Parameters

Use the Advanced Quantitation Assay Parameters to create a custom assay.

ilable Assays:	Assay Parameters				
Basic Quantitation	Single analyte	O Multiple analy Replicates p	<b>yte</b> per sensor type:	1	
1 Anti-GST -Standard range	Step Type	Time (s)	Shake (rpm)	Step Options	Insert
	Sample	<ul> <li>120</li> </ul>	1000	Online	Remove
	Detection	120	1000	Reuse position	Hemove
Anti-Penta-HIS -Standard range					
High sensitivity Human IgG quantitation					
Human IgG Quantitation					Maus Lin
Immunogenicity - Direct detection					wove op
Murine IgG Quantitation	1				> Move Dow
Protein L -Standard range					
Standard Assay	[✓] Regeneration	Time (s):	Shake speed	(rpm):	
Basic Quantitation with Regeneration     Acti Human Eab CH1 (EAD) with regeneration	Regeneration:	5	1000 🚖		
High constitutive assay with regeneration			1000		
Protein L Standard range	Neutralization:	5 💌	1000		
			Regeneration	cycles:	
Advanced Quantitation	Between assa	ay steps:	3 📫		
Immunogencity - Enzyme Linked	Pre-condition	eeneore	3 *		
Residual Protein A		20112012	J 👻		
Standard Assay	Post-condition	sensors	3		
- <u>x</u>					
≣⊒ Three Step Assay	and a second s				

Figure 5-30: Assay Parameters-Advanced Quantitation

- 1. Select the type of Analyte.
  - Single analyte select to use one biosensor per sample well.
  - Multiple analytes select to use multiple biosensors per sample well.
    - Replicates per sensor type select the number of replicates for each sensor type.
- 2. Select the desired step options.
  - Insert click insert to add a step.
  - Remove select a step and then click Remove to remove a step.
  - Move Up select a step and then click Move Up to move a step up one row.
  - Move Down select a step and then click Move Down to move a step up one row.
- 3. Adjust the Time and Shake speed (rpm) of each step.
  - Time select the duration time of the step.
  - Shake speed select the shake speed in rpm for the step.
- 4. Regeneration Incubate the biosensor in the regeneration buffer to remove the bound analyte.
- 5. Neutralization Incubate the biosensor in the neutralization buffer after the regeneration step.
- 6. Between assay steps Regeneration cycles select the number of cycles of regeneration and neutralization to perform in between assays.
- 7. Pre-condition sensors Perform a set of regeneration or neutralization steps before the start of the experiment. These settings are like the time and rpm settings for the regeneration steps. For example, an acidic pre-conditioning buffer maximizes the binding competency of Protein A biosensors.
- 8. Post-condition sensors Perform the selected number of regeneration cycles on the biosensors prior to re-racking for storage.
- 9. Step option Reagent wells can be reused.
  - Reuse Position define a single position for a reagent. This position is used for all assays in the experiment

- Use x1 through Use x10 define the number of times the reagent in a position can be used. After the selected number of times, that position is no longer used in the experiment. You must define enough reagent positions in the plate to complete the experiment. For example, if the experiment has six assays:
  - You can define two reagent positions on the place and select use x3.
  - Or you can define three reagent positions on the plate and select use x2.
- Distribute usage (auto) define multiple positions in the plate for the reagent. The software automatically distributes the assays, so the defined reagent positions are used equally. For example, if the experiment has six assays and there are two defined reagent positions, the software will use each position three times.

**NOTICE:** Preview the application of the Reuse Position setting to ensure your settings. Select the Review Experiment tab and step through the experiment.

# Assigning Biosensors to Samples

After the sample plate is defined, assign biosensors to the samples.

## Biosensor Assignment in Single-Analyte Experiments

In a single analyte experiment, only one biosensor type is assigned to each sample and only one analyte is analyzed per experiment.

**NOTICE:** For single analyte experiments, the **Single Analyte** option must be selected in the **Assay Parameters** dialog box. For more information, please see "Managing Assay Parameter Settings" on page 107.

Click the **Sensor Assignment** tab, or click the  $\rightarrow$  arrow to access the Sensor Assignment window (see Figure 5-31).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map**.



Figure 5-31: Sensor Assignment Window for Basic Quantitation without Regeneration

- 1. There are two ways to assign biosensors:
  - Select a column(s) in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list).
  - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 5-32).



The wells in the **Sensor Type** column are populated with the selected biosensor type.

Figure 5-32: Changing Biosensor Types

2. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

- 3. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column automatically populates with the lot number entered.
- 4. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it is not available for display as a legend.

5. Optional for the Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2 instrument only: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 5-33).



Figure 5-33: Replace Sensors in Tray After Use Check Box

**NOTICE:** Biosensors can be regenerated up to a max of 11 times per experiment.

## Biosensor Assignment in Multiple Analyte Experiments

In a multiple analyte experiment, more than one biosensor type is assigned to the same sample, allowing multiple analytes to be analyzed in a single experiment.

**NOTICE:** For multiple analyte experiments, the Multiple Analyte option must be selected in the Assay Parameters dialog box. For more information, please see "Managing Assay Parameter Settings" on page 107.

Click the **Sensor Assignment** tab, or click the  $\rightarrow$  arrow to access the Sensor Assignment window (see Figure 5-31).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 5-34, one replicate had been previously selected with the **Multiple Analyte** assay parameter option.



Figure 5-34: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

There are two ways to assign biosensors:

- Select a set of wells in the Sensor Tray Map, right-click and select a biosensor type from the drop-down list.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the dropdown list (see Figure 5-35).



Figure 5-35: Changing Biosensor Types

### Biosensor Assignment Using Heterogeneous Biosensor Trays

The default Tray Format is Heterogeneous. Heterogeneous biosensor trays contain a mixture of biosensor types.

**NOTICE:** When using this Heterogeneous option, the order of biosensor types in each tray must be identical.

- If Heterogeneous Trays does not appear next to the Tray Format button, click the button. The Tray Format dialog box appears (see Figure 5-36).
- 2. Select Heterogeneous and click OK.

Heterogeneous	Sensor trays may contain various senso but all sensor trays used are identical.	or types,
Homogeneous	A different sensor tray is used for each	sensor type.
Sensors:	Anti-Human IgG Fc	Add
		Remove
		Change
		Move Up
		Move Down

Figure 5-36: Tray Format Dialog Box

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to use (see Figure 5-37).

The Sensor Type column updates accordingly.



Figure 5-37: Populating the Sensor Tray Map with First Biosensor Type

4. Select the sensors in the **Sensor Tray Map** that contain the second biosensor type, right-click and select the second biosensor type (see Figure 5-38).

The Sensor Type column updates accordingly.



Figure 5-38: Populating the Sensor Tray Map with Second Biosensor Type

5. Repeat this sensor selection and assignment process for all other biosensor types in the experiment. The software automatically updates the number of biosensor trays needed and biosensor assignments in all trays according to the column assignments made in Tray 1.

In the example shown in Figure 5-39, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays are needed for the experiment.



Figure 5-39: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

6. To view or change the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected show and biosensor assignments can be changed as needed (see Figure 5-40).

Sens	ior Tray leplace sensors in tray after use	Senso Tray	r Tray: 1 V of 2 Tra	y Format H	eterogeneous trays
	1 2 3 4 5 6 7 8 9 10 11 12	Tray	or Type	Lot Number	Information
A		85	Pilagein G		
B		C5	Protein G		
		D5	Protein G		
		E5	Protein G		
D		F5	Protein G		



7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

- 8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for that biosensor type automatically populate with the lot number entered.
- 9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it is not available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace** sensors in tray after use check box (see Figure 5-41).



Figure 5-41: Replace Sensors in Tray After Use Check Box

NOTICE: Biosensors can be regenerated up to a max of 11 times per experiment.

### Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.

**NOTICE:** Using the Homogeneous option requires switching trays during the experiment.

#### 1. Click Tray Format.

The Tray Format dialog box appears and the Sensors box populates with the default biosensor type.

A Pormat     A Pormat     A Pormat	Sensor travs may contain various sensor types.
_	but all sensor trays used are identical.
Homogeneous	A different sensor tray is used for each sensor type.
Sensors:	Anti-Human IgG Fc Add
	Remove
	Change
	Move Up
	Move Down
	OK Cancel

Figure 5-42: Tray Format Dialog Box

2. Select Homogeneous. Click Add to select the first biosensor type.

Tray Format		<b>X</b>
C Heterogeneous	Sensor trays may contain various sensor ty but all sensor trays used are identical.	pes,
Homogeneous	A different sensor tray is used for each sensor	or type.
Sensors:	Anti-Human IgG Fc	Anti-Mouse IgG Fv
		Protein A
		Protein G
		Protein L
		SA (Streptavidin)
		Residual Protein A
		Anti-Penta-HIS
		Custom
	OK Cancel	

Figure 5-43: Selecting a Biosensor Type in the Tray Format Dialog Box

- 3. Repeat this step to add any additional biosensor types used in the experiment. To remove a biosensor type, select a biosensor type in the **Sensor** box and click **Remove**.
- 4. Adjust the order of biosensor types as needed by selecting the biosensor type in the **Sensor** box and clicking **Move Up** or **Move Down**.

The order of biosensor types listed in the **Sensor** box is used as the default tray assignment.

Tray Format		X
Heterogeneous	Sensor trays may contain various sensor ty but all sensor trays used are identical.	pes,
Homogeneous	A different sensor tray is used for each sense	or type.
Sensors:	Protein A Protein G	Add
	The second	Remove
		Change
		Move Up Move Down
	OK Cancel	]

Figure 5-44: Biosensor Types List Order in Sensor Box

### 5. Click OK.

The software automatically calculates the number of biosensor trays needed and assign biosensors types to each tray.

In the example shown in Figure 5-45, Protein A and Protein G biosensor types are used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) are needed for the experiment. The Tray 1 Sensor Tray Map appears by default.



Figure 5-45: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

6. To view the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

Sensor Tray ☑ Replace sensors in tray after use	Sensor Tray: Tray 1 v of 4 Tray Format Homogeneous trays
1       2       3       4       5       6       7       8       9       10       11       12         A       1       2       3       4       5       6       7       8       9       10       11       12         B       1       1       1       10       11       12       11       12         B       1       1       10       11       12       10       11       12         B       1       1       10       10       11       12       11       12         B       1       1       10       10       10       10       11       12         C       1       10       10       10       10       10       10       10         E       1       10       10       10       10       10       10       10       10       10       10       10       10       11       12       10	Tray 1     or Type     Lot Number     Information       Tray 2     or Type     Lot Number     Information       Tray 4     A     Tray 4     A       Tray 4     A     Tray 4     A       Tray 4     A     Tray 4     A       Fast 3     Frotein A     A       C5     Protein A     D       D5     Protein A     F       F5     Protein A     A       G5     Protein A     A       H5     Protein A     A       A6     Protein A     B
Remove Fill Fill Plate Print	C6 Protein A

The **Sensor Tray Map** and table for the tray selected appears.

Figure 5-46: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

8. Optional: Double-click in any cell in the Lot Number column to enter a biosensor lot number.

All wells in the **Lot Number** column for the biosensor type selected automatically populates with the lot number entered.

9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it is not available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace** sensors in tray after use check box (see Figure 5-47).



Figure 5-47: Replace Sensors in Tray After Use Check Box

**NOTICE:** Regnerate biosensors no more than 11 times per experiment.

## **Biosensor Regeneration**

For Basic Quantitation with Regeneration experiments only, the Sensor Assignment tab includes the Regenerations parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The number of regeneration cycles determines the minimum number of cycles required for two sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by two sensors is represented by a pie chart (Figure 5-48).



Figure 5-48: Fractional Use of Regeneration and Neutralization Wells

## Using Partial Biosensor Trays

If you are using a partial tray of biosensors (some biosensors are missing), specify the missing columns in the **Sensor Tray Map**:

1. Select the column(s) without biosensors and click **Remove**, or right-click the selection and select **Remove**.

If the number of specified biosensors in the **Sensor Assignment** tab is less than the number required to perform the assay, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay.

2. The example in Figure 5-49 shows that Tray 1 is a partial tray that does not contain enough biosensors for the assay. To view the additional biosensor tray that is required, select Tray 2 from the Sensor Tray drop-down list (Figure 5-49 top). The Sensor Tray Map displays the additional biosensors required for the assay (Figure 5-49 bottom).

Sensor Tray												
✓ Replace sensors in tray after use												
	1	2	3	4	5	6	7	8	9	10	11	12
A							$\otimes$	$\otimes$	$\otimes$	$\otimes$	$\otimes$	$\boxtimes$
B							$\otimes$	$\otimes$	$\otimes$	$\otimes$	$\otimes$	$\boxtimes$
C							$\boxtimes$	$\otimes$	$\otimes$	$\otimes$	$\boxtimes$	$\boxtimes$
D							$\boxtimes$	$\boxtimes$	$\boxtimes$	$\otimes$	$\boxtimes$	$\boxtimes$
E							$\boxtimes$	$\otimes$	$\boxtimes$	$\boxtimes$	$\otimes$	$\boxtimes$
F							$\boxtimes$	$\otimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$
G							$\boxtimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$
Η							$\boxtimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$	$\boxtimes$
Lege	end:		Unas	ssigne	ed ser	nsors		× 1	Missin	g sen	sors	
R	emove	•		Fill		F	ill Plat	е			Pri	nt
Sar	neor Tr	-										
	Repla	ce se	nsors	in tra	y afte	ruse						
	1	2	3	4	5	6	7	8	9	10	11	12
A												
E	3											

D E F G H

Legend: Unassigned sensors

ove Fill Fill Plate

Figure 5-49: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray

Print...

Missing sensors

To restore biosensors that have been removed, select the columns to restore and click **Fill**. To restore all sensors on the plate, click **Fill Plate**.

**NOTICE:** If multiple biosensor trays are used, only the first biosensor tray can be a partial tray. During the experiment, the software prompts you to insert the appropriate tray in the Octet<sup>®</sup> instrument.

# **Reviewing Experiments**

NOTICE: For optimal results, ensure total assay time is less than 3 hours.

Before running an experiment, you can review the sample plate layout and the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window, move the slider left or right to highlight the biosensors and samples in an assay, or click the  $\epsilon$  arrows to select an assay.



Figure 5-50: Review Experiment Window

# Saving Experiments

After a run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment method:

- 1. Click the **Save Method File** button 📩 , or on the main menu, click **File** > **Save Method File**. To save more than one open experiment, click the **Save All Methods Files** button 🚓 .
- 2. In the Save dialog box, enter a name and location for the file, and click Save.

**NOTICE:** If you edit a saved experiment and want to save it without overwriting the original file, select **File** > **Save Method File As** and enter a new name for the experiment.

### Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment is available on the menu bar. To view templates click **Experiment > Templates > Quantitation > Experiment Name** (see Figure 5-51).

Follow the previous steps to save an experiment to the Template folder located at C:\Program Files\Sartorius\Octet-BLIDiscovery\TemplateFiles.

**IMPORTANT:** Do not change the location of the Template folder. If the Template folder is not at the factory-set location, the software may not function properly.

Experiment Instrument Wind	ow Help					
✓ New Experiment Wizard Edit Assay Parameters	Ctrl+N					
Edit Sensor Types						
Set Plate Temperature						
Templates	+	Kinetics	- + I			
Skip Step		Quantitation	+	Advanced Quantitation	+	
Stop				Basic Quantitation	•	Anti-hIgG biosensor_16CH_96W.fmf
				Basic Quantitation with Regeneration	•	Anti-hIgG biosensor_8CH_96W.fmf
						Anti-mIgG biosensor_16CH_96W.fmf
						Anti-mIgG biosensor_8CH_96W.fmf
						Anti-Penta-HIS Dilution Factor Scouting_96W.fmf
						Anti-Penta-HIS Spike Recovery Assay_96W.fmf
						DirectDetectionImmunogenicity_16CH_96W.fmf
						DirectDetectionImmunogenicity_8CH_96W.fmf
						Protein A biosensor_16CH_96W.fmf
						Protein A biosensor_8CH_96W.fmf
						Protein A or G biosensor_16CH_96W.fmf
						Protein A or G biosensor_8CH_96W.fmf
						Protein L biosensor_16CH_96W.fmf
						Protein L biosensor_8CH_96W.fmf

Figure 5-51: Experiments in the Template Folder

# Running a Quantitation Experiment

**IMPORTANT:** Before starting an experiment, ensure that the biosensors are properly rehydrated. The biosensor product insert has the instructions for preparing the biosensors.

## Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

- 1. Open the Octet<sup>®</sup> instrument door (lift the handle up).
- 2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 5-52).
- 3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 5-52).



Figure 5-52: Biosensor Stage (left) and Sample Stage (right)

**IMPORTANT:** Ensure that the bottom of the sample plate and biosensor tray are flat on each stage.

- 4. Close the Octet<sup>®</sup> instrument door.
- 5. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

## Starting an Experiment

To start the experiment:

#### 1. Click the **Run Experiment** tab (see Figure 5-53).

Plate Definition 2 Sensor Assignment	3 Review Experiment 4 Run Experiment	
Data File Location and Names		
Assay type:	Basic Quantitation Standard Assay	Prior to pressing "Go" confirm the Ass
Quantitation data repository:	C:\data	
Experiment run name (sub directory):	hlgG ProG Q	<b>→</b>
Plate name/barcode (file prefix):	201030	
Auto-increment file ID start:	1	
Data files will be stored as follows:		Total experiment time:
C:\data\higG ProG Q\201030_003.frd		
Run Settings		
Start after (s): 600	Upen runtime charts automatically	
Shake sample plate while waiting	Set plate temperature (°C): 30	A T
General Information User name: Description:	Machine name: DESKTOP-0EHTC34	

Figure 5-53: Run Experiment Window

2. Confirm the defaults or enter new settings. See "Run Experiment Window Settings" on page 133 for more information on experimental settings.

NOTICE: If you delay the experiment start, you have the option to shake the plate until the experiment starts.

3. To start the experiment, click GO .

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you selected the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time and the experiment progress (see Figure 5-54).

NOTICE: For more details about the Runtime Binding Chart, see "Managing Runtime Binding Charts" on page 136.



Figure 5-54: Runtime Binding Chart

4. Optional: Click View > Instrument Status to view the log file (see Figure 5-55).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



**WARNING:** Do not open the Octet<sup>®</sup> instrument door when an experiment is in progress. If the door is opened the data from the active acquisition step is lost. The data acquired in previous steps is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



**WARNING:** N'ouvrez pas la porte de l'instrument Octet<sup>®</sup> lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



**WARNING:** Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

🖸 Instrument Status 📃 🗖 💌				X
14:47:39	Sensor 7: Integration Time = 1.0 ms			
14:47:39	Sensor 8: Integration Time = 1.0 ms			
14:47:40	Picking sensors completed location A1			
14:47:40	Plate temperature = 30 C			
014:47:40	Ready to move to sample location A1			
014:47:40	Moving to sample location A1			
014:47:41	Arrived at sample location A1			
014:47:41	Waiting to start sample location A1			
14:47:41	Processing sample location A1			
014:47:51	Sample completed location A1			
-14:47:51	Waiting to start new step			
-14:47:51	Starting new step			
014:47:52	Ready to move to sample location A2			-
014:47:52	Moving to sample location A2			-
014:47:53	Arrived at sample location A2			
0 14:47:53	Waiting to start sample location A2			
14:47:53	Processing sample location A2			
				-
<b>▲</b>	III		•	
Auto scroll to bottom Save to File				

Figure 5-55: Instrument Status Log

## Run Experiment Window Settings

The following Data File Location and Name settings are available on the Run Experiment Tab:

Table 5-8: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click <b>Browse</b> to select another data location.
	<b>NOTICE:</b> Save the data to the local machine first, then transfer to a network drive.
Experiment Run name (sub- directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software generates one data file for each biosensor.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following Run Settings are available on the Run Experiment Tab:

Table 5-9: Run Settings

Item	Description
Delayed experiment start	Specifies a time delay for the start of the experiment.Enter the number of seconds to wait before the experiment starts after you click <b>co</b> .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while wait- ing	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automati- cally	Displays the <b>Runtime Binding Chart</b> for the current biosensor during data acquisi- tion.
Automatically save runtime chart	Saves an image (.jpg) of the <b>Runtime Binding Chart</b> . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in <b>File &gt; Options</b> . The factory set default temperature is 30 °C.
	<b>NOTICE:</b> If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet <sup>®</sup> BLI Discovery software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.

Optimize the signal to noise ratio of the assay by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet<sup>®</sup> system per second and is reported in Hertz (per second).

- A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate.
- A lower acquisition rate allows the software enough time to perform more averages of the collected data.

Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.
#### Table 5-10: Advanced Settings

Item	Description
Acquisition rate	<b>NOTICE:</b> For the Octet <sup>®</sup> R2, Octet <sup>®</sup> R4, or Octet <sup>®</sup> K2 system, acquisition rate settings are available on the Plate Definition Tab.
	<ul> <li>High concentration quantitation (10 Hz, averaging by 5) — The average of 5 data frames is reported as one data point. 10 data points are reported per second.</li> </ul>
	<ul> <li>High sensitivity quantitation (2 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second.</li> </ul>
	<ul> <li>Standard quantitation (5 Hz, averaging by 20)—The average of 20 data frames is reported as one data point. Five data points are reported per second.</li> </ul>
Sensor offset (mm)	Recommended sensor offset for quantitation—3 mm.
	<b>NOTICE:</b> For more details on optimizing the sensor offset and acquisition rate please contact your local Sartorius representative.
Default	Sets acquisition rate and sensor offset to the defaults.

#### The following General Settings are available on the Run Experiment Tab:

#### Table 5-11: General Settings

Item	Description
Machine name	The computer name that controls the $Octet^{\circledast}$ instrument and acquires the data.
User name	The user logon name.
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

# Stopping an Experiment

To stop an experiment in progress, click  $\bigotimes$  or click **Experiment** > Stop.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.

**NOTICE:** After the experiment is run, the software automatically saves the experiment method (.fmf).

# Managing Runtime Binding Charts

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 5-56). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active sensors are color-coded (A=green, B=magenta, C=orange, D=purple, E=olive, F= black, G=red, H=blue) within the **Sensor Tray Map**. Used sensors that are inactive are colored black. Active sample columns are colored green. Each data acquisition step is represented by **Sample Column X** in the **Current Binding Charts** box.

To selectively display acquisition data for a particular acquisition step:

- 1. Click the corresponding Sample Column number.
- 2. Select a sub-set of sensors for a displayed column under Sensors to Chart box (see Figure 5-56).

**IMPORTANT:** Do not close the Runtime Binding Chart window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet<sup>®</sup> BLI Discovery software saves the Runtime Binding Chart as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor causes this data not to be included in the bitmap image generated at the end of the run.



Figure 5-56: Runtime Binding Chart Window

# Opening a Runtime Binding Chart

After an experiment is run, you can open and review the Runtime Binding Chart at any time:

#### 1. Click File > Open Experiment.

2. In the dialog box that appears, select an experiment folder and click Select.

## Viewing Reference-Subtracted Data

Display reference-subtracted data during acquisitions that include reference biosensors by clicking the Subtract reference sensors check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the Sensor Assignment tab
- During acquisition in the Runtime Binding Chart Sensors to Chart box
- During analysis in the Data Selection tab

#### Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the Sensors to Chart list or the Sensor Tray, right-click a biosensor and select Reference.



Figure 5-57: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor shows with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 5-58).

2. Click the Subtract reference sensors check box, see Figure 5-58.



Figure 5-58: Subtract Reference Sensors check box in the Runtime Binding Chart

**NOTICE:** Subtracting reference data in the Runtime Binding Chart only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be re-done in data analysis if needed.

# Viewing Inverted Data

The data displayed in the Runtime Binding Chart can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the Flip Data check box (see Figure 5-59). Deselect the box to return to the default data display.



Figure 5-59: Data Inverted Using Flip Data Function

# Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

# Scaling a Runtime Binding Chart

To scale the Runtime Binding Chart:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, select Fullscale or Autoscale.

# Adding a Runtime Binding Chart Title

To add a Runtime Binding Chart title:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, enter a graph title or subtitle.

# Selecting a Runtime Binding Chart Legend

To select a Runtime Binding Chart legend:

- 1. Right-click the chart and select **Properties**.
- 2. In the **Runtime Graph Properties** dialog box (see Figure 5-60), select one of the following legends:
  - Sensor Location
  - Sample ID
  - Sensor Information
  - Concentration/Dilution

Runtime Graph Properti	ies X
Title:	
Subtitle:	
Legend	
Sensor Location	Sensor Information
Sample ID	Concentration / Dilution
	OK Cancel

Figure 5-60: Selecting a Runtime Binding Chart Legend

**NOTICE:** Text for **Sample ID**, **Sensor Information**, or **Concentration/Dilution** is taken from the **Plate Definition** and **Sensor Assignment** tabs, and must be entered before the experiment is started.

3. Click OK.

# Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window** > **New Window**.

# Exporting or Printing the Runtime Binding Chart

To export the Runtime Binding Chart as a graphic or data file:

- 1. Right-click the chart and select **Export Data**.
- 2. In the **Exporting** dialog box (see Figure 5-61), select the export options and click **Export**.

Export							Taut / Data	
O EWF	O WN		змр	O JPG	O PNG		I ext / Data	
Export Des	stination							
ClipBoa	ard							
File		Prov						
) File		Brow	se					
<ul><li>File</li><li>Printer</li></ul>		Brow	se					
<ul> <li>File</li> <li>Printer</li> </ul>	2	Brow	se					
<ul> <li>File</li> <li>Printer</li> <li>Export Size</li> </ul>	9	Brow	rse Ilimeter	rs Olno	thes OF	Points		
<ul> <li>File</li> <li>Printer</li> <li>Export Size</li> </ul>	e	Brow	llimeter	rs Olno	ches © F	Points		

Figure 5-61: Exporting Dialog Box

Table 5-12: Runtime Binding Chart Export Options (Sheet 1 of 2)

Task	Export	Option	Export Destination	Result
	Text/Data	EMF, WMF, BMP, JPG, or PNG		
Save the bind- ing data	$\checkmark$		Click File > Browse to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosen- sor. Open the file with a text editor such as Notepad.
Export the Run- time Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.
Copy the Run- time Binding Chart		$\checkmark$	Clipboard	Copies the chart to the system clip- board

#### Table 5-12: Runtime Binding Chart Export Options (Sheet 2 of 2)

Task	Export	Option	Export Destination	Result
Print the Run- time Binding Chart		$\checkmark$	Printer	Opens the Print dialog box.

# Managing Experiment Method Files

After you run an experiment, the Octet<sup>®</sup> BLI Discovery software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it as needed.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message appears

#### Table 5-13: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > Open Method File 🔂	Select and open a method file (.fmf)
File > Save Method File 📥 or 🖄	Saves one method file or all method files. Saves a method file before the exper- iment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

# Custom Quantitation Assays

# Defining a Custom Assay

To define a custom assay:

1. Click Experiment > Edit Assay Parameters.

The Edit Assay Parameters dialog box appears; see Figure 5-62.

Edit Assay Parameters			×
Available Assays:			
Basic Quantitation	Name:	Standard Assay	
	Description:	Basic Quantitation - Standard Assav (Read Only)	
Anti-GST -High sensitivity			
Anti-GST -Standard range	Assay Param	eters	
Anti-HIS (HIS2) Quantitation	Single an	alvte O Multiple analvte	
Anti-Penta-HIS -High sensitivity		Replicates per sensor type: 1	
III High sensitivity Human IgG guantitation			
		Time (s): Shake speed (rom):	
	Quantitation	· 120 A 400 A	
My Basic Quant Assay			
Protein L -Standard range			
Standard Assay			
Basic Quantitation with Regeneration			
Anti-Human Fab-CH I (FAB) with regeneration			
High sensitivity assay with Regen Assay			
Protein L -Standard range			
Standard Assay			
Advanced Quantitation			
Immunogencity - Enzyme Linked			
Standard Assay			
····· <b>I</b> I Three Step Assay			
			_
aray indicates a built-in assay and cannot be modified or de	eleted.		

Figure 5-62: Edit Assay Parameters Dialog Box

- 2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
- 3. Click **Duplicate**, Figure 5-63.
- 4. In the New Assay dialog box (see Figure 5-63 top), enter an Assay name.
- 5. Optional: In the Assay Description, enter information about the assay.
- 6. Click Save.

The new assay appears in the directory tree of available assays (see Figure 5-63 bottom).

Available Assays:		~
basic Quantitation	Name:	Standard Assay
Anti-FLAG Quantitation	Description:	Basic Quantitation - Standard Assay (Read Only)
	Assay Param	eters
Anti-HIS (HIS2) Quantitation	Single an	alyte O Multiple analyte
Anti-Penta-HIS -High sensitivity		Replicates per sensor type: 1
Anti-Penta-HIS -Standard range		
High sensitivity Human IgG quantitation		
		Time (s): Shake speed (rpm):
Murine IgG Quantitation	Quantitation	120 🗘 400 🗘
Standard Assay		
Basic Quantitation with Regeneration		
	New As	ay X
Protein L -Standard range	Enter	Assay Information
Standard Assay	Assay	name: My Basic Quant Assay
	Assav	description: Enter a short description of the assau here
Residual Protein A		
Inree Step Assay		
		OK Cancel
Gray indicates a built-in assay and cannot be modified or de	eleted.	
Duplicate Remove		Save Cancel
Edit Assay Parameters		×
Available Assays:		
Basic Quantitation	Name:	My Basic Quant Assay
	Description:	Enter a short description of the assay here.
Anti-GST -Standard range	Assay Param	sters
Anti-HIS (HIS2) Quantitation	Single an	alvte O Multiple apalvte
Anti-Penta-HIS -High sensitivity		Replicates per sensor type: 1
		Replicates per sensor type: 1
EArti-Penta-HIS -High sensitivity     EArti-Penta-HIS -Standard range     EArti-Penta-HIS -Standard range     EA High sensitivity Human IgG quantitation     EA Human IgG Quantitation		Replicates per sensor type: 1
Anti-Penta-HIS -High sensitivity     BArti-Penta-HIS -Standard range     BA High sensitivity Human IgG quantitation     BA Human IgG Quantitation     BA Human IgG Quantitation     BA Human IgG Quantitation		Time (s): Shake speed (rpm):
Anti-Penta-HIS -High sensitivity     BArti-Penta-HIS -Standard range     BArti-Penta-HIS -Standard range     BA High sensitivity Human IgG quantitation     BA Human IgG Quantitation     BA Munine IgG Quantitation     BA Munine IgG Quantitation     BA Munine IgG Quantitation	Quantitation	Time (s):     Shake speed (rpm):       120     400
Anti-Penta-HIS -High sensitivity     BA Anti-Penta-HIS -Standard range     BA Anti-Penta-HIS -Standard range     BA High sensitivity Human IgG Quantitation     BA Human IgG Quantitation     BA Murine IgG Quantitation	Quantitation	Time (s):     Shake speed (rpm):       120     400
Anti-Penta-HIS -High sensitivity     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Direct detection     Anti-Penta-HIS	Quantitation	Time (s):     Shake speed (rpm):       120     400
Anti-Penta-HIS -High sensitivity     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Direct detection     Anti-Penta I-Standard range     Anti-Penta-HIS - Figure - Anti-Penta I-Standard - Assay     Anti-Penta-HIS - Anti-Penta I-Standard - Assay     Anti-Penta-HIS - Anti-Penta I-Standard - Assay	Quantitation	Time (s):     Shake speed (rpm):       120     400
Anti-Penta-HIS -Fligh sensitivity     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Direct detection     Anti-Penta-Istandard range     Anti-Penta-Istandard range	Quantitation	Replicates per sensor type:     1       Time (s):     Shake speed (rpm):       120     400
Anti-Penta-HIS -High sensitivity     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Punan IgG Quantitation     Anti-Punan     AntigG Quantitation     Anti-Punan     Anti-Punan     Anti-Punan	Quantitation	Time (s):     Shake speed (rpm):       120     400
Arti-Penta-HIS -High sensitivity     Arti-Penta-HIS -Standard range     Arti-Penta-HIS -Standard range     High sensitivity Human IgG Quantitation     Arti-Penta-HIS -Direct detection     Arti-Penta-HIS -Direct detection     Arti-Penta-Randard range     Standard Assay     My Basic Quantitation     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB)     Arti-Human Fab-CH1 (FAB)	Quantitation	Time (s):     Shake speed (rpm):       120     400
Arti-Penta-HIS -High sensitivity     Arti-Penta-HIS -Standard range     Arti-Penta-HIS -Standard range     High sensitivity Human IgG quantitation     Arti-Penta-HIS -Standard range     Murine IgG Quantitation     Arti-Penta-HIS -Direct detection     Arti-Penta-Standard range     My Basic Quantitation     Arti-Penta-Standard range     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB)     Arti-Penta-Standard range     Advanced Quantitation     Advanced Quantitation     Advanced Quantitation     Advanced Quantitation     Advanced Resolute Protein A	Quantitation	Replicates per sensor type: 1 Time (s): Shake speed (rpm): 120 400
Arti-Penta-HIS -High sensitivity     Arti-Penta-HIS -Standard range     Arti-Penta-HIS -Standard range     High sensitivity Human IgG quantitation     Arti-Penta-HIS -Standard range     Murine IgG Quantitation     Arti-Penta-HIS -Direct detection     Arti-Penta-Standard range     My Basic Quantitation     Arti-Penta-Standard range     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB)     Arti-Human Fab-CH1 (FAB)     Arti-Human Fab-CH1 (FAB)	Quantitation	Replicates per sensor type: 1 Time (s): Shake speed (rpm): 120 400
Arti-Penta-HIS -High sensitivity     Arti-Penta-HIS -Standard range     Arti-Penta-HIS -Standard range     High sensitivity Human IgG quantitation     Arti-Penta-HIS -Standard range     Murine IgG Quantitation     Arti-Penta-HIS -Direct detection     Arti-Penta-Standard range     My Basic Quantitation     Arti-Hotin L-Standard range     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB)     Arti-Human Fab-CH	Quantitation	Replicates per sensor type: 1 Time (s): Shake speed (rpm): 120 400
Arti-Penta-HIS -High sensitivity     Arti-Penta-HIS -Standard range     High sensitivity Human IgG quantitation     High sensitivity Human IgG quantitation     Arti-Penta-HIS -Standard range     Murine IgG Quantitation     Arti-Human Factor range     Standard Assay     My Basic Quantitation     Arti-Human Factor range     Advanced Quantitation     Arti-Human Factor range     Arti-Human	Quantitation	Replicates per sensor type: 1 🗢 Time (s): Shake speed (rpm): 120 💽 400 💽
Arti-Penta-HIS -High sensitivity     Arti-Penta-HIS -Standard range     High sensitivity Human IgG quantitation     High sensitivity Human IgG quantitation     Arti-Penta-HIS -Standard range     Murine IgG Quantitation     Arti-Human IgG Quantitation     Arti-Human IgG Quantitation     Arti-Penta-Standard range     Standard Assay     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB) with regeneration     Arti-Human Fab-CH1 (FAB)     Three Step Assay     Arta-Fatigment Assay     Arti-Fatigment	Quantitation	Replicates per sensor type: 1 Time (s): Shake speed (rpm): 120 400
Image: Standard angle         Image: Arti-Penta-HIS -Standard range         Image: Arti-Penta-HIS -Standard range         Image: High sensitivity Human IgG quantitation         Image: High sensitivity Human IgG quantitation         Image: Arti-Penta-HIS -Standard range         Image: Arti-Penta-HIS -Standard range         Image: Arti-Penta-HIS -Standard range         Image: Arti-Penta-HIS -Standard range         Image: Arti-Human Fab-CH1 (FAB) with regeneration         Image: Protein L -Standard range         Image: Protein L - Standard Assay         Image: Protein A - Protein A         Image: Protein A - Protein A         Imad	Quantitation	Replicates per sensor type: 1 +

Figure 5-63: Defining a New Assay

# Editing Assay Parameters

To edit assay parameters:

- 1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 5-63 bottom).
- 2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
- 3. Click **Save** to accept the new parameter values. The new assay is added to the system.

NOTICE: Not all parameters are available for all assays.

#### Basic Quantitation Assay Parameters

Edit Assay Parameters		×
Available Assays:		
Basic Quantitation	Name:	My Basic Quant Assay
	Description:	Enter a short description of the assay here.
Anti-GST -High sensitivity	A	
	Assay Param	eters
Anti-Human Fab-CH1 (FAB)	Single and	alyte 🔿 Multiple analyte
		Replicates per sensor type:
Human IgG Quantitation		
Immunogenicity - Direct detection		Time (s): Shake speed (rpm):
Wy Basic Quant Assav	Quantitation	120 🗬 400 🗬
Protein L -Standard range		
Standard Assay		
Basic Quantitation with Regeneration		
Anti-Human Fab-CH1 (FAB) with regeneration		
Ingri sensitivity assay with regeneration		
Standard Assav		
Advanced Quantitation		
Immunogencity - Enzyme Linked		
Residual Protein A		
Standard Assay		
Inree Step Assay		
Gray indicates a built-in assay and cannot be modified or de	eleted.	
Duplicate Remove		Save Cancel

Figure 5-64: Assay Parameters-Basic Quantitation Assay

#### Table 5-14: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analy- sis.
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

## Basic Quantitation with Regeneration Assay Parameters

Edit Assay Parameters					×
Available Assays:					
Available Assays:         Available Assays:         Basic Quantitation         Basic Anti-EAG Quantitation         Basic Anti-EAG Quantitation         Basic Anti-Strandard range         Basic Anti-HIS (HIS2) Quantitation         Basic Anti-His (HIS2) Quantitation         Basic Anti-Horta-HIS -High sensitivity         Basic Anti-Penta-HIS -Standard range         Basic Anti-Penta-HIS -Standard range         Basic Quantitation         Basic Quantitation         Basic Quantitation         Basic Quant Assay         Protein L -Standard range         Basic Quantitation with Regeneration         Basic Quant Assay	Name: Description: Assay Param Single an Quantitation Regeneratio Neutralizatio Neutralizatio Betu Pre- Pos	My Basic Quant with Enter a short descrip eters alyte Multiple a Replicat Time (s): 120 • 120 • 15 • 120 • 15 • 120 • 12	1 Regen Assay         tion of the assay here.         analyte         tes per sensor type:         1         Shake speed (rpm):         400         400         400         2         Regeneration cycles:         3         3         3	¢.	
Advanced Quantitation  Advanced Quantitation	leted.			Cause	Count
Duplicate Remove				Save	Cancel

Figure 5-65: Assay Parameters-Basic Quantitation with Regeneration

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analysis.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regener- ated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

#### Table 5-15: Assay Parameters—Basic Quantitation with Regeneration

### Advanced Quantitation Assay Parameters

Use the Advanced Quantitation Assay Parameters to create a custom assay.

bic rissuys.					
Basic Quantitation	Name: Custom C	antitation			
Anti-FLAG Quantitation	Description:				
Anti-GST -High sensitivity					
Anti-GST -Standard range	Assay Parameters				
Anti-HIS (HIS2) Quantitation	Single analyte (	) Multiple analy	te		
Anti-Human Fab-CHT (FAB)		Replicates p	er sensor type:	1	
Anti-Penta-HIS -High sensitivity					
Anti-renta-nio-standard range	Step Type	lime (s)	Shake (rpm)	Step Options	Insert
High sensitivity Human Igo quantitation	Sample	<ul> <li>120</li> </ul>	1000	Online	Remove
Immunocepicity - Direct detection	Detection	120	1000	Reuse position	Tieniove.
Murine InG Quantitation					
My Basic Quant Assay					
Pertoin L. Chandred and an					
Protein L-Stangarg range					
Standard Assav					Marca Un
Standard Assay sic Quantitation with Regeneration					Move Up
Standard Assay sic Quantitation with Regeneration Anti-Human Fab-CH1 (FAB) with regeneration	4				Move Up
Protein L-standard range Standard Assay ic Quantitation with Regeneration Anti-Human Fab-CH1 (FAB) with regeneration High sensitivity assay with regeneration	<				Move Up
otein L -Standard range andard Assay Quantitation with Regeneration tit-Human Fab-CH1 (FAB) with regeneration igh sensitivity assay with regeneration otein L -Standard range	< ✓ Regeneration	līme (s):	Shake speed	(mm):	Move Up
oten L - Standard range andard Assay Quanttation with Regeneration til-Human Fab-CH1 (FAB) with regeneration gh sensitivity assay with regeneration otein L -Standard range andard Assay	< ✓ Regeneration : Regeneration;	Time (s):	Shake speed	(rpm):	Move Up
err L-Standard range dnard Assay -Human Fab-CH1 (FAB) with regeneration -Buman Fab-CH1 (FAB) with regeneration tein L-Standard range ndard Assay Basic Quant with Regen Assay	< ✓ Regeneration Regeneration:	Time (s):	Shake speed	(mpm):	Move Up
kern L-Standard range Juantitation with Regeneration U-thiman Fab-CH (FAB) with regeneration is teni L-Standard range midraf Assay Basic Quart with Regen Assay ced Quart tation	<ul> <li>✓ Regeneration</li> <li>Regeneration:</li> <li>Neutralization:</li> </ul>	Time (s): 5 ★ 5 ★	Shake speed	(mpm):	Move Up
oten L - Standard range Quartitation with Regeneration ti-Human Fab-CH1 (FAB) with regeneration di enstituty asasy with regeneration otein L - Standard range andraf Asasy y Basic Quart with Regen Assay ced Quartitation munogenchy - Enzyme Linked	<ul> <li>✓ Regeneration</li> <li>Regeneration:</li> <li>Neutralization:</li> </ul>	Time (s): 5 +	Shake speed 1000 -	(rpm): cycles:	Move Up Move Dow
oten L - Standard range Quartitation with Regeneration ti-Human Fab-CH (T AB) with regeneration of a sensitivity assay with regeneration oten L - Standard range andard Assay y Basic Quart with Regen Assay ced Quartitation munogenchy - Enzyme Linked scioual Protein A	<ul> <li>Regeneration</li> <li>Regeneration:</li> <li>Neutralization:</li> <li>Between assay</li> </ul>	Time (s): 5 ★ 5 ★	Shake speed 1000 + 1000 + Regeneration 3 +	(rpm): cycles:	Move Up Move Dow
oten L Standard range Quartitution with Regeneration tit-Human Fab-CHI (FAB) with regeneration gin sensitivity assay with regeneration otein L Standard range andard Assay y Basic Quart with Regen Assay need Quartitation munogencity - Enzyme Linked eaidual Protein A andard Assay	<     Regeneration Regeneration Regeneration Between assay	Time (s): 5 🔹 5 🔹	Shake speed	(rpm): cycles:	Move Up
Toten L Sundard Tange Sandard Asay C Quartitation with Regeneration Yigh sensitivity assay with regeneration Yigh sensitivity assay with regeneration Yinetin L -Standard range Standard Assay Wy Basic Quart with Regen Assay mored Quartitation munogencity - Enzyme Linked Needual Protein A Standard Assay Time Step Assay	<ul> <li>✓ Regeneration</li> <li>Regeneration: [</li> <li>Neutralization: [</li> <li>Between assay</li> <li>□ Pre-condition set</li> </ul>	Time (s): 5 • 5 • steps: msors	Shake speed           1000         •           1000         •           Regeneration         3         •           3         •         •	(pm): cycles:	Move Up

Figure 5-66: Assay Parameters-Advanced Quantitation

- 1. Select the type of Analyte.
  - Single analyte select to use one biosensor per sample well.
  - Multiple analytes select to use multiple biosensors per sample well.
    - Replicates per sensor type select the number of replicates for each sensor type.
- 2. Select the desired step options.
  - Insert click insert to add a step.
  - Remove select a step and then click Remove to remove a step.
  - Move Up select a step and then click Move Up to move a step up one row.
  - Move Down select a step and then click Move Down to move a step up one row.
- 3. Adjust the Time and Shake speed (rpm) of each step.
  - Time select the duration time of the step.
  - Shake speed select the shake speed in rpm for the step.
- 4. Regeneration Incubate the biosensor in the regeneration buffer to remove the bound analyte.
- 5. Neutralization Incubate the biosensor in the neutralization buffer after the regeneration step.
- 6. Between assay steps
  - Regeneration cycles select the number of cycles for a biosensor before reuse or storage.
  - Pre-condition sensors Perform a set of regeneration or neutralization steps before the start of the experiment. These settings are like the time and rpm settings for the regeneration steps. For example, an acidic pre-conditioning buffer maximizes the binding competency of Protein A biosensors.
  - Post-condition sensors Re-racked biosensors in a regenerated state for storage.
- 7. Step option Reagent wells can be reused.

- Reuse Position define a single position for a reagent. This position is used for all assays in the experiment
- Use x1 through Use x10 define the number of times the reagent in a position can be used. After the selected number of times is used, that position is no longer used in the experiment. You must define enough reagent positions in the plate to complete the experiment. For example, if the experiment has six assays:
  - You can define two reagent positions on the place and select use x3.
  - Or you can define three reagent positions on the plate and select use x2.
- Distribute usage (auto) define multiple positions in the for the reagent. The software automatically distributes the assays, so the defined reagent positions are used equally. For example, if the experiment has six assays and there are two defined reagent positions, the software will use each position three times.

**NOTICE:** Preview the application of the Reuse Position setting to ensure your settings. Select the Review Experiment tab and step through the experiment.

# Selecting a Custom Assay

Select a custom assay when you define a sample plate.

To select a custom assay:

1. In the **Plate Definition** tab, click **Modify** in the **Assay Settings** box.

The <b>Edi</b>	<b>it Assay Parameters</b> dia	log box appears (see Figure 5-67).	
1 Plate Definition 2	Sensor Assignment 3 Review Experiment 4	Run Experiment	
In this step, a First, check t Acquisition Rate: Assay Settings Assay: Basic	all the information about the sample plate and its wells the assay settings. Then highlight one or more wells or Randard (5.0 Hz)	will be entered. n the sample plate, and right-click to enter/modify well data. Plate 1 Table (96 wells) Concentration units: µg/ml V Export Import Print Well Sample ID Replicate Group Type Conc (µg/ml) Dilution Factor Informa	$\leftarrow \rightarrow$
Stan Singl	dard Assay le analyte		
Time	(s): Shake speed (rpm):		
Quantitation: 120	Assay Parameters		×
Plate 1 (96 wells)	Available Assays:	Assay Parameters	
A B C C C C C C C C C C C C C C C C C C	Basic Quartitation  Anti-FLAG Quartitation  Anti-FLAG Quartitation  Anti-GST -High sensitivity  Anti-GST -High sensitivity  Anti-Human Fab-CH1 (FAB)  Anti-Penta-H1S -High sensitivity  Anti-Penta-H1S -High sensitivity  Anti-Penta-H1S -Standard range  High sensitivity Human IgG quantitation  High sensitivity Human IgG quantitation  Mutine IgG Quartitation  Mutine IgG Quartitation  Mutine IgG Quartitation  Mutine IgG Quartitation  Standard range  Anti-L -Standard range  Anti-L -Standard range  Anti-Human Landard range  Anti-Human IgG quantitation  Anti-Human IgG Quartitation  Anti-Human IgG	Single analyte Multiple analyte Replicates per sensor type: 1 + Time (a): Shake speed (rpm): Quantitation: 120 + 400 +	
	Gray indicates a built-in assay.	OK Cancel	

Figure 5-67: Selecting a Custom Assay

2. Select the custom assay from the directory tree and click **OK**.

# Multi-Step Advanced Quantitation Experiments

The multi-step selection interface for Advanced Quantitation methods increases the flexibility to add more assay steps prior to the Sample or Detection steps. In addition, all steps in an Advanced Quantitation assay may be viewed and analyzed in the Octet<sup>®</sup> Analysis Studio software.

After starting the Octet<sup>®</sup> system and the Octet<sup>®</sup> BLI Discovery software, follow the steps below to set up and run an Advanced Quantitation experiment. You can start an Advanced Quantitation experiment using one of the following options:

- Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run.
- On the menu bar, click Experiment > Templates > Quantitation > Advanced Quantitation.

These options are explained further in "Starting an Experiment Using the Experiment Wizard" on page 85.

**NOTICE:** The Sample plate and the Reagent plate are now referred to as "Plate 1" and "Plate 2" in the software.

1. To add or edit assay steps in Tab 1 (Plate Definition), click **Modify** in Assay Settings to display the Assay Parameters window. Click on the **Step Type** drop-down list or highlight the parameter you want to change:

	Concent	ation units:	ua/mi	~	Export	Import	Print	
Advanced Quantitation	Wall	Sample ID	Poplica	to Group	Tuno	Cono (un/ml)	Dilution Ex	atau 🔺
Standard Assay Mod		Sample ID	neplica	te croup	Standard	cone (µg/mi)	Dilution Fa	
Single analyte					Stanuaru		iva.	
Assay Parameters								×
Available Accave:	Assay Parame	ers						
Anti-GST -Standard range			Wala analis					
1 Anti-HIS (HIS2) Quantitation	Single and	nyte O Mit Bi	enlicates ne	te er sensor hu	ner 1			
			-			-		
Anti-Penta-HIS -High sensitivity	Step Type		lime (s)	Shake (ŋ	om) Step	Options	Insert	
Anti-Penta-HIS -Standard range	Sample	· ·	120	1000	Onlin	e	Remove	
High sensitivity Human IgG quantitation	Detection	n	120	1000	Reus	e position		
Human IgG Quantitation								
Immunogenicity - Direct detection								
Munne IgG Quantitation								_
Protein L -Standard range							Move Up	>
Standard Assay								_
Basic Quantitation with Regeneration	<					>	Move Dov	vn
Anti-Human Fab-CH1 (FAB) with regeneration	Regene	ration						
High sensitivity assay with regeneration		Time	[s]:	Shake sp	eed (rpm):			
My Basic Quant with Regen Assay	Regenerati	on: 5	Y	1000	¥			
	Neutralizati	on: 5	*	1000	*			
Standard Assay				Begener	ation cucle	e.		
Advanced Quantitation	Datus			negener		0.		
	Betwe	en assay step	S:	3	-			
Immunogencity - Enzyme Linked	Pre-co	ndition sensor	3	3	*			
Residual Protein A	Post-o	andition senso	19	2	*			
El Residual Protein A El Standard Assay	Post-c	ondition senso	rs	3	× ·			

Figure 5-68: Assay Parameters Window.

To add or remove steps, click the **Insert** or **Remove** buttons. Individual steps may be re-organized using the **Move Up** or **Move Down** buttons. Click **OK** to save any changes.

- 2. Continue with the plate layout and sample well designation in Tab 1. For more details see "Defining the Sample Plate" on page 86, "Managing Sample Plate Definitions" on page 104 and "Managing Assay Parameter Settings" on page 107.
- 3. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with "Assigning Biosensors to Samples" on page 112 before running the Advanced Quantitation method.

### Chapter 6:

# Quantitation Experiments: Octet<sup>®</sup> R8, Octet<sup>®</sup> RED96e, and Octet<sup>®</sup> QKe

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# Introduction

Quantitation experiment determine the analyte concentration of a sample using a reference set of standards. After starting the Octet<sup>®</sup> system hardware and the Octet<sup>®</sup> BLI Discovery software, follow the steps (in Table 6-1) to set up and analyze a quantitation experiment. The appropriate biosensor product insert has the instructions for preparing the biosensors.

 Table 6-1:
 Starting and Analyzing a Quantitation Experiment

Software		Step	See
Octet <sup>®</sup> BLI Discovery	1.	Select a quantitation experiment in the <b>Experiment wizard</b> or open a method file (.fmf).	"Starting and Analyzing a Quantitation Experiment" on page 154
	2.	Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 156
	3.	Confirm or edit the assay settings.	"Managing Assay Parameter Settings" on page 174
	4.	Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 178
	5.	Run the experiment.	"Running a Quantitation Experiment" on page 195
Octet <sup>®</sup> Analysis Studio	6.	Analyze the binding data.	Octet <sup>®</sup> Analysis Studio Software User
Octet Analysis Studio 12.2	7.	Generate a report.	Guide

**IMPORTANT:** Using 96-well half-area plates on the Octet<sup>®</sup> R8, Octet<sup>®</sup> RED96, or Octet<sup>®</sup> RED96e system results in non-optimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

**NOTICE:** Before starting an experiment, check the plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet<sup>®</sup> BLI Discovery software is closed, the plate temperature resets to the default startup value specified in the **Options** dialog box when the software is relaunched.

You can start a quantitation experiment by one of the following methods:

- · Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking File > Open Method File. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run. For more details on method files see, "Managing Experiment Method Files" on page 206.
- On the menu bar, click Experiment > Templates > Quantitation.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message displays.

# Starting an Experiment Using the Experiment Wizard

To start an experiment using the **Experiment Wizard**:

- 1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button **New Experiment Wizard** (**Ctrl+N**) from the **Main Menu**.
- 2. In the Experiment Wizard, select New Quantitation Experiment (see Figure 6-1, left).
- 3. Select a type of quantitation experiment (see Table 6-2 for options).

Table 6-2: Quantitation Experiment Selection

Quantitation Experiment	Description
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regenera- tion	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two- or three-step quantitation assay that enables signal amplifica- tion for higher detection sensitivity.

4. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

) 🕅	New Quantitation Experiment Basic Quantitation Advanced Quantitation with Regeneration Advanced Quantitation New Kinetics Experiment Basic Kinetics Eptope Binning	Available Templates for - Octet R8  Protein A G or L biosensors_BCH_96W fmf Protein A G or L biosensors_high sensitivity_8CH_96W fmf	
	Recent Methods	Basic Quantitation Experiment - Protein A G or L biosensor 2CH_96W	• •
		1         Plate Definition         2         Sensor Assignment         3         Review Experiment         4         Run Experiment           Image: In this step, all the information about the sample plate and its wells will be entered.         Image:	$\rightarrow$
		Acquisition Rate: Standard (5.0 Hz) V Plate 1 Table (96 wells) Concentration units: ug/ml V Export Import Print	
		Assay settings Assay: Basic Quantitation Madify Well Sample ID Replicate Group Type Conc (µg/ml) Dilution Factor A	
		Standard Assay Single analyte	
		Time (s): Shake speed (rpm): DB1 Standard n/a	
		Quantitation: 120 400 C1 Standard n/a	
		Plate 1 (96 wells) D1 Standard n/a	
		Modify E1 Standard n/a	
		▼ 1 2 3 4 5 6 7 8 9 10 11 12 ● F1 Standard n/a	
		A O O O O O O O O O O O O O O O O O O O	
		O H1 Standard n/a	
		A Standard n/a	
		C Standard n/a	
		G G G G G G G G G G G G G G G G G G G	
		Sandard Control Disactioned B3 Disknown r/a	
		D3 Unknown n/a	
		E3 Unknown n/a	
		F3 Unknown n/a	
		G3 Urknown n/a	

Figure 6-1: Selecting an Experiment Type in the Experiment Wizard

5. Click the  $\rightarrow$  arrow.

The **Experiment** dialog box appears (Figure 6-1, right).

# Defining the Sample Plate

Table 6-3 lists the steps to define a sample plate.

#### Table 6-3: Defining a Sample Plate

	Step	See Page
1.	Designate the samples.	157
2.	Annotate the samples (optional).	167
З.	Save the sample plate definition (optional).	171

## **Designating Samples**

Each well may be designated as a **Standard**, **Unknown**, **Control**, or **Reference**. A well may also remain **Unassigned** or be designated as **Reserved** by the system for Basic Quantitation with Regeneration and Advanced Quantitation experiments.

**NOTICE:** It is important to define all of the wells used in the assay. Only wells that are selected and defined using one of the sample types in Table 6-4 are included in the assay.

#### Table 6-4: Types of Sample Wells

lcon	Description
Standard	Contains an analyte of known concentration. Data from the well is used to generate a standard curve during analysis.
Unknown	Contains an analyte of unknown concentration. The concentration of the analyte is calculated from the well data and the standard curve.
Control	<ul> <li>A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis.</li> <li>Positive Control: A control sample that contains analyte of known concentration</li> </ul>
	Negative Control: A control sample known not to contain analyte
Reference	Provides a baseline signal which serves as a reference signal for <b>Unknowns</b> , <b>Controls</b> , and <b>Standards</b> . The reference signal can be subtracted during data acquisition in the <b>Runtime Binding Chart</b> and during data analysis.
Unassigned	Not used during the experiment.
Reserved	Used by the system during Basic Quantitation with Regeneration experiments and Advanced Quantitation multi-step experiments for <b>Regeneration</b> (R), <b>Neutralization</b> (N), <b>Detection</b> (D), or <b>Capture Antibody</b> (C). Reserved wells are not available for use as <b>Standards</b> , <b>Unknowns</b> , <b>Controls</b> , or <b>References</b> .

#### **Reserved Wells**

In a Basic Quantitation with Regeneration or an Advanced Quantitation experiment, the **Sample Plate Map** includes gray wells. These wells are reserved by the system and specify the location of particular sample types.

Reserved samples cannot be removed from the sample plate, but you can change their column location. To change the location of a reserved column ((\*, (\*), (\*), or (\*)) right-click a column header in the **Sample Plate Map** and select **Regeneration**, **Neutralization**, **Detection**, or **Capture Antibody**.

#### Table 6-5: Reserved Well Requirements

Reserved Well	Must Contain
Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

#### Table 6-5: Reserved Well Requirements

Reserved Well	Must Contain
Detection	Secondary antibody or precipitating substrate that is used with an enzyme-antibody conju- gate to amplify the analyte signal. Sample concentrations are computed using the binding data from the detection wells.
Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of interest onto the biosensor.

#### Basic Quantitation with Regeneration

Advanced Quantitation

	1 2 3 4 5 6 7 8 9 10 11 12	1 2 3 4 5 6 7 8 9 10 11 12
A	$\bigcirc \bigcirc $	
В	$\bigcirc \bigcirc $	
С		
D		
Е	$\bigcirc \bigcirc $	
F	$\bigcirc \bigcirc $	
G		G
Η	$\bigcirc \bigcirc $	$ H \bigcirc \bigcirc$

Figure 6-2: Default Locations for Reserved Wells in a 96-Well Sample Plate Map

# Selecting Wells in the Sample Plate Map

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 6-3, left). To select nonadjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 6-3, center).
- Click a well or draw a box around a group of wells (Figure 6-3, right).



Figure 6-3: Selecting Wells in the Sample Plate Map

NOTICE: Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

# Designating Standards

To designate standards:

- 1. In the **Sample Plate Map**, select the wells to define as standards.
- Click the Standard button below the Sample Plate Map (see Figure 6-4), or right-click and select Standard.
   The standards are marked in the plate map and the Sample Plate Table is updated.
- 3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

Plate Definition 2 Sensor Assignment 3 Review Experiment	4 Run Exp	eriment					
In this step, all the information about the sample plate and its we First, check the assay settings. Then highlight one or more wells	lls will be en on the sam	tered. ble plate, and r	ight-click to enter/mo	dify well da	ta.		
cquisition Rate: Standard (5.0 Hz) V	Plate 17	able (96 wells			_		
ssay Settings	Concen	ration units:	µg/ml ∨	Export	Import	Print	
ay: Basic Quantitation Modify	Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Fact	or Information
Single analyte	🔵 A1			Standard		n/a	
Time (s): Shake speed (rpm):	🔵 B1			Standard		n/a	
ntitation: 120 400	🔵 C1			Standard		n/a	
e 1 (96 wells)	O D1			Standard		n/a	
Modify	🔵 E1			Standard		n/a	
1 2 3 4 5 6 7 8 9 10 11 12	F1			Standard		n/a	
	🔵 G1			Standard		n/a	
	O H1			Standard		n/a	
	A2			Standard		n/a	
	O B2			Standard		n/a	
$\bigcirc \bigcirc $	O C2			Standard		n/a	
	O D2			Standard		n/a	
	E2			Standard		n/a	
$\circ \circ $	F2			Standard		n/a	
$\bigcirc \bigcirc $	O G2			Standard		n/a	
	O H2			Standard		n/a	
	A3			Standard		n/a	
) Standard Ocontrol Olnassigned	O B3			Standard		n/a	
Unknown 🔴 Reference 🔘 Reserved	🔵 C3			Standard		n/a	
	O D3			Standard		n/a	
	E3			Standard		n/a	
	🔵 F3			Standard		n/a	
	🔵 G3			Standard		n/a	
	H3			Standard		n/a	

Figure 6-4: Plate Definition Window–Designating Standards

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box appears (see Figure 6-5).

Plate 1 (96 wells)	Modify	
A C C C C C C C C C C C C C C C C C C C		
C Control	Set Well Data	×
	Well Information	
E OOC Set Well Data	Sample ID:	Starting value (ug/ml): 200
F OOC Clear Data		Sorias aparetar:
G Copy to Clipboard	Replicate Group:	Series operator: / V
H C Extended Sample Types		Series operand: 2
Standard Control Ulas	Well Information:	
		CRight CLeft
		O     O     Down     O     O     O     Up     O
	Concentration (µg/ml):	OK Cancel

Figure 6-5: Sample Plate Map–Setting a Dilution Series

- 2. Select the **Dilution Series** option and enter the starting concentration value.
- 3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 6-6).



Figure 6-6: Concentration Representation in Dilution Series

4. Click OK.

The Sample Plate Table displays the standard concentrations entered.

#### Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

#### 1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

**NOTICE:** A range of wells can be selected clicking and dragging, holding the Shift key and using the arrow keys to select sections of the plate, or by holding the Ctrl key to select specific wells.

The Set Well Data dialog box appears (see Figure 6-7).

Plate 1 (96 wells)	Modify		
A O O Standard B O O Standard C O Unknown C C C Standard			
	Set Well Data		×
E OOC Set Well Data	Well Information Sample ID:	Dilution Series Starting value (µg/ml): 1	
G Copy to Clipboard H C Copy to Clipboard Extended Sample Types	Replicate Group:	Series operator: /	~
Standard Control Un	Well Information:	Dilution orientation	
			Up
	Concentration (µg/ml): 200 200 200 200 200 200 200 200 200 20	ОК	Cancel

Figure 6-7: Sample Plate Map—Assigning a Standard Concentration

- 2. Enter the starting concentration value. If a range of cells was selected, all cells update with the specified value.
- 3. Click **OK**. The Sample Plate Table displays the standard concentrations entered.

#### Editing an Individual Standard Concentration

To enter or edit an individual standard concentration, in the **Conc** column of the **Sample Plate Table**, double-click the value and enter a new value (see Figure 6-8).

Sample	e Plate Table –							
Conce	ntration units:	µg/ml  ▼	Expo	ort	Impo	rt		
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc	(µg/ml)	<b>Dilution Factor</b>	Information	
🔵 A1			Standard	1		n/a		
🔵 B1			Standard	200		n/a		
🔵 C1			Standard	100	Un	do		
🔵 D1			Standard	50	<b>C</b>			
🔵 E1			Standard	25	Cu	t		
🔵 F1			Standard	10	Co	ру		
🔵 G1			Standard	5	Pas	ste		
🔵 H1			Standard	2.5	De	lete		
🔵 A2			Standard	1				
🔵 В2			Standard	200	Sel	ect All		
🔵 C2			Standard	100	Ric	ht to left Readin	a order	
🔵 D2			Standard	50	i tig			
🔵 E2			Standard	25	She	ow Unicode cont	trol characters	
🔵 F2			Standard	10	Ins	ert Unicode cont	trol character	+
🔵 G2			Standard	5	On	on IME		
🔵 H2			Standard	2.5	Op	CITIVIL .		
🔵 A3			Standard	1	Ree	conversion		
🔵 ВЗ			Standard	200		n/a		

Figure 6-8: Sample Plate Table–Shortcut Menu of Edit Commands

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

# Designating Unknowns

To designate unknowns in the **Sample Plate Map**, select the wells to define as unknown, right-click and select **Unknown**. The unknown wells are marked in the plate map and the sample plate table is updated (see Figure 6-9).

1 Plate Definition	n 2 Sensor Assignm	ent 3 Review Experiment	4 Run Exp	eriment					
in this First, c	step, all the information a heck the assay settings.	about the sample plate and its we Then highlight one or more wells	Is will be ent on the samp	ered. ble plate, and ri	ght-click to enter/mo	dify well da	ita.		
Acquisition Rate	Standard (5.0 Hz)	~	Plate 1 T	able (96 wells)					
Assay Settings			Concent	ration units:	$\mu g/ml$ $\sim$	Export	Import	Print	
Assay:	Basic Quantitation	Modify	Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	Informatic ^
	Standard Assay Single analyte		O D2			Standard	50	n/a	
	Time (s): Shał	ke speed (rpm):	E2			Standard	25	n/a	
Quantitation:	120 400		F2			Standard	10	n/a	
Plate 1 (96 wells	)		🔵 G2			Standard	5	n/a	
		Modify	O H2			Standard	2.5	n/a	
1 2	3 4 5 6 7	7 8 9 10 11 12	A3			Standard	1	n/a	
			🔵 ВЗ			Standard	200	n/a	
	Standar	rd	🔵 C3			Standard	100	n/a	
BOO	Unknov	wn N	O D3			Standard	50	n/a	
	Control	3	🔵 E3			Standard	25	n/a	
	O O Negativ	ve Control	<b>F</b> 3			Standard	10	n/a	
	Positive	Control	🔵 G3			Standard	5	n/a	
	Referen	ice	🔵 НЗ			Standard	2.5	n/a	
	Set Wel	I Data	A4			Unknown	n/a		
GOO		ata	O B4			Unknown	n/a		
HÕÕ		dld	O C4			Unknown	n/a		
	Copy to	o Clipboard	🔵 D4			Unknown	n/a		
Standar	d 🤇 🗸 Extende	ed Sample Types	🔵 E4			Unknown	n/a		
Unknow	n Reference	Reserved	🔵 F4			Unknown	n/a		
		_	🔵 G4			Unknown	n/a		
			O H4			Unknown	n/a		
			A5			Unknown	n/a		

Figure 6-9: Plate Definition Window–Designate Unknown Wells

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

#### Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

- 1. In the **Sample Plate Map**, select the unknown wells (see Figure 6-9).
- 2. Right-click and select Set Well Data.

The Set Well Data dialog box appears (see Figure 6-10).

Plate 1 (96 wells)			
		Modify	
	7 8 9 10	11 12	
	00000		
BOOOC			
	Unknown		
	Control		
	Negative Control		
	Positive Control		
	Reference		
GOOOC	Set Well Data		
	Clear Data 😡	Set Well Data	×
🔵 Standard 🥚 (	Copy to Clipboard		_
🔵 Unknown 🛛 🔴 Re 🧹	Extended Sample Ty	Well Information Dilution Series	
		Sample ID: Starting value:1	
		Series operator: / V	
		Series operand: 2	
		Well Information	
		Dilution Factor: 2	
		Dilution Factor: Unknowns only	

Figure 6-10: Sample Plate Map–Setting a Dilution Factor or a Serial Dilution

To assign a dilution factor to selected wells:

- 1. In the Set Well Data dialog box (see Figure 6-10), select the Dilution Factor option.
- 2. Enter the dilution factor value and click OK.

To assign a serial dilution to selected wells:

- 1. In the Set Well Data dialog box (see Figure 6-10), select the Dilution series option.
- 2. Enter the starting dilution, select a series operator, and enter a series operand.
- 3. Select the appropriate dilution orientation: (see Figure 6-11).



Figure 6-11: Concentration Representation in Dilution Series

4. Click OK.

The Sample Plate Table displays the dilution factors entered.

#### Editing a Dilution Factor in the Sample Plate Table

To edit a dilution factor in the **Sample Plate Table**:

- 1. In the **Set Well Data** dialog box (see Figure 6-12), double-click a cell in the **Dilution Factor** column for the desired unknown.
- 2. Enter the new value (the default dilution factor is 1)

Concent	tration units:	µg/ml 🗸 🗸	Export	Import	Print		
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution	Factor	Informatic ^
D2			Standard	50	n/a		
) E2			Standard	25	n/a		
) F2			Standard	10	n/a		
) G2			Standard	5	n/a		
) H2			Standard	2.5	n/a		
) A3			Standard	1	n/a		
<b>B</b> 3			Standard	200	n/a		
C3			Standard	100	n/a		
D3			Standard	50	n/a		
) E3			Standard	25	n/a		
<b>F</b> 3			Standard	10	n/a		
🔵 G3			Standard	5	n/a		
🔵 НЗ			Standard	2.5	n/a		
) A4			Unknown	n/a	2		
<b>B4</b>			Unknown	n/a	2	Undo	)
) C4			Unknown	n/a	2	Cut	
D4 (			Unknown	n/a	2	Copy	
) E4			Unknown	n/a	2	Paste	
) F4			Unknown	n/a	2	Delet	•
) G4			Unknown	n/a	2	Delet	с
			Unknown	n/a	2	Selec	t All
A5			Unknown	n/a	2	Right	to left Reading or
<b>B</b> 5			Unknown	n/a	2	Show	Unicode control c
C5 (			Unknown	n/a	2	Incot	Unicode control e
2 77				'	2	insen	Control C

Figure 6-12: Sample Plate Table–Shortcut Menu of Edit Commands

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

# Designating Controls or Reference Wells

Controls are samples of known concentration and are not used to generate a standard curve. A reference well contains sample matrix only, and is used to subtract non-specific binding of the sample matrix to the biosensor. During data analysis, data from reference wells can be subtracted from standards and unknowns to correct for background signal.

- To designate controls, select the control wells and click **Control** (below the **Sample Plate Map)**, or right-click and select **Control**. Positive and Negative Control types can also be assigned using this menu.
- To designate reference wells, select the reference wells and click the **Reference** button below the **Sample Plate Map**, or right-click the selection and choose **Reference**.

The wells are marked in the Sample Plate Map and the Sample Plate Table is updated (see Figure 6-13).



Figure 6-13: Designate Controls or Reference Wells

**NOTICE:** Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

# Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it is not available for display as a legend.

#### Annotating Wells in the Sample Plate Map

To annotate one or more wells:

- 1. In the Sample Plate Map, select the samples to annotate, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 6-14), enter the Sample ID and/or Well Information and click OK.

Plate 1 (96 wells)	Modify		
A C Control C C C Control C C C Control C C C C C C C C C C C C C C C C C C C	10 11 12		×
G Copy to Clipboard Extended Sample Types Standard Control Ulna Unknown Reference Res	Well Information Sample ID:	Dilution Series         Starting value (µg/ml):         Series operator:         Series operand:         Dilution orientation	
	Concentration (µg/ml):	Image: Stress of the stress	eft Ip Cancel

Figure 6-14: Adding Sample Annotations from the Sample Plate Map

#### Annotating Wells in the Sample Plate Table

To annotate an individual well in the Sample Plate Table:

- 1. Double-click the table cell for **Sample ID** or **Well Information**.
- 2. Enter the desired information in the respective field (see Figure 6-15).

NOTICE: A series of Sample IDs may also be assembled in Excel and pasted into the Sample Plate Table.

Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	Information
) E3	IgG Standard		Standard	25	n/a	Sample Diluer
) F3	IgG Standard		Standard	10	n/a	Sample Diluer
) G3	IgG Standard		Standard	5	n/a	Sample Diluer
) нз	IgG Standard		Standard	2.5	n/a	Sample Diluer
A4	Ab1		Unknown	n/a	2	Sample Diluer
) B4	Ab2		Unknown	n/a	2	Sample Diluer
) C4	Ab3		Unknown	n/a	2	Sample Diluer
) D4	Ab4		Unknown	n/a	2	Sample Diluer
) E4	Ab5		Unknown	n/a	2	Sample Diluer
) F4	Ab6		Unknown	n/a	2	Sample Diluer
) G4	Ab7		Unknown	n/a	2	Sample Diluer
) H4	Ab8		Unknown	n/a	2	Sample Diluer
) A5	Ab1		Unknown	n/a	2	Sample Diluer
) B5	Ab2		Unknown	n/a	2	Sample Diluer
) C5	Ab3		Unknown	n/a	2	Sample Diluer
) D5	Ab4		Unknown	n/a	2	Sample Diluer
) E5	Ab5		Unknown	n/a	2	Sample Diluer
) F5	Ab6		Unknown	n/a	2	Sample Diluer
) G5	Ab7		Unknown	n/a	2	Sample Diluer
) H5	Ab8		Unknown	n/a	2	Sample Diluer
) A6	Ab1		Unknown	n/a	2	Sample Diluer
) B6	Ab2		Unknown	n/a	2	Sample Diluer
) C6	Ab3		Unknown	n/a	2	Sample Diluer
) D6	Ab4		Unknown	n/a	2	Sample Diluer
) E6	Ab5		Unknown	n/a	2	Sample Diluer
) F6	Ab6		Unknown	n/a	2	Sample Diluer
) G6	Ab7		Unknown	n/a	2	Sample Diluer
) H6	Ab8		Unknown	n/a	2	Sample Diluer
) A7	hlgG		Control	10	n/a	
<b>B</b> 7	hlgG		Control	10	n/a	
C7	hlgG		Control	10	n/a	
D7	hlgG		Control	10	n/a	
) E7	hlgG		Control	10	n/a	

Figure 6-15: Adding Sample Annotations in the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

## **Replicate Groups**

After samples are assigned to a **Replicate Group**, the statistics for all samples in that group are calculated automatically. The average binding rate, average concentration and corresponding standard deviation as well CV% are presented in the **Results** table for each group (see Figure 6-16).

Sensor	Replicat	BR Avg	BR SD	BR CV	Conc. Avg	Conc. SD	Conc. CV
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2

Figure 6-16: Replicate Group Result Table Statistics

NOTICE: Replicate Group information can also be entered in the Results table.

Assigning Replicate Groups in the Sample Plate Map To assign **Replicate Groups** in the **Sample Plate Map**:

- 1. Select the samples to group, right-click and select **Set Well Data**.
- 2. In the Set Well Data dialog box (see Figure 6-17), enter a name in the Replicate Group box and click OK.

Set Well Data		×
Well Information	Dilution Series	
Sample ID:	Starting value (µg/ml): 1	
IgG Standard	Series operator:	~
Replicate Group:	Series operand: 2	
Well Information:	Dilution orientation	
Sample Diluent	■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■	ft
	Down	p
Concentration (µg/ml): 200 Standards only	ОК С	ancel

Figure 6-17: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software only recognizes and calculates statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

**NOTICE:** When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they are treated as separate groups. Statistics for these groups are calculated separately for each biosensor type.

Wells in the **Sample Plate Map** show color-coded outlines as a visual indication of which wells are in the same group (see Figure 6-18).



Figure 6-18: Replicate Groups in Sample Plate Map

The Sample Plate Table updates with the Replicate Group names entered (see Figure 6-19).

ample	Plate Table					
Concentration units:		µg/ml ▼	Export	Import		
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	
🔵 A1	lgG Standard	200	Standard	200	n/a	
🔵 B1	lgG Standard	100	Standard	100	n/a	
🔵 C1	lgG Standard	50	Standard	50	n/a	
🔵 D1	lgG Standard	25	Standard	25	n/a	
🔵 E1	lgG Standard	10	Standard	10	n/a	
🔵 F1	lgG Standard	5	Standard	5	n/a	
🔵 G1	lgG Standard	2.5	Standard	2.5	n/a	
🔵 H1	lgG Standard	1	Standard	1	n/a	Ξ
🔵 A2	lgG Standard	200	Standard	200	n/a	
<b>)</b> B2	lgG Standard	100	Standard	100	n/a	
🔵 C2	lgG Standard	50	Standard	50	n/a	
🔵 D2	lgG Standard	25	Standard	25	n/a	
<b>)</b> E2	lgG Standard	10	Standard	10	n/a	
<b>)</b> F2	lgG Standard	5	Standard	5	n/a	
🔵 G2	lgG Standard	2.5	Standard	2.5	n/a	_
🔵 H2	lgG Standard	1	Standard	1	n/a	
<b>)</b> A3	lgG Standard	200	Standard	200	n/a	
<b>B</b> 3	lgG Standard	100	Standard	100	n/a	
C3 🕻	lgG Standard	50	Standard	50	n/a	
<b>D</b> 3	lgG Standard	25	Standard	25	n/a	
<b>)</b> E3	lgG Standard	10	Standard	10	n/a	
<b>)</b> F3	lgG Standard	5	Standard	5	n/a	
🔵 G3	lgG Standard	2.5	Standard	2.5	n/a	
🔵 НЗ	lgG Standard	1	Standard	1	n/a	
<b>A</b> 4	Ab1	Ab1	Unknown	n/a	2	
<b>B</b> 4	Ab2	Ab2	Unknown	n/a	2	
C4	Ab3	Ab3	Unknown	n/a	2	
<b>D</b> 4	Ab4	Ab4	Unknown	n/a	2	
<b>E</b> 4	Ab5	Ab5	Unknown	n/a	2	
<b>F</b> 4	Ab6	Ab6	Unknown	n/a	2	
<b>G</b> 4	Ab7	Ab7	Unknown	n/a	2	-

Figure 6-19: Replicate Groups in Sample Plate Table
#### Assigning Replicate Groups in the Sample Plate Table

#### To assign Replicate Groups in the Sample Plate Table:

- 1. Double-click the desired cell in the **Replicate Group** table column.
- 2. Enter a group name (see Figure 6-20).

Sample	Plate Table —				
Concentration units:		µg/ml   ▼	Export	Import	
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	Dilution Factor 🔺
🔵 A1	lgG Standard	200	Standard	200	n/a
🔵 B1	lgG Standard	100	Standard	100	n/a
🔵 C1	lgG Standard	50	Standard	50	n/a
🔵 D1	lgG Standard	25	Standard	25	n/a
🔵 E1	lgG Standard	10	Standard	10	n/a
🔵 F1	lgG Standard	5	Standard	5	n/a
🔵 G1	lgG Standard	2.5	Standard	2.5	n/a

Figure 6-20: Add Replicate Group from the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The Octet<sup>®</sup> BLI Analysis software only recognizes and calculates statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

**NOTICE:** When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they are treated as separate groups. Statistics for these groups are calculated separately for each biosensor type.

## Managing Sample Plate Definitions

NOTICE: After you define a sample plate, you can export and save the plate definition for future use.

#### Exporting a Plate Definition

To export a plate definition:

1. In the Sample Plate Table (see Figure 6-21), click Export.

Sample	Plate Table —				
Concentration units:		μg/ml 🛛 🔻	Export	Import	
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	Dilution Factor 🔺
🔵 A1	lgG Standard	200	Standard	200	n/a
🔵 B1	lgG Standard	100	Standard	100	n/a
🔵 C1	lgG Standard	50	Standard	50	n/a
🔵 D1	lgG Standard	25	Standard	25	n/a
🔵 E1	lgG Standard	10	Standard	10	n/a
🔵 F1	lgG Standard	5	Standard	5	n/a

Figure 6-21: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 6-22), select a folder, enter a name for the plate (.csv), and click **Save**.



Figure 6-22: Export Plate Definition Window

#### Importing a Plate Definition

To import a plate definition:

1. In the Sample Plate Table (see Figure 6-23), click Import.

S	Sample	Plate Table				
	Concer	ntration units:	μg/ml   ▼	Export	Import	
	Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	Dilution Factor 🔺
	🔵 A1	lgG Standard	200	Standard	200	n/a
	🔵 B1	lgG Standard	100	Standard	100	n/a
	🔵 C1	lgG Standard	50	Standard	50	n/a
	🔵 D1	lgG Standard	25	Standard	25	n/a
	🔵 E1	lgG Standard	10	Standard	10	n/a
	🔵 F1	lgG Standard	5	Standard	5	n/a

Figure 6-23: Import Button in Sample Plate Table

2. In the Import Plate Definition window (see Figure 6-24), select the plate definition (.csv), and click Open.

🖽 Import Plate 1 Definition					×
$\leftarrow \rightarrow \land \uparrow$ his F	PC → Local Disk (C:) → data	~	ට 🔎 Search	data	
Organize 🔻 New folder					?
A Ouick access	Name	Date modified	Туре	Size	
	96 standard plate.csv	1/8/2021 10:14 AM	CSV File	1 KB	
This PC					
3D Objects					
Desktop					
Documents					
🖶 Downloads					
👌 Music					
E Pictures					
Videos					
Local Disk (C:)					
🔲 DVD Drive (D:) O					
· · · · · · · · · · · · · · · · · · ·					
File <u>n</u> am	e: 96 standard plate.csv		CSV Files (*.e)	:sv)	$\sim$
			<u>O</u> pen	Cance	:I

Figure 6-24: Import Plate Definition Window

**NOTICE:** You can also create a .csv file for import. Figure 6-25 shows the appropriate column information layout.

	А	В	С	D	E	F	G	
1	PlateWells	96						
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information	=
3	A1	IgG Standard	200	Standard	200		Sample Diluent	
4	B1	IgG Standard	100	Standard	100		Sample Diluent	
5	C1	IgG Standard	50	Standard	50		Sample Diluent	
6	D1	IgG Standard	25	Standard	25		Sample Diluent	
7	E1	IgG Standard	10	Standard	10		Sample Diluent	
8	F1	IgG Standard	5	Standard	5		Sample Diluent	
9	G1	IgG Standard	2.5	Standard	2.5		Sample Diluent	
10	H1	IgG Standard	1	Standard	1		Sample Diluent	
11	A2	IgG Standard	200	Standard	200		Sample Diluent	-
I	< < > > > 96 standard plate / □ / □ / □ · · · · · · · · · · · · · ·							

Figure 6-25: Example Sample Plate File (.csv)

#### Printing a Sample Plate Definition

To print a plate definition:

1. In the Sample Plate Map (see Figure 6-26), click Print.

Plate 1	Table (96 wel	ls) –								
Conce	ntration units:		µg/ml	$\sim$	Exp	ort	Import		Print	
Well	Sample ID	Re	plicate	Group	Туре	Conc	(µg/ml)	Dilu	tion Factor I	ni

Figure 6-26: Sample Plate Print Button

The associated **Sample Plate Table** information prints.

# Managing Assay Parameter Settings

### Modifying Assay Parameter Settings

You can modify the assay parameter settings during sample plate definition. However, the changes are only applied to the current experiment. To save modified parameter settings, you must define a new assay. For details on creating a new assay, see "Custom Quantitation Assays" on page 206.

### Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box appears (Figure 6-27). The settings available are experiment-dependent.



Figure 6-27: Modifying Assay Parameters

#### Basic Quantitation Assay Parameters

Available Assavs:	Assay Parameters
Waldwind Fadya     Basic Quantitation     Basic Quantitation     Anti-GST -High sensitivity     Anti-GST -Standard range     Anti-H15 (HIS2) Quantitation     Anti-H15 (HIS2) Quantitation     Anti-Penta-HIS -High sensitivity     Anti-Penta-HIS -Standard range     High sensitivity Human IgG quantitation     High sensitivity -Direct detection     Murine IgG Quantitation     Protein L -Standard range     Standard Assay	Single analyte Replicates per sensor type:   1   Time (s):   Shake speed (rpm):   Quantitation:   120

Figure 6-28: Assay Parameters-Basic Quantitation AssayBasic Quantitation with Regeneration Assay Parameters

Table 6-6: Basic	Quantitation Assay Parameters
------------------	-------------------------------

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analy- sis.
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

vailable Assays:	Assay Parameters	
Basic Quantitation with Regeneration Anti-Human Fab-CH1 (FAB) with regeneration High sensitivity assay with regeneration	Single analyte O Multiple analyte Replicates per sensor type: 1	
— <u>B</u> } Protein L – Standard range —B} Standard Assay	Time (s):       Shake speed (pm):         Quantitation:       120       400	
indicates a built-in assay.	OK Cancel	

Figure 6-29: Assay Parameters-Basic Quantitation with Regeneration

Table 6-7: Assay Parameters	-Basic Quantitation	with Regeneration
-----------------------------	---------------------	-------------------

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analysis.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regener- ated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

#### Advanced Quantitation Assay Parameters

Use the Advanced Quantitation Assay Parameters to create a custom assay.

vailable Assays:	Assay Parameters	
Basic Quantitation	Single analyte     O Multiple analyte     Replicates per sensor type:     1	
Anti-GST -Standard range	Step Type Time (s) Shake (rpm) Step Options In	sert
Anti-HIS (HIS2) Quantitation	Sample - 120 1000 Online	
Anti-Human Fab-CH1 (FAB)	Detection 120 1000 Reuse position	nove
Human IgG Quantitation		
Immunogenicity - Direct detection	MOV	le up
Murine IgG Quantitation	Move	Dowr
Protein L -Standard range		_
Standard Assay	Regeneration Time (s): Shake speed (mm):	
Basic Quantitation with Regeneration	Regeneration: 5 1000	
Anti-Human Fab-CHT (FAb) with regeneration		
Protein L -Standard range	Neutralization: 5 v 1000 v	
Standard Assay	Regeneration cycles:	
Advanced Quantitation	Between assay steps: 3	
Immunogencity - Enzyme Linked	Pre-condition sensor	
Residual Protein A		
	Post-condition sensors 3	
🚺 Standard Assay		

Figure 6-30: Assay Parameters—Advanced Quantitation

- 1. Select the type of Analyte.
  - Single analyte select to use one biosensor per sample well.
  - Multiple analytes select to use multiple biosensors per sample well.
    - Replicates per sensor type select the number of replicates for each sensor type.
- 2. Select the desired step options.
  - Insert click insert to add a step.
  - Remove select a step and then click Remove to remove a step.
  - Move Up select a step and then click Move Up to move a step up one row.
  - Move Down select a step and then click Move Down to move a step up one row.
- 3. Adjust the Time and Shake speed (rpm) of each step.
  - Time select the duration time of the step.
  - Shake speed select the shake speed in rpm for the step.
- 4. Regeneration Incubate the biosensor in the regeneration buffer to remove the bound analyte.
- 5. Neutralization Incubate the biosensor in the neutralization buffer after the regeneration step.
- 6. Between assay steps
  - Regeneration cycles select the number of cycles for a biosensor before reuse or storage.
  - Pre-condition sensors Perform a set of regeneration or neutralization steps before the start of the experiment. These settings are like the time and rpm settings for the regeneration steps. For example, an acidic pre-conditioning buffer maximizes the binding competency of Protein A biosensors.
  - Post-condition sensors Re-racked biosensors in a regenerated state for storage.
- 7. Step option Reagent wells can be reused.
  - Reuse Position define a single position for a reagent. This position is used for all assays in the experiment

- Use x1 through Use x10 define the number of times the reagent in a position can be used. After the selected number of times is used, that position is no longer used in the experiment. You must define enough reagent positions in the plate to complete the experiment. For example, if the experiment has six assays:
  - You can define two reagent positions on the place and select use x3.
  - Or you can define three reagent positions on the plate and select use x2.
- Distribute usage (auto) define multiple positions in the plate for the reagent. The software automatically distributes the assays, so the defined reagent positions are used equally. For example, if the experiment has six assays and there are two defined reagent positions, the software will use each position three times.

NOTICE: Preview the application of the Reuse Position setting to ensure your settings. Select the Review Experiment tab and step through the experiment.

# Assigning Biosensors to Samples

After the sample plate is defined, biosensors must be assigned to the samples.

#### Biosensor Assignment in Single-Analyte Experiments

In a single analyte experiment, only one biosensor type is assigned to each sample and only one analyte is analyzed per experiment.

**NOTICE:** For single analyte experiments, the Single Analyte option must be selected in the Assay Parameters dialog box. For more information, please see "Managing Assay Parameter Settings" on page 174.

Click the Sensor Assignment tab, or click the  $\rightarrow$  arrow to access the Sensor Assignment window (see Figure 6-31).

The software generates a color-coded Sensor Tray Map and Sample Plate Map that shows how the biosensors are assigned to the samples by default.



Figure 6-31: Sensor Assignment Window for Basic Quantitation without Regeneration

- 1. Assign biosensors in one of two ways:
  - Select a column(s) in the Sensor Tray Map, right-click and select a biosensor type from the drop-down list.
  - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 6-32).



Figure 6-32: Changing Biosensor Types

 All wells in the Sensor Type column will automatically populate with the biosensor type selected, (Figure 6-32). To designate reference biosensors, select the desired biosensors in the Sensor Tray Map, right-click and select Reference. The reference biosensors are marked with an R.

**NOTICE:** Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

- 3. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column will automatically populate with the lot number entered.
- 4. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

5. Optional for the Octet<sup>®</sup> RED96 and the Octet<sup>®</sup> RED96e instrument only: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 6-33).



Figure 6-33: Replace Sensors in Tray After Use Check Box

**NOTICE:** Biosensors can be regenerated up to a max of 11 times per experiment.

#### Biosensor Assignment in Multiple Analyte Experiments

In a multiple analyte experiment, more than one biosensor type is assigned to the same sample, allowing multiple analytes to be analyzed in a single experiment.

**NOTICE:** For multiple analyte experiments, the Multiple Analyte option must be selected in the Assay Parameters dialog box. For more information, please see "Managing Assay Parameter Settings" on page 174.

Click the Sensor Assignment tab, or click the  $\rightarrow$  arrow to access the Sensor Assignment window (see Figure 6-34).

The software generates a color-coded Sensor Tray Map and Sample Plate Map that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 6-34, one replicate had been selected with the Multiple Analyte assay parameter option.



Figure 6-34: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

There are two ways to assign biosensors:

- Select a column in the Sensor Tray Map, right-click and select a biosensor type from the drop-down list.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the dropdown list (see Figure 6-35).



Figure 6-35: Changing Biosensor Types

#### Biosensor Assignment Using Heterogeneous Biosensor Trays

The default **Tray Format** is **Heterogeneous**. Heterogeneous biosensor trays contain a mixture of biosensor types.

NOTICE: When using this Heterogeneous option, the order of biosensor types in each tray must be identical.

- If Heterogeneous Trays is not displayed next to the Tray Format button, click the button. The Tray Format dialog box appears (see Figure 6-36).
- 2. Select Heterogeneous and click OK.

Heterogeneous	Sensor trays may contain various sensor typ but all sensor trays used are identical.	pes,
Homogeneous	A different sensor tray is used for each sensor	or type.
Sensors:	Anti-Human IgG Fc	Add
		Remove
		Change
		Move Up
		Move Down

Figure 6-36: Tray Format Dialog Box

The Tray 1 Sensor Tray Map appears by default.

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to be used (see Figure 6-37).

The **Sensor Type** column will update accordingly.



Figure 6-37: Populating the Sensor Tray Map with First Biosensor Type

4. Select the columns in the **Sensor Tray Map** that should contain the second biosensor type, right-click and select the second biosensor type (see Figure 6-38).



The Sensor Type column will update accordingly.

Figure 6-38: Populating the Sensor Tray Map with Second Biosensor Type

 Repeat this column selection and assignment process for all other biosensor types to be used in the experiment. The software will automatically update the number of biosensor trays needed and biosensor assignments in all trays according to the column assignments made in Tray 1. In the example shown in Figure 6-39, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays will be needed for the experiment.



Figure 6-39: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

6. To view or change the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown and biosensor assignments can be changed as needed (see Figure 6-40).

Sensor Tray ☑ Replace sensors in tray after use	Sensor Tray: Tray 3 v of 3 Tray Format Heterogeneous trays
1       2       3       4       5       6       7       8       9       10       11       12         A       1 <td>Irrey 1     or Type     Lot Number     Information       Irrey 2     h A       B1     Protein A       C1     Protein A       D1     Protein A       E1     Protein A       G1     Protein A       G1     Protein A       H1     Protein A       B2     Protein A</td>	Irrey 1     or Type     Lot Number     Information       Irrey 2     h A       B1     Protein A       C1     Protein A       D1     Protein A       E1     Protein A       G1     Protein A       G1     Protein A       H1     Protein A       B2     Protein A
Legend: Unassigned sensors 🕅 Missing sensors	C2 Protein A D2 Protein A
Remove Fill Fill Plate Print	E2 Protein A



7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

- 8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered.
- 9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace** sensors in tray after use check box (see Figure 6-41).



Figure 6-41: Replace Sensors in Tray After Use Check Box

**NOTICE:** Biosensors can be regenerated up to a max of 11 times per experiment.

#### Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.

NOTICE: Using the Homogeneous option will necessitate switching trays during the experiment.

1. Click Tray Format, see Figure 6-40

The **Tray Format** dialog box appears (Figure 6-42) and the **Sensors** box is populated with the default biosensor type.

Heterogeneous	Sensor trays may contain various sensor but all sensor trays used are identical.	types,
Homogeneous	A different sensor tray is used for each ser	nsor type.
Sensors:	Anti-Human IgG Fc	Add
		Remove
		Change
		Move Up
		Move Down

Figure 6-42: Tray Format Dialog Box

2. Select Homogeneous. Click Add to select the first biosensor type (see Figure 6-43).

Tray Format  Heterogeneous  Homogeneous	Sensor trays may contain various sensor ty but all sensor trays used are identical. A different sensor tray is used for each sensor	pes,
Sensors:	Anti-Human IgG Fc	Anti-Mouse IgG Fv Protein A Protein G Protein L SA (Streptavidin) Residual Protein A Anti-Penta-HIS Custom
	OK Cancel	]

Figure 6-43: Selecting a Biosensor Type in the Tray Format Dialog Box

- 3. Repeat this step to add any additional biosensor types that will be used in the experiment. To remove a biosensor type, select a biosensor type in the **Sensor** box and click **Remove**.
- 4. Adjust the order of biosensor types as needed by selecting the biosensor type in the **Sensor** box and clicking **Move Up** or **Move Down**.

The order of biosensor types listed in the **Sensor** box will be used as the default tray assignment (see Figure 6-44).

Heterogeneous	Sensor trays may contain various senso but all sensor trays used are identical.	or types,
Homogeneous	A different sensor tray is used for each s	ensor type.
Sensors:	Protein A Protein G	Add
		Remove
		Change
		Move Up
		Move Down

Figure 6-44: Biosensor Types List Order in Sensor Box

5. Click OK.

The software will automatically calculate the number of biosensor trays needed and assign biosensors types to each tray.

In the example shown in Figure 6-45, Protein A and Protein G biosensor types will be used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) will be needed for the experiment. The Tray 1 **Sensor Tray Map** will be appears by default.

Image: Province and easymed to samples.         Province and samples.         Province and samples.             Image: Province and	1 Plate Definition 2 Sensor Assignment 3 Review Experiment	4 Run Experiment
Sensor Tray       Tray format       Homogeneous trays         A       2       3       4       5       6       7       8       10       11       12         A       2       3       4       5       6       7       8       10       11       12         A       1       2       3       4       5       6       7       8       10       11       12         A       1       2       3       4       5       6       7       8       10       11       12         A       1       2       3       4       5       6       7       8       10       11       12         A       1       1       1       12       1       1       12       1       12       12       12       12       12       11       12	In this step, sensors are assigned to samples. If you have a partial sensor tray it can be accomodated by select Only the first sensor tray can be a partial plate.	ing the missing sensors and clicking 'Remove'.
Replace sensors in tray after use       Image of 2       Tray Format       Homogeneous trays         1       2       4       6       7       8       10       11       12         A       1       2       4       6       7       8       10       11       12         A       1       2       3       6       7       8       10       11       12         A       1	Sensor Tray	Sensor Tray:
1       2       3       4       5       6       7       8       9       10       11       12         A       Image: Amount of the stress of the s	Replace sensors in tray after use	Iray 1 V of 2 Iray Format Homogeneous trays
A Protein A   B C   C C   C C   C C   C C   C C   F C   C C   F C   C C   F C   C C   F C   C C   F C   C C   F C   C C   F C   C C   F C   C C   F C   C C   F C   F C   F C   F C   F C   F C   F C   F C   F C   F C   F C   F C   F C   C C   F C   C C   F C   C C   F C   C C   F C   C C   C C   C C   C C   C C   C C   C C   C C   C C   C C   C C   C C   C C   C C   C C   C C </td <td></td> <td>Well Sensor Type Lot Number Information</td>		Well Sensor Type Lot Number Information
B Potein A   C Potein A   F Potein A   G Potein A   F Potein A   G Potein A   G Potein A   G Potein A   H Potein A   Legend: Unassigned sensors   Plate 1 (96 wells)   1 2   1 2   1 2   2 3   5 7   9 10   1 12   3 4   5 7   9 10   1 12   1 2   3 5   7 9   10 11   12 3   4 5   7 9   10 11   12 3   4 5   7 9   10 11   12 14   12 15   12 16   10 11   12 16   10 11   12 16   12 16   12 16   12 17   12 16   13 10   14 10   15 10   16 10   17 10   18 10   19 11   12 12   14 10   15 10   16 11   17 10   18 </td <td></td> <td>A1 Protein A</td>		A1 Protein A
C1 Potein A   D D   F D   G D   H D   C Potein A   C Potein A   C Potein A   D Potein A   D Potein A   D Potein A   D Potein A   C Potein A   D Potein A   S Potein A   C Potein A   S Potein		B1 Protein A
D1 Protein A   F D1   G D1   F D1   G D1   H D1   C Protein A   Remove Fill   Fill Fill Plate   Plate 1 (96 wells)   1 2   1 2   1 2   3 4   6 0   1 2   1 2   2 4   5 7   8 0   0 0   0 0   0 0   1 2   1 2   1 2   1 2   1 2   1 2   1 3   1 0   1		C1 Protein A
E Potein A   F Potein A   G Potein A   H Potein A   Legend: Unassigned sensors   Missing sensors Missing sensors   Plate 1 (36 wells)   1 2   1 2   1 2   2 3   5 7   9 10   1 2   2 7   9 10   1 12   3 5   7 9   10 11   12 3   5 7   9 10   11 12   12 3   12 3   13 Protein A   14 Protein A   15 Protein A   16 Protein A   17 Protein A   18 Protein A   19 10   10 11   10 11   12 14   12 15   12 15   12 16   13 Protein A   14 Protein A   15 Protein A   16 10   17 10   18 10   19 11   10 11   12 16   13 Protein A   14 Protein A   15 Protein A   16 Protein A   17 Protein A   18 Protein A		D1 Protein A
F1 Protein A   G G   H G   Legend: Unassigned sensors   Remove Fill   Fill Fill Plate   Pitet 1 (96 wells)   1 2   1 2   4 5   6 7   9 10   1 2   4 5   7 9   10 11   12 3   4 5   7 9   10 11   12 7   12 3   5 7   9 10   11 12   13 7   14 7   15 7   16 10   17 10   18 10   19 10   10 10   10 10   10 10   11 12   12 11   12 10   12 10   12 10   12 11   13 10   14 10   15 70   16 11   17 10   10 11   12 10   12 12   12 10   12 12   13 70   14 70   15 70   16 10   17 10   18 70   19 10 <td></td> <td>E1 Protein A</td>		E1 Protein A
F G1 Protein A   H Protein A   Legend: Unassigned sensors   Remove Fill   Fill Fill Plate   Pitat 1 (96 wells)   1 2   4 5   7 9   1 2   4 5   7 9   1 2   4 5   7 9   1 1   2 4   5 7   9 0   1 7   6 0   7 9   1 1   1 7   1		F1 Protein A
G H   Legend: Unassigned sensors   Remove Fill   Fill Fill Plate   Plate 1 (96 wells)   1 2   6 7   9 10   1 2   4 6   7 9   10 7   8 7   9 10   12 3   4 6   7 9   12 3   5 7   9 10   12 7   12 7   12 3   5 7   9 10   12 7   12 3   5 7   9 10   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   12 7   13 7   14 7   15 7   16 11   17 7   18 7   19 7   10 7   11 10   11 12   12 10   13 7   14 7   15 7   16 7  <		G1 Protein A
A2 Protein A   Legend: Unassigned sensors   Remove Fill   Flate 1 (96 wells)     1 2   6 7   9 10   1 2   4 6   7 9   1 2   7 9   1 2   2 Protein A   2 Protein A   3 Protein A   4 2   9 1   12 3   4 5   7 9   1 2   1 2   4 5   7 9   1 1   1 1   1 1   1 2   1 2   2 9   1 2   3 4   4 7   1 1   1 1   1 1   1 1   2 1   3 1   4 1   5 1   6 1   10 1   11 1   12 1   13 1   14 1   15 1   16 1   17 1   18 1   19 1   10 1   10 1   10 1   11 1   12 1   13 1 <td></td> <td>H1 Protein A</td>		H1 Protein A
H B2   Legend: Unassigned sensors   Remove Fill   Flate 1 (96 wells)     1 2   1 2   4 6   7 9   1 2   7 9   1 2   7 9   1 2   7 9   1 2   7 9   1 2   7 9   1 2   7 9   1 2   7 9   1 2   7 9   1 2   7 9   1 2   1 3   7 9   1 2   2 70tein A   10 10   2 70tein A   12 10   13 70tein A   14 10   15 70tein A   16 10   17 10   18 10   19 10   10 10   10 10   10 10   10 10   10 10   10 10   11 10   12 10   13 10   14 10   15 10   16 10   17 10   18 10   19 10   10 10   10 10 <tr< td=""><td></td><td>A2 Protein A</td></tr<>		A2 Protein A
Legend:       Unassigned sensors       Missing sensors         Remove       Fill       Fill Plate       Print         Plate 1 (96 wells)       Image: Content of the print       F2       Protein A         1       2       3       5       6       7       9       10       11       12         A       Image: Content of the print       Image: Content of the print       F2       Protein A       F2         B       Image: Content of the print       Image: Content of the print       F2       Protein A       F2         B       Image: Content of the print       F2       Protein A       F3       Frotein A         Content of the print       Image: Content of the print       F3       Protein A       F3         F       Image: Content of the print       F3       Protein A       F3         F       Image: Content of the print of th		B2 Protein A
Legend:       Unassigned sensors       Missing sensors         Remove       Fill       Fill Plate       Print         Plate 1 (96 wells)       1       2       3       4       6       7       8       9       10       11       12         A       Image: Constraint of the sensors       Image: Constraint of the sens       Imag		C2 Protein A
Remove       Fill       Fill Plate       Print         Plate 1 (96 wells)       1       2       3       5       6       7       9       10       11       12         A       Image: Control of the stress of the s	Legend: Unassigned sensors XX Missing sensors	D2 Protein A
Plate 1 (96 wells)       F2       Protein A         1       2       3       5       6       7       8       1       12         A       Image: Contract of the state of the sta	Remove Fill Fill Plate Print	E2 Protein A
1       2       3       4       5       6       7       8       9       10       11       12         A       Image: Constraint of the structure of	Plate 1 (96 welle)	F2 Protein A
1       2       3       4       5       6       7       8       9       10       11       12         A       Image: Constraint of the structure of	Tide T (30 Weila)	G2 Protein A
A A3   B A3   C A3   Potein A   D A3   Potein A   B A3   Potein A   C A3   Potein A   C A3   Potein A   A3   Potein A   A43   Potein A   A4   Protein A   A4   Protein A   A4   Protein A   B4   Potein A   B4   Potein A   B4   Potein A	1 2 3 4 5 6 7 8 9 10 11 12	H2 Protein A
B    Image: Constraint of the second s		A3 Protein A
C       3       Protein A         D       0       0       0         E       0       0       0         F       0       0       0         G       0       0       0         H       0       0       0         Legend:       Unassigned samples       Unassigned samples		B3 Protein A
D       D		C3 Protein A
E    Protein A      F    Protein A      G    Protein A      H    Protein A      Legend:    Unassigned samples		D3 Protein A
F    Protein A      G    G      H    C      Legend:    Unassigned samples		E3 Protein A
F       G       G       Protein A         H       G       G       G       H         Legend:       Unassigned samples       Unassigned samples       G		F3 Protein A
G       G		G3 Protein A
G       A4       Protein A         H       C       Protein A         Legend:       Unassigned samples       D4		H3 Protein A
H C4 Protein A Legend: Unassigned samples B4 Protein A D4 Protein A		A4 Protein A
Legend:         Unassigned samples           C4         Protein A           D4         Protein A		B4 Protein A
Legend: Unassigned samples D4 Protein A		C4 Protein A
	Legend: Unassigned samples	D4 Protein A

Figure 6-45: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

6. To view the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The Sensor Tray Map and table for the tray selected appear (see Figure 6-46).



Figure 6-46: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

8. Optional: Double-click in any cell in the Lot Number column to enter a biosensor lot number.

All wells in the **Lot Number** column for the biosensor type selected will automatically populate with the lot number entered.

9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace** sensors in tray after use check box (see Figure 6-47).

Sensor Tray Replace sensors in tray after use	Senso Tray	rTray: 1 ✓ of 2 1	Fray Format H	omogeneous trays
1       2       3       4       5       6       7       8       9       10       11       12         A       1       1       1       1       1       1       1       1       1       12         B       1	Tray           Tray           A1           B1           C1           D1           E1           F1           G1           H1           A2           B2           C2           D2           E2	or Type Lotein A Protein A	Lot Number	

Figure 6-47: Replace Sensors in Tray After Use Check Box

NOTICE: Biosensors can be regenerated up to a max of 11 times per experiment.

#### Biosensor Regeneration

For Basic Quantitation with Regeneration experiments only, the **Sensor Assignment** tab includes the **Regenerations** parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The specified number of regeneration cycles determines the minimum number of cycles required for each column of sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by each column of sensors is represented by a pie chart (Figure 6-48).

1 Plate Definition 2 Sensor Assignment 3 Review Experiment	4 Run Experiment
In this step, sensors are assigned to samples. If you have a partial sensor tray it can be accomodated by selec Only the first sensor tray can be a partial plate. Sensor Tray	lecting the missing sensors and clicking 'Remove'.
Replace sensors in tray after use	
	Well Sensor Type Lot Number Information
A 2 2 1	A1 Protein A
B 2 2 1	B1 Protein A
	C1 Protein A
	D1 Protein A
	E1 Protein A
E 2 2 1	F1 Protein A
	G1 Protein A
	H1 Protein A
	A2 Protein A
	B2 Protein A
	C2 Protein A
	D2 Protein A
Remove Fill Fill Plate Print	E2 Protein A
Plate 1 (96 wells)	F2 Protein A
	G2 Protein A
1 2 3 4 5 6 7 8 9 10 11 12	H2 Protein A
	A3 Protein A
	B3 Protein A
	C3 Protein A
	D3 Protein A
	E3 Protein A
	F3 Protein A
	G3 Protein A
	H3 Protein A

Figure 6-48: Fractional Use of Regeneration and Neutralization Wells

## Using Partial Biosensor Trays

If you are using a partial tray of biosensors (some biosensors are missing), specify the missing columns in the **Sensor Tray Map**:

1. Select the column(s) without biosensors and click **Remove**, or right-click the selection and select **Remove**.

If the number of specified biosensors in the **Sensor Assignment** tab is less than the number required to perform the assay, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay.

2. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 6-49). In the example shown, Tray 1 is a partial tray that does not contain enough biosensors for the assay. To designate a second tray, select Tray 2 from the **Sensor Tray** drop-down list (Figure 6-49, top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 6-49, bottom).





To restore biosensors that have been removed, select the columns to restore and click **Fill**. To restore all sensors on the plate, click **Fill Plate**.

**NOTICE:** If multiple biosensor trays are used, only the first biosensor tray can be a partial tray. During the experiment, the software prompts you to insert the appropriate tray in the Octet<sup>®</sup> instrument.

## **Reviewing Experiments**

Before running an experiment, you can review the sample plate layout and the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window, move the slider left or right to highlight the biosensors and samples in an assay, or click the (  $\leftarrow \rightarrow$  ) arrows to select an assay.



Figure 6-50: Review Experiment Window

# Saving Experiments

After a run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment method:

- 1. Click the **Save Method File** button, 📩 or on the main menu, click **File** > **Save Method File**. To save more than one open experiment, click the **Save All Methods Files** button 🚓 .
- 2. In the Save dialog box, enter a name and location for the file, and click Save.

**NOTICE:** If you edit a saved experiment and want to save it without overwriting the original file, select **File** > **Save Method File As** and enter a new name for the experiment.

#### Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available on the menu bar. To view templates click **Experiment > Templates > Quantitation > Experiment Name (**see Figure 6-51).

Follow the steps above to save an experiment to the Template folder located at C:\ProgramFiles\Sartorius\Octet-BLIDiscovery\TemplateFiles.

**IMPORTANT:** Do not change the location of the Template folder. If the Template folder is not at the factory-set location, the software may not function properly.

New Experiment Wizard Edit Assay Parameters Edit Sensor Types	Ctrl+N				
Set Plate Temperature	- 1				
Templates	•	Kinetics	•]		
Skin Sten		Quantitation	Advanced Quantitation	- + J	
Stop	1		Basic Quantitation	•	Anti-hIgG biosensor_16CH_96W.fmf
Stop			Basic Quantitation with Regeneration	•	Anti-hIgG biosensor_8CH_96W.fmf
					Anti-mIgG biosensor_16CH_96W.fmf
					Anti-mIgG biosensor_8CH_96W.fmf
					Anti-Penta-HIS Dilution Factor Scouting_96W.fmf
					Anti-Penta-HIS Spike Recovery Assay 96W.fmf
					DirectDetectionImmunogenicity 16CH 96W.fmf
					DirectDetectionImmunogenicity 8CH 96W.fmf
					Protein A biosensor 16CH 96W.fmf
					Protein A biosensor 8CH 96W.fmf
					Protein A or G biosensor 16CH 96W fmf
					Protein A or G biosensor, 8CH, 96W fmf
					Protein L biosensor 16CH 96W fmf
					Protein L biosensor_SCH_06W/fmf

Figure 6-51: Experiments in the Template Folder

# Running a Quantitation Experiment

**IMPORTANT:** Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare the biosensors, see the appropriate biosensor product insert.

#### Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

- 1. Open the Octet<sup>®</sup> instrument door (lift the handle up).
- 2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 6-52).
- 3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 6-52).



Figure 6-52: Biosensor Stage (left) and Sample Stage (right)

**IMPORTANT:** Ensure that the bottom of the sample plate and biosensor tray are flat on each stage.

4. Octet<sup>®</sup> RED96e and Octet<sup>®</sup> R8 only, optional. Cover the microplate with the evaporation cover to prevent evaporation from samples during analysis and lengthen the experiment time (only applies to the Octet<sup>®</sup> RED96e and the Octet<sup>®</sup> R8 instruments). For more information, see "Microplate Evaporation Cover" on page 64.

**IMPORTANT:** Ensure that the push bar is installed near the biosensor pickers in the Octet<sup>®</sup> RED96e system prior to using the evaporation cover. The evaporation cover must be used with the push bar, otherwise the biosensors can crash into the cover.

- 5. Close the Octet<sup>®</sup> instrument door.
- 6. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you're cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

#### Starting an Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow  $\rightarrow$  to access the Run Experiment window (see Figure 6-53).

Data File Location and Names			
Assay type:	Basic Quantitation Standard Assay		Phor to pressing "Go" confirm the Assay
Quantitation data repository:	C:\data		
Experiment run name (sub directory):	Experiment_1	<b>→</b>	
Plate name/barcode (file prefix):	201102		
Auto-increment file ID start:	1		
Data files will be stored as follows:			Total experiment time: 0:20:00
Delayed experiment start Start after (s): 600	Open runtime charts automatica	elly chart	
Shake sample plate while waiting	Set plate temperature (°C):	30	
General Information User name:	Machine name: DESKTOP-0EH	TC34	

Figure 6-53: Run Experiment Window

2. Confirm the defaults or enter new settings. See "Run Experiment Window Settings" on page 198 for more information on experimental settings.

**NOTICE:** If you delay the experiment start, you have the option to shake the plate until the experiment starts. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you're cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

- 3. Optional if you are using a microplate evaporation cover. Hold plate at temperature after run is pertinent when you are running very long experiments with the evaporation cover. If you are running a 10-12 hour assay and want to ensure that the plate temperature remains at the set plate temperature, then check Hold plate at temperature after run. If it is acceptable for the plate to go back to room temperature post-run, then leave that option unchecked.
- 4. To start the experiment, click **GO**

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you selected the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time and the experiment progress (see Figure 6-54).

NOTICE: For more details about the Runtime Binding Chart, see "Managing Runtime Binding Charts" on page 201.



Figure 6-54: Runtime Binding Chart

5. Optional: Click **View** > **Instrument Status** to view the log file (see Figure 6-55).

The experiment temperature is recorded at the beginning of each experiment and each time the manifold picks up a new set of biosensors. Instrument events, such as, biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



**WARNING:** Do not open the Octet<sup>®</sup> instrument door when an experiment is in progress. If the door is opened the data from the active acquisition step is lost. The data acquired in previous steps is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



**WARNING:** N'ouvrez pas la porte de l'instrument Octet<sup>®</sup> lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



**WARNING:** Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

Instrument Status	
14:47:39 Sensor 7: Integration	Time = 1.0 ms
14:47:39 Sensor 8: Integration	Time = 1.0 ms
14:47:40 Picking sensors comple	eted location A1
14:47:40 Plate temperature = 3	0 C
14:47:40 Ready to move to san	nple location A1
014:47:40 Moving to sample loca	tion A1
14:47:41 Arrived at sample locat	ion A1
014:47:41 Waiting to start sample	e location A1
14:47:41 Processing sample loca	ation A1
014:47:51 Sample completed loca	ation A1
—14:47:51 Waiting to start new s	tep
— 14:47:51 Starting new step	
014:47:52 Ready to move to san	nple location A2
014:47:52 Moving to sample loca	tion A2
014:47:53 Arrived at sample locat	ion A2
014:47:53 Waiting to start sample	e location A2
14:47:53 Processing sample loca	ition A2
	<b>~</b>
•	4
Auto scroll to bottom	Save to File

Figure 6-55: Instrument Status Log

## Run Experiment Window Settings

The following Data File Location and Name settings are available on the Run Experiment Tab:

Table 6-8: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click Browse to select another data location.
	<b>NOTICE:</b> Save the data to the local machine first, then transfer to a network drive.
Experiment Run name (sub- directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software generates one data file for each biosensor.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following **Run Settings** are available on the **Run Experiment** Tab:

Table 6-9: Run Settings

Item	Description	
Delayed experiment start	Specifies a time delay for the start of the experiment.Enter the number of seconds to wait before the experiment starts after you click <b>go</b> .	
Start after	Enter the number of seconds to delay the start of the experiment.	
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.	
Open runtime charts automatically	Displays the <b>Runtime Binding Chart</b> for the current biosensor during data acquis tion.	
Automatically save runtime chart	Saves an image (.jpg) of the <b>Runtime Binding Chart</b> . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.	
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If no selected, the plate temperature is set to the default temperature specified in <b>Fil</b> e <b>Options</b> . The factory set default temperature is 30 °C.	
	<b>NOTICE:</b> If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet <sup>®</sup> BLI Discovery software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.	

The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet<sup>®</sup> system per second and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios.

Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

Table 6-10: Advanced Settings

Item	Description
Acquisition rate: • Octet <sup>®</sup> QKe	• High sensitivity quantitation (0.3 Hz, averaging by 40)—The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds.
	<ul> <li>Standard quantitation (0.6 Hz, averaging by 5)—The average of five data frames is reported as one data point. One data point is reported every 1.6 seconds.</li> </ul>
Acquisition rate: • Octet <sup>®</sup> RED96	<b>NOTICE:</b> For the Octet <sup>®</sup> RED, Octet <sup>®</sup> RED96, Octet <sup>®</sup> RED96e, and Octet <sup>®</sup> R8 sys- tems, acquisition rate settings are available on the Plate Definition Tab.
<ul> <li>Octet<sup>®</sup> RED96e</li> <li>Octet<sup>®</sup> R8</li> </ul>	<ul> <li>High concentration quantitation (10 Hz, averaging by 5) — The average of 5 data frames is reported as one data point. 10 data points are reported per second.</li> </ul>
	<ul> <li>High sensitivity quantitation (2 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second.</li> </ul>
	<ul> <li>Standard quantitation (5 Hz, averaging by 20)—The average of 20 data frames is reported as one data point. Five data points are reported per second.</li> </ul>
Sensor offset (mm)	Recommended sensor offset for quantitation—3 mm.
	<b>NOTICE:</b> For more details on optimizing the sensor offset and acquisition rate please contact your local Sartorius representative.
Default	Sets acquisition rate and sensor offset to the defaults.

#### The following General Settings are available on the Run Experiment Tab:

 Table 6-11: General Settings

Item	Description
Machine name	The computer name that controls the $Octet \circledast$ instrument and acquires the data.
User name	The user logon name.
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

#### Stopping an Experiment

To stop an experiment in progress, click 🛞 or click **Experiment** > **Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.

NOTICE: After the experiment is run, the software automatically saves the experiment method (.fmf).

# Managing Runtime Binding Charts

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 6-56). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

The Runtime Binding Chart is updated at the start of each experimental step. The active biosensor column is colorcoded (A=green, B=magenta, C=orange, D=purple, E=olive, F= black, G=red, H=blue) within the Sensor Tray Map. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each data acquisition step is represented by Sample Column X in the Current Binding Charts box.

To selectively display acquisition data for a particular acquisition step:

- 1. Click the corresponding **Sample Column** number.
- 2. Select a sub-set of sensors for a displayed column under Sensors to Chart box (see Figure 6-56).

**IMPORTANT:** Do not close the Runtime Binding Chart window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet<sup>®</sup> BLI Discovery software saves the Runtime Binding Chart as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.



Figure 6-56: Runtime Binding Chart Window

#### Opening a Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

- 1. Click File > Open Experiment.
- 2. In the dialog box that appears, select an experiment folder and click **Select**.

### Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data during acquisition in the chart by clicking the **Subtract reference sensors** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the Sensor Assignment tab
- During acquisition in the Runtime Binding Chart Sensors to Chart box
- During analysis in the **Data Selection** tab

#### Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the Sensors to Chart list or the Sensor Tray, right-click a biosensor and select Reference (see Figure 6-57).



Figure 6-57: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray (**see Figure 6-58).

2. Click the Subtract reference sensors check box (see Figure 6-58).



Figure 6-58: Subtract Reference Sensors check box in the Runtime Binding Chart

**NOTICE:** Subtracting reference data in the Runtime Binding Chart only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be re-done in data analysis if needed.

### Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 6-59). Uncheck the box to return to the default data display.



Figure 6-59: Data Inverted Using Flip Data Function

## Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

#### Scaling a Runtime Binding Chart

To scale the Runtime Binding Chart:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, select Fullscale or Autoscale.

#### Adding a Runtime Binding Chart Title

#### To add a Runtime Binding Chart title:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, enter a graph title or subtitle.

### Selecting a Runtime Binding Chart Legend

To select a Runtime Binding Chart legend:

- 1. Right-click the chart and select **Properties**.
- 2. In the **Runtime Graph Properties** dialog box (see Figure 6-60), select one of the following legends:
  - Sensor Location
  - Sample ID
  - Sensor Information
  - Concentration/Dilution

Runtime Graph Properties			
Title:			
Subtitle:			
Legend Sensor Location	Sensor Information		
Sample ID	© Concentration / Dilution		
	OK		

Figure 6-60: Selecting a Runtime Binding Chart Legend

**NOTICE:** Text for Sample ID, Sensor Information, or Concentration/Dilution is taken from the Plate Definition and Sensor Assignment tabs, and must be entered before the experiment is started.

3. Click OK.

#### Viewing Multiple Runtime Binding Charts

To view multiple charts of the same experiment click **Window** New Window to open a copy of chart of the experiment that you can modify to view different assays from the same experiment.

#### Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

- 1. Right-click the chart and select **Export Data**.
- 2. In the **Exporting** dialog box (see Figure 6-61), select the export options and click **Export**.

xporting					X
Export EMF	© WMF	O BMP	) JPG	O PNG	🔘 Text / Data
Export De	estination				
ClipBo OlipBo	ard				
File		Browse			
Printer					
Export Siz	ze				
		Millimete	rs 🔘 In	ches 💿 Poir	nts
	Width: 152.4	400 /	101.600	Millimeters	Export
	DPI: 300	-	🔲 Large Fo	nt	Cancel

Figure 6-61: Exporting Dialog Box

 Table 6-12: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	√		Click <b>File &gt; Browse</b> to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Run- time Binding Chart to a graphic file		✓	Click <b>File &gt; Browse</b> to select a folder and enter a file name.	Creates a graphic image.
Copy the Run- time Binding Chart		$\checkmark$	Clipboard	Copies the chart to the system clip- board
Print the Runtime Binding Chart		$\checkmark$	Printer	Opens the Print dialog box.

# Managing Experiment Method Files

After you run an experiment, the Octet<sup>®</sup> BLI Discovery software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it for your needs.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 6-13: Managing Experiment Method Files

Menu Bar Command/Toolbar Button	Description
File > Open Method File 🗂	Enables you to select and open a method file (.fmf)
File > Save Method File 📩 or 🚖	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwrit- ten.

## Custom Quantitation Assays

#### Defining a Custom Assay

To define a custom assay:

1. Click Experiment > Edit Assay Parameters.

The Edit Assay Parameters dialog box appears; see Figure 6-62.
Edit Assay Parameters			×
Available Assays:			
Basic Quantitation Name:		Standard Assay	
Anti-FLAG Quantitation Descrip	ption:	Basic Quantitation - Standard Assay (Read Only)	
Anti-GST -Standard range	v Param	atom	=
Anti-HIS (HIS2) Quantitation	iy i alalii	eters	
Anti-Human Fab-CH1 (FAB)	ingle an	alyte O Multiple analyte	
Anti-Penta-HIS -High sensitivity		Heplicates per sensor type: 1	
Anti-Penta-HIS -Standard range			
High sensitivity Human IgG quantitation			
Human IgG Quantitation			
Murine IoG Quantitation		lime (s): Shake speed (rpm):	
Qua	intitation	120 🖶 400 🖶	
Protein L -Standard range			
Standard Assay			
Basic Quantitation with Regeneration			
Anti-Human Fab-CH1 (FAB) with regeneration			
High sensitivity assay with regeneration			
Protein L -Standard range			
Standard Assay			
Diagonal Advanced Quantitation			
Custom Quantitation			
Immunogencity - Enzyme Linked			
Residual Protein A			
Three Step Assay			
- nice step nady			
Gray indicates a built-in assay and cannot be modified or deleted.			
Duplicate Remove		Save Cancel	

Figure 6-62: Edit Assay Parameters Dialog Box

- 2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
- 3. Click Duplicate.
- 4. In the **New Assay** dialog box (see Figure 6-63, top), enter an **Assay name**.
- 5. Optional: In the **Assay Description**, enter information about the assay.
- 6. Click Save.

The new assay appears in the directory tree of available assays (see Figure 6-63, bottom).

Edit Assay Parameters		×				
Available Assays:						
Basic Quantitation	Name:	Standard Assay				
	Description:	Basic Quantitation - Standard Assay (Read Only)				
Anti-GST -Standard range Assay Parameters						
	Single ar	nalyte O Multiple analyte				
Anti-Penta-HIS -High sensitivity		Replicates per sensor type: 1				
	0	Time (s): Shake speed (rpm):				
	Quantitation	1. 120 - 400 -				
Basic Quantitation with Regeneration						
Anti-Human Fab-CH1 (FAB) with regeneration	New A	ssay X				
	Enter	r Assau Information				
Standard Assay	Assa	v name: My Basic Quant Assay				
Advanced Quantitation						
Residual Protein A	Assay	y description: Enter a short description of the assay here.				
		OK Cancel				
Gray indicates a built-in assay and cannot be modified or de	eleted.					
Duplicate Remove		Save Cancel				
Edit Assay Parameters						
Available Assavs						
Basic Quantitation		Name: My Basic Quant Assay				
Anti-FLAG Quantitation		Description: Enter a short description of the assay here.				
		Assay Parameters				
Anti-HIS (HIS2) Quantitation		Single analyte     O Multiple analyte				
		Replicates per sensor type: 1				
Anti-Penta-HIS -Standard range	-View					
	auon					
Immunogenicity - Direct detection		Time (s): Shake speed (pm):				
Protein L -Standard range		Quantitation: 120 400 +				
Basic Quant Assay						
Anti-Human Fab-CH1 (FAB) with re-	generation					
High sensitivity assay with regeneration in the sensitivity assay wi	ation					
Standard Assay						
Advanced Quantitation						
Residual Protein A						
Standard Assay						
Gray indicates a built-in assay and cannot be	e modified or de	leted.				
Duplicate Remove		Save Cancel				

Figure 6-63: Defining a New Assay

### Editing Assay Parameters

To edit assay parameters:

- 1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 6-63).
- 2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
- 3. Click **Save** to accept the new parameter values. The new assay is added to the system.

NOTICE: Not all parameters are available for all of the assays.

#### Basic Quantitation Assay Parameters

Edit Assay Parameters			×
Available Assays:			
basic Quantitation	Name:	My Basic Quant Assay	
Anti-FLAG Quantitation	Description:	Enter a short description of the assay here.	
Anti-GST -High sensitivity			
Anti-GST -Standard range	Assay Param	leters	
Anti-Human Fab-CH1 (FAB)	Single ar	alyte 🔿 Multiple analyte	
Anti-Penta-HIS -High sensitivity		Replicates per sensor type: 1	×
High sensitivity Human IgG quantitation			
Human IgG Quantitation			
Murine Information		Time (s): Shake speed (rpm):	
Multile Igo Guantiation	Quantitation	: 120 🚔 400 🚔	
1 Protein L -Standard range			
Standard Assay			
Basic Quantitation with Regeneration			
Anti-Human Fab-CH1 (FAB) with regeneration			
High sensitivity assay with regeneration			
Standard Assay			
Advanced Quantitation			
Immunogencity - Enzyme Linked			
Standard Assay			
Implain Three Step Assay			
Gray indicates a built-in assay and cannot be modified or del	eted.		
Duplicate Remove			Save Cancel

Figure 6-64: Assay Parameters-Basic Quantitation Assay

#### Table 6-14: Basic Quantitation Assay Parameters (Sheet 1 of 2)

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.

#### Table 6-14: Basic Quantitation Assay Parameters (Sheet 2 of 2)

Parameter	Description
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analy- sis.
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

### Basic Quantitation with Regeneration Assay Parameters

Edit Assay Parameters		;	<
Available Assays:			
Basic Quantitation	Name:	My Basic Quant Assay	
Anti-FLAG Quantitation     Anti-GST -High sensitivity	Description:	Enter a short description of the assay here.	
Anti-GST -Standard range	Assay Para	meters	-
Anti-GST - Standard range     Anti-HS (HIS2) Quantitation     Anti-Human Fab-CH1 (FAB)     Anti-Penta-HIS - His sensitivity     Anti-Penta-HIS - His sensitivity     Anti-Penta-HIS - His sensitivity     Anti-Penta-HIS - His sensitivity     Anti-Penta-HIS - Standard range     Anti-Human IgG Quantitation     Murine IgG Quantitation     Anti-Human Fab-CH1 (FAB) with regeneration     Anti-Human Fab		nalyte Multiple analyte Replicates per sensor type: 1 Time (s): Shake speed (rpm): n: 120 ↓400 ↓	
Gray indicates a built-in assay and cannot be modified or de	leted.		
Duplicate Remove		Save Cancel	

Figure 6-65: Assay Parameters–Basic Quantitation with Regeneration

 Table 6-15: Assay Parameters-Basic Quantitation with Regeneration (Sheet 1 of 2)

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analysis.

Parameter	Description
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regener- ated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

 Table 6-15: Assay Parameters—Basic Quantitation with Regeneration (Sheet 2 of 2)

#### Advanced Quantitation Assay Parameters

Use the Advanced Quantitation Assay Parameters to create a custom assay.



Figure 6-66: Assay Parameters-Advanced Quantitation

- 1. Select the type of Analyte.
  - Single analyte select to use one biosensor per sample well.
  - Multiple analytes select to use multiple biosensors per sample well.
    - Replicates per sensor type select the number of replicates for each sensor type.
- 2. Select the desired step options.
  - · Insert click insert to add a step.

- Remove select a step and then click Remove to remove a step.
- Move Up select a step and then click Move Up to move a step up one row.
- Move Down select a step and then click Move Down to move a step up one row.
- 3. Adjust the Time and Shake speed (rpm) of each step.
  - Time select the duration time of the step.
  - Shake speed select the shake speed in rpm for the step.
- 4. Regeneration Incubate the biosensor in the regeneration buffer to remove the bound analyte.
- 5. Neutralization Incubate the biosensor in the neutralization buffer after the regeneration step.
- 6. Between assay steps
  - Regeneration cycles select the number of cycles for a biosensor before reuse or storage.
  - Pre-condition sensors Perform a set of regeneration or neutralization steps before the start of the experiment. These settings are like the time and rpm settings for the regeneration steps. For example, an acidic pre-conditioning buffer maximizes the binding competency of Protein A biosensors.
  - Post-condition sensors Re-racked biosensors in a regenerated state for storage.
- 7. Step option Reagent wells can be reused.
  - Reuse Position define a single position for a reagent. This position is used for all assays in the experiment
  - Use x1 through Use x10 define the number of times the reagent in a position can be used. After the selected number of times is used, that position is no longer used in the experiment. You must define enough reagent positions in the plate to complete the experiment. For example, if the experiment has six assays:
    - You can define two reagent positions on the place and select use x3.
    - Or you can define three reagent positions on the plate and select use x2.
  - Distribute usage (auto) define multiple positions in the for the reagent. The software automatically distributes the assays, so the defined reagent positions are used equally. For example, if the experiment has six assays and there are two defined reagent positions, the software will use each position three times.

**NOTICE:** Preview the application of the Reuse Position setting to ensure your settings. Select the Review Experiment tab and step through the experiment.

### Selecting a Custom Assay

You can select a custom assay when you define a sample plate.

To select a custom assay:

1. In the **Plate Definition** tab, click **Modify** in the **Assay Settings** box.

The Edit Assay Parameters dialog box appears (see Figure 6-67).



Figure 6-67: Selecting a Custom Assay

2. Select the custom assay from the directory tree and click OK.

# Multi-Step Advanced Quantitation Experiments

The multi-step selection interface for Advanced Quantitation methods increases the flexibility to add more assay steps prior to the Sample or Detection steps. In addition, all steps in an Advanced Quantitation assay may be viewed and analyzed in the software.

After starting the Octet<sup>®</sup> system and the Octet<sup>®</sup> BLI Discovery software, follow the steps below to set up and run an Advanced Quantitation experiment. You can start an Advanced Quantitation experiment using one of the following options:

- · Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run.
- On the menu bar, click Experiment > Templates > Quantitation > Advanced Quantitation.

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These options are explained further in "Starting an Experiment Using the Experiment Wizard" on page 155.

**NOTICE:** The Sample plate and the Reagent plate are now referred to as "Plate 1" and "Plate 2" in the software.

1. To add or edit assay steps in Tab 1 (Plate Definition), click **Modify** in Assay Settings to display the Assay Parameters window. Click on the **Step Type** drop-down list or highlight the parameter you want to change:

isitio	ion Rate: Standard (5.0 Hz)	Plate 1 T Concent	able (96 wells) ration units:	ua/ml	~ E	xport	Import	Print	
/ 5e /:	Advanced Quantitation Modify	Well	Sample ID	Replica	te Group 1	· Гуре	Conc (µg/ml)	Dilution Factor	^
	Standard Assay Single analyte	🔵 A1	1		S	itandard		n/a	
Γ									×
le	Assay Farameters								
πο	Available Assays:	Assay Parame	ters						
	Anti-GST -Standard range	Single and	alyte 🔿 Mul	tiple analy	te				
	Anti-HIS (HIS2) Quantitation		Re	plicates p	er sensor type	: 1	-		
	Anti-Human Fab-CH1 (FAB)     Anti-Renta-HIS -High sensitivity	Step Type		Time (s)	Shake (rpr	n) Step	Options	Insert	
		Sample	• 1	20	1000	Online	•		
	High sensitivity Human IgG quantitation	Detection	n	20	1000	Reuse	e position	Remove	
	Immunogenicity - Direct detection								
1									
	My Basic Quant Assay							Move Up	
1	Frotein L -Standard range     Standard Assay								
	Basic Quantitation with Regeneration	<					>	Move Down	
2	Anti-Human Fab-CH1 (FAB) with regeneration	Regene	eration						
			Time (	s):	Shake spe	ed (rpm):			
	My Basic Quant with Regen Assay	Hegenerati	on: 5	w	1000				
(	Protein L -Standard range	Neutralizati	on: 5	*	1000 🌲				
	Standard Assay				Regenerati	ion cycles			
	Advanced Quantitation	Betwe	een assav steps		3 1				
	Immunogencity - Enzyme Linked								
2	■ Residual Protein A	Pre-co	nation sensors		۰ ۲				
		Post-c	ondition sensor	S	3 🌲				
	1 Three Sten Accov								

Figure 6-68: Assay Parameters Window.

To add or remove steps, click the **Insert** or **Remove** buttons. Individual steps may be re-organized using the **Move Up** or **Move Down** buttons. Click **OK** to save any changes.

- Continue with the plate layout and sample well designation in Tab 1. For more details see "Defining the Sample Plate" on page 156, "Managing Sample Plate Definitions" on page 171 and "Managing Assay Parameter Settings" on page 174.
- 3. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with "Assigning Biosensors to Samples" on page 178 before running the Advanced Quantitation method.

### Chapter 7:

# Quantitation Experiments: Octet<sup>®</sup> RH16, Octet<sup>®</sup> RH96, and Octet<sup>®</sup> QK384

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# Introduction

A quantitation experiment enables you to determine analyte concentration within a sample using a reference set of standards. After starting the Octet<sup>®</sup> system hardware and the Octet<sup>®</sup> BLI Discovery software, follow the steps (in Table 7-1) to set up and analyze a quantitation experiment.

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

Software		Step	See
Octet <sup>®</sup> BLI Discovery	1.	Select a quantitation experiment in the <b>Experiment Wizard</b> or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 217
Octet BLI Discovery 12.1 (CFR11)			
	2.	Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 219
	3.	Define a or import a reagent plate (optional) for a Basic Quantitation with Regeneration experiment or an Advanced Quantitation experiment).	"Working with a Reagent Plate" on page 243
	4.	Confirm or edit the assay settings.	"Modifying Assay Parameter Settings" on page 245
	5.	Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 249
	6.	Run the experiment.	"Running a Quantitation Experiment" on page 266
Octet <sup>®</sup> Analysis	7.	Analyze the binding data.	Octet <sup>®</sup> Analysis Studio Software User Guide
Studio Octet Analysis Studio 12.1 (CFR 11)	8.	Generate a report.	

 Table 7-1:
 Setting Up and Analyzing a Quantitative Experiment

# Starting a Quantitation Experiment

**NOTICE:** Before starting an experiment, check the plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet<sup>®</sup> BLI Discovery software is closed, the plate temperature will reset to the default startup value specified in the Options dialog box when the software is relaunched.

You can start a quantitation experiment using one of the following options:

- · Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking File > Open Method File. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run. For more details on method files see "Managing Experiment Method Files" on page 279.
- On the menu bar, click Experiment > Templates > Quantitation.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

### Starting an Experiment Using the Experiment Wizard

To start an experiment using the **Experiment Wizard**:

- 1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button and click **Experiment > New Experiment Wizard** (**Ctrl+N**) from the **Main Menu**.
- 2. In the Experiment Wizard, select New Quantitation Experiment (see Figure 7-1, left).
- 3. Select a type of quantitation experiment (see Table 7-2 for options).

Table 7-2: Quantitation Experiment Selection

Quantitation Experiment	Description
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regenera- tion	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two-or three-step quantitation assay that enables signal amplifica- tion for higher detection sensitivity.

4. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

Experiment wizard							
Choose an option to start							
New Quantitation Exception 1	Available Templates for - Octet HTX						
	Blank Experiment	CLI 2Y204W forf					
O Basic Quantitation	- Protein A G or L biosensors_16	CH_2X96W.fmf					
Basic Quantitation with Regeneration	Protein A G or L biosensors_80	H_96W.fmf					
Advanced Quantitation	Protein A G or L biosensors_hig	h sensitivity_16CH_2X384W.tmt h sensitivity_8CH_96W.fmf					
0.1111	risten rie bioschools_ng	schalling_och_ootrain					
Now Kestics Experiment							
188K							
<ul> <li>Basic Kinetics</li> </ul>							
Epitope Binning							
L							
		$\rightarrow$					
Papart Methoda	4						
TROOM PROPOSITION	Thate Definition	n 2 Sensor Assignment 3 Review	Experiment 4 Hun E	xperiment			
	In this	step, all the information about the sample pl	ate and its wells will be e	ntered.			
	L-J-J First,	check the assay settings. Then highlight one	or more wells on the sa	mple plate, and right-click to enter/r	nodify well data		
Decel Lleed Cettin	Pand Hand	10 shannala Arish sanaživitu)	Plate 1	Table (384 wells)			
Read Head Setting	9 <u> </u>	To charmers (right sensitivity)	Conce	ntration units: µg/ml 🗸	Export	Import	Print
	Acquisition Rat	Standard (5.0 Hz)	✓ Well	Sample ID Replicate Group	Туре	Conc (ua/ml)	Dilution Fa
	Assay Settings	Paris Constitution with Parameters	A22	n/a	Regeneration	n/a	n/a
	Assay.	Standard Assay	Modify B22	n/a	Neutralization	n/a	n/a
		Single analyte	C22	n/a	Regeneration	n/a	n/a
	Quantitation:	lime (s): Shake speed (rpm): 120 400	D22	n/a	Neutralization	n/a	n/a
	Regeneration:	5 400	E22	n/a	Regeneration	n/a	n/a
	3 cycles per rec	5 400 eneration	F22	n/a	Neutralization	n/a	n/a
	Pre-conditioning	Enabled	G22	n/a	Regeneration	n/a	n/a
			H22	n/a	Neutralization	n/a	n/a
	Plate 1 (384 w	(s)	Modify 122	n/a	Regeneration	n/a	n/a
	12345	6 7 8 9 10111213141516171819202	J22	n/a	Neutralization	n/a	n/a
	A00000		© © K22	n/a	Regeneration	n/a	n/a
	BOOOOC		8 8 L22	n/a	Neutralization	n/a	n/a
	00000		<u>ଭ</u> ଁ ଭ M22	n/a	Regeneration	n/a	n/a
			ଷ ଷ ଭ ଭ	n/a	Neutralization	n/a	n/a
	G0000		ଁ ୦୦୦୦	n/a	Regeneration	n/a	n/a
			8 8 P22	n/a	Neutralization	n/a	n/a
	100000		8 8 A24	n/a	Regeneration	n/a	n/a
	100000		824 B24	n/a	Neutralization	n/a	n/a
	MOOOOO		© © C24	n/a	Regeneration	n/a	n/a
	00000		ିତ୍ର D24	n/a	Neutralization	n/a	n/a
		000000000000000000000000000000000000000	<u>∞</u> ∞ E24	n/a	Regeneration	n/a	n/a
	Standa	d 🔵 Control 🔵 Unassigne	d F24	n/a	Neutralization	n/a	n/a
	Unknov	in 🥚 Reference 🔿 Reserved	G24	n/a	Regeneration	n/a	n/a
		v	104	n/a	Neutralization	0/2	n/a

Figure 7-1: Selecting an Experiment Type in the Experiment Wizard (for Octet® RH16)

5. Click the  $\rightarrow$  arrow.

The **Experiment** window appears (Figure 7-1, right).

- 6. Octet<sup>®</sup> RH96 Only. Open Tab 1(Plate Definition) for Read Head configuration and plate(s) layout. The default Read Head setting is 96 channels, which dips 96 biosensors simultaneously for a given assay step.
- 7. Click on the drop-down list for Read Head to select 96, 48, 32, 16 or 8 channels (Figure 7-2) as the new Read Head setting. An individual assay is defined as a series of steps or dips starting with pick up of the biosensors, followed by the assay steps, and ending with ejection of biosensors back into the biosensor tray or disposal chute. A Quantitation method file may contain multiple assays.



Figure 7-2: Selecting Read Head Channels

# Defining the Sample Plate

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher (Figure 7-3).



Figure 7-3: Sample Plate Renamed Plate 1 in Software Versions 8.0 and Higher

The general steps for defining a sample plate for all models are listed in Table 7-3. Information specific for each model follow the table.

#### Table 7-3: Defining a Sample Plate

	Step
1.	Select the instrument read head configuration (8 or 16 channels).
2.	Select the sample plate format (96 or 384 wells).
З.	Designate the samples.
4.	Annotate the samples (optional).
5.	Save the sample plate definition (optional).

### Read Head Configuration and Plate Layout

### Octet<sup>®</sup> RH16 and Octet<sup>®</sup> QK384

The Octet<sup>®</sup> read head contains the collection optics. If the read head is set to 8 channels, one column of 8 biosensors interrogate 8 plate wells. If the read head is set to 16 channels, two columns of biosensors interrogate 16 wells in a column format. (Figure 7-1).

The read head configuration and the plate format (96 or 384 wells) determine the plate layout (Figure 7-4 and Figure 7-5).

#### 8 Channel Read Head



#### 16 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

8 Channel Read Head

Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.

Figure 7-4: Color-Coded Wells: How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head



#### Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

#### 16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.

Figure 7-5: Color-Coded Wells: How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head

**NOTICE:** Keep the read head configuration in mind when laying out the sample plate. While reading a 384-well sample plate, both the 8 channel and 16 channel read heads can freely step through the plate by either moving left or right to step across columns or step one row up or down.

### Octet<sup>®</sup> RH96

The Octet<sup>®</sup> RH96 system has a user-selectable Read Head for monitoring 8, 16, 32, 48, or 96 wells in parallel so you can tailor your assay design to maximize either throughput or detection sensitivity.

The 96 biosensor mode uses multiplexer switching to read 96 wells simultaneously either in a 96- or 384-well plate, with similar sensitivity as the Octet<sup>®</sup> QK384 system. Large sample sets are analyzed in the shortest amount of time using this Read Head setting, which is also ideal for rapid, whole plate analysis and biosensor loading in multi-step assays.

Figure 7-6 shows the biosensor layout in a 96- and 384-well plate with the 96-channels Read Head setting. Biosensors interrogate 96 wells in 12 columns at the same time.



Figure 7-6: Biosensor Layout in 96- and 384-well Plates Using 96-channels Read Head Setting.

**NOTICE:** A column of 16 wells is read in two sets of interrogations. Biosensors interrogate 8 wells in a column at a time: rows A, C, E, G, I, K, M and O are read first followed by rows B, D, F, H, J, L, N and P.

The 32 and 48 biosensor modes also use multiplexer switching to read 32 and 48 wells in parallel, with sensitivity equivalent to the Octet<sup>®</sup> QK384 system. Cross-blocking experiments as large as 32 x 32 or larger may be accomplished with the 32 or 48 biosensor modes combined with 384-well tilted-bottom plates in a shorter amount of time compared to other Octet<sup>®</sup> systems.

In Figure 7-7, biosensors interrogate 32 wells in 4 columns at a time or 48 wells in 6 columns at a time. Columns 1, 3, 5 and 7 are interrogated at the same time, and so on for the 32-channels setting. Columns 1, 3, 5, 7, 9 and 11 are interrogated at the same time, and so on for the 48-channel setting:



Figure 7-7: Biosensor Layout in 384-well Plates Using 32 (left) and 48 (right) Channels Read Head Setting.

The 8 and 16 biosensor modes provide high sensitivity for measuring small molecule binding interactions and protein quantitation down to 50 ng/mL, similar to the Octet<sup>®</sup> RED96e and Octet<sup>®</sup> RH16 systems. These two modes are best for assays requiring a wide dynamic range or fine signal resolution, and may be combined with the other Read Head options in a single experiment.



Figure 7-8: Zoomed View of Closely Overlaid Traces Shows Fine Signal Resolution for Human IgG Quantitation Assay with Protein A Biosensors

#### 8 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

#### 16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.

Figure 7-9: Color-Coded Wells: How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

#### 8 Channel Read Head





16 Channel Read Head

Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so

Figure 7-10: Color-Coded Wells: How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head

### Changing the Plate Format

#### NOTICE:

Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet <sup>®</sup> BLI Discovery software versions 8.0 and higher.

The default plate format can be changed from 96-well plate to 384-well plate by selecting File > Options and Default Sample Plate(s).

To change the sample plate format:

- 1. Click the **Modify** button above the plate map.
- 2. In the Modify Plates box, select 96 Well or 384 Well format.



Figure 7-11: Changing the Sample Plate Format

**NOTICE:** In Basic Quantitation with Regeneration and Advanced Quantitation experiments, a reagent plate format option is also available. Please refer to "Working with a Reagent Plate" on page 243 for more information.

### Designating Samples

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

Each well may be designated as a **Standard**, **Unknown**, **Control**, or **Reference**. A well may also remain **Unassigned** or be designated as **Reserved** by the system for Basic Quantitation with Regeneration and Advanced Quantitation experiments.

**NOTICE:** It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 7-4 will be included in the assay.

Table 7-4: Types of Sample Wells

lcon	Description
Standard	Contains an analyte of known concentration. Data from the well is used to generate a standard curve during analysis.
Unknown	Contains an analyte of unknown concentration. The concentration of the analyte is calculated from the well data and the standard curve.
Control	A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis.
	Positive Control: A control sample that contains analyte of known concentration
	Negative Control: A control sample known not to contain analyte
Reference	Provides a baseline signal which serves as a reference signal for <b>Unknowns</b> , <b>Controls</b> , and <b>Stan- dards</b> . The reference signal can be subtracted during data acquisition in the <b>Runtime Binding</b> <b>Chart</b> and during data analysis.
Unassigned	Not used during the experiment.
Reserved	Used by the system during Basic Quantitation with Regeneration experiments and Advanced Quantitation multi-step experiments for <b>Regeneration</b> (R), <b>Neutralization</b> (N), <b>Detection</b> (D), or <b>Capture Antibody</b> (C). Reserved wells are not available for use as <b>Standards</b> , <b>Unknowns</b> , <b>Controls</b> , or <b>References</b> .

#### **Reserved Wells**

In a Basic Quantitation with Regeneration or an Advanced Quantitation experiment, the **Sample Plate Map** includes gray wells. These wells are reserved by the system and specify the location of particular sample types. The default location of the reserved wells depends on the sample plate format (96 or 384-wells) and the Octet<sup>®</sup> instrument read head configuration (8 or 16 channels).

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Reserved samples cannot be removed from the sample plate, but you can change their column location. To change the location of a reserved column ((\*), (\*), (\*), (\*), or (\*)) right-click a column header in the **Sample Plate Map** and select **Regeneration**, **Neutralization**, **Detection**, or **Capture Antibody**.

 Table 7-5: Reserved Well Requirements

Reserved Well	Must Contain						
Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).						
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.						
Detection	Secondary antibody or precipitating substrate that is used with an enzyme-antibody conjugate to amplify the analyte signal. Sample concentrations are computed using the binding data from the detection wells.						
Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of inter- est onto the biosensor.						



Figure 7-12: Default Locations for Reserved Wells in 96-well (top) and 384-well Sample Plate Maps (bottom)

### Selecting Wells in the Sample Plate Map

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

There are several ways to select wells in the Sample Plate Map:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 7-13, left). To select nonadjacent columns, hold the Ctrl key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 7-13, center).
- · Click a well or draw a box around a group of wells (Figure 7-13, right).



Figure 7-13: Selecting Wells in the Sample Plate Map

**NOTICE:** Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

### Designating Standards

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in the Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

To designate standards:

- 1. In the **Sample Plate Map**, select the wells to define as standards. Alternatively, for 384-well plates, you can sort the plate in the table based on rows, columns, quadrant-rows and quadrant-columns by right-clicking on the sample table **Well** heading and selecting the desired sorting option.
- 2. Click the **Standard** button below the **Sample Plate Map** (see Figure 7-14), or right-click and select **Standard**.

The standards are marked in the plate map and the **Sample Plate Table** is updated.

3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

Plate Definition	2 Sensor Assignment 3 Re	view Experiment	4 Run Expe	eriment				
In this step,	all the information about the sar	nple plate and its wel	ls will be ente	ered.				
First, check	the assay settings. Then highlig	ht one or more wells	on the samp	le plate, and r	ght-click to enter/mo	dify well da	ta.	
ead Head:	16 channels (high sensitivity)	~	Plate 1 Ta	able (384 well	3)	-		-
couisition Rate:	Standard (5.0 Hz)	~	Concentr	ration units:	µg/ml ∨	Export	Import	Print
seav Settings			Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor
say: Ba	sic Quantitation with Regeneration	Modify	🔘 A1			Standard		n/a
Sta	ndard Assay ole analyte	woully	<u>C</u> C1			Standard		n/a
Tin	ne (s): Shake speed (np	m);				Standard		n/a
antitation: 120	400		<u>O G1</u>			Standard		n/a
generation: 5 utralization: 5	400					Standard		n/a
ycles per regenera	tion		О К1			Standard		n/a
-conditioning Enal	bled		O M1			Standard		n/a
			01			Standard		n/a
ate 1 (384 wells)		V Modify	🔘 A3			Standard		n/a
4 2 2 4 5 6 7	8 0 4044424244464647404	00004000004	🔘 C3			Standard		n/a
<b>o</b> _ <b>r</b>	0 9 1011121314131017101	00808	🔵 E3			Standard		n/a
OC O Star	ndard	0000	🔵 G3			Standard		n/a
💍 🔵 Unk	nown		O 13			Standard		n/a
🔍 🌔 Cor	ntrol	00808	🔵 КЗ			Standard		n/a
o 🕻 Ň Neg	jative Control	DO BOB	O M3			Standard		n/a
🚬 🕑 Pos	itive Control	0000	0 03			Standard		n/a
💽 🔵 Refe	erence	႞ႄၜၴၜၜၴၜ	A22		n/a	Regenera	n/a	n/a
			B22		n/a	Neutraliza	n/a	n/a
	eneration	jõõõõ	C22		n/a	Regenera	n/a	n/a
	itralization		D22		n/a	Neutraliza	n/a	n/a
Set	Well Data	DONON	E22		n/a	Regenera	n/a	n/a
S Clea	ar Data	igned	F22		n/a	Neutraliza	n/a	n/a
	ute Clintered	ved	G22		n/a	Regenera	n/a	n/a
Cop	by to Clipboard	veu	H22		n/a	Neutraliza	n/a	n/a
<ul> <li>Extension</li> </ul>	ended Sample Types		122		n/a	Regenera	n/a	n/a

Figure 7-14: Plate Definition Window–Designating Standards

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

1. In the Sample Plate Map, select the standard wells, right-click and select Set Well Data.

The Set Well Data dialog box appears (see Figure 7-15).

Plate 1 (384 wells)	~	Modify		
1       2       3       4       5       6       7       8       9       101112131415167         A       Standard       Standard       0       0       0       101112131415167         C       Unknown       E       O       Control       6       8       Negative Control         F       O       Control       F       Positive Control       1       1       6       Reference         K       O       Reference       Reference       1				
N Neutralization		Set Well Data		×
Clear Data Clear Data Copy to Clipboard Extended Sample Types	Jnassigned Reserved	Well Information Sample ID: Replicate Group:	Dilution Series       Starting value (µg/ml):       Series operator:       Series operand:       Series operand:	⊻ _
		Well Information: Concentration (µg/ml): Standards only	Dillution orientation	t incel

Figure 7-15: Sample Plate Map–Setting a Dilution Series

- 2. Select the **Dilution Series** option and enter the starting concentration value.
- 3. Select a series operator, enter an operand, and select the appropriate dilution orientation.



Figure 7-16: Concentration Representation in Dilution Series

4. Click OK.

The Sample Plate Table displays the standard concentrations entered.

#### Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

1. In the Sample Plate Map, select the standard wells, right-click and select Set Well Data.

**NOTICE:** A range of wells can be selected clicking and dragging, holding the Shift key and using the arrow keys to select sections of the plate, or holding the Ctrl key to select specific wells.

The Set Well Data dialog box appears (see Figure 7-17).

Plate 1 (384 wells)	<ul> <li>✓ Modify</li> </ul>				
1     2     3     4     5     6     7     8     9     101112131415161       1     2     3     4     5     6     7     8     9     101112131415161       2     Standard     0     Unknown     0     0     0       2     Control     0     Regative Control     0     0       3     Positive Control     0     Reference       4     R     Regeneration					
N Neutralization	Set Well	Data			×
Clear Data Clear Data Copy to Clipboard Extended Sample Types	Reserved Replic	formation e ID: ate Group: nformation:		Dilution Series Starting value (µg/ml): Series operator: Series operand: Dilution orientation	1 / ~ ~ 2
	Conce Standa	ntration (µg/ml): ards only	200	OK	Cancel

Figure 7-17: Sample Plate Map–Assigning a Standard Concentration

- 2. Select the **By value** option and enter the starting concentration value. If a range of cells was selected, all cells will update with the specified value.
- 3. Click **OK**. The **Sample Plate Table** displays the standard concentrations entered.

### Editing an Individual Standard Concentration

To enter or edit an individual standard concentration, in the **Conc** column of the **Sample Plate Table**, double-click the value and enter a new value (see Figure 7-18).

Concer	ntration units:	μg/ml 🔻	Expo	rt	Impo	rt		
Well	Sample ID	<b>Replicate Group</b>	Туре	Cond	: (µg/ml)	<b>Dilution Factor</b>	Informatio	
🔵 A1			Standard	200		n/a		
🔵 C1			Standard	100		n/a		
🔵 E1			Standard	50		nla		
🔵 G1			Standard	25	Und	lo		
🔵 11			Standard	10	Cut			
🔵 K1			Standard	5	Cut			
🔵 M1			Standard	2.5	Cop	y		
01			Standard	1	Pas	te		
🔵 A3			Standard	200	Dele	ete		
🔵 C3			Standard	100	Cal			
🔵 E3			Standard	50	Sele	ect All		
🔵 G3			Standard	25	Ria	nt to left Reading	order	
🔵 13			Standard	10	Sho	w Unicode contr	, ol characters	
🔵 КЗ			Standard	5	5110		or characters	
🔵 МЗ			Standard	2.5	Inse	ert Unicode contr	ol character	
03			Standard	1	Ope	en IME		
					Rec	onversion		

Figure 7-18: Sample Plate Table–Shortcut Menu of Edit Commands

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

### Designating Unknowns

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

To designate unknowns in the **Sample Plate Map**, select the wells to define as unknown, right-click and select **Unknown**. The unknown wells are marked in the plate map and the **Sample Plate Table** is updated (see Figure 7-19).

Read Head:	16 channels (high sensitivity)	~	- Plate 1 Ta Concentr	able (384 wells ration units:	) ua/ml v	Export	Import	Print
Acquisition Rate:	Standard (5.0 Hz)	~	Well	Sample ID	Replicate Group	Туре	Conc (µa/ml)	Dilution Factor
ssay Settings			→ H1			Standard		n/a
ssay: E S	Basic Quantitation Standard Assay	Modify	0 11			Standard		n/a
S	ingle analyte		J1			Standard		n/a
)uantitation: 1	ime (s): Shake speed (npm): 20 400		🔾 к1			Standard		n/a
			L1			Standard		n/a
						Standard		n/a
Plate 1 (384 wells)	) ~	Modify	○ N1			Standard		n/a
123456	7 8 9 10111213141516171819202	1222324	01			Standard		n/a
			O P1			Standard		n/a
	<b>0000000000000000000000000000000000000</b>	0000	A2			Unknown	n/a	
	Standard	0000	🔘 В2			Unknown	n/a	
	Unknown		🔵 C2			Unknown	n/a	
ି <b>ଁ ଁ ଁ ଁ ଁ</b> ଁ	Control	0000	🔵 D2			Unknown	n/a	
8888	Negative Control		🔵 E2			Unknown	n/a	
	Positive Control	0000	🔵 F2			Unknown	n/a	
	Reference	0000	🔵 G2			Unknown	n/a	
000	Set Well Data		🔘 H2			Unknown	n/a	
	Clear Data	0000	<u> </u>			Unknown	n/a	
Star		ad .	🔵 J2			Unknown	n/a	
	Copy to Clipboard		🔘 К2			Unknown	n/a	
	Extended Sample Types d		O L2			Unknown	n/a	

Figure 7-19: Plate Definition Window–Designate Unknown Wells

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

- 1. In the Sample Plate Map, select the unknown wells (see Figure 7-19).
- 2. Right-click and select Set Well Data.

The Set Well Data dialog box appears (see Figure 7-20).

Plate 1 (384 wells) V	dify	
1       2       3       4       5       6       7       8       9       1011121314151617181920212222         A       Image: Constraint of the second		
N Set Well Data	Set Well Data	×
Standard     Copy to Clipboard       Unknown     Extended Sample Types	Well Information     Dilution Series       Sample ID:     Starting value:       Replicate Group:     Series operator:	1 / 2
	Well Information:	Content Conten
	Dilution Factor: 2 V Unknowns only OK	Cancel

Figure 7-20: Sample Plate Map–Setting a Dilution Factor or a Serial Dilution

To assign a dilution factor to selected wells:

- 1. In the **Set Well Data** dialog box (see Figure 7-20), select the **By Value** option.
- 2. Enter the dilution factor value and click **OK**.

To assign a serial dilution to selected wells:

- 1. In the **Set Well Data** dialog box (see Figure 7-20), select the **Dilution series** option.
- 2. Enter the starting dilution, select a series operator, and enter a series operand.
- 3. Select the appropriate dilution orientation (see Figure 7-21).



Figure 7-21: Concentration Representation in Dilution Series

4. Click OK.

The Sample Plate Table displays the dilution factors entered.

Editing a Dilution Factor in the Sample Plate Table

To edit a dilution factor in the **Sample Plate Table**:

- 1. In the **Sample Plate Table** (see Figure 7-22), double-click a cell in the **Dilution Factor** column for the desired unknown.
- 2. Enter the new value (the default dilution factor is 1).

Sample	Plate Table –								
Conce	ntration units:	μg/ml 🛛 🔻	Expo	ort Impo	t				
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	Dilu	tion Factor	Information		
🔵 A3			Standard	200	n/a				
🔘 C3			Standard	100	n/a				
🔵 E3			Standard	50	n/a				
🔵 G3			Standard	25	n/a				
🔘 I3			Standard	10	n/a				
🔵 КЗ			Standard	5	n/a				
🔘 МЗ			Standard	2.5	n/a				
<b>O</b> 03			Standard	1	2				
🔵 A5			Unknown	n/a	1	Undo			
🔵 C5			Unknown	n/a	1	Cut			
🔵 E5			Unknown	n/a		cui			
🔵 G5			Unknown	n/a	1	Сору			
<b>(</b> ) 15			Unknown	n/a	1	Paste			
🔘 К5			Unknown	n/a	1	Delete			
🔵 M5			Unknown	n/a		C			
05			Unknown	n/a	1	Select All			
🔿 A7			Unknown	n/a		Right to le	eft Reading o	rder	
🔿 C7			Unknown	n/a		Show Uni	code control	character	
🔵 E7			Unknown	n/a		3100 011	code control	character	2
🔵 G7			Unknown	n/a		Insert Uni	code control	cnaracter	
0 17			Unknown	n/a		Open IMF			
🔘 K7			Unknown	n/a		Poconvor	sion		
O M7			Unknown	n/a		Reconver	SIUT		

Figure 7-22: Sample Plate Table—Shortcut Menu of Edit Commands

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

### Designating Controls or Reference Wells

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

Controls are samples of known concentration that are not used to generate a standard curve. A reference well contains sample matrix only, and is used to subtract non-specific binding of the sample matrix to the biosensor. During data analysis, data from reference wells can be subtracted from standards and unknowns to correct for background signal.

- To designate controls, select the control wells and click **Control** (below the **Sample Plate Map)**, or right-click and select **Control**. Positive and Negative Control types can also be assigned using this menu.
- To designate reference wells, select the reference wells and click the **Reference** button below the **Sample Plate Map**, or right-click the selection and choose **Reference**.

The wells are marked in the **Sample Plate Map** and the **Sample Plate Table** is updated.

1 Plate Definition 2 Sense	or Assignment	3 Review Experiment	4	Run Expe	eriment						
In this step, all the in First, check the assa	formation about t y settings. Then	he sample plate and its v highlight one or more we	vells w Ils on t	ill be ente the samp	ered. le plate, and ri	ght-click to e	nter/mo	dify well da	ta.		
Read Head: 16 chann	nels (high sensitiv	ity) ~	l rf	Plate 1 Ta	able (384 wells	s)					
Acquisition Poto: Chandrad			i	Concentr	ation units:	µg/ml	$\sim$	Export	Import	Print	
Acquisition nate. Standard	I (3.0 HZ)	Ý	1   [	Well	Sample ID	Replicate	Group	Туре	Conc (µg/ml)	Dilution Factor	^
Assay Settings Assay: Basic Quanti	tation			○ M4				Unknown	n/a		
Standard As	say	Modify		N4				Unknown	n/a		
Single analyt	e			04				Unknown	n/a		
Quantitation: 120	Shake spe 400	ed (npm):		P4				Unknown	n/a		
				▲ A5				Control		n/a	1
				O 85				Control		n/a	i –
Plate 1 (384 wells)		✓ Modify		C5				Control		n/a	1
1 2 3 4 5 6 7 8 9 10	1112131415161	718192021222324		O D5				Control		n/a	1
				C E5				Control		n/a	
c 🖸 🖉 🖉 🔵 St	andard	Ĕ		O F5				Control		n/a	
	nknown	R		G5				Control		n/a	
F00000000000	ontrol N	Ď		отно Н5				Control		n/a	
	ہم egative Control	Ы		0 15				Control		n/a	
	sitive Control	ĝ		Ŭ ☐ J5				Control		n/a	
	ference	Б		<u>с</u> к5				Control		n/a	
	t Wall Data	P.		<u> </u>				Control		n/a	1
		Б		ом м5				Control		n/a	
	ear Data	P		O N5				Control		n/a	
C	opy to Clipboar	d H		0 05				Control		n/a	
Standard 🗸 Ex	tended Sample	Types		O P5				Control		n/a	
Unknown		neserveu		A6				Unknown	n/a		
				BE				Unknown	n/a		
								Upknown	n/2		

Figure 7-23: Designate Controls or Reference Wells

**NOTICE:** Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

### Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

#### Annotating Wells in the Sample Plate Map

To annotate one or more wells:

- 1. In the Sample Plate Map, select the samples to annotate, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 7-24), enter Sample ID and/or Well Information and click OK.

Plate 1 (384 wells)	~ Modify	
1       2       3       4       5       6       7       8       1011121314151617         A       Standard       O       Unknown       0	8 192021 222324 	
Regeneration	Set Well Data	×
Set Well Data Clear Data Copy to Clipboard Extended Sample Types	Well Information     Dilution Series       Sample ID:     Image: Sample ID:       IgG Standard     Starting value         Rese     Replicate Group:	μg/ml): 1 : / ~ t 2
	Well Information:	stion Right COC Left Down
	Concentration (µg/ml):	OK Cancel

Figure 7-24: Adding Sample Annotations from the Sample Plate Map

#### Annotating Wells in the Sample Plate Table

To annotate an individual well in the Sample Plate Table:

- 1. Double-click the table cell for **Sample ID** or **Well Information**.
- 2. Enter the desired information in the respective field (see Figure 7-25).

**NOTICE:** A series of Sample IDs may also be assembled in Excel and pasted into the Sample Plate Table.

	Sample F	Plate Table —				_		
Concentration units:		ration units:	μg/ml 👻	Export.	. Import			
	Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	<b>Dilution Factor</b>	Information	
	🔵 A1	hlgG		Standard	200	n/a	human IgG	
	🔵 C1			Standard	100	n/a		
	🔵 E1			Standard	50	n/a		
	🔵 G1			Standard	25	n/a		
	🔵 l1			Standard	10	n/a		
	🔵 K1			Standard	5	n/a		

Figure 7-25: Adding Sample Annotations in the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

### **Replicate Groups**

When samples are assigned to a **Replicate Group**, the software will automatically calculate statistics for all samples in that group. The average binding rate, average concentration and corresponding standard deviation as well CV% are presented in the **Results** table for each group (see Figure 7-26).

Sensor	Replicat	BR Avg	BR SD	BR CV	Conc. Avg	Conc. SD	Conc. CV
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2

Figure 7-26: Replicate Group Result Table Statistics

NOTICE: Replicate Group information can also be entered in the Results table.

Assigning Replicate Groups in the Sample Plate Map To assign **Replicate Groups** in the **Sample Plate Map**:

- 1. Select the samples to group, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 7-27), enter a name in the Replicate Group box and click OK.

Well Information		Dilution Series	
Sample ID:	[	Starting value:	1
Ab1		Series operator:	/ ~
Replicate Group: 9		Series operand:	2
Well Information:		Dilution orientation	
Sample Diluent		Right	SSSS O Left
		Down	
Dilution Factor:	2		

Figure 7-27: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software only recognizes and calculates statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

**NOTICE:** When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 7-28).



Figure 7-28: Replicate Groups: Sample Plate Map

The Sample Plate Table will update with the Replicate Group names entered (see Figure 7-29).

Sample F	Plate Table —						
Concen	tration units:	µg/ml ▼	Export	Import			
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	<b>Dilution Factor</b>	Information	
🔵 A1	hlgG	1	Standard	200	n/a	human IgG	
🔵 C1	hlgG	2	Standard	100	n/a	human IgG	
🔵 E1	hlgG	3	Standard	50	n/a	human IgG	
🔵 G1	hlgG	4	Standard	25	n/a	human IgG	
🔵 l1	hlgG	5	Standard	10	n/a	human IgG	
🔵 K1	hlgG	6	Standard	5	n/a	human IgG	
🔵 M1	hlgG	7	Standard	2.5	n/a	human IgG	
01 🜔	hlgG	8	Standard	1	n/a	human IgG	
🔵 A3	hlgG	1	Standard	200	n/a	human IgG	
🔵 C3	hlgG	2	Standard	100	n/a	human IgG	Ξ
🔵 E3	hlgG	3	Standard	50	n/a	human IgG	
🔵 G3	hlgG	4	Standard	25	n/a	human IgG	
🔘 I3	hlgG	5	Standard	10	n/a	human IgG	
🔘 КЗ	hlgG	6	Standard	5	n/a	human IgG	
🔘 МЗ	hlgG	7	Standard	2.5	n/a	human IgG	
<b>O</b> 03	hlgG	8	Standard	1	n/a	human IgG	
🔵 A5	Ab1	9	Unknown	n/a	2	Sample Diluent	
🔵 C5	Ab2	10	Unknown	n/a	2	Sample Diluent	
🔵 E5	Ab3	11	Unknown	n/a	2	Sample Diluent	
🔵 G5	Ab4	12	Unknown	n/a	2	Sample Diluent	
🔵 IS	Ab5	13	Unknown	n/a	2	Sample Diluent	
🔵 K5	Ab6	14	Unknown	n/a	2	Sample Diluent	
🔵 M5	Ab7	15	Unknown	n/a	2	Sample Diluent	
<b>O</b> 05	Ab8	16	Unknown	n/a	2	Sample Diluent	
🔵 A7	Ab9	9	Unknown	n/a	2	Sample Diluent	
🔵 C7	Ab10	10	Unknown	n/a	2	Sample Diluent	
🔵 E7	Ab11	11	Unknown	n/a	2	Sample Diluent	
🔵 G7	Ab12	12	Unknown	n/a	2	Sample Diluent	

Figure 7-29: Replicate Groups in Sample Plate Table

## Assigning Replicate Groups in the Sample Plate Table

### To assign **Replicate Groups** in the **Sample Plate Table**:

- 1. Double-click the desired cell in the **Replicate Group** table column.
- 2. Enter a group name (see Figure 7-30).

c!	Sample F	Plate Table						
Concentration units:			µg/ml ▼	Export	Import			
	Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	<b>Dilution Factor</b>	Information	
	🔵 КЗ	hlgG		Standard	5	n/a	human IgG	
	🔵 МЗ	hlgG		Standard	2.5	n/a	human IgG	
	<b>O</b> 03	hlgG		Standard	1	n/a	human IgG	
	🔵 A5	Ab1	9	Unknown	n/a	2	Sample Diluent	
	🔵 C5	Ab2		Unknown	n/a	2	Sample Diluent	
	🔵 E5	Ab3		Unknown	n/a	2	Sample Diluent	
	🔵 G5	Ab4		Unknown	n/a	2	Sample Diluent	

Figure 7-30: Add Replicate Group from the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The Octet<sup>®</sup> BLI Analysis software will only recognize and calculate statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

**NOTICE:** When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

# Managing Sample Plate Definitions

NOTICE: After you define a sample plate, you can export and save the plate definition for future use.

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

### Exporting a Plate Definition

To export a plate definition:

1. In the Sample Plate Table (see Figure 7-31), click Export.

-Sample F	Plate Table			-			
Concentration units:		µg/ml ▼	Export	Import			
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	<b>Dilution Factor</b>	Information	
🔵 A1	hlgG	1	Standard	200	n/a	human IgG	
🔵 C1	hlgG	2	Standard	100	n/a	human IgG	
🔵 E1	hlgG	3	Standard	50	n/a	human IgG	
🔵 G1	hlgG	4	Standard	25	n/a	human IgG	
🔵 l1	hlgG	5	Standard	10	n/a	human IgG	
🔵 K1	hlgG	6	Standard	5	n/a	human IgG	

Figure 7-31: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 7-31), select a folder, enter a name for the plate (.csv), and click **Save**.

Export Plate 1 Definition					×
$\leftarrow$ $\rightarrow$ $\checkmark$ $\uparrow$ $\square$ $\rightarrow$ This PC $\rightarrow$ Loc	al Disk (C:) → data	~	ۍ ې	earch data	
Organize 🔻 New folder					?
<ul> <li>OneDrive</li> <li>Name</li> </ul>	^	Date modified	Туре	Size	
💻 This PC		No items match your search	1.		
3D Objects					
E Desktop					
Documents					
🖶 Downloads					
👌 Music					
Pictures					
📕 Videos					
Local Disk (C:)					
DVD Drive (D:) O					
· · · · · · · · · · · · · · · · · · ·					
File name: 384 standard plate					~
Save as type: CSV Files (*.csv)					~
<ul> <li>Hide Folders</li> </ul>			2	ave Canc	ei

Figure 7-32: Export Plate Definition Window

### Importing a Plate Definition

To import a plate definition:

1. In the **Sample Plate Table** (see Figure 7-33), click **Import**.

	Sample F	Plate Table				1		
Concentration units: µg		tration units:	µg/ml ▼	Export	Import			
	Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	<b>Dilution Factor</b>	Information	
	🔵 A1	hlgG	1	Standard	200	n/a	human IgG	
	🔵 C1	hlgG	2	Standard	100	n/a	human IgG	
	🔵 E1	hlgG	3	Standard	50	n/a	human IgG	
	🔵 G1	hlgG	4	Standard	25	n/a	human IgG	
	🔘 l1	hlgG	5	Standard	10	n/a	human IgG	
	🔵 К1	hlgG	6	Standard	5	n/a	human IgG	

Figure 7-33: Import Button in Sample Plate Table

2. In the **Import Plate Definition** window (see Figure 7-35), select the plate definition (.csv), and click **Open**.

🗐 Import Plate 1 Defi	nition					×
import nate i ben	intion					
$\leftrightarrow \rightarrow \land \uparrow$	> This PC	C > Local Disk (C:) > data	~	ල 🔎 Search	data	
Organize 🔻 Nev	w folder					?
- Ouick access	<u>^</u> 1	Name	Date modified	Туре	Size	
Quick access		384 standard plate.csv	1/8/2021 10:14 AM	CSV File	1 KB	
OneDrive						
This PC						
illi 3D Objects						
📃 Desktop						
Documents						
🖶 Downloads						
👌 Music						
E Pictures						
🔚 Videos						
🏪 Local Disk (C:)	)					
🛄 DVD Drive (D:)	0					
<b>a</b>	~					_
	File <u>n</u> ame	384 standard plate.csv		✓ CSV Files (*.c	sv)	$\sim$
				<u>O</u> pen	Cancel	

Figure 7-34: Import Plate Definition Window

**NOTICE:** You can also create a .csv file for import. Figure 7-35 shows the appropriate column information layout.

	А	В	С	D	E	F	G	
1	PlateWells	384						=
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information	_
3	A1	hlgG	1	Standard	200		human IgG	
4	C1	hlgG	2	Standard	100		human IgG	
5	E1	hlgG	3	Standard	50		human IgG	
6	G1	hIgG	4	Standard	25		human IgG	
7	11	hlgG	5	Standard	10		human IgG	
8	K1	hlgG	6	Standard	5		human IgG	
9	M1	hIgG	7	Standard	2.5		human IgG	
10	01	hlgG	8	Standard	1		human IgG	-
4	▶ ▶ 384	0 method expo	ort 🧖					

Figure 7-35: Example Sample Plate Definition File (.csv)

### Printing a Sample Plate Definition

To print a plate definition:

1. In the Sample Plate/Plate 1 Map (see Figure 7-36), click Print.

	Plate 1 Table (384 wells)										
Concentration units:		ration units:	$\mu g/ml$ $\sim$	µg/ml ∨ Export Impo		Print					
	Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	^				
	🔵 A1		1	Standard		n/a					

Figure 7-36: Sample Plate/Plate 1 Print Button

The associated Sample Plate Table information will print.
## Working with a Reagent Plate

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher (Figure 7-37).



Figure 7-37: Reagent Plate Renamed Plate 2 in Software Versions 8.0 and Higher

## Reagent Plate for Octet<sup>®</sup> RH16 and Octet<sup>®</sup> QK384

You can include an optional reagent plate in a Basic Quantitation with Regeneration or Advanced Quantitation experiment. Using a reagent plate enables higher sample throughput since no reagents are included in the sample plate. A reagent plate can contain:

- Regeneration and neutralization reagents for Basic Quantitation with Regeneration experiments
- Buffers, enzyme solutions, and detection reagents for Advanced Quantitation experiments

An experiment can include any combination of sample and reagent plate formats (96- or 384-well). However, a reagent plate can include only reagent wells (regeneration, neutralization, detection). Wells for standards, unknowns, controls and references can not be assigned to the reagent plate.

**NOTICE:** Reagent plates can only contain reagents. Standards, unknown samples, controls and references must be assigned to the sample plate.

**NOTICE:** The reagent plate format (96- or 384-well) and the read head configuration (8 or 16 channels) determine the reagent plate layout. For more details, see "Read Head Configuration and Plate Layout" on page 219.

### Reagent Plate for the Octet<sup>®</sup> RH96

The Octet<sup>®</sup> RH96 system supports a second sample plate which may contain any combination of sample wells and reagent wells.

#### To define a reagent plate

- 1. Select the **Reagent Plate** radio button above the plate map to display the **Reagent Plate Map** (Figure 7-38).
- 2. Click Modify to display the Modify Plates dialog box.

243

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6

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0

Modify Plates		×
Plate 1	384 Wells	$\sim$
Plate 2	96 Wells	$\sim$
ОК	Cancel	1

Figure 7-38: Modifying the Reagent Plate

- 3. Select a reagent plate format (96 Well or 384 Well) and click OK.
- 4. In the **Reagent Plate Map**, right-click a column to use and make a selection on the shortcut menu that appears:
  - Advanced Quantitation-Select Detection.
  - Basic Quantitation with Regeneration-Select Regeneration or Neutralization. Repeat this step to set both the regeneration and neutralization reagent columns.

The Reagent Plate Map then shows where to dispense the reagents in the plate (Figure 7-39).



96-well Format Reagent Plate

384-well Format Reagent Plate

Figure 7-39: Example Reagent Plate Layouts for an Advanced Quantitation Experiment-16 Channel Read Head

To remove well designations, select the column(s) and click Unassigned, or right-click and choose Clear Data.

#### Saving a Reagent Plate Definition

Exporting and saving a reagent plate definition is done in the same manner as you would for sample plates. For details "Managing Sample Plate Definitions" on page 240.

#### Printing a Reagent Plate Definition

To print a plate definition:

#### In the Reagent Plate/Plate 2 Map (see Figure 7-40), click Print. 1

Plate 2	Table (384 w	ells)							-	_	
Conce	ntration units:		µg/ml	$\sim$	Exp	ort	Import		Print		
Well	Sample ID	Re	plicate	Group	Туре	Conc	(µg/ml)	Dilu	ition Factor	Informa	2

Figure 7-40: Reagent Plate/Plate 2 Print Button

## Managing Assay Parameter Settings

#### Modifying Assay Parameter Settings

You can modify the assay parameter settings during sample plate definition. However, the changes are only applied to the current experiment. To save modified parameter settings, you must define a new assay. For details on creating a new assay, see "Custom Quantitation Assays" on page 280.

### Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box will appear (Figure 7-41). The settings are experiment dependent.

1         Flate Definition         2         Sensor Assignment         3         Review Experiment         4           Image: Sensor Assignment         4         Image: Sensor Assignment         3         Review Experiment         4           Image: Sensor Assignment         4         Image: Sensor Assignment         3         Review Experiment         4           Image: Sensor Assignment         4         Image: Sensor Assignment         3         Review Experiment         4           Image: Sensor Assignment         4         Image: Sensor Assignment         3         Review Experiment         4           Image: Sensor Assignment         4         Image: Sensor Assignment         3         Review Experiment         4           Image: Sensor Assignment         4         Image: Sensor Assignment         4 <th>Run Experiment is will be entered. on the sample plate, and right-click to enter/modify well data.</th> <th><math>\leftarrow</math> <math>\rightarrow</math></th>	Run Experiment is will be entered. on the sample plate, and right-click to enter/modify well data.	$\leftarrow$ $\rightarrow$
Read Head:     16 channels       Acquisition Rate:     Standard (5.0 Hz)       Assay:     Basic Quartitation with Regeneration Standard Assay Single analyte	Well         Sample ID         Replicate Group         Type         Concertulation         Import         Print           A22         n/a         Regeneration         n/a         n/a         n/a         n/a	
Time (s):         Shake speed (pm):           Quaritation:         120         400           Respensation:         5         400           Neutrilization:         5         400           3 cycles per regeneration:         F         400           Pre-conditioning Enabled         Modfy         1         2         3 4 5 6 7 8 9 101112131415161718192021222324         Modfy           1 2 3 4 5 6 7 8 9 101112131415161718192021222324         6	Assay Parameters       Available Assays:     Assay Parameters       Available Assays:     Assay Parameters       Image: Sand Quantitation with Regeneration     Single analyte       Image: Applicates per sensor type:     1       Image: Applicate Sand Quart with Regeneration     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration:     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration:     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration:     Image: Applicates per sensor type:       Image: Applicate Sand Quart with Regeneration:     Image: Applicates per sensor type:       Image: Applicate Sand Quart With Regeneration:     Image: Applicates per sensor type:       Image: Applicate Sand Quart With Regeneration:     Image: Applicates per sensor type:       Image: Applicate Sand Quart With Regeneration:     Image: Applicates per sensor type:       Image: Applicate Sand Quart Applicates per sensor type:     Image: Applicates per sensor type:       Image: Applicate Sand Quart Applicates per sensor type:     Image: Appl	ead: mels
N Standard Unknown Standard Unknown Standard Control Reference Control Con	Pre-condition sensors 3 0 Post-condition sensors 3 0	

Figure 7-41: Modifying Assay Parameters.

#### Basic Quantitation Assay Parameters

Assay Parameters		×
Available Assays:	Assay Parameters	
Basic Quantitation     Basic Quantitation     B Anti-FLAG Quantitation     D Anti-GST -High sensitivity     B Anti-GST -Standard range     D Anti-HIS (HIS2) Quantitation	Single analyte O Multiple analyte Replicates per sensor type: 1	
P1 Anti-Human Fab-CH1 (FAB)     P2 Anti-Penta-HIS -High sensitivity     P1 Anti-Penta-HIS -Standard range     P2 High sensitivity Human IgG quantitation     P2 Human IgG Quantitation     P1 Immunogenicity - Direct detection     P2 Immunogenicity - Direct detection     P2 Protein L_Standard range     P3 Standard Assay	Time (s): Shake speed (rpm): Quantitation: 120 + 400 +	
Gray indicates a built-in assay.	OK Cancel	

Figure 7-42: Assay Parameters-Basic Quantitation Assay

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analy- sis.
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

#### Basic Quantitation with Regeneration Assay Parameters

Assay Parameters		$\times$
Available Assays:	Assay Parameters	
Basic Quantitation with Regeneration Anti-Human Fab-CH1 (FAB) with regeneration High sensitivity assay with regeneration	Single analyte     O Multiple analyte     Replicates per sensor type:     1	
	Time (s):     Shake speed (rpm):       Quantitation:     120	
	Regeneration:     5     400       Neutralization:     5     400	
	Regeneration cycles: Between assay steps: 3	
	Pre-condition sensors 3	
	Post-condition sensors 3	

**Figure 7-43:** Assay Parameters—Basic Quantitation with Regeneration

Table 7-7: Assay Parameters-	-Basic Quantitation	with Regeneration
------------------------------	---------------------	-------------------

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analysis.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regener- ated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

#### Advanced Quantitation Assay Parameters

Use the Advanced Quantitation Assay Parameters to create a custom assay.

vailable Assays:	Assay Parameters				
Basic Quantitation	Single analyte	l <b>ultiple analy</b> Replicates p	<b>te</b> er sensor lype:	1	
Anti-GST -Standard range	Step Type	Time (s)	Shake (rpm)	Step Options	Insert
Anti-HIS (HIS2) Quantitation	Sample -	120	1000	Online	Demous
	Detection	120	1000	Reuse position	Remove
Anti-Penta-HIS -High sensitivity					
Anti-Penta-HIS -Standard range					
High sensitivity Human IgG quantitation					
Human IgG Quantitation					Move Up
Immunogenicity - Direct detection					move op
Pertoin L. Standard range	<				Move Down
Standard Assay	Regeneration				
Basic Quantitation with Regeneration	Time	(s):	Shake speed	(npm):	
Anti-Human Fab-CH1 (FAB) with regeneration	Regeneration: 5	-	1000 🚔		
	Neutralization: 5		1000		
Protein L -Standard range			Personantian	ouoloo:	
Standard Assay			Regeneration	cycles:	
	Between assay ste	DS:	3		
Advanced Quantitation		re .	3 🌲		
Advanced Quantitation	Fre-condition sense				
Advanced Quantitation     Immunogencity - Enzyme Linked     Residual Protein A	Post-condition sense	ors	3 🔺		
Advanced Quantitation  Munogencity - Enzyme Linked  Residual Protein A  Standard Sasay  Time Stan Acazy (	Post-condition sense	ors	3		

Figure 7-44: Assay Parameters-Advanced Quantitation

- 1. Select the type of Analyte.
  - Single analyte select to use one biosensor per sample well.
  - Multiple analytes select to use multiple biosensors per sample well.
    - Replicates per sensor type select the number of replicates for each sensor type.
- 2. Select the desired step options.
  - Insert click insert to add a step.
  - Remove select a step and then click Remove to remove a step.
  - Move Up select a step and then click Move Up to move a step up one row.
  - Move Down select a step and then click Move Down to move a step up one row.
- 3. Adjust the Time and Shake speed (rpm) of each step.
  - Time select the duration time of the step.
  - Shake speed select the shake speed in rpm for the step.
- 4. Regeneration Incubate the biosensor in the regeneration buffer to remove the bound analyte.
- 5. Neutralization Incubate the biosensor in the neutralization buffer after the regeneration step.

- 6. Between assay steps
  - Regeneration cycles select the number of cycles for a biosensor before reuse or storage.
  - Pre-condition sensors Perform a set of regeneration or neutralization steps before the start of the experiment. These settings are like the time and rpm settings for the regeneration steps. For example, an acidic pre-conditioning buffer maximizes the binding competency of Protein A biosensors.
  - Post-condition sensors Re-racked biosensors in a regenerated state for storage.
- 7. Step option Reagent wells can be reused.
  - Reuse Position define a single position for a reagent. This position is used for all assays in the experiment
  - Use x1 through Use x10 define the number of times the reagent in a position can be used. After the selected number of times is used, that position is no longer used in the experiment. You must define enough reagent positions in the plate to complete the experiment. For example, if the experiment has six assays:
    - You can define two reagent positions on the place and select use x3.
    - Or you can define three reagent positions on the plate and select use x2.
  - Distribute usage (auto) define multiple positions in the for the reagent. The software automatically distributes the assays, so the defined reagent positions are used equally. For example, if the experiment has six assays and there are two defined reagent positions, the software will use each position three times.

**NOTICE:** Preview the application of the Reuse Position setting to ensure your settings. Select the Review Experiment tab and step through the experiment.

## Assigning Biosensors to Samples

After you define the sample plate, assign biosensors to the samples.

**NOTICE:** When using a 96-well plate with the 8 channel read head, do not put biosensors in columns 2, 4, 6, 8, 10, and 12 if the biosensors will be returned to the biosensor tray and not discarded. If the biosensors will be ejected, biosensors can be placed in all columns.

#### Biosensor Assignment in Single-Analyte Experiments

In a single analyte experiment, only one biosensor type is assigned to each sample and only one analyte is analyzed per experiment.

**NOTICE:** For single analyte experiments, the Single Analyte option must be selected in the Assay Parameters dialog box. For more information, please see "Managing Assay Parameter Settings" on page 245.

Click the **Sensor Assignment** tab, or click the **GO** arrow to access the Sensor Assignment window (see Figure 7-45).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default.

1 Plate Definition 2 Sensor Assignment 3 Review Experiment	4 Run	Experiment		
In this step, sensors are assigned to samples. If you have a partial sensor tray it can be accomodated by select Only the first sensor tray can be a partial plate.	ing the	missing sensors and clic	king 'Remove'.	
	147-11	C T	Lat Number	L.C
	Well	Sensor Type	Lot Number	Information
	A1	Anti-Human IgG FC		
	01	Anti-Human IgG FC		
	D1	Anti-Human IgG Fc		
	E1	Anti-Human IgG Fc		
	E1	Anti-Human IgG FC		
	C1	Anti-Human Igo Fo		
		Anti-Human IgG Fo		
	Δ2	Anti-Human IgG Fo		
	R2	Anti-Human IgG Fc		
	C2	Anti-Human IgG Fc		
Legend: Unassigned sensors 🕅 Missing sensors	D2	Anti-Human IgG Fc		
Pamaua Eill Eil Dista Drint	F2	Anti-Human IgG Fc		
	F2	Anti-Human IgG Fc		
Plate 1 (204 malle)	G2	Anti-Human IgG Fc		
1 2 3 4 5 6 7 8 9 101112131415161718192021222324	H2	Anti-Human IgG Fc		
	A3	Anti-Human IgG Fc		
	B3	Anti-Human IgG Fc		
	C3	Anti-Human loG Fc		
	D3	Anti-Human IgG Fc		
	E3	Anti-Human loG Fc		
	F3	Anti-Human IgG Fc		
	G3	Anti-Human IgG Fc		
	H3	Anti-Human IgG Fc		
	A4	Anti-Human IgG Fc		
	B4	Anti-Human IgG Fc		
P0000000000000000000000000000000000000	C4	Anti-Human IgG Fc		
Legend: Unassigned samples	D4	Anti-Human IgG Fc		

Figure 7-45: Sensor Assignment Window for Basic Quantitation without Regeneration

- 1. Assign biosensors in one of two ways:
  - Select column(s) in the Sensor Tray Map, right-click and select a biosensor type from the drop-down list.
  - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 7-46).

All wells in the **Sensor Type** column are automatically populate with the biosensor type selected.



Figure 7-46: Changing Biosensor Types

2. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

- 3. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column will automatically populate with the lot number entered.
- 4. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (**Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

 Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the Replace sensors in tray after use check box (see Figure 7-47).



Figure 7-47: Replace Sensors in Tray After Use Check Box

**NOTICE:** Do not regenerate biosensors more than S11 times per experiment.

#### Biosensor Assignment in Multiple Analyte Experiments

In a multiple analyte experiment, more than one biosensor type is assigned to the same sample, allowing multiple analytes to be analyzed in a single experiment.

**NOTICE:** For multiple analyte experiments, the Multiple Analyte option must be selected in the Assay Parameters dialog box. For more information, please see "Managing Assay Parameter Settings" on page 245.

Click the **Sensor Assignment** tab, or click the  $\rightarrow$  arrow to access the Sensor Assignment window (see Figure 7-45).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 7-45, **one** replicate had been previously selected with the **Multiple Analyte** assay parameter option.





There are two ways to assign biosensors:

- Select a column in the Sensor Tray Map, right-click and select a biosensor type from the drop-down list.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the dropdown list (see Figure 7-49).



Figure 7-49: Changing Biosensor Types

#### Biosensor Assignment Using Heterogeneous Biosensor Trays

The default Tray Format is Heterogeneous. Heterogeneous biosensor trays contain a mixture of biosensor types.

**NOTICE:** When using this Heterogeneous option, the order of biosensor types in each tray must be identical.

1. If Heterogeneous Trays is not displayed next to the **Tray Format** button, click the button.

The Tray Format dialog box appears (see Figure 7-50).

2. Select Heterogeneous and click OK.

	X
Sensor trays may contain various sensor ty but all sensor trays used are identical.	rpes,
A different sensor tray is used for each sense	or type.
Anti-Human IgG Fc	Add
	Remove
	Change
	Move Up
	Move Down
OK Cancel	
	Sensor trays may contain various sensor ty but all sensor trays used are identical. A different sensor tray is used for each sensor Anti-Human IgG Fc OK Cancel

Figure 7-50: Tray Format Dialog Box

The Tray 1 Sensor Tray Map appear by default.

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to be used (see Figure 7-51).

The Sensor Type column will update accordingly.



Figure 7-51: Populating the Sensor Tray Map with First Biosensor Type

4. Select the columns in the **Sensor Tray Map** that should contain the second biosensor type, right-click and select the second biosensor type (see Figure 7-53).

The Sensor Type column will update accordingly.



Figure 7-52: Populating the Sensor Tray Map with Second Biosensor Type

5. Repeat this column selection and assignment process for all other biosensor types to be used in the experiment. The software will automatically update the number of biosensor trays needed and biosensor assignments in all trays according to the column assignments made in Tray 1.

In the example shown in Figure 7-53, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays will be needed for the experiment.



Figure 7-53: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

6. To view or change the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown and biosensor assignments can be changed as needed (see Figure 7-54).

Sensor Tray ☑ Replace sensors in tray after use	Sensor Tray: Tray 2 ✓ of 2 Tray Format Heterogeneous trays
Construction       Construction         1       2       3       4       5       6       7       8       9       10       11       12         A       1       2       3       4       5       6       7       8       9       10       11       12         B       1       1       1       1       10       10       11       12         C       1       1       1       10       11       12       10<	Tray 1     of 2     Tray Format     Heterogeneous trays       Tray 1     or Type     Lot Number     Information       Tray 2     or Type     Lot Number     Information       Tray 1     Playein A     Information       1     Protein A     Information       2     Protein A     Information       82     Protein A     Information
Legend:     Unassigned sensors       Remove     Fill       Fill     Fill Plate	C2         Protein A           D2         Protein A           E2         Protein A           E2         Protein A

Figure 7-54: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

- 8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered.
- 9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 7-47).



Figure 7-55: Replace Sensors in Tray After Use Check Box

NOTICE: Biosensors can be regenerated up to a max of 11 times per experiment.

#### Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.

**NOTICE:** Using the Homogeneous option will necessitate switching trays during the experiment.

1. Click Tray Format.

The **Tray Format** dialog box appears (see Figure 7-56) and the **Sensors** box will be populated with the default biosensor type.

	X
Sensor trays may contain various sen but all sensor trays used are identical.	sor types,
A different sensor tray is used for each	n sensor type.
Anti-Human IgG Fc	Add
	Remove
	Change
	Move Up
	Move Down
OK Cano	cel
	Sensor trays may contain various sen but al sensor trays used are identical. A different sensor tray is used for each Anti-Human IgG FC OK Canc

Figure 7-56: Tray Format Dialog Box

2. Select Homogeneous. Click Add to select the first biosensor type (see Figure 7-57).

Tray Format			
Heterogeneous	Sensor trays may contain various sensor typ but all sensor trays used are identical.	oes,	
Homogeneous	A different sensor tray is used for each senso	or type.	
Sensors:	Anti-Human IgG Fc	Ant	i-Mouse IgG Fv
		Pro	tein A
		Pro	tein G
		Pro	tein L
		SA	(Streptavidin)
		Res	idual Protein A
		Ant	i-Penta-HIS
		Cus	stom
	OK Cancel		

Figure 7-57: Selecting a Biosensor Type in the Tray Format Dialog Box

- 3. Repeat this step to add any additional biosensor types that will be used in the experiment. To remove a biosensor type, select a biosensor type in the **Sensor** box and click **Remove**.
- 4. Adjust the order of biosensor types as needed by selecting the biosensor type in the **Sensor** box and clicking **Move Up** or **Move Down**.

The order of biosensor types listed in the **Sensor** box will be used as the default tray assignment (see Figure 7-58).

ray Format				×
Heterogeneous	Sensor trays may contain var but all sensor trays used are i	ious sensor ty dentical.	pes,	
Homogeneous	A different sensor tray is used	for each sense	or type.	_
Sensors:	Protein A		Add	
	Protein G		Remove	
			Kelliove	
			Change	
			Move Up	
			Move Down	
			1	
	OK	Cancel	J	

Figure 7-58: Biosensor Types List Order in Sensor Box

5. Click OK.

The software will automatically calculate the number of biosensor trays needed and assign biosensors types to each tray.

In the example shown in Figure 7-59, Protein A and Protein G biosensor types will be used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) will be needed for the experiment. The Tray 1 **Sensor Tray Map** appears by default.





6. To view the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.



The Sensor Tray Map and table for the selected tray appear (see Figure 7-54).

Figure 7-60: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.

**NOTICE:** Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

- 8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for the biosensor type selected will automatically populate with the lot number entered.
- 9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for particular cell.

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** For greater clarity, annotation text may be displayed as the legend of the Runtime Binding Chart during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace** sensors in tray after use check box (see Figure 7-47).



Figure 7-61: Replace Sensors in Tray After Use Check Box

NOTICE: Biosensors can be regenerated up to a max of 11 times per experiment.

### **Biosensor Regeneration**

For Basic Quantitation with Regeneration experiments only, the **Sensor Assignment** tab includes the **Regenerations** parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The specified number of regeneration cycles determines the minimum number of cycles required for each column of sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by each column of sensors is represented by a pie chart (Figure 7-62)



Figure 7-62: Fractional Use of Regeneration and Neutralization Wells

### Using Partial Biosensor Trays

If you are using a partial tray of biosensors (some biosensors are missing), specify the missing columns in the **Sensor Tray Map**:

1. Select the column(s) without biosensors and click **Remove**, or right-click the selection and select **Remove**.

If the number of specified biosensors in the **Sensor Assignment** tab is less than the number required to perform the assay, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay.

2. To view the additional biosensor tray that is required for the assay, select Tray 2 from the Sensor Tray drop-down list (Figure 7-63). In the example shown, Tray 1 is a partial tray that does not contain enough biosensors for the assay. To designate a second tray, select Tray 2 from the Sensor Tray drop-down list (Figure 7-63 top). The Sensor Tray Map will then display the additional biosensors required for the assay (Figure 7-63 bottom).



Figure 7-63: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray

To restore biosensors that have been removed, select the columns to restore and click **Fill**. To restore all sensors on the plate, click **Fill Plate**.

**NOTICE:** If multiple biosensor trays are used, only the first biosensor tray can be a partial tray. During the experiment, the software prompts you to insert the appropriate tray in the Octet<sup>®</sup> instrument.

## **Reviewing Experiments**

Before running an experiment, you can review the sample plate layout and the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window, move the slider left or right to highlight the biosensors and samples in an assay, or click the  $\epsilon$  arrows to select an assay.



Figure 7-64: Review Experiment Window

## Saving Experiments

After a run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment method:

- 1. Click the **Save Method File** button 📩, or on the main menu, click **File** > **Save Method File**. To save more than one open experiment, click the **Save All Methods Files** button 🐴.
- 2. In the Save dialog box, enter a name and location for the file, and click Save.

**NOTICE:** If you edit a saved experiment and want to save it without overwriting the original file, select File > Save Method File As and enter a new name for the experiment.

#### Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, click **Experiment > Templates > Quantitation > Experiment Name** (see Figure 7-65).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\Sartorius\Octet-BLIDiscovery\TemplateFiles.

**IMPORTANT:** Do not change the location of the Template folder. If the Template folder is not at the factory-set location, the software may not function properly.

Experiment	Instrument Window	/ Help					
<ul> <li>New Exp</li> </ul>	eriment Wizard	Ctrl+N					
Edit Ass	ay Parameters						
Edit Sen	sor Types						
Set Plate	Temperature						
Templat	es	•	Kinetics	- <b>F</b> _			
Skip Ste	n		Quantitation	+	Advanced Quantitation	- + <u> </u>	
Stop		1			Basic Quantitation	•	Anti-hIgG biosensor_16CH_96W.fmf
- Citch		_			Basic Quantitation with Regeneration	•	Anti-hIgG biosensor_8CH_96W.fmf
							Anti-mIgG biosensor_16CH_96W.fmf
							Anti-mIgG biosensor_8CH_96W.fmf
							Anti-Penta-HIS Dilution Factor Scouting_96W.fmf
							Anti-Penta-HIS Spike Recovery Assay_96W.fmf
							DirectDetectionImmunogenicity_16CH_384W.fmf
							DirectDetectionImmunogenicity_16CH_96W.fmf
							DirectDetectionImmunogenicity_8CH_96W.fmf
							Protein A biosensor_16CH_96W.fmf
							Protein A biosensor_8CH_96W.fmf
							Protein A or G biosensor_16CH_96W.fmf
							Protein A or G biosensor_8CH_96W.fmf
							Protein L biosensor_16CH_96W.fmf
							Protein L biosensor_8CH_96W.fmf

Figure 7-65: Experiments in the Template Folder

# Running a Quantitation Experiment

**IMPORTANT:** Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare the biosensors, see the appropriate biosensor product insert.

#### Loading the Biosensor Tray, Sample and Reagent Plates

To load the biosensor tray, sample plate, and reagent plate:

- 1. If the instrument door is closed, click the Present Stage icon ( 🔺 ) to present the instrument stage.
- 2. Place the biosensor tray, sample plate, and reagent plate on the appropriate stage so that well A1 is located at the upper right corner (see Figure 7-66):
  - a. Place the rehydration plate and biosensor tray on the biosensor stage (left platform).
  - b. Place the sample plate on the sample stage (middle platform).
  - c. Optional: Place the reagent plate on the reagent stage (right platform) if you are using a reagent plate.



Figure 7-66: Octet<sup>®</sup> Instrument Stage Platform

**IMPORTANT:** Ensure that the bottom of the sample plate, reagent plate, biosensor tray and rehydration plate are flat on each stage.

- 3. Click  $\blacktriangle$  to close the Octet<sup>®</sup> instrument door.
- 4. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

### Starting an Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow  $\rightarrow$  to access the Run Experiment window (see Figure 7-67).

ata File Location and Names			
Assay type:	Basic Quantitation Standard Assay	'	for to pressing "Go" confirm the Assay.
Quantitation data repository:	C:\data		
Experiment run name (sub directory):	Experiment_1	<b>→</b>	
Plate name/barcode (file prefix):	201102		
2nd Plate name/barcode:			
Auto-increment file ID start:	1	1	otal experiment time: 0:09:20
Data files will be stored as follows:			
C:\data\Experiment_1\201102_001.frd C:\data\Experiment_1\201102_002.frd C:\data\Experiment_1\201102_003.frd			
Run Settings			
Delayed experiment start	Open runtime charts automatically		
Start after (s): 600	Automatically save runtime cl	art	
Start after (s): 600	Automatically save runtime ch Set plate temperature (°C):	art	
Start after (s): [600 ] Shake sample plate while waiting Present stage at end of experiment	✓ Automatically save runtime ch ✓ Set plate temperature (°C): 3	art V	
Start after (s): 600 Shake sample plate while waiting Present stage at end of experiment dvanced Settings	Automatically save runtime cf     Set plate temperature (°C):	art T	
Start after (s): 600 Shake sample plate while waiting Present stage at end of experiment dvanced Settings jensor offset (mm): 3 ~	Automatically save runtime of Set plate temperature (°C):	ert	
Start after (s): 600 Start after (s): 600	Automatically save runtime of     Set plate temperature ("C):	ərt i	
Start after (s): 600 Start after (s): 600 Shake sample plate while waiting Present stage at end of experiment dvanced Settings Sensor offset (mm): 3 Warning: changing th If you are unsure of how to ieneral Information	Automatically save runtime of     Set plate temperature ("C):	efault	
Start after (s): 600	Automatically save runtime of     Set plate temperature ("C):      distance to sensor tip from bottom of well  distance to sensor tip from bottom of well  Machine name:  DESKTOP-0EHTC	art Default	

Figure 7-67: Run Experiment Window–Octet<sup>®</sup> RH16

2. Confirm the defaults or enter new settings. See "Run Experiment Window Settings" on page 270 for more information on experimental settings.

**NOTICE:** If you delay the experiment start, you have the option to shake the plate until the experiment starts.

3. To start the experiment, click **GO**.

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window appears with the binding data in real-time and the experiment progress (see Figure 7-68).

NOTICE: For more details about the Runtime Binding Chart, see "Managing Runtime Binding Charts" on page 273.



Figure 7-68: Runtime Binding Chart

4. Optional: Click View > Instrument Status to view the log file (see Figure 7-69).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



**WARNING:** Do not open the Octet<sup>®</sup> instrument door when an experiment is in progress. If the door is opened the data from the active acquisition step is lost. The data acquired in previous steps is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



**WARNING:** N'ouvrez pas la porte de l'instrument Octet<sup>®</sup> lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



**WARNING:** Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

Instrument Status			X
14:47:39 Sensor 7: Integ	ration Time = 1.0 ms		
14:47:39 Sensor 8: Integ	ration Time = 1.0 ms		
14:47:40 Picking sensors	completed location A1		
14:47:40 Plate temperatu	re = 30 C		
14:47:40 Ready to move	to sample location A1		
14:47:40 Moving to samp	le location A1		
014:47:41 Arrived at samp	le location A1		
0 14:47:41 Waiting to start	sample location A1		
14:47:41 Processing sam	ole location A1		
014:47:51 Sample complet	ed location A1		
-14:47:51 Waiting to start	new step		
-14:47:51 Starting new ste	p		
14:47:52 Ready to move	to sample location A2		=
0 14:47:52 Moving to samp	le location A2		-
14:47:53 Arrived at samp	le location A2		
14:47:53 Waiting to start	sample location A2		
14:47:53 Processing sam	ole location A2		
			9
Auto scroll to bottom		Save	to File

Figure 7-69: Instrument Status Log

### Run Experiment Window Settings

The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

ltem	Description
Assay type	The name of the selected assay.
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click <b>Browse</b> to select another data location.
	<b>NOTICE:</b> Save the data to the local machine first, then transfer to a network drive.
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software gener- ates one data file for each biosensor.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/bar- code	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

Table 7-8: Data File Location and Name

#### The following **Run Settings** are available on the **Run Experiment** Tab:

#### Table 7-9: Run Settings

Item	Description
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click <b>GO</b> .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the <b>Runtime Binding Chart</b> for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the <b>Runtime Binding Chart</b> . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in <b>File &gt; Options</b> . The factory set default temperature is 30 °C.
	<b>NOTICE:</b> If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet <sup>®</sup> BLI Discovery software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.

The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet<sup>®</sup> system per second and is reported in Hertz (per second).

- Higher acquistion rates generate more data points per second and monitor faster binding events bettern than slower acquisition rates.
- With a lower acquisition rate, the software can perform more averages of the collected data.

Typically, more averaging leads to reduced noise and better signal-to-noise ratios. Determine the frequency setting based on the binding rate, the amount of signal generated in the assay, and experimentation with the settings.

#### The following **Advanced Settings** are available for the Octet<sup>®</sup> RH96 system:

Table 7-10: Advanced Settings Octet<sup>®</sup> RH96

Item	Description
Acquisition rate	<b>NOTICE:</b> For the Octet <sup>®</sup> RH96 system, acquisition rate settings are available on the Plate Definition Tab.
	<ul> <li>High concentration quantitation (10 Hz, averaging by 5) — The average of 5 data frames is reported as one data point. 10 data points are reported per second.</li> </ul>
	<ul> <li>High sensitivity quantitation (2.0 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second.</li> </ul>
	<ul> <li>Standard quantitation (5.0 Hz, averaging by 20)—The average of 50 data frames is reported as one data point. Five data points are reported per second.</li> </ul>
Sensor off set (mm)	Recommended sensor offset: Quantitation—3 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

#### The following **Advanced Settings** are available for the Octet<sup>®</sup> QK384 system:

Table 7-11: Advanced Settings Octet<sup>®</sup> QK384

Item	Description
Acquisition rate	<ul> <li>High sensitivity quantitation (0.3 Hz, averaging by 40)—The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds.</li> </ul>
	<ul> <li>Standard quantitation (0.6 Hz, averaging by 5)—The average of 5 data frames is reported as one data point. One data point is reported every 1.6 seconds.</li> </ul>
Sensor off set (mm)	Recommended sensor offset: Quantitation—3 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

#### The following General Settings are available on the Run Experiment Tab:

#### Table 7-12: General Settings

Item	Description
Machine name	The computer name that controls the $Octet^{\circledast}$ instrument and acquires the data.
Username	The user logon name.
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

#### Stopping an Experiment

To stop an experiment in progress, click  $\bigotimes$  or click **Experiment** > **Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.

NOTICE: After the experiment is run, the software automatically saves the experiment method (.fmf).

## Managing Runtime Binding Charts

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 7-70). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active biosensor column is colorcoded (A=green, B=magenta, C=orange, D=purple, E=olive, F= black, G=red, H=blue) within the **Sensor Tray Map**. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each data acquisition step is represented by **Sample Column X** in the **Current Binding Charts** box.

To selectively display acquisition data for a particular acquisition step:

- 1. Click the corresponding **Sample Column** number.
- 2. Select a sub-set of sensors for a displayed column in the Sensors to Chart box (see Figure 7-70).

**IMPORTANT:** Do not close the Runtime Binding Chart window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet<sup>®</sup> BLI Discovery software saves the Runtime Binding Chart as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.



Figure 7-70: Runtime Binding Chart Window

#### Opening a Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

- 1. Click File > Open Experiment.
- 2. In the dialog box that appears, select an experiment folder and click **Select**.

### Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data during acquisition in the chart by clicking the **Subtract reference sensors** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the Sensor Assignment tab
- During acquisition in the Runtime Binding Chart Sensors to Chart box
- During analysis in the **Data Selection** tab

#### Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the Sensors to Chart list or the Sensor Tray, right-click a biosensor and select Reference (see Figure 7-71).



Figure 7-71: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an R in the Sensors to Chart list and Sensor Tray (see Figure 7-74).

2. Click the Subtract reference sensors check box (see Figure 7-74).



Figure 7-72: Subtract Reference Sensors check box in the Runtime Binding Chart

**NOTICE:** Subtracting reference data in the Runtime Binding Chart only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be re-done in data analysis if needed.

### Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 7-73). Uncheck the box to return to the default data display.



Figure 7-73: Data Inverted Using Flip Data Function

### Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the area on the chart that you want to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

#### Scaling a Runtime Binding Chart

#### To scale the Runtime Binding Chart:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, select Fullscale or Autoscale.

### Adding a Runtime Binding Chart Title

To add a Runtime Binding Chart title:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, enter a graph title or subtitle.

#### Selecting a Runtime Binding Chart Legend

To select a Runtime Binding Chart legend:

- 1. Right-click the chart and select Properties.
- 2. In the Runtime Graph Properties dialog box (see Figure 7-74), select one of the following legends:
  - Sensor Location
  - Sample ID
  - Sensor Information
  - Concentration/Dilution

Runtime Graph Properties							
Title:							
Subtitle:							
Legend		1					
<ul> <li>Sensor Location</li> </ul>	Sensor Information						
Sample ID	Concentration / Dilution						
		4					
	OK Cancel						

Figure 7-74: Selecting a Runtime Binding Chart Legend

**NOTICE:** Text for Sample ID, Sensor Information, or Concentration/Dilution is taken from the Plate Definition and Sensor Assignment tabs, and must be entered before the experiment is started.

3. Click OK.

### Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window** > **New Window**.

#### Exporting or Printing the Runtime Binding Chart

To export the Runtime Binding Chart as a graphic or data file:

- 1. Right-click the chart and select **Export Data**.
- 2. In the **Exporting** dialog box (see Figure 7-75), select the export options and click **Export**.

Export					
EMF	<b>WMF</b>	BMP	◎ JPG	O PNG	🔘 Text / Data
Export D	estination				
ClipBo	pard				
) File		Browse			
Printe	r				
Printe Export Si	r ze				
Printe Export Si	r ze	<ul> <li>Millimeter</li> </ul>	rs 🔘 Ir	nches 🔘 Poi	ints
Printe Export Si	r ze Width: 152.4	Millimeter     H00	rs O Ir 101.600	nches ⑦Poi	ints Export

Figure 7-75: Exporting Dialog Box

Table 7-13: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click <b>File &gt; Browse</b> to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Run- time Binding Chart to a graphic file		$\checkmark$	Click <b>File</b> > <b>Browse</b> to select a folder and enter a file name.	Creates a graphic image.
Copy the Runtime Binding Chart		$\checkmark$	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		$\checkmark$	Printer	Opens the Print dialog box.
# Managing Experiment Method Files

After you run an experiment, the Octet<sup>®</sup> BLI Discovery software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it for your needs.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 7-14: Managing Experiment Method Files

Menu Bar Command/Toolbar Button	Description
File > Open Method File	Enables you to select and open a method file (.fmf)
File > Save Method File 📩 or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

# Custom Quantitation Assays

### Defining a Custom Assay

To define a custom assay:

1. Click Experiment > Edit Assay Parameters.

The Edit Assay Parameters dialog box appears (see Figure 7-76).

Edit Assay Parameters		>	<					
Available Assays:								
Basic Quantitation	Name:	Standard Assay						
Anti-FLAG Quantitation	Description:	Basic Quantitation - Standard Assay (Read Only)	٦I					
Anti-GST -High sensitivity			-					
	Assay Faram	aers	_					
Anti-Human Fab-CH1 (FAB)	Single and	alyte 🔘 Multiple analyte						
		Replicates per sensor type:						
High sensitivity Human IgG quantitation								
Immunogenicity - Direct detection		Time (s): Shake speed (rpm):						
Munne IgG Quantitation	Quantitation	120 🔶 400 🚖						
Init Basic Quant Assay								
Basic Quantitation with Regeneration								
Anti-Human Fab-CH1 (FAB) with regeneration								
High sensitivity assay with regeneration								
Protein L -Standard range								
Standard Assay								
Advanced Quantitation								
Custom Quantitation								
Immunogencity - Enzyme Linked								
El Residual Protein A								
1 Three Step Assay								
inco ordy , and ,								
Gray indicates a built-in assay and cannot be modified or de	eleted.							
Duplicate		Save Cancel						

Figure 7-76: Edit Assay Parameters Dialog Box

- 2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
- 3. Click Duplicate.
- 4. In the **New Assay** dialog box (see Figure 7-77 top), enter an **Assay name**.
- 5. Optional: In the **Assay Description**, enter information about the assay.

#### 6. Click Save.

The new assay appears in the directory tree of available assays (see Figure 7-77 bottom).

Edit Assay Parameters				×					
Available Assays:									
Basic Quantitation	Name:	Standard Assay							
	Description:	Basic Quantitation							
	Assay Paran	neters							
	Single ar	Single analyte     O Multiple analyte     Realizates per expect type:							
Anti-Penta-HIS -High sensitivity		нерію	ates per sensor (ype:						
High sensitivity Human IgG quantitation									
Human IgG Quantitation									
	Quantitation	Time (s):	Shake speed (rpm):						
Protein L -Standard range									
Basic Quantitation with Regeneration									
Anti-Human Fab-CH1 (FAB) with regeneration	New As	isay		×					
High sensitivity assay with regeneration     Figure 1 - Standard range	Enter	Assay Information							
El Standard Assay	Assav	name: N	Ny Basic Quant Assay						
Advanced Quantitation									
	Assay	description:	nter a short description of the assay here.						
		L							
			OK Can	cel					
Gray indicates a built-in assay and cannot be modified or d	eleted.								
Duplicate Remove			Save	Cancel					
Edit Assay Parameters				×					
Available Assays									
Basic Quantitation		Name: My	Basic Quant Assay						
Anti-FLAG Quantitation		Description: Ent	er a short description of the assay here.						
		Assay Parameters							
Anti-HIS (HIS2) Quantitation		Single analyte	O Multiple analyte						
			Replicates per sensor type: 1						
Anti-Penta-HIS -Standard range									
	ation								
Immunogenicity - Direct detection			Time (s): Shake speed (rpm):						
		Quantitation:	120 🔶 400 🖨						
Basic Quant Assay									
Anti-Human Fab-CH1 (FAB) with re	generation								
High sensitivity assay with regener	ation								
Standard Assay									
Advanced Quantitation									
Residual Protein A									
Standard Assay									
Gray indicates a built-in assay and cannot b	e modified or del	eted.							
Duplicate Remove				Save Cancel					

Figure 7-77: Defining a New Assay

### Editing Assay Parameters

To edit assay parameters:

- 1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 7-77 bottom).
- 2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
- 3. Click Save to accept the new parameter values. The new assay is added to the system.

**NOTICE:** Not all parameters are available for all of the assays.

#### Basic Quantitation Assay Parameters

Edit Assay Parameters			×			
Available Assays:						
Basic Quantitation	Name:	My Basic Quant Assay				
Anti-FLAG Quantitation	Description:	Enter a short description of the assay here.				
	Accay Param	atare				
Anti-HIS (HIS2) Quantitation	Assay Faran	eters				
	Single an	alyte O Multiple analyte				
Anti-Penta-HIS -High sensitivity		Heplicates per sensor type:				
Anti-Penta-HIS -Standard range						
High sensitivity Human igo quantitation						
Immunogenicity - Direct detection		Time (a): Shake anead (mm):				
Murine IgG Quantitation	Quantitation					
	Guanacation					
Protein L -Standard range						
Basic Quantitation with Receneration						
Anti-Human Fab-CH1 (FAB) with regeneration						
Protein L -Standard range						
Immunogencity - Enzyme Linked						
Standard Assay						
Implain Three Step Assay						
Gray indicates a built-in assay and cannot be modified or de	leted.					
Duplicate Remove		Save	Cancel			

Figure 7-78: Assay Parameters-Basic Quantitation Assay

#### Table 7-15: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analy- sis.
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

#### Basic Quantitation with Regeneration Assay Parameters

Available Assays:           Image: Description         Name:         My Basic Quant with Regen Assay	
Description Name: My Basic Quant with Regen Assay	
Bit         Anti-FLAG Quantitation         Description:         Enter a short description of the assay here.	
EXAMPLAST I High sensitivity     EXAMPLAST I High sensitivity     Anonu Parameters	_
Atti-IIS (HIS2) Quantitation	
Anti-Human Fab-CH1 (FAB)     Single analyte Multiple analyte	
Heplicates per sensor type: 1	
The first standard range	
Bi High sensitivity Human IgG quantitation Imme (s). Snake speed upm).	
■E2 Murine loG Quantitation Regeneration: 5 ↓ 400 ↓	
₩y Basic Quant Assay Neutralization: 5 400	
Protein L -Standard range	
E Standard Assay Regeneration cycles:	
Basic Quantitation with Regeneration Between assay steps: 3	
Anti-Human Fab-CH1 (FAB) with regeneration     Pre-condition sensors     3     ✓	
El Protein L-Standard range	
Ear Forcen - Standard range	
Wy Basic Quant with Regen Assay	
Advanced Quantitation	
I Residual Protein A	
E Standard Assay	
····· <u>································</u>	
Gray indicates a built-in assay and cannot be modified or deleted.	
Duplicate Remove Save Cancel	

Figure 7-79: Assay Parameters-Basic Quantitation with Regeneration

 Table 7-16: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description					
Single analyte	For single-analyte experiments using only one biosensor type per sample well.					
Multiple analyte and Repli- cates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.					
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).					
	<b>NOTICE:</b> A subset of data points may be selected for processing during data analysis.					
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.					
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.					
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.					
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regener- ated state.					
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.					

#### Advanced Quantitation Assay Parameters

Use the Advanced Quantitation Assay Parameters to create a custom assay.

Edit Assay Parameters						×
Available Assays:						
Basic Quantitation	Name:	Custom Quan	titation			
	Description:					
Anti-GST -Standard range	Assay Param	eters				
Anti-HIS (HIS2) Quantitation     Anti-Human Fab-CH1 (FAB)     Anti-Human Fab-CH1 (FAB)     Anti-Penta-HIS -High sensitivity	Single ar	nalyte ⊖M F	<b>ultiple analy</b> eplicates p	<b>te</b> er sensor type:	1	
Anti-Penta-HIS -Standard range	Step Typ	e	Time (s)	Shake (rpm)	Step Options	Insert
High sensitivity Human IgG quantitation	Sample	e 🔹	120	1000	Online	Remove
Human igo Quantitation	Detect	ion	120	1000	Reuse position	Hemove
Standard Assay						Move Up
Basic Quantitation with Regeneration						Maura Daura
Anti-Human Fab-CHT (FAB) with regeneration	<					> Move Down
Fight sensitivity assay with regeneration	Reger	neration Time	(-).	Challen and	()-	
Standard Assay	Receptor	tion: 5	(S):	1000	(rpm):	
My Basic Quant with Regen Assay	negenera	uon. 5	<b>•</b>			
advanced Quantitation	Neutraliza	tion: 5	-	1000 🚔		
Immunogencity - Enzyme Linked				Regeneration	cycles:	
Residual Protein A	Betv	veen assay step	S:	3		
Standard Assay		ondition senso	re .	3 🔺		
Three Step Assay		onulion senso	2	J 🔻		
	Post-	condition sense	ors	3		
Gray indicates a built-in assay and cannot be modified or del	eted.					
Duplicate Remove					Save	Cancel

Figure 7-80: Assay Parameters-Advanced Quantitation

- 1. Select the type of Analyte.
  - Single analyte select to use one biosensor per sample well.
  - Multiple analytes select to use multiple biosensors per sample well.
    - Replicates per sensor type select the number of replicates for each sensor type.
- 2. Select the desired step options.
  - Insert click insert to add a step.
  - **Remove** select a step and then click **Remove** to remove a step.
  - Move Up select a step and then click Move Up to move a step up one row.
  - Move Down select a step and then click Move Down to move a step up one row.
- 3. Adjust the Time and Shake speed (rpm) of each step.
  - **Time** select the duration time of the step.
  - Shake speed select the shake speed in rpm for the step.
- 4. Regeneration Incubate the biosensor in the regeneration buffer to remove the bound analyte.
- 5. Neutralization Incubate the biosensor in the neutralization buffer after the regeneration step.

#### 6. Between assay steps

- Regeneration cycles select the number of cycles for a biosensor before reuse or storage.
- **Pre-condition sensors** Perform a set of regeneration or neutralization steps before the start of the experiment. These settings are like the time and rpm settings for the regeneration steps. For example, an acidic pre-conditioning buffer maximizes the binding competency of Protein A biosensors.
- Post-condition sensors Re-racked biosensors in a regenerated state for storage.
- 7. Step option Reagent wells can be reused.
  - Reuse Position define a single position for a reagent. This position is used for all assays in the experiment
  - Use x1 through Use x10 define the number of times the reagent in a position can be used. After the selected number of times is used, that position is no longer used in the experiment. You must define enough reagent positions in the plate to complete the experiment. For example, if the experiment has six assays:
    - You can define two reagent positions on the place and select **use x3**.
    - Or you can define three reagent positions on the plate and select **use x2**.
  - Distribute usage (auto) define multiple positions in the for the reagent. The software automatically distributes the assays, so the defined reagent positions are used equally. For example, if the experiment has six assays and there are two defined reagent positions, the software will use each position three times.

**NOTICE:** Preview the application of the Reuse Position setting to ensure your settings. Select the Review Experiment tab and step through the experiment.

# Selecting a Custom Assay

You can select a custom assay when you define a sample plate.

To select a custom assay:

1. In the **Plate Definition** tab, click **Modify** in the **Assay Settings** box.

The Edit Assay Parameters dialog box appears (see Figure 7-81).

ead Head: 16 cha	nnels V Plat	te 1 Tal	ble (384 wells)	ng /ml	Export	Import	Print	
equisition Rate: Standa	ard (5.0 Hz)	/ell	Sample ID Re	eplicate Gr		Conc (ua/mi	Dilution Factor In	
isay Settings isay: Basic Qua Standard / Single ana	ntitation Assay Iyte						]]	
Time (s): uantitation: 120	Assay Parameters							
	Available Assays:		Assay Para	meters				
1 2 3 4 5 6 7 8 9           1 2 3 4 5 6 7 8 9           0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Anti-FLAG Quantitation     Anti-GST -High sensitivity     Anti-GST -High sensitivity     Anti-GST -Standard range     Anti-His (HIS2) Quantitation     Anti-His (HIS2) Quantitation     Anti-Penta-HIS -High sensitivity     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     Anti-Penta-HIS -Standard range     My Basic Quantitation     By Protein L -Standard range     By Standard Assay		Quantitatio	Time ( n: 120	Replicates p	er sensor type: hake speed (rpm): 100	2 \$	16 channels

Figure 7-81: Selecting a Custom Assay

2. Select the custom assay from the directory tree and click **OK**.

# Multi-Step Advanced Quantitation Experiments

# Octet<sup>®</sup> RH16 and Octet QK<sup>®</sup> 384

The multi-step selection interface for Advanced Quantitation methods increases the flexibility to add more assay steps prior to the Sample or Detection steps. In addition, all steps in an Advanced Quantitation assay may be viewed and analyzed in the Octet<sup>®</sup> Analysis Studio software.

After starting the Octet<sup>®</sup> system and the Octet<sup>®</sup> BLI Discovery software, follow the steps below to set up and run an Advanced Quantitation experiment. You can start an Advanced Quantitation experiment using one of the following options:

- · Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Quantitation > Advanced Quantitation**.

These options are explained further in "Starting an Experiment Using the Experiment Wizard" on page 217.

**NOTICE:** The Sample plate and the Reagent plate are now referred to as "Plate 1" and "Plate 2" in the software.

1. To add or edit assay steps in Tab 1 (Plate Definition), click **Modify** in Assay Settings to display the Assay Parameters window. Click on the **Step Type** drop-down list or highlight the parameter you want to change:



Figure 7-82: Assay Parameters Window.

To add or remove steps, click the **Insert** or **Remove** buttons. Individual steps may be re-organized using the **Move Up** or **Move Down** buttons. Click **OK** to save any changes.

- Continue with the plate layout and sample well designation in Tab 1. For more details see "Defining the Sample Plate" on page 219, "Managing Sample Plate Definitions" on page 240 and "Managing Assay Parameter Settings" on page 245.
- 3. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with "Assigning Biosensors to Samples" on page 249 before running the Advanced Quantitation method.

# Octet<sup>®</sup> RH96

The Advanced Quantitation application combines the flexibility of the user-selectable Read Head with easier visualization of all the steps in a quantitation assay, including multiple steps preceding the Detection or Sample step. Users can configure the initial assay steps with a Read Head of 8, 16, 32, 48 or 96 biosensors, separately from the later detection steps. Analysis from 8 or 16 biosensors provides the greatest sensitivity and finer signal resolution whereas data acquisition from 32, 48 or 96 biosensors provides higher throughput.

Two new tabs, Sensor Loading and Plate Definition, provide individual control for preliminary assay steps, apart from the detection steps. An Advanced Quantitation Method file (\*.fmf) may contain assays with two different Read Head configurations. An example of this would be to immobilize 96 biosensors all at once, re-rack all 96 biosensors, and then analyze 16 biosensors at a time for the entire biosensor tray. Quantitation analysis will be performed on the default Detection step type, typically the last assay step in Plate Definition.

After starting the Octet<sup>®</sup> RH96 system and the Octet<sup>®</sup> BLI Discovery software, follow the steps below to set up and run an Advanced Quantitation experiment with user-selectable Read Head configurations. For information on how to connect the Octet<sup>®</sup> instrument to the computer and starting the software, please refer to Chapter 3, "Getting Started" on page 7.

You can start an Advanced Quantitation experiment using one of the following options:

- Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click Experiment > Templates > Quantitation > Advanced Quantitation.

These options are explained further in "Starting an Experiment Using the Experiment Wizard" on page 217.

- 1. Open Tab 1 (Sensor Loading) to configure the Read Head for the preliminary assay steps that will have a different setting from the later steps or Detection step. The default Read Head configuration is 96 channels which dips 96 biosensors simultaneously for the Sensor Loading steps.
- 2. Click on the drop-down list for Read Head to select 96, 48, 32, 16 or 8 channels as the new Read Head setting for all of these early assay steps (Figure 7-83).



Figure 7-83: Selecting a New Read Head Setting

3. To add or edit the Sensor Loading steps, click **Modify** in Sensor Loading Settings to bring up the Sensor Loading tab in the Assay Parameters window. Click on the drop-down list for Step Type or highlight the parameter you want to change. Click **OK** to complete the changes (Figure 7-84).

1 Senso	r Loading	2 Plate Definition	3 Sensor Assignment	4	Review Experi	ment 5	Run Experin	ment				
Ļ	In this ste First, cheo	p, all the information ab ok the assay settings.	oout the sensor loading re Then highlight one or more	agent e wells	positions will be s on the sample	e entered. plate, and	d right-click to	enter/m	odify wel	l data.		
Read He	ad:	48 channels (high th	roughput)	$\sim$	Plate 1 Tab	le (384 we	ells)					1
Acquisitio	on Rate:	Standard (0.6 Hz)		$\sim$	Concentral	ion units:	µg/ml	$\sim$	Expo	rt Import	Print	
- Sensor L	oading Set	tings			Well Sa	mple ID	Replicate (	Group	Туре	Conc (µg/ml)	Dilution Factor	Informa
Assay:	AS	dvanced Quantitation tandard Assay	Mod	ify								
	Assay Par	ameters				_						×
	Available	Assays:		S	ensor Loading	Assay Pa	arameters					
		Anti-GST -Standard ran Anti-HIS (HIS2) Quantit	ge 🔨									Re 48
		Anti-Human Fab-CHT (r Anti-Penta-HIS -High se	nsitivity		Step Type		Time (s	s) Sha	ke (rpm	1)		1
Plate 1	/	Anti-Penta-HIS -Standa High sensitivity Human	rd range IgG quantitation	Ī	Activation		120	400				Re
AOD		mmunogenicity - Direct	detection		(none)		1					
BOC	- <u>-</u>	Aurine IgG Quantitation	1		Sample							
D O C		My Basic Quant Assay Protein L -Standard ran	ae .		Load	N						
FOU		Standard Assay	-		Activati	on 😡						
GOG	Basic	Quantitation with Reg	eneration		Buffer X	[						Mc
100		high sensitivity assay w	ith regeneration		Buffer \	r						Mov
1 OC	- E I	My Basic Quant with Re	egen Assay		Quench	1						_
LOG		Protein L -Standard ran	ge									
NOC	Adva	Inced Quantitation										
000	E (	Custom Quantitation										
		mmunogencity - Enzym Residual Protein A	ie Linked									
		Standard Assay										
$\bigcirc$		Threa Stan Accou	×									
	•			- I I								
	Gray indica	ites a built-in assay.			ОК	0	Cancel					

Figure 7-84: Modifying Sensor Loading Parameters

4. Continue with the plate layout and sample well designation for the Sensor Loading steps.

**NOTICE:** All sample types such as Standards, Unknowns, Controls and References can now be loaded in either plate positions 1 or 2, or both.

- 5. Proceed to Tab 2 (Plate Definition) to configure the Read Head for the later steps or Detection step that will have a different setting from the preliminary Sensor Loading step(s). The default Read Head configuration will be the same setting previously selected in Tab 1 (Sensor Loading).
- 6. Click on the drop-down list for Read Head to select 96, 48, 32, 16 or 8 channels as the new Read Head setting for all of these later assay steps:

Sensor Loading	2 Plate Definition 3 Sensor Assignment 4 Review Experiment 5 Run Experiment
In this s First, ch	tep, all the information about the sample plate and its wells will be entered. leck the assay settings. Then highlight one or more wells on the sample plate, and right-click to enter the sample plate.
Read Head:	48 channels (high throughput) V Plate 1 Table (384 wells)
Acquisition Rate:	96 channels (high throughput) 48 channels (high throughput) 20 channels (high throughput) Well Sample ID Replicate Gro
Assay Settings — Assay:	Ad B channels (high sensitivity)
Sample Detection	Single analyte Time (s): Shake speed (rpm): 120 1000 120 1000

Figure 7-85: Selecting a New Read Head Setting

7. To add or edit the later steps or detection step, click **Modify** in Assay Settings to bring up the Assay Parameters tab in the Assay Parameters window. Click on the drop-down list for Step Type or highlight the parameter you want to change. Click **OK** to complete the changes:



Figure 7-86: Modifying Assay Parameters

**NOTICE:** Quantitation analysis will be performed on the default Detection step type, typically the last assay step in Plate Definition.

- 8. Continue with the plate layout and sample well designation for the Plate Definition assay steps. For more details see "Defining the Sample Plate" on page 219, "Managing Sample Plate Definitions" on page 240 and "Managing Assay Parameter Settings" on page 245.
- 9. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with "Assigning Biosensors to Samples" on page 249 before running the Advanced Quantitation method.

### Chapter 8:

# Kinetics Experiments: Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, and Octet<sup>®</sup> K2

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# Introduction

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet<sup>®</sup> system hardware and the Octet<sup>®</sup> BLI Discovery software, follow the steps (in Table 8-1) to set up and analyze a kinetics experiment.

**NOTICE:** Use the kinetics templates in the experiment wizard rather than creating a custom experiment to avoid acquiring data that cannot be analyzed by the Octet<sup>®</sup> Analysis Studio Software.

Software		Step	See
Octet <sup>®</sup> BLI Discovery	1.	Select a kinetics experiment in the <b>Experiment Wizard</b> or open a method file (.fmf).	"Starting a Basic Kinetics Experiment" on page 297
	2.	Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 298
	3.	Specify assay steps.	"Defining a Kinetic Assay" on page 313
	4.	Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 326
	5.	Run the experiment.	"Running a Kinetics Experiment" on page 334
Octet <sup>®</sup> Analysis Studio	6.	View and process the raw data.	Octet <sup>®</sup> Analysis Studio Software User Guide
Rel I	7.	Analyze the data.	

Table 8-1: Setting Up and Analyzing a Kinetic Experiment

**NOTICE:** Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet<sup>®</sup> BLI Discovery software is closed, the plate temperature will reset to the default startup value specified in the Options window when the software is relaunched.

# Starting a Basic Kinetics Experiment

**IMPORTANT:** Using 96-well half-area plates on the Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2 system will result in nonoptimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

You can start a kinetics experiment using one of the following options:

- · Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking File > Open Method File. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run. For more details on method files see "Managing Experiment Method Files" on page 346.
- On the menu bar, click Experiment > Templates > Kinetics.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

#### Starting an Experiment Using the Experiment Wizard

- 1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button (, or click **Experiment > New Experiment Wizard** (**Ctrl+N**) from the **Main Menu**.
- 2. In the Experiment Wizard, click New Kinetics Experiment (Figure 8-1, left).

#### NOTICE:

Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2 method templates are not compatible with other Octet<sup>®</sup> instruments. Use the kinetics templates in the experiment wizard rather than creating a custom experiment to avoid acquiring data that cannot be analyzed by the Octet<sup>®</sup> Analysis Studio Software.

- 3. All available Kinetics templates for your system are displayed, the options are:
  - Click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.
  - · Select a template.
  - If none of the templates are suitable for your experiment, select **Blank Experiment** to create a custom one.
- 4. Click the arrow button ( $\rightarrow$ ). The Basic Kinetics Experiment window appears (Figure 8-1, right).

s Experiment Wizard		- • •	]		
choose an option to stat     Choose an option to stat     Basic Quantitation Experiment     Basic Quantitation     Basic Quantitation     Advanced Quantitation     Advanced Quantitation     New Kinetics Experiment	Available Templates for - Octet R8 Bomleoude kinetics - AHC biosen Bomleoude kinetics - AHC biosen Bomleoude kinetics - AHC biosen Bomleoude kinetics - SAB biosens Bomleoude kinetics - SA biosens Bomleoude kinetics - SA biosens Description - Small Molecule and Fragment Kine	sor isor or tics - SSA biosensor			
Recent Methods	Image: Basic Kine           Image: Plane De           Image: Plane De	tics Experiment finition 2 Assay Definition 3 Se this step, all the information about the is foljabit on ce or more wells on the sample	nsor Assignment 4 F sample plate and its wells e plate, and right-click to	Review Experiment <b>5</b> Run Experime s will be entered. enter/modify well data.	ent
	Plate 1 (95	2     3     4     5     6     7     8     9       2     3     4     5     6     7     8     9       2     3     4     5     6     7     8     9       2     3     4     5     6     7     8     9       2     3     4     5     6     7     8     9       3     4     5     6     7     8     9       3     4     5     6     7     8     9       3     4     5     6     7     8     9       4     6     6     6     7     8     9       4     6     6     6     7     6     9       4     6     6     7     6     7     6       6     6     6     6     6     6     6       6     6     6     6     6     6     6       6     6     6     6     6     6     6       6     6     6     6     6     6     6       6     6     6     6     6     6     6       6     6     6 <th>10 11 12</th> <th>Plate 1 Table</th> <th>Concentration ( Molar concentr up Type Conc (µg/ml)</th>	10 11 12	Plate 1 Table	Concentration ( Molar concentr up Type Conc (µg/ml)

Figure 8-1: Starting a Kinetics Experiment with the Experiment Wizard

# Defining the Sample Plate

To define a sample plate do the following:

	Step	See Page
1.	Designate the samples.	299
2.	Save the sample plate definition (optional).	310

### **Designating Samples**

**NOTICE:** It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 8-2 will be included in the assay.

Table 8-2 displays the well types that can be assigned to a plate map.

Table	8-2:	Tvpes	of Sa	elam	Wells
10010	·	19000	0100	inpic	110113

lcon	Description
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for sys- tem drift.
Controls	<ul> <li>A control sample, either positive or negative, of known analyte composition.</li> <li>Positive Control: A control sample that contains analyte of known concentration</li> <li>Negative Control: A control sample known not to contain analyte</li> </ul>
₿Buffer	Any type of buffer. For example, the buffer in a baseline, or dissociation step.
(A) Activation	Activation reagent. Makes the biosensor competent for binding.
(Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
(Load	Ligand to be immobilized (loaded) on the biosensor surface.
🛞 Wash	Wash buffer.
Regeneration	Regeneration reagents dissociate the analyte from the ligand.
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

### Selecting Wells in the Sample Plate Map

**NOTICE:** For the Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2 system, wells in sample plate are restricted to rows AB, CD, EF and GH. Sample wells cannot be designated in row pairs BC, DE and FG.

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag. To select non-adjacent columns, hold the **Ctrl** key and click the column header (Figure 8-2 left).
- Click a row header or select adjacent row headers by click-hold-drag (Figure 8-2, center).
- Click a well or draw a box around a group of wells (Figure 8-2, right).



Figure 8-2: Selecting Wells in the Sample Plate Map

**NOTICE:** Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

# Designating Well Types

In the Sample Plate Map, select the wells, right-click and select a sample type (see Figure 8-25).



Figure 8-3: Designating a Well Type in the Plate Definition Window

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 8-4).



Figure 8-4: Clearing Sample Data from a Sample Plate

### Entering Sample Information

**NOTICE:** You must specify sample (analyte) concentration and molecular weight, otherwise the Octet<sup>®</sup> BLI Discovery software cannot compute a K<sub>D</sub> value. If the sample concentration is not specified, only k<sub>d</sub> and k<sub>obs</sub> are calculated. You can also annotate any well with Sample ID or Well Information, and assign Replicate Groups.

Assigning Molecular Weight and Molar Concentration

- 1. In the Sample Plate Map, select the sample wells, right-click and select Set Well Data.
- 2. In the **Set Well Data** dialog box, enter the analyte molecular and molar concentration (Figure 8-5).

Set Well Data	×
Well Information Sample ID:	Dilution Series  Apply to:  Concentration
Replicate Group:	Molar Concentration Starting value (µg/ml):
Well Information:	Series operator: / ✓ Series operand: 2
	Dilution orientation
Well Data - Sample only Molecular Weight (kD): 150	Down Up
Molar Concentration (nM):     66.67       Concentration (µg/ml):     1	OK Carol
	UK Cancel

Figure 8-5: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

The information displays in the Sample Plate Table (see Figure 8-6).

<i>,</i>					Conce	entration units:	µg/ml	
					Molar	concentration units:	nM	,
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information	Τ
🕑 F3			Buffer					
🖻 G3			Buffer					
B H3			Buffer					
🔵 A4			Sample		150	66.67		
<b>B</b> 4			Sample		150	33.33		
🔵 C4			Sample		150	16.67		
🔵 D4			Sample		150	8.333		
🔵 E4			Sample		150	4.167		
🔵 F4			Reference					
🔵 G4			Reference					
🔵 H4			Reference					ſ
🔵 A5			Sample		150	66.67		
<b>B</b> 5			Sample		150	33.33		
🔵 C5			Sample		150	16.67		
D5			Sample		150	8.333		
🔵 E5			Sample		150	4.167		
🔵 F5			Reference					
🔵 G5			Reference					
🔵 H5			Reference					
🔵 A6			Sample		150	66.67		
<b>B</b> 6			Sample		150	33.33		
🔵 C6			Sample		150	16.67		
🔵 D6			Sample		150	8.333		
<b>)</b> E6			Sample		150	4.167		
<b>F</b> 6			Reference					1

#### 3. In the **Sample Plate Table**, select the sample concentration units and the molar concentration units.

Figure 8-6: Entering Molecular Weight and Molar Concentration from the Plate Table

#### Assigning User-Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. In the Sample Plate Map, select the desired wells, right-click and select Set Well Data.

**NOTICE:** A range of wells can be selected clicking and dragging, holding the Shift key and using the arrow keys to select sections of the plate, or holding the Ctrl key to select specific wells.

The Set Well Data dialog box appears (see Figure 8-7).

2. Select the **By value** option and enter the starting concentration value. If a range of cells was selected, all cells will update with the specified value.

Set Well Data			×
Well Information		Dilution Series	
			Molar Concentration
Replicate Group:		Starting value (µg/ml):	1
Well Information:		Series operator:	/ ~
		Series operand:	2
		Dilution orientation	
		●● ● ●	
Well Data - Sample only		Down	
Molecular Weight (KD).	150		
Molar Concentration (nM):	66.67		
Concentration (µg/ml):	1	C	OK Cancel

Figure 8-7: Sample Plate Map-Assigning Sample Concentrations by Value

3. Click OK. The Sample Plate Table will display the entered concentration.

#### Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

- In the Sample Plate Map, select the wells, right-click, and select Set Well Data. The Set Well Data dialog box appears (see Figure 8-8)
- 2. Select the **Dilution Series** option and enter the starting concentration value.

Well Information	Dilution Series	
Sample ID:	Apply to:	Concentration
		Molar Concentration
Replicate Group:	Starting value (µg/	(ml): 10
Well Information:	Series operator:	/ ~
	Series operand:	2
	Dilution orientation	n
		ht 😪 😂 🔿 Left
Well Data - Sample only	Dov	wn 🕺 O Up
Molecular Weight (kD): 150		
Molar Concentration (nM):		
Concentration (ug/ml):		

Figure 8-8: Sample Plate Map-Assigning Sample Concentrations Using Dilution Series

3. Select a series operator, enter an series operand, and select the appropriate dilution orientation (see Figure 8-9).



Figure 8-9: Concentration Representation in Dilution Series

#### 4. Click OK.

The Sample Plate Table displays the standard concentrations.

#### Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

#### Annotating Wells in the Sample Plate Map

To annotate one or more wells:

- 1. In the Sample Plate Map, select the samples to annotate, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 8-10), enter the Sample ID and/or Well Information and click OK.

Well Information		Dilution Series	[
Sample ID:	$\checkmark$	Apply to:	Concentration
Analyte 1			O Molar Concentration
Replicate Group:		Starting value (µg/m	l): 10
M-II I-fti		Series operator:	/ ~
1x Kinetics Buffer		Series operand:	2
		Dilution orientation	
		Right	Cleft
Well Data - Sample only		Down	
Molecular Weight (kD): 150		ōō	ěě
Molar Concentration (nM):			
Concentration (ug/ml):			

Figure 8-10: Add Sample Annotations from the Sample Plate Map

#### Annotating Wells in the Sample Plate Table

To annotate an individual well in the Sample Plate Table:

- 1. Double-click the table cell for **Sample ID** or **Well Information**.
- 2. Enter the desired information in the respective field (see Figure 8-11).

NOTICE: A series of Sample IDs may also be assembled in Excel and pasted into the Sample Plate Table.

sample	Plate Lable					Concentrat	ion units:	µg/ml 🛛 🔻
						Molar conc	entration units:	nM 🔻
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information	
📵 G3	Dissociation		Buffer				1X Kinetics Buffer	
B H3	Dissociation		Buffer				1X Kinetics Buffer	
🔵 A4	Association		Sample	10	150	66.67	1X Kinetics Buffer	
🔵 В4	Association		Sample	5	150	33.33	1X Kinetics Buffer	
🔘 C4	Association		Sample	2.5	150	16.67	1X Kinetics Buffer	
🔵 D4	Association		Sample	1.25	150	8.333	1X Kinetics Buffer	
🔵 E4	Association		Sample	0.625	150	4.167	1X Kinetics Buffer	
🔵 F4	Association		Reference				1X Kinetics Buffer	
🔵 G4	Association		Reference				1X Kinetics Buffer	
🔵 H4	Association		Reference				1X Kinetics Buffer	

Figure 8-11: Add Sample Annotations in the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

### Replicate Groups

Replicate Groups enable data to be organized into custom groups during data analysis (see Figure 8-12).

Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.	
20	x		C2	SA (Streptavidin)		3	C3	
21	x		C2	SA (Streptavidin)		3	C3	
22	x		D2	SA (Streptavidin)		4	D3	
23	x		D2	SA (Streptavidin)		4	D3	
24	x		E2	SA (Streptavidin)		5	E3	
25	x		E2	SA (Streptavidin)		5	E3	
26	x		F2	SA (Streptavidin)		6	F3	
27	x		F2	SA (Streptavidin)		6	F3	
28	x		G2	SA (Streptavidin)		6	G3	
29	x		G2	SA (Streptavidin)		6	G3	
30	x		H2	SA (Streptavidin)		6	H3	
31	x		H2	SA (Streptavidin)		6	H3	Ξ
32	x		A3	SA (Streptavidin)		1	A3	
33	x		A3	SA (Streptavidin)		1	A3	
34	x		B3	SA (Streptavidin)		2	B3	
35	x		B3	SA (Streptavidin)		2	B3	
36	x		C3	SA (Streptavidin)		3	C3	
37	x		C3	SA (Streptavidin)		3	C3	
38	x		D3	SA (Streptavidin)		4	D3	
20	v		אח	SA (Strentsvidin)		4	D3	Ψ.
•	11						Þ	

Figure 8-12: Replicate Group Color-Coding

NOTICE: Replicate Group information can also be entered in the software.

Assigning Replicate Groups in the Sample Plate Map

#### To assign **Replicate Groups** in the **Sample Plate Map**:

- 1. Select the samples you wish to group, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 8-13), enter a name in the Replicate Group box and click OK.

Well Information	Dilution Serie	s	
Sample ID:	Apply to:	Cond	centration
Analyte 1		🔘 Mola	r Concentration
Replicate Group:	Starting va	lue (μg/ml):	10
1	Series ope	rator:	/ ~
1x Kinetics Buffer	Series ope	rand:	2
	Dilution or	ientation	
		Right 88	Se OLeft
Well Data - Sample only	••	Down	OUp
Molecular Weight (kD): 150		ě	ě.
Molar Concentration (nM):			
Concentration (ug/ml):			

Figure 8-13: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 8-14).



Figure 8-14: Replicate Group in the Sample Plate Map

The Sample Plate Table will update with the Replicate Group names entered (see Figure 8-15)

	Concentration units:						μg/ml
					M	olar concentration un	its: nM
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
<b>)</b> A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
<b>0</b> B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
🔵 C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
🔵 D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
🔵 E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
🔵 F4	Association	6	Reference				1X Kinetics Buffer
🔵 G4	Association	6	Reference				1X Kinetics Buffer
🔵 H4	Association	6	Reference				1X Kinetics Buffer
🔿 A5	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
🔵 B5	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
🔿 C5	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
🔿 D5	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
🔵 E5	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
🔵 F5	Association	6	Reference				1X Kinetics Buffer
🔵 G5	Association	6	Reference				1X Kinetics Buffer
🔵 H5	Association	6	Reference				1X Kinetics Buffer

Figure 8-15: Replicate Groups in Sample Plate Table

#### Assigning Replicate Groups in the Sample Plate Table

#### To assign Replicate Groups in the Sample Plate Table:

- 1. Double-click the desired cell in the **Replicate Group** table column.
- 2. Enter a group name (see Figure 8-16).

Sample	Plate Table				Co	oncentration units:	μg/ml	•
					М	olar concentration un	its: nM	•
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information	
🔿 A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer	
🔵 B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer	
🔵 C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer	
🔵 D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer	
🔵 E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer	
🔵 F4	Association	6	Reference				1X Kinetics Buffer	
🔵 G4	Association	6	Reference				1X Kinetics Buffer	
🔴 H4	Association	6	Reference				1X Kinetics Buffer	

Figure 8-16: Add Replicate Group from the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

# Editing the Sample Table

#### Changing Sample Well Designations

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection (see Figure 8-17).

o ampio	, late i dbio							Concentration units:	µg/ml	•
				Sample				Molar concentration units:	nM	•
₩ell	Sample ID	Replicat	ŏ	Reference	.D)	Molar Conc (nM)	Information			
B A1			~	Control						
B B1			0	Neesting Central						
B C1			0	Negative Control						
B D1			C	Positive Control						
B E1			₿	Buffer						
B F1			(8)	Activation						
B G1			0	Quench						
B H1				Load						
L A2			W	Wash						
🛈 B2			R	Regeneration						
L C2			N	Neutralization						
L D2			0		_					
L E2				Set Well Data						
(L) F2				Clear Data						
L G2				Convito Clinhoard						
L H2				Estanded Complete						
O A3			V	Extended sample Types						
🔵 ВЗ				Sample						
C 1 0				Cample						

Figure 8-17: Sample Plate Table–Well Designation

#### Editing Sample Information

To edit sample data in the Sample Plate Table, double-click a value and enter a new value (see Figure 8-18).

					C	Concentration units:	μg/ml	•	
					M	Iolar concentration un	its: nM	•	
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information		
B) H3	Dissociation		Buffer				1X Kinetics Buffer		
) A4	Association	1	Sample	10	150	66.67	1Y Kinotice Buffor	-	
<b>)</b> B4	Association	2	Sample	5	150	Undo			
) C4	Association	3	Sample	2.5	150	Cut			
D4 🜔	Association	4	Sample	1.25	150	Сору			
) E4	Association	5	Sample	0.625	150				
🕽 F4	Association	6	Reference			Paste			
<b>G</b> 4	Association	6	Reference			Delete			
H4	Association	6	Reference			5 I III			
) A5	Association	1	Sample	10	150	Select All			
) B5	Association	2	Sample	5	150	Right to left Readir	na order		
) C5	Association	3	Sample	2.5	150	Chow Unicode control character			
D5 🜔	Association	4	Sample	1.25	150				
) E5	Association 5 Samp		Sample	0.625	150	Insert Unicode con	troi character		
<b>F</b> 5	Association	6	Reference			Open IMF			
G5	Association	6	Reference						

Figure 8-18: Sample Plate Table-Editing Sample Data

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the right-click menu used to designate sample types.

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# Managing Sample Plate Definitions

NOTICE: After you define a sample plate, you can export and save the plate definition for future use.

### Exporting a Plate Definition

To export a plate definition:

1. In the Sample Plate Map, click Export (see Figure 8-19).



Figure 8-19: Sample Plate Map-Export Button

2. In the **Export Plate Definition** window (see Figure 8-20), select a folder, enter a name for the plate (.csv), and click **Save**.



Figure 8-20: Export Plate Definition Window

### Importing a Plate Definition

To import a plate definition:

1. In the Sample Plate Definition window (see Figure 8-19: on page 310), click Import.



Figure 8-21: Sample Plate Map- Import Button

2. In the Import Plate Definition window (see Figure 8-22), select the plate definition (.csv), and click Open.



Figure 8-22: Import Plate Definition Window

**NOTICE:** You can also create a .csv file for import. Figure 8-23 shows the appropriate column information layout.

	Α	В	С	D	E	F	G	Н	
1	PlateWells	96							
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information	=
3	A1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
4	B1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
5	C1	Kinetics Buffer		Buffer				<b>1X</b> Kinetics Buffer	
6	D1	Kinetics Buffer		Buffer				<b>1X</b> Kinetics Buffer	
7	E1	Kinetics Buffer		Buffer				<b>1X</b> Kinetics Buffer	
8	F1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
9	G1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
10	H1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
11	A2	Loading		Load				12.5 ug/ml ProA	
12	B2	Loading		Load				12.5 ug/ml ProA	Ŧ
14 -	i ▶ ₩ 96 s	tandard plate ⁄ 🖅							1

Figure 8-23: Example Plate Definition File (.csv)

### Printing a Sample Plate Definition

To print a plate definition:

1. In the Sample Plate Map (see Figure 8-24), click Print.



Figure 8-24: Sample Plate Print Button

# Defining a Kinetic Assay

After you define the sample plate, you must define the assay.

To define a kinetic assay, do the following steps.

# Defining Step Types

	Step	See Page
1.	Define the step types.	313
2.	Build the assay by assigning a step type to a column(s) in the sample plate.	317
3.	Save the sample plate definition (optional).	310

Table Figure 8-3 lists an example of the step types used to define a kinetic assay. Use these examples as starting point for creating your steps.

#### Table 8-3: Sample Step Types for Kinetic Assays

Step Type	Step Description
Association	Calculates the $k_{ m obs}$ or the $k_{ m a}$ . Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the <i>k</i> <sub>d</sub> . Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the base- line step directly precedes an association step, perform the baseline step at 1,000 rpm.
	<b>IMPORTANT:</b> A kinetics assay must include a baseline step followed by a set of association/ dissociation steps to be analyzed. The software recognizes the baseline/association/dissocia- tion step series during processing. Data cannot be processed if this sequence is not included in the assay setup.
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor.
	<b>NOTICE:</b> This step may be performed offline (outside the Octet $^{ extsf{B}}$ instrument).
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.
Regeneration	Used when employing a reagent to chemically regenerate biosensors and remove bound ana- lyte.
Custom	Can be used for an activity not included in any of the above step types.

#### Creating Step Types

Click the **Assay Definition** tab, or click the  $\rightarrow$  arrow to access the Assay Definition window (see Figure 8-25). The **Step Data List** has the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay steps:

- 1. Click Add.
- 2. In the **Assay Step Definition** dialog box (Figure 8-25), multiple assay steps can be added at the same time. For each step, specify the step information:
  - a. Choose a step type.
  - b. Set the step time and shake speed
    - Time range: 2 to 48,000 seconds
    - Shake speed: Off 0 rpm or On range: 100 to 1,500 rpm.
- 3. The step name can be edited after it has been added to the Step Data List table. Edit the step name by double clicking the table cell.

I Plate Definition         2 Assa           In this step, the ass Select a group of set	ay Definition <b>3</b> Sensor Assign ay steps will be assembled from the ensors and append the currently s	ment <b>4</b> ne Step Da selected st	Review Experim ata List. ep into the curren	ent <b>5</b> Ru tassaywith a	un Experimen a double clicł	t <, or right c	lick for n	nore opt	tions.		
Plate 1 (96 wells)			-Sten Data List -					Tim	ie in (s), Shak	e speed	in (rpm)
			Add	Сору	Remove	Re	generatio	on Para	ms Thre	shold Pa	arams
		12	Name	Time Sha	ake speed	Туре		Thres	hold		
			→ Baseline	600 100	0	🛌 Baseli	ne				
		Add Ste	p Definitions							×	
				Name			Time (s)	)	Shake speed	(rpm)	
EBLBO			Association	Associa	ition		600	•	1000 🗘		
FBLBO			Dissociation	Dissoci	ation		600	▲ ▼	1000 🖨		
GBLBO			Baseline	Baselin	e		600	<b>•</b>	1000 🖨		Table
HBLBO			Loading	Loading	)		600		1000 🖨		mment
Assayed samples	O Unassigned samples		Activation	Activati	on		600	-	1000 🖨		
			Quenching	Quench	ning		600	-	1000 🖨		
		🗆 🕏	Regeneration	Regene	eration		30	* *	1000 🖨		
		□羣	Custom	Custom			600		1000 \$		
			Dip	Dip			600	-	1000		
				(	ОК	Cano	el		Defa	ults	

Figure 8-25: Creating an Assay Step Type

- 4. Apply a threshold to the step:
  - a. In the Step Data List, click the Threshold check box.

The Threshold Parameters dialog box appears (see Figure 8-26).

b. Set the threshold parameters (refer to Table 8-4 for the parameter definitions).
1 Plate Definition         2 Assay Definition         3 Se           In this step, the assay steps will be asser Select a group of sensors and append th	nsor Assignment 4 Review Experiment 5 nbled from the Step Data List. he currently selected step into the current assay with	Run Experiment
Plate 1 (96 wells)	Step Data List Add Copy Threshold Parameters Assay steps designated as "Threshold" will term criteria is reached. Active Channels: Set All Clear All Channel 1 Channel 2 The step is terminated when: (a) the threshold is achieved on ALL channels (b) the threshold is achieved on ANY ONE char OK Cancel	Time in (s), Shake speed in (pm)         Remove       Regeneration Params       Threshold Params         Image: Threshold Params       Image: Threshold Params       Image: Threshold Params         Image: Threshold remove and the step time elapses or the threshold termination       Image: Threshold (mm):       1.00         Image: Threshold (mm):       1.00       Image: Threshold (mm):       1.00         The threshold is achieved when:       Image: Threshold (mm):       1.00         Image: Threshold (mm):       1.00       Image: Threshold (mm):       1.00         Image: Threshold (mm):       1.00       Image: Threshold (mm):       Image: Threshold

Figure 8-26: Setting Assay Step Threshold Parameters

**NOTICE:** If thresholds are applied, the step is terminated when either the step time elapses or the threshold termination criteria is reached.

Table 8-4	: Threshold	Parameters
-----------	-------------	------------

ltem	Description
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step:
	The threshold is achieved on ALL channels
	The threshold is achieved on ANY ONE channel
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

5. Click **OK** to save the newly-defined step. The new step type appears in the **Step Data List**.

6. Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 8-27).

-Step I	Data List				
A	dd Copy		Remove	generation Params	Threshold Params
	Name	Time	Shake speed	Туре	Threshold
	Baseline	10	1000	🛌 Baseline	
	Loading	20	1000	🖌 Loading	
	Wash	15	1000	藉 Custom	
	Association	30	1000	🞽 Association	
	Long Dissociation	2000	1000	L Dissociation	
•	Regeneration	24	1000	💈 Regeneration	
	Activation	25	1000	🛧 Activation	

Figure 8-27: Step Data List with Step Types

7. To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

#### Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the **Step Data List**.

To define a step type by editing an existing one:

1. Double-click the cell in the step's **Name, Time** or **Shake speed** column and then enter a new value. Or, right-click the cell to display a shortcut menu of editing commands (see Figure 8-28, left).

**NOTICE:** Keyboard commands can also be used (Ctrl+x=cut, Ctrl+c=copy, Ctrl+v=paste, Ctrl+z=undo).

2. Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 8-28, right)



Figure 8-28: Editing a Step Value (left) or Step Type (right)

-

### Building an Assay

After creating the different step types that the assay will use, step types are assigned to sets of wells in the Sample Plate or Reagent Plate maps.

To build an assay:

- 1. Select a step type in the **Step Data List**.
- 2. In the **Sample Plate Map**, double-click the set of wells associated with the selected step type. For information about sample plate wells, mouse over a well to view a tool tip (see Figure 8-29).



Figure 8-29: Tool Tip of Well Information

The selected wells are marked with hatching (for example, 🍘) and the step appears in the Assay Steps List (see Figure 8-30) with an associated Assay Time.

1 Plate Definition 2 Assay Definition	3 Sensor Assignment	<b>4</b> Re	view Experiment	5 6	Run Experiment			
In this step, the assay steps will b Select a group of sensors and ap	be assembled from the Step opend the currently selected	Data l distepii	ist. nto the current as	say with	a double click, o	r right click for more	options.	
Plate 1 (96 wells)		St	ep Data List				Time in (s), Sha	ke speed in (rpm)
	0 0 40 44 42		Add	Сору	Remove	Regeneration P	arams Th	reshold Params
			Name	Time	Shake speed	Туре	Threshold	
		E	Baseline	60	1000	🛌 Baseline		
ROCOOO			Association	600	1000	🞽 Association		
			Dissociation	600	1000	L Dissociation		
			Loading	600	1000	🖌 Loading		
EÖÖÖÖÖÖÖ	ŏŏŏŏŏ		Baseline2	600	1000	🛌 Baseline		
FÕÕÕÕÕÕÕ	ÕÕÕÕÕ							
GOOOOOO	00000	A	say Steps List					
HOOOOOO	ăăăăăă		New Assay Mo	ove Up	Move Down	Remove Repli	icate Edit Si	tep Info Table
	00000	1	Assay No. Sar	nple S	Step Name Ste	ep Type Sensor	Type A	ssay Time Cor
Assayed samples	nassigned samples	1	1 A1	E	laseline 🔹 🛌	Baseline SA (Strep	tavidin) 👻 🕻	):01:30

Figure 8-30: Assigning a Step Type to a Column in the Sample Plate

3. Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 8-32).

NOTICE:

For Octet<sup>®</sup> R2, or Octet<sup>®</sup> K2: All assay steps, within an assay or in a different assay, are restricted within row pairs AB, CD, EF and GH. Steps within an assay are restricted to the same row pair. If the selected step is outside the row, then it will be added as a new assay (see Figure 8-31).

For Octet<sup>®</sup> R4: All assay steps, within an assay or in a different assay, are restricted within row quadrants ABCD or EFGH. Steps within an assay are restricted to the same quadrant. If the selected step is outside the row, then it will be added as a new assay (see Figure 8-31).



Figure 8-31: Adding a Step Outside a Pair Adds it as a New Assay for Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2;

New A:	ssay Mo	ove Up Move Down	Remove Re	eplicate Edit !	itep	
Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time	
1	1	Baseline	🛌 Baseline	SA (Streptavidin)		
1	2	Loading	🖌 Loading	SA (Streptavidin)		
1	7	Wash	🗱 Custom	SA (Streptavidin)		
1	3	Association	🞽 Association	SA (Streptavidin)		
1	8	Long Dissociation	📐 Dissociation	SA (Streptavidin)		
1	10	Regeneration	💈 Regeneration	SA (Streptavidin)	0:35:23 🔪	
2	1	Baseline	🔙 Baseline	SA (Streptavidin)		
2	2	Loading	🖌 Loading	SA (Streptavidin)	Total A	sav Time
2	7	Wash	🙀 Custom	SA (Streptavidin)	Totalita	July Inn
2	4	Association	🞽 Association	SA (Streptavidin)		
2	8	Long Dissociation	📐 Dissociation	SA (Streptavidin)	0:35:15	
3	1	Baseline 🔹	🖵 Baseline	SA (Streptavidin) 💌		
3	2	Loading	🖌 Loading	SA (Streptavidin)		
3	7	Wash	🐺 Custom	SA (Streptavidin)		
3	5	Association	🞽 Association	SA (Streptavidin)		
3	8	Long Dissociation	📐 Dissociation	SA (Streptavidin)		
3	10	Regeneration	🔁 Regeneration	SA (Streptavidin)	0:35:23	

Figure 8-32: Experiment and Assay Time Updates as Steps Are Added to the Assay

*IMPORTANT:* If you intend to analyze the data from a sample using the Inter-step correction feature in the Octet<sup>®</sup> BLI Discovery software, the assay must use the same well to perform baseline and dissociation for the sample.

#### Adding a Regeneration Step

1. In the Sample Plate Map, assign wells as Regeneration or Neutralization (Figure 8-33).



Figure 8-33: Regeneration Step

2. Click Add (Figure 8-34) to display the Add Step Definition dialog box (Figure 8-35).

S	tep (	Data List					
	A	dd 📐 💷 C	ору	Remove	Regeneration Params	Threshold	Params
		Name	Time	Shake speed	Туре	Threshold	
		Baseline	60	1000	🛌 Baseline		
•	•	Regeneration	30	400	💈 Regeneration		

Figure 8-34: Add Button

Add Step	Definitions			×
		Name	Time (s)	Shake speed (rpm)
	Association	Association	600 🚔	1000 🚔
	Dissociation	Dissociation	600 🚔	1000
🗖 🗖	Baseline	Baseline	600 🚔	1000
	Loading	Loading	600 🚔	1000
	Activation	Activation	600 🚔	1000
	Quenching	Quenching	600 🚔	1000
🔽 🕏	Regeneration	Regeneration	30	1000
□ 7	Custom	Custom	600 🚔	1000
🖻 🗵	Dip	Dip	600 🚔	1000
		OK Can	cel	Defaults

Figure 8-35: Add Step Definition Dialog Box

- 3. Select **Regeneration** and click **OK**.
- 4. Click **Regeneration Params** (Figure 8-36).

- 9	itep [	Data List					
	A	dd 🛛 🔂 📿 C	ору	Remove	Regeneration Params	Threshold F	Params
ſ		Name	Time	Shake speed	Туре	Threshold	
		Baseline	60	1000	🛌 Baseline		
	•	Regeneration	30	400	💈 Regeneration		

Figure 8-36: Regeneration Params Button

The **Regeneration Parameters** dialog box (Figure 8-37) appears, where you can edit Regeneration parameters as needed.

Regeneration Paramet	ters	
Step Name:	Regeneration	
	Time (s)	Shake speed (rpm):
Regeneration:	5 🚔	400
Neutralization:	5 🚔	0
Regeneration cycles:	3	
Total step time:	30 s	OK Cancel

Figure 8-37: Regeneration Parameters Dialog Box

#### Replicating Steps within an Assay

To copy steps and add them to an assay:

- 1. In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 8-38, step rows 1-4 are selected).
  - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
  - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
- 2. In the **Replicate Steps** dialog box (see Figure 8-38):
  - a. If you select **Append to current assay**: The Offset Steps option is not automatically selected. If you select it, only the horizontal option is available. The vertical option is not available because Octet<sup>®</sup> R2, or Octet<sup>®</sup> K2 kinetic assays are restricted to a row pair. If a vertical offset is required, then replicate the steps as a new assay instead.
  - b. If you select **Add as a new assay**: The Offset Steps options is not automatically selected. If you select it, both vertical and horizontal offsets are allowed.
- 3. Select and set the options in the Offset steps box as appropriate. (For more details on offset options, see Table 8-5.)



Figure 8-38: Replicating Assay Steps by Appending

#### 4. Click OK. The step(s) appear at the end of the assay in the Assay Steps List.

Table 8-5: Replicate Steps Options .

Item	Description
Add as a new assay	Adds the replicate step(s) as a new assay to the Assay Steps List.
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
All steps	Applies the offset to the sample and reagent steps in the plate.
Sample and Reagent steps will be adjusted horizontally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter 1. Enter 0 to apply the step(s) to the same columns.
Octet® K2 and Octet® R2	Adjust Sample and Reagent steps vertically for two rows
Octet® R4	Adjust Sample and Reagent steps vertically by four rows.

#### Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensors:

- 1. Select two wells in the **Sample Plate Map**.
- 2. Right-click to view the shortcut menu and select Start New Assay (see Figure 8-39).
- 3. Add steps to the assay as described earlier.



Figure 8-39: Start New Assay

#### Inserting or Adding an Assay Step

To insert an assay step:

- 1. Select a step in the Step Data List.
- 2. In the Assay Steps List, select the row above where you want to insert the step.
- In the Sample Plate Map, right-click the column to which the step will be applied and select Insert Assay Step. The step is inserted into the Assay Steps List.

To add an assay step:

- 1. Select a step type in the **Step Data List**.
- 2. In the **Sample Plate Map**, right-click the column to which the step will be applied, and select **Add Assay Step**.

The step is added to the end of the **Assay Steps List**.

#### Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow (**•**) for any step in the assay and select a sensor type from the drop-down list (Figure 8-40). The biosensor type will automatically update for every assay step.

Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time
1	1	Baseline	🛌 Baseline	SA (Streptavidin	)
1	2	Loading	📝 Loading	SA (Streptavidin	)
1	7	Wash	🗱 Custom	SA (Streptavidin	)
1	3	Association -	<ul> <li>Association</li> </ul>	SA (Streptavidin	) -
1	8	Long Dissociation	▶ Dissociation	SA (Stree	atavidin)
1	10	Regeneration	💈 Regeneration	AHC (An	ti-hlaG Ec Cantur
2	1	Baseline	닖 Baseline	Anti CCI	r ngo re cuptan
2	2	Loading	📝 Loading	Anti-OSI	
2	7	Wash	🗱 Custom	Anti-Hui	man Fab-CHI (FAI
2	4	Association	🗶 Association	Anti-FLA	AG (FLG)
2	8	Long Dissociation	📐 Dissociation	APS (Am	inopropyls (ane)
3	1	Baseline	닖 Baseline	AR (Ami	ne Reactive)
3	2	Loading	🔟 Loading	AR2G (A	mine Reactive 2nd
3	7	Wash	🙀 Custom	SSA (Sup	er Streptavidin)
3	5	Association	🗶 Association	AMC (Ar	nti-mIgG Fc Captu
3	8	Long Dissociation	L Dissociation	Ni-NTA	
3	10	Regeneration	💈 Regeneration	Custom	

Figure 8-40: Selecting an Assay Sensor Type

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.

#### Editing an Assay

To edit the step type or the biosensor type:

#### 1. In the Assay Steps List:

- To change the step type, click the **Step Name** arrow (➡) and select a step name from the drop-down list (Figure 8-41, top).
- To change the biosensor type, click the Sensor Type arrow (
   for any step in the assay and select a sensor type from the drop-down list (Figure 8-41, bottom). The biosensor type will automatically update for every assay step.

**NOTICE:** The Step Name drop-down list includes only the step types defined in the Step Data List.

ssay S	Sample	Step Name	Step Type	Sensor Type	Assay Time	
1	1	Baseline	🛌 Baseline	SA (Streptavidin)		
2	2	Loading	📝 Loading	SA (Streptavidin)		
7	7	Wash	🙀 Custom	SA (Streptavidin)		
3	3	Association -	🗶 Association	SA (Streptavidin) 👻		
8	3	Baseline	n	SA (Streptavidin)		
1	10	Loading	pn	SA (Streptavidin)	0:35:23	
1	1	Wash		SA (Streptavidin)		
2	2	vvdsii		SA (Streptavidin)		
7	7	Association		SA (Streptavidin)		
4	4	Long Dissoci	ation	SA (Streptavidin)		
8	3	Regeneration		SA (Streptavidin)	0:35:15	
1	1	Baseline	🔚 Baseline	SA (Streptavidin)		
2	2	Loading	🔟 Loading	SA (Streptavidin)		
7	7	Wash	🕁 Custom	SA (Streptavidin)		
5	5	Association	K Association	SA (Streptavidin)		
8	3	Long Dissociation	▶ Dissociation	SA (Streptavidin)		
1 ay Step ew Assi	10 ps List ay Mo	Regeneration	Regeneration       Remove     Remove	SA (Streptavidin) plicate Edit	0:35:23 Step	
1 ay Step aw Assi say S	10 ps List ay Mo Sample	Regeneration           we Up         Move Down           Step Name	Regeneration       Remove     Remove       Step Type	SA (Streptavidin) splicate Edit Sensor Type	0:35:23 Step Assay Time	
1 ay Step ew Assa ssay 9 1	10 ps List ay Mc Sample	Regeneration           we Up         Move Down           Step Name         Baseline	Regeneration     Remove Re     Step Type     Baseline	SA (Streptavidin) eplicate Edit Sensor Type SA (Streptavidin)	0:35:23 Step Assay Time	
1 say Step ew Assi ssay 9 1 2	10 ps List ay Mo Sample 1 2	Regeneration           we Up         Move Down           Step Name         Baseline           Loading	Regeneration     Remove Re     Step Type     Baseline     Loading	SA (Streptavidin) eplicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin)	0:35:23 Step Assay Time	
1 say Step ew Assi ssay 9 1 2 7 7	10 ps List ay Mo Sample 1 2 7	Regeneration we Up Move Dowr Step Name Baseline Loading Wash	Regeneration  Remove Re  Step Type  Baseline  C Loading  Custom Custom	SA (Streptavidin) eplicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin)	0:35:23 Step Assay Time	
1 ay Step ew Assi ssay 9 1 2 7 3	10 ps List ay Mo Sample 1 2 7 3	Regeneration ave Up Move Down Step Name Baseline Loading Wash Association	Regeneration  Remove  Re  Step Type  Baseline  Cooding  Custom  Association	SA (Streptavidin) eplicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)	0:35:23 Step Assay Time	
1 say Step ew Assi ssay 5 1 2 7 3 8	10 ps List ay Mo Sample 1 2 7 3 3	Regeneration We Up Move Dowr Step Name Baseline Loading Wash Association Cong Dissociation	Regeneration  Remove Re  Step Type  Baseline  Coding  Custom  Association  Dissociation	SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)	0:35:23 Step Assay Time	
1 say Step ew Assi ssay \$ 1 2 7 3 8 8 1	10 ps List ay Mo Sample 1 2 7 3 3 10	Regeneration We Up Move Down Step Name Baseline Loading Wash Association Regeneration		SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-F	0:35:23 Step Assay Time idin) IgG Fc Capture)	
ay Step ew Assa ssay s 1 2 7 3 8 1 1	10 ps List ay Mo Sample 1 2 7 3 3 10	Regeneration We Up Move Dowr Step Name Baseline Loading Wash Association Regeneration Baseline	Regeneration  Remove Re  Step Type  Baseline  Custom  Custom  Regeneration  Baseline  Baseline  Custom  Regeneration  Regeneration  Baseline  Custom  Regeneration  Rege	SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) + SA (Streptavidin) AHC (Anti-F Anti-GST	0:35.23 Step Assay Time idin) algG Fc Capture)	
1 say Step ew Asso ssay 9 1 2 7 3 8 1 1 2 2 1 2	10 ps List ay Mo Sample 1 2 7 3 3 10 1 2	Regeneration  Ave Up Move Down  Step Name  Baseline Loading  Wash  Long Dissociation  Regeneration  Baseline Loading	Regeneration  Remove Re  Step Type  Baseline  Custom  Custom  Regeneration  Baseline  Custom  Custom	SA (Streptavidin) eplicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-F Anti-GST Anti-Humar	0:35.23 Step Assay Time idin) IgG Fc Capture) n Fab-CH1 (FAB)	
ay Step ew Assa ssay 9 7 3 8 1 1 2 7 7 3 7 7 7 7 7	10 ps List ay Mo Sample 1 2 7 3 3 3 10 1 2 7 7	Regeneration We Up Move Down Step Name Baseline Loading Wash Cong Dissociation Regeneration Baseline Loading Wash	Regeneration    Remove Re	SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-H Anti-GST Anti-FILMG (	0:35:23 Step Assay Time idin) IgG Fc Capture) h Fab-CH1 (FAB) El G)	
ay Step ew Asse ssay \$ 1 2 7 3 8 1 2 2 7 4 4	10 ps List ay Mc Sample 1 2 7 3 10 1 2 7 4	Regeneration Ave Up Move Down Step Name Baseline Loading Vash Association Regeneration Baseline Loading Wash Association	Regeneration  Remove  Re  Step Type  Step Type  Step Type  Custom  Custom  Association  Regeneration  Baseline  Custom  Custom  Custom  Association  Association  Regeneration  Association  Regeneration  Regener	SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-H Anti-FLAG ( OBS (Amino-	0:35:23 Step Assay Time idin) IgG Fc Capture) n Fab-CH1 (FAB) FLG)	
1 say Step ew Asssay 5 1 2 7 7 7 7 8 8 1 1 2 7 7 4 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	10 ps List (Mc Sample 1 2 7 3 3 10 1 2 7 4 3 3	Regeneration  Ave Up Move Down  Step Name  Baseline Loading  Wash  Long Dissociation  Regeneration  Baseline Loading  Wash  Association  Loading  Wash  Association  Long Dissociation  Long Dissociation	Regeneration	SA (Streptavidin) eplicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-Pt Anti-GST Anti-Human Anti-FLAG ( APS (Amino APS (Amino	0:35:23 Step Assay Time idin) IgG Fc Capture) n Fab-CH1 (FAB) FLG) propyIst one)	
1 ay Step ew Ass 5 3 3 8 8 1 1 2 2 7 7 7 4 8 8 8 1 1 1 2 2 7 7 7 7 7 1 1 2 2 1 1 1 2 2 1 1 1 2 2 1 1 1 1	10 ps List 3 3 3 10 1 2 7 4 3 1 1 2 7 4 3 1 1 2 7 4 3 1 1 2 7 4 3 1 1 2 7 1 4 3 1 1 2 7 1 4 1 3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Regeneration		SA (Streptavidin) eplicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-Pt Anti-GST Anti-Humar Anti-FLAG ( APS (Amino AR (Amino f	0:35:23 Step Assay Time idin) IgG Fc Capture) n Fab-CH1 (FAB) FLG) propyls[*ne) Reactive)	
1 ay Step ew Asse 1 2 7 7 7 3 3 8 8 1 1 2 2 7 7 4 8 8 1 2 2 7 7 1 2 2 7 7 7 7 7 7 7 7 7 7 7 7	10 ps List ay Mo Sample 1 2 7 3 3 10 1 2 7 4 3 1 2 7 4 3 1 2 2 7 4 3 1 2 2 7 4 3 1 2 2 7 4 3 3	Regeneration  Step Mare Down  Step Name Baseline Loading Wash Regeneration Baseline Loading Wash Association Loading Wash Association Long Dissociation Baseline Loading Comp Dissociation Baseline Comp Dissociation Baselin	Regeneration   Remove Re  Step Type  Baseline  Custom  Custom  Regeneration  Regener	SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-Pt Anti-GST Anti-Humar Anti-FLAG ( APS (Amino AR (Amino ft AR2G (Amin	0:35:23 Step Assay Time Assay Time idin) ilgG Fc Capture) n Fab-CH1 (FAB) FLG) propy/store) Reactive 2nd Gen)	
1 say Step lew Assay 5 1 2 7 7 7 3 3 3 8 8 8 1 1 1 2 7 7 4 8 8 8 1 1 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	10 ps List ay Mc Sample 1 2 7 3 3 10 1 2 7 4 3 1 2 7 4 3 1 2 7 4 3 3 1 2 2 7 4 3 3 1 2 2 7 4 3 3 3 3 1 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3	Regeneration  Step Name Baseline Loading Wash Log Dissociation Regeneration Baseline Loading Wash Association Long Dissociation Baseline Loading Wash Association Baseline Loading Wash Association Baseline Loading Wash		SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-H Anti-GST Anti-Humar Anti-FLAG ( APS (Amino AR (Amino f AR2G (Amino SSA (Super S	0:35:23 Step Assay Time Assay Time idin) idin) ilgG Fc Capture) n Fab-CH1 (FAB) FLG) propyls[one) Reactive) the Reactive 2nd Gen) Streptavidin)	
1 say Step iew Assay 5 1 2 7 7 3 8 8 8 1 1 2 7 7 4 8 8 1 2 7 7 7 5 5	10 solution so	Regeneration	Remove Re  Step Type  Baseline  Custom  Cust	SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-F Anti-FLAG ( APS (Amino AR (Amino F AR2G (Amino SSA (Super S AMC (Anti-F	0:35:23 Step Assay Time Assay Time idin) lgG Fc Capture) n Fab-CH1 (FAB) FLG) propyls[*ne) Reactive) the Reactive 2nd Gen) Streptavidin) mIgG Fc Capture)	
1 say Step iew Assay 5 1 2 7 7 3 8 8 1 1 2 7 7 4 8 8 1 2 7 7 7 5 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	10 ps List ay Mc Sample 1 2 7 3 3 3 3 3 4 3 1 2 7 4 3 1 2 7 5 3 3 3 3 3 3 3 3 3 3 3 3 3	Regeneration	Regeneration     Remove     Re     Step Type     Baseline     Loading     Custom     Cissociation     Baseline     Loading     Custom     Custom     Loading     Custom     Dissociation     Baseline     Loading     Custom     Dissociation     Baseline     Loading     Custom     Loading     Custom     Loading     Custom     Loading     Custom     Dissociation	SA (Streptavidin) splicate Edit Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-FLAG ( APS (Amino AR (Amine F AR2G (Amino SSA (Super S AMC (Anti-I- Ni-NTA	0:35:23 Step Assay Time Assay Time idin) lgG Fc Capture) n Fab-CH1 (FAB) FLG) propyls[*qne) Reactive) re Reactive 2nd Gen) Streptavidin) mIgG Fc Capture)	
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1 ay Step ew Ass. 5 5 5 5 5 7 7 7 7 8 8 1 1 2 2 7 7 4 8 8 1 1 2 2 7 7 5 8 8 1 1 2 2 7 7 7 7 7 7 8 8 1 1 2 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	10 solution so	Regeneration  Xet Up Move Down  Step Name Baseline Loading  Wash Association Regeneration Baseline Loading  Wash Association Long Dissociation Baseline Loading Wash Association Long Dissociation Baseline Loading Wash Association Baseline		SA (Streptavidin) splicate Sensor Type SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) AHC (Anti-FLAG ( APS (Amino AR (Amino F AR2G (Amino SSA (Super S AMC (Anti-I- Ni-NTA Custom anti-penta h	0:35:23 Step Assay Time Assay Time idin) UgG Fc Capture) n Fab-CH1 (FAB) FLG) propyls[*qne) Reactive) ne Reactive 2nd Gen) Streptavidin) mIgG Fc Capture) is	

Figure 8-41: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

To reorder or remove an assay step:

- 1. Select a step (row) in the Assay Steps List.
- 2. Click the **Move Up**, **Move Down**, or **Remove** button located above the list.

*IMPORTANT:* An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. Octet<sup>®</sup> BLI Analysis software recognizes the baseline/association/dissociation set of steps.

#### Adding an Assay Through Replication

A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

- 1. In the Assay Steps List, select the steps to copy and click Replicate.
  - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
  - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
- 2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 8-42).



Figure 8-42: Adding a Replicate Assay to a Plate

- 3. Click the **Offset steps** check box and set the options as appropriate (see Table 8-5 on page 321 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the **Offset steps** option. If the replicate assay uses a different sample column, select **Offset steps** and the appropriate options.
  - Sample and Reagent Steps offsets all wells in the assay by the value specified.
- 4. Click OK. The new assay appears in the Assay Steps List.
- 5. Continue to add assay steps as needed.

# Assigning Biosensors to Samples

After you define the sample plate and assay(s), click the **Sensor Assignment** tab, or click the arrow  $\rightarrow$  to access the Sensor Assignment window. The color-coded **Sensor Tray** and **Sample Plate Map** show the locations of the biosensors associated with the samples, Figure 8-43.

**NOTICE:** If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 8-43, the experiment includes three assays in the same wells. The use of those wells by three different biosensors is indicated by the pie chart colors.

**NOTICE:** The Sensor Type for the assay must be first be defined in the Assay Steps List on the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



Figure 8-43: Sensor Assignment Window

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 8-44).



Figure 8-44: Tool Tip of Well Information

#### Replacing the Biosensors in the Biosensor Tray

After an assay is competed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 8-45).



Figure 8-45: Replace Sensors in Tray After Use Check Box

NOTICE: Do not regenerate biosensors more than 11 times per experiment.

#### Entering Biosensor Information

To enter information about a biosensor:

- Optional: Double-click in any cell in the Lot Number column to enter the biosensor lot number. All wells in the Lot Number column for that biosensor type will automatically populate with the lot number entered (see Figure 8-46).
- 2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 8-46).

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

Well	Sensor Type	Lot Number	Information		
A1	SA (Streptavidin)	10102020	Default		
B1	SA (Streptavidin)	10102020		Undo	
C1	SA (Streptavidin)	10102020			
D1	SA (Streptavidin)	10102020		Cut	
E1	SA (Streptavidin)	10102020		Сору	
F1	SA (Streptavidin)	10102020		Paste	
G1	SA (Streptavidin)	10102020		Delete	
H1	SA (Streptavidin)	10102020		Delete	
A2	SA (Streptavidin)	10102020		Select All	
B2	SA (Streptavidin)	10102020		Diskton left Desidien ender	
C2	SA (Streptavidin)	10102020		Right to left Reading order	
D2	SA (Streptavidin)	10102020		Show Unicode control characters	
E2	SA (Streptavidin)	10102020		Insert Unicode control character	۲
F2	SA (Streptavidin)	10102020		0 11/5	
G2	SA (Streptavidin)	10102020		Open IME	
H2	SA (Streptavidin)	10102020		Reconversion	

Figure 8-46: Entering or Editing Biosensor Information

#### Changing the Biosensor Location

If you prefer to not use the default biosensor locations, you can select other locations to use. There are two ways to do this:

- Method 1—In the Sensor Tray Map, Remove the sensor locations you do not want to use. The software automatically selects the next available location(s).
- Method 2-Remove all sensor locations from the Sensor Tray Map, then select the locations you want to use.

#### Method 1

- 1. In the **Sensor Tray Map** (see Figure 8-47), select the locations you do not want to use and click **Remove**. Or, right-click the selection and select **Remove** (Figure 8-47 left). The software automatically selects the next available biosensor locations in the tray (Figure 8-47 right).
- 2. Click Fill Plate to return the Sensor Tray Map to the default layout.



Figure 8-47: Changing Biosensor Location (Method 1)

#### Method 2

- 1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 2 top left). Or, right-click the select tion and select **Remove**. All columns will be shown as **Missing** (Figure 2 top right).
- 2. Select the sensor locations to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 2 bottom left). The software fills the selected columns in the tray (Figure 2 bottom right).



Figure 8-48: Changing Biosensor Location (Method 2)

#### 3. Click Fill Plate to return the Sensor Tray Map to the default layout.

#### Using Heterogeneous Trays

If heterogeneous biosensor trays will be used, the well location of each biosensor type in the tray can be identified in the **Assay Definition Tab**. Assignment of biosensors that will not be used in the assay enables the software to autoassign the biosensors that will be used in the assay by biosensor type.

The biosensor type can be changed per assay by selecting the desired biosensor type in the drop down list under sensor type in the Assay Steps List in the **Assay Definition Tab** (Figure 8-49).

New A:	ssay	Move U	p Move Dow	n Remove R	eplicate   Edit Step   Info Table
Assay	No.	Sample	Step Name	Step Type 🄇	Sensor Type Assay Time Comment
1	1	A1	Baseline 👻	🛌 Baseline	AHC (Anti-higG F -
1	2	A2	Loading	🖌 Loading	SA (Streptavidin)
1	3	A1	Baseline	🛌 Baseline	SAX (High Precision Strentsvidin)
1	4	A3	Baseline	🛌 Baseline	ALIC (Anti-his C Es Contum)
1	5	A4	Association	🞽 Association	AHC (Anti-nigo Pc Capture)
1	6	A3	Dissociation	📐 Dissociation	Anti-GS1
2	1	C1	Baseline	🛏 Baseline	Anti-Human Fab-CH1 (FAB)
2	2	C2	Loading	🖌 Loading	Anti-FLAG (FLG)
2	3	C1	Baseline	🔜 Baseline	APS (Aminopropylsilane)
2	4	C3	Baseline	Baseline	AR (Amine Reactive)
2	5	C4	Association	Association	AR2G (Amine Reactive 2nd Gen)
2	6	C3	Dissociation	📐 Dissociation	SSA (Super Streptavidin)
3	1	E1	Baseline	닖 Baseline	AMC (Anti-mIgG Fc Capture)
3	2	E2	Loading	🖌 Loading	Ni-NTA
3	3	E1	Baseline	🛌 Baseline	Custom
3	4	E3	Baseline	🛌 Baseline	Custom
3	5	E4	Association	🞽 Association	AHQ
3	6	E3	Dissociation	📐 Dissociation	Protein A
4	1	G1	Baseline	😓 Baseline	SA (Streptavidin)
4	2	G2	Loading	🖌 Loading	SA (Streptavidin)
4	3	G1	Baseline	🔜 Baseline	SA (Streptavidin)
4	4	G3	Baseline	🛌 Baseline	SA (Streptavidin)
4	5	G4	Association	🖌 Association	SA (Streptavidin)
4	6	G3	Dissociation	Dissociation	SA (Streptavidin) 0:36:45

Figure 8-49: Assay Steps List – Changing the Biosensor Type

#### Changing the Biosensor Type

The biosensor type used in each assay can be modified and must be selected in the Assay Definition window.

To change the biosensor type:

- 1. Click the Assay Definition Tab.
- 2. In the Assay Steps List, click the cell in the Sensor Type column to change.
- 3. Select from the drop-down list (see Figure 8-49).

**IMPORTANT:** Ensure that the biosensor types selected in the Assay Definition window have been correctly assigned in the Sensor Assignment window or the experiment cannot be run.

#### Using Partial Biosensor Trays

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 8-50) includes three assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 8-50 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 8-50 bottom). If necessary, change the location of these biosensors.



Figure 8-50: Example Experiment Using Two Biosensor Trays

**NOTICE:** Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet<sup>®</sup> instrument.

#### **Reference Biosensors**

To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.

**NOTICE:** Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

# **Reviewing Experiments**

NOTICE: For optimal results, ensure total assay time is less than 3 hours.

Before running an experiment, you can review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window (Figure 8-51), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the  $\leftarrow$  arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.



# Saving Experiments

After an experiment is run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment:

1. Click Save Method File ( d), or on the main menu, click File > Save Method File.

If there is more than one open experiment and you want to save all of them, click Save All Methods Files 🐴.

2. In the Save dialog box, enter a name and location for the file, and click Save.

**NOTICE:** If you edit a saved experiment and want to save it without overwriting the original file, click File > Save Method File As and enter a new name for the experiment.

### Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment > Templates > Kinetics > Experiment Name** (Figure 8-52).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\Sartorius\OctetBLIDiscovery\TemplateFiles.

**IMPORTANT:** Do not change the location of the Template folder. If the Template folder is moved from the factoryset location, the software may not function properly.



Figure 8-52: Saved Experiments in the Template Folder

# Running a Kinetics Experiment

**IMPORTANT:** Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.

### Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

- 1. Open the Octet<sup>®</sup>instrument door (lift the handle up).
- 2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 8-53).
- 3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 8-53).



Figure 8-53: Biosensor Stage (left) and Sample Stage (right)

**IMPORTANT:** Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.

- 4. Close the Octet<sup>®</sup> instrument door.
- 5. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

## Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow ( $\rightarrow$ ) to access the Run Experiment window (see Figure 8-54).

					Prior to pressing "Go" confirm the Assay.
Kinetics data repository:	C	D:\data			
Experiment run name (si	ub directory):	Experiment_1		-	<b>→</b>
Plate name/barcode (file	e prefix):	201103			
Auto-increment file ID st	art:	-			
Data files will be stored	as follows:				Total experiment time: 2:07:30
C:\data\Experiment_1\ C:\data\Experiment_1\ C:\data\Experiment_1\ 	201103_001.frd 201103_002.frd 201103_003.frd				
Run Settings					
Start	ster (s): 600 💭	Set plate	time chars automatica omatically save runtime temperature (°C):	chart 25 🛓	
Advanced Settings					
Acquisition rate:	andard kinetics (5	.0 Hz)	~	Default	JIL
Warr If you are	ing: changing these unsure of how to us	settings could affect ass these settings, please c	ay signal-to-noise. onsult the User Guide		
General Information		Machine name:	DESKTOP-0EH	TC34	
General Information User name: Description:				^	
General Information User name: Description: AHC example method - Analyte titration series v	full characterization /ith reference chanr	nel		~	

- Figure 8-54: Run Experiment Window
- 2. Confirm the default settings or enter new settings. See "Run Experiment Window Settings" on page 337 for more information on experimental settings.

**NOTICE:** If you delay the experiment start, you have the option to shake the plate until the experiment starts.

3. To start the experiment, click GO.

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 8-55).

**NOTICE:** For more details about the Runtime Binding Chart, see "Managing the Runtime Binding Chart" on page 339.



Figure 8-55: Runtime Binding Chart

4. Optional: Click **View** > **Instrument Status** to view the log file (see Figure 8-56).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



Figure 8-56: Instrument Status Log



**WARNING:** Do not open the Octet<sup>®</sup> instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



**WARNING:** N'ouvrez pas la porte de l'instrument Octet<sup>®</sup> lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



**WARNING:** Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

### Run Experiment Window Settings

The following Data File Location and Name settings are available on the Run Experiment Tab:

Table 8-6: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Kinetics data repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click <b>Browse</b> to select another data location.
	<b>NOTICE:</b> Save the data to the local machine first, then transfer to a network drive.
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/bar- code	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

#### The following **Run Settings** are available on the **Run Experiment** Tab:

#### Table 8-7: Run Settings

Item	Description
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the <b>Runtime Binding Chart</b> for the current biosensor during data acquisition.
Automatically save run- time chart	Saves an image (.jpg) of the <b>Runtime Binding Chart</b> . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in <b>File &gt; Options</b> . The factory set default temperature is 30 °C.
	<b>NOTICE:</b> If the actual plate temperature is not equal to the set plate temperature, a warn- ing displays and the Octet <sup>®</sup> BLI Discovery software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.

The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet<sup>®</sup> system per minute and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. The choice of a setting should be determined based upon consideration of the binding rate and the amount of signal generated in your assay, and some experimentation with the settings.

### Table 8-8: Advanced Settings Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, or Octet<sup>®</sup> K2 System

Item	Description
Acquisition rate	<ul> <li>High sensitivity kinetics (2 Hz, averaging by 50): - The average of 50 data frames is reported as one data point. Two data points are reported per second.</li> </ul>
	<ul> <li>Standard kinetics (5 Hz, averaging by 20 - The average of 20 data frames is reported as one data point.</li> </ul>
Default	Sets acquisition rate and sensor offset to the defaults.

### Stopping an Experiment

To stop an experiment in progress, click  $\bigotimes$  or click **Experiment** > **Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.

NOTICE: After the experiment is run, the software automatically saves the experiment method (.fmf).

# Managing the Runtime Binding Chart

If the **Open runtime charts automatically** check box is selected in the Run Experiment window (Figure 8-57), the Runtime Binding Charts are automatically displayed when data acquisition starts. The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active set of biosensors is colorcoded (A=green, B=magenta, C=orange, D=purple, E=olive, F= black, G=red, H=blue) within the **Sensor Tray Map**. Used sets of sensor columns that are inactive are colored black. Active sample columns are colored green. Each assay in the experiment is represented by **Assay X** in the **Current Binding Charts** box.

To selectively display data for particular assay:

- 1. Click the corresponding Assay number.
- 2. Select a subset of sensors for a displayed column under Sensors to Chart box (see Figure 8-57).

**IMPORTANT:** Do not close the Runtime Binding Chart window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet<sup>®</sup> BLI Discovery software saves the Runtime Binding Chart as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.



Figure 8-57: Runtime Binding Chart Window

### Opening the Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

1. Click **File > Open Experiment**.

2. In the dialog box that appears, select an experiment folder and click Select.

### Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the Sensor Assignment tab
- During acquisition in the Runtime Binding Chart Sensors to Chart box
- During analysis in the Data Selection tab

#### Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the Sensors to Chart list or the Sensor Tray, right-click a biosensor and select Reference (see Figure 8-58).



Figure 8-58: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray (**see Figure 8-59).

2. Click the Subtract reference sensors check box (see Figure 8-59).



Figure 8-59: Subtract Reference Sensors check box in the Runtime Binding Chart

**NOTICE:** Subtracting reference data in the Runtime Binding Chart only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be repeated during data analysis if needed.

### Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 8-60). Uncheck the box to return to the default data display.



Figure 8-60: Data Inverted Using Flip Data Function

### Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 8-61), right-click a step and select **Align to Step <number>**.

To remove the step alignment, right-click the step and select Unaligned.



Figure 8-61: Runtime Binding Chart–Aligning the Data to a User-Selected Step

### Aligning Data to a Specific Time

1. To align the binding data to a specific time, in the **Runtime Binding Chart** (see Figure 8-62), right-click and select **Align at time**.



Figure 8-62: Runtime Binding Chart-Aligning the Data to a User-Specified Time

The Align at Time dialog box appears (Figure 8-63).

Align at Time	×
Timer (s):	320
ОК	Cancel

Figure 8-63: Align at Time Dialog Box

2. Enter the time point you want to align to and click **OK**. The binding chart will then align to the time point specified.

To remove the time alignment, right-click and select Unaligned.

### Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.

**NOTICE:** If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.

To extend the duration of the active step:

1. In the chart window, click the **Extend Current Step** button.

2. In the **Extend Current Step** dialog box (see Figure 8-64), enter the number of seconds to extend the step and click **OK**.

Extending this s	tep will prevent data a	nalysis if these se	nsors are to b	e used in
Parallel Referer	ice, Double Reference,	or Average Refer	ence subtract	ion methods.
Assay 1, Step 2	1			
Duration (s):	600			
				( <u></u>

Figure 8-64: Extend Current Step Dialog Box

### Terminating a Step to Begin the Next Step

To terminate a step and begin the next step in the assay:

- 1. In the chart window, click the **Go to Next Step** button.
- 2. In the **Data Acquisition** dialog box, click **OK**.

### Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

### Scaling a Runtime Binding Chart

#### To scale the Runtime Binding Chart:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, select Fullscale or Autoscale.

### Adding a Runtime Binding Chart Title

To add a Runtime Binding Chart title:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, enter a graph title or subtitle.

### Selecting a Runtime Binding Chart Legend

To select a Runtime Binding Chart legend:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box (see Figure 8-65), select one of the following legends:
  - Sensor Location
  - Sample ID
  - Sensor Information
  - Concentration/Dilution

Runtime Graph Propert	ies
Title:	
Subtitle:	
Legend	Sensor Information
Sample ID	Concentration / Dilution
	OK Cancel

Figure 8-65: Selecting a Runtime Binding Chart Legend

**NOTICE:** Text for Sample ID, Sensor Information, or Concentration/Dilution is taken from the Plate Definition and Sensor Assignment tabs, and must be entered before the experiment is started.

3. Click OK.

### Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window** > **New Window**.

### Exporting or Printing the Runtime Binding Chart

To export the Runtime Binding Chart as a graphic or data file:

- 1. Right-click the chart and select **Export Data**.
- 2. In the **Exporting** dialog box (see Figure 8-66), select the export options and click **Export**.

xporting						×
Export	© WMF	O BMP	) JPG	O PNG	) Te	xt/Data
Export De	stination ard					
) File		Browse				
Printer						
-Export Siz	e					
	Width: 152	400 /	ers 01	nches OP Millimeters	oints	Export
	DPI: 300	•	Large Fo	ont		Cancel

Figure 8-66: Exporting Dialog Box

Table 8-9: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	$\checkmark$		Click <b>File</b> > <b>Browse</b> to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Run- time Binding Chart to a graphic file		$\checkmark$	Click <b>File</b> > <b>Browse</b> to select a folder and enter a file name.	Creates a graphic image.
Copy the Run- time Binding Chart		$\checkmark$	Clipboard	Copies the chart to the system clip- board

lable 8-9: Runtime Binding Chart Export Options (Continued)									
Task	Export Option		Export Destination	Result					
Print the Runtime Binding Chart	Ņ	(	Printer	Opens the Print dialog box.					

# Managing Experiment Method Files

After you run an experiment, the Octet<sup>®</sup> BLI Discovery software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. A read-only copy of the method used for an experiment is automatically saved in the experiment folder. Open a method (.fmf) and edit it as needed.

NOTICE: When using the 21 CFR Part 11 version of the Octet® BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 8-10: Managing Experiment Method Files

Menu Bar Command/Toolbar Button	Description				
File > Open Method File 📥	Enables you to select and open a method file (.fmf)				
File > Save Method File 📥 or	Saves one method file or all method files. Saves a method file before the experi- ment is run.				
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.				

# Epitope Binning

The goal of a typical epitope binning or cross-blocking experiment is to identify antibodies which bind to different or identical epitopes on the antigen. Antibodies are tested two at a time for competitive binding to one antigen. By competing antibodies against one another in a pairwise and combinatorial format, antibodies with distinct blocking behaviors can be discriminated and assigned to "bins". The end result is a matrix of pairwise binders and blockers.

An epitope binning or cross-blocking experiment must be run as a kinetic experiment with repeating steps in the Octet<sup>®</sup> BLI Discovery software.

**NOTICE:** Sartorius highly recommends using the Loading, Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

After starting the Octet<sup>®</sup> system and the Octet<sup>®</sup> BLI Discovery software, follow the steps in Table 8-11 to set up and run an epitope binning experiment.

Table 8-11: Octet <sup>®</sup> BLI Discovery Steps for Epitope Binning Assay
--

Octet <sup>®</sup> Software	Functions						
	<ol> <li>Select Epitope Binning under New Kinetics Experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf).</li> </ol>						
Octet BLI Discovery 12.1 (CFR11)	<b>NOTICE:</b> In the Experiment Menu, the Templates command allows users to pick from a so of predefined method templates for Kinetic, Quantitation, or Epitope Binning experiment Users may also modify existing method templates to suit their experimental conditions are save as a new method file and new method file name.						
	2. Define a sample plate or open a sample plate definition.						
	3. Specify assay steps.						
	4. Assign biosensors to samples.						
	5. Run the experiment.						

### Starting an Experiment

You can start a kinetics experiment using one of the following options:

- Launch the Experiment Wizard by clicking Experiment > New Experiment Wizard, and selecting New Kinetics Experiment and Epitope Binning.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click Experiment > Templates > Epitope Binning.
- 6. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

### Tab1(Plate Definition)

**NOTICE:** For the Octet<sup>®</sup> K2 system, wells in sample plate are restricted to rows AB, CD, EF and GH. Sample wells cannot be designated in row pairs BC, DE and FG.

- 1. Designate layouts for the plate by selecting wells in the plate map and designating sample types. There are several ways to select sample wells in the plate map:
  - Click a column header or select adjacent column headers by click-hold-drag.
  - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
  - · Click a row header or select adjacent row headers by click-hold-drag.
  - Click a well or draw a box around a group of wells.
- 2. Designate well types by right-clicking on selected wells and assigning a sample type:





- 3. Enter sample information by selecting the table for the plate. There are several ways to enter sample information:
  - Select an individual well in the plate table.
  - · Click-drag-hold several wells in the plate table, right-click and choose Set Well Data.

**NOTICE:** Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.

**NOTICE:** More information on sample information and annotation can be found in "Entering Sample Information" on page 301.

#### Tab 2 (Assay Definition)

After completing the plate layout, an Epitope Binning Assay can be defined by building a kinetic assay.

- 1. Click on Tab 2 (Assay Definition).
- 2. Add assay step types in the Step Data List:

a. Click the **Add** button. The Add Step Definition box appears:

Step Data	List	Сори	Bemove	Begeneration F	Params	eshold Params
Nar Bas	me eline	<b>Time</b> 120	Shake speed	Type Baseline	Threshold	
Add Step	Definitio	ons				×
	Associa Dissocia Baselin Loading Activat Quench Regene Custor Dip	ation e g ion hing eration	Name Association Dissociation Baseline Loading Activation Quenching Regenerati Custom Dip	on	Time (s)           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -           600         -	Shake speed (rpm)         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -         1000       -
			ОК	Canc	el	Defaults

Figure 8-68: Add Step Definition Box

- b. Choose a step type.
- c. Optional: edit step name.
- d. Set the step time and shake speed.
- e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:



Figure 8-69: Regeneration Parameters Box

- f. Optional: assign a threshold. See "Creating Step Types" on page 314 for more information.
- 3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).

**NOTICE:** Sartorius highly recommends using the Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

- a. Select a step type in the Step Data List.
- b. In the plate map, double-click the columns that you want associated with that step type.
- c. The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

1 Plate Definition 2 Assay Definition 3 Sensor Assignment	4 Review	Experi	ment 5	Run E	cperime	ent					
In this step, the assay steps will be assembled from the Step	Data List. step into th		nt seesu wit	th a dou	ible cli	ek orright eliek	for more options				
	otop into ui	o oano	n abbay m			ore, or right order			Time in (s) Sha	ke sneed in (	(mm)
Plate 1 (96 wells)	Step Da	Step Data List								ipiny	
	Add	i	Сору		Regeneration Params		Threshold Params	i - 1			
	1	Name			Time	me Shake speed Type		Threshold			
	→	→         Sensor Check         30         1000           Antigen Immobilization         300         1000           Baseline         30         1000			30 1000 300 1000	1000	⊨ Baseline ✓ Loading				
B <b>@@@@@@@</b> 00000				ion		1000					
	l.			1000	Baseline						
	Saturating mAb (1st Ab)			tAb)	600	1000	Association				
FOODOOOOOOO	(	Compet	ing mAb (2n	nd Ab)	300	1000	Association	ם י			
	Accay	tene li	et								
GOOOOOOOOOOOOOOO	New Assav Move Up				Move Down Remove Replicate		Replicate	Edit Step Info Tab	le		
HD00000000000000	Assay	No.	Sample	Step	Name		Step Type	Sensor Type	Assay Time	Comment	^
Assayed samples O Unassigned samples	1	1	A1	Sensor	Check	· •	👡 Baseline	SA (Streptavidin) 👻	1		-
	1	2	A2	Antiger	n Immo	bilization	🖊 Loading	SA (Streptavidin)			-
	1	3	A3	Baselin	e		Baseline	SA (Streptavidin)			
	1	4	A4	Saturat	ing mA	b (1st Ab)	Association	SA (Streptavidin)			
	1	5	A5	Baselin	e		💑 Baseline	SA (Streptavidin)			
	1	6	A6	Compe	ting m/	Ab (2nd Ab)	Association	SA (Streptavidin)	0:22:50		
	2	1	A1	Sensor	Check	c .	- Baseline	SA (Streptavidin)			
	2	2	A2	Antiger	n Immo	bilization	🖌 Loading	SA (Streptavidin)			
	2	3	A3	Baselin	e		Baseline	SA (Streptavidin)			

Figure 8-70: Assay Steps List

- d. Select the correct biosensor from the Sensor Type drop-down list.
- e. Repeat the previous steps to define other steps in the assay.
- f. New assays may be added by clicking the **New Assay** button in the Assay Steps List:

(	Assay St New As	epsitist ssay M	ove Up	Move Down	Remove	Replicate	Edit Step			
	Assay sample Plate		Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time		
	1	A14	1	Baseline	🔙 Baseline	SA (Streptavidin)	32 channels	no	0:01:20	
	2	A14	1	Baseline	🖕 Baseline	SA (Streptavidin)	32 channels	no	0:01:20	
	3	A14	1	Baseline 🔹	🔙 Baseline	SA (Streptavidin) 💌	32 channels	no	0:01:20	

Figure 8-71: New Assay Button

**NOTICE:** More information on assay step editing in Tab 2 (Assay Definition) can be found in "Creating Step Types" on page 314.

#### Tab 3 (Sensor Assignment):

After completing the assay definition, click on Tab 3 (Sensor Assignment) to verify sensor type(s) for the epitope binning experiment.

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.
**NOTICE:** Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in "Assigning Biosensors to Samples" on page 326.

Replacing Biosensors in the Biosensor Tray. After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the checkbox, "Replace sensors in tray after use".



Figure 8-72: Replace Sensors in Tray After Use Check Box

## Tab 4 (Review Experiment)

NOTICE: For optimal results, ensure total assay time is less than 3 hours.

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:

1 Plat	e Defin	ition 2	Assay Definition 3 Sens	or Assignment 4 Rev	lew Experiment 5 Run Experiment
	In this	step, you c	can review the steps that mak	e up the experiment, mov	ing the alder to change the active step. Assay 2 600 s Step 4 1000 gm
L					of 2:17:00
Sens	or Tray				Plate 1 (96 wells)
A		2 3	4 5 6 7 8	9 10 11 12	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
В					
C					
D				$\otimes$	
E		$\otimes$		$\otimes$	
IF.		20			
G					
ŭ	H		1000 1000 1000 1000 1000 1000 1000 1000		
		~~~~			
Lege	nd:	Unass	igned sensors 🛛 🕅 Mis	sing sensors	Legend: Unassigned   Samples  Pie charts
Assa	y No.	Sample	Step Name	Step Type S ^	
1	1	A1	Sensor Check	Baseline S.	
1	2	A2	Antigen Immobilization	🖌 Loading S.	
1	3	A3	Baseline	Baseline S.	
1	4	A4	Saturating mAb (1st Ab)	Association S.	
1	5	A5	Baseline	Baseline S.	
1	6	A6	Competing mAb (2nd Ab)	Association S.	
2	1	A1	Sensor Check	🖙 Baseline 🛛 S.	
2	2	A2	Antigen Immobilization	🖌 Loading S.	
2	3	A3	Baseline	Baseline S.	
2	4	A4	Saturating mAb (1st Ab)	Association S.	
2	5	A5	Baseline	Baseline S.	
2	6	A7	Competing mAb (2nd Ab)	Association S.	
2	- 1	A1	Conner Charle	I Passins C	

Figure 8-73: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

#### Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

#### 1. Select File > Save Method File.

2. In the Save dialog box, enter a name and location for the file, and click **Save**.

## Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

- 1. Open the Octet<sup>®</sup> instrument door (lift the handle up).
- 2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 8-74).
- 3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 8-74).



Figure 8-74: Biosensor Stage (left) and Sample Stage (right)

**IMPORTANT:** Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.

- 4. Close the Octet<sup>®</sup> instrument door.
- 5. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

## Tab 5 (Run Experiment)

- 1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
- 2. To start the experiment, click the **GO** button:

File Location and Names	$\leftarrow$ o
	Prior to pressing "Go" confirm the Assay.
tics data repository: C.\data	
eriment run name (sub directory): Experiment_1	
e name/barcode (file prefix): 201105	
o-increment file ID start: 1	
a files will be stored as follows:	Total experiment time: 2:17:00
Jata Experiment_1201105_001/rd Jata Experiment_1201105_002/rd Jata Experiment_1201105_003/rd	
Settings	
Delayed experiment start Start after (s): 600 brake sample plate while waiting ✓ Set plate temperature (°C): 30 ✓	
anced Settings uiation rate: Standard kinetics (5.0 Hz) V Default	
Warning: changing these settings could affect assay signal to noise. If you are unsure of how to use these settings, please consult the User Guide	
and Information	
rname: DESKTOP-0EHTC34	
enption:	

Figure 8-75: GO Button

## Chapter 9:

# Kinetics Experiments: Octet<sup>®</sup> R8, Octet<sup>®</sup> RED96e, and Octet<sup>®</sup> QKe

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## Introduction

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet<sup>®</sup> system hardware and the Octet<sup>®</sup> BLI Discovery software, follow the steps (in Table 9-1) to set up and analyze a quantitation experiment.

NOTICE: Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm

Software		Step	See
Octet <sup>®</sup> BLI Discovery	1.	Select a kinetics experiment in the <b>Experi-</b> <b>ment Wizard</b> or open a method file (.fmf).	"Starting a Basic Kinetics Experiment" on page 356
	2.	Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 358
	З.	Specify assay steps.	"Defining a Kinetic Assay" on page 371
	4.	Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 383
	5.	Run the experiment.	"Running a Kinetics Experiment" on page 391
Octet <sup>®</sup> Analysis Studio	6.	View and process the raw data.	Octet <sup>®</sup> Analysis Studio Software
Octet Analysis Studio 12.2	7.	Analyze the data.	User Guide

that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet<sup>®</sup> BLI Discovery software is closed, the plate temperature will reset to the default startup value specified in the Options window when the software is relaunched.

## Starting a Basic Kinetics Experiment

**IMPORTANT:** Using 96-well half-area plates on the Octet<sup>®</sup> RED96 and Octet<sup>®</sup> RED96e, and Octet<sup>®</sup> R8 system will result in non-optimal system performance. Sartorius cannot guarantee results within the optimal performance specifications of the system when these plates are used.

Start a kinetics experiment using one of the following options:

- Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking File > Open Method File. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run. For more details on method files see "Managing Experiment Method Files" on page 404.

• On the menu bar, click Experiment > Templates > Kinetics.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

## Starting an Experiment Using the Experiment Wizard

- 1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button (A), or click **Experiment > New Experiment Wizard** (**Ctrl+N**) from the **Main Menu**.
- 2. In the Experiment Wizard, click New Kinetics Experiment (see Figure 9-1, left).
- 3. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.
- 4. Click the arrow button( $\rightarrow$ ). The **Basic Kinetics Experiment** window displays (Figure 9-1, right).





## Defining the Sample Plate

The steps to define a sample plate include:

	Step	See Page
1.	Designate the sample.	358
2.	Managing sample plate definitions.	368

## Designating Samples

**NOTICE:** It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 9-2 will be included in the assay.

Table 9-2 displays the well types that can be assigned to a plate map.

Table 9-2: Types of Sample Wells

lcon	Description
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.
Controls	<ul> <li>A control sample, either positive or negative, of known analyte composition.</li> <li>Positive Control: A control sample that contains analyte of known concentration</li> <li>Negative Control: A control sample known not to contain analyte</li> </ul>
Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
Activation	Activation reagent. Makes the biosensor competent for binding.
(Q) Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
Load	Ligand to be immobilized (loaded) on the biosensor surface.
₩ Wash	Wash buffer.
Regeneration	Regeneration reagents dissociate the analyte from the ligand.
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

## Selecting Wells in the Sample Plate Map

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag. To select non-adjacent columns, hold the **Ctrl** key and click the column header (Figure 9-2 left).
- Click a row header or select adjacent row headers by click-hold-drag (Figure 9-2, center).
- Click a well or draw a box around a group of wells (Figure 9-2, right).



Figure 9-2: Selecting Wells in the Sample Plate Map

**NOTICE:** Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

## Designating Well Types

In the Sample Plate Map, select the wells, right-click and select a sample type (see Figure 9-25).



Figure 9-3: Designating a Well Type in the Plate Definition Window

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 9-4).



Figure 9-4: Clearing Sample Data from a Sample Plate

## Entering Sample Information

**NOTICE:** You must specify sample (analyte) concentration and molecular weight, otherwise the Octet<sup>®</sup> BLI Discovery software cannot compute a K<sub>D</sub> value. If the sample concentration is not specified, only k<sub>d</sub> and k<sub>obs</sub> are calculated. You can also annotate any well with Sample ID or Well Information, and assign Replicate Groups.

Assigning Molecular Weight and Molar Concentration

- 1. In the Sample Plate Map, select the sample wells, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box, enter the analyte molecular and molar concentration (Figure 9-5).

et Well Data			×
Well Information Sample ID:		Dilution Series	Concentration
Replicate Group:			Molar Concentration
		Starting value (µg/ml): Series operator:	1
Well Information:		Series operand:	2
		Dilution orientation	
Well Data - Sample only			
Molecular Weight (kD):	150		
Molar Concentration (nM):	66.67		
Concentration (µg/ml):	1	(	OK Cancel

Figure 9-5: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

The information displays in the Sample Plate Table (see Figure 9-6).

3. In the **Sample Plate Table**, select the sample concentration units and the molar concentration units.

						Conce	ntration units:	µg/ml	
						Molar	concentration units:	nM	•
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	M	W (kD)	Molar Conc (nM)	Information	
B) F3			Buffer						
🖻 G3			Buffer						
B) H3			Buffer						
<b>)</b> A4			Sample		15	0	66.67		
<b>)</b> B4			Sample		15	0	33.33		
🔵 C4			Sample		15	0	16.67		
D4			Sample		15	0	8.333		
<b>)</b> E4			Sample		15	0	4.167		
🕽 F4			Reference						
🔵 G4			Reference						
🔵 H4			Reference						
🔵 A5			Sample		15	0	66.67		
🔵 B5			Sample		15	0	33.33		
🔵 C5			Sample		15	0	16.67		
D5 🔵			Sample		15	0	8.333		
🔵 E5			Sample		15	0	4.167		
🔵 F5			Reference						
🔵 G5			Reference						1
🔵 H5			Reference						
🔵 A6			Sample		15	0	66.67		
D B6			Sample		15	0	33.33		
C6 🜔			Sample		15	0	16.67		
D6 🜔			Sample		15	0	8.333		
🔵 E6			Sample		15	0	4.167		
D F6			Reference						

Figure 9-6: Entering Molecular Weight and Molar Concentration from the Plate Table

Assigning User-Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. In the Sample Plate Map, select the desired wells, right-click and select Set Well Data.

**NOTICE:** A range of wells can be selected clicking and dragging, holding the Shift key and using the arrow keys to select sections of the plate, or holding the Ctrl key to select specific wells.

The Set Well Data dialog box appears (see Figure 9-7).

2. Select the **By value** option and enter the starting concentration value. If a range of cells was selected, all cells will update with the specified value.

Set Well Data X								
Well Information Sample ID:	Dilution Series							
	O Molar Concentration							
Replicate Group:	Starting value (µg/ml): 1							
Well Information:	Series operator: 🗸 🗸 🗸							
	Series operand: 2							
	Dilution orientation							
	Right Right Left							
Well Data - Sample only	Down Down							
Molecular Weight (kD): 150								
Molar Concentration (nM): 66.67		1						
Concentration (µg/ml):	OK Cancel	1						

Figure 9-7: Sample Plate Map-Assigning Sample Concentrations by Value

3. Click OK. The Sample Plate Table will display the entered concentration.

## Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

- In the Sample Plate Map, select the wells, right-click, and select Set Well Data. The Set Well Data dialog box appears (see Figure 9-8)
- 2. Select the **Dilution Series** option and enter the starting concentration value.

Set Well Data	×
Well Information Sample ID:  Replicate Group:	Dilution Series  Apply to: Oldar Concentration Starting value (µg/ml):
Well Information:	Series operand: 2 Dilution orientation Dilution orientation Dilution orientation Dilution orientation Dilution orientation Dilution orientation Dilution orientation
Well Data - Sample only       Molecular Weight (kD):       150       Molar Concentration (nM):	O     O     Down     O     O     O     Up
Concentration (µg/ml):	OK Cancel

Figure 9-8: Sample Plate Map–Assigning Sample Concentrations Using Dilution Series

3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 9-9).



Figure 9-9: Concentration Representation in Dilution Series

4. Click OK.

The Sample Plate Table displays the standard concentrations.

### Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

#### Annotating Wells in the Sample Plate Map

To annotate one or more wells:

- 1. In the Sample Plate Map, select the samples to annotate, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 9-10), enter the Sample ID and/or Well Information and click OK.

Set Well Data		×
Well Information Sample ID: Analyte 1 Replicate Group:	Dilution Series	Concentration Molar Concentration
Well Information:	Starting Value (µg/mi). Series operator: Series operand: Dilution orientation	2
Well Data - Sample only       Molecular Weight (kD):       150       Molar Concentration (nM):	Geographic Content of Conten	
Concentration (µg/ml):	0	Cancel

Figure 9-10: Add Sample Annotations from the Sample Plate Map

#### Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

- 1. Double-click the table cell for **Sample ID** or **Well Information**.
- 2. Enter the desired information in the respective field (see Figure 9-11).

**NOTICE:** A series of Sample IDs may also be assembled in Excel and pasted into the Sample Plate Table.

Jampie						Concentrat	ion units:	µg/ml ▼
						Molar conc	entration units:	nM 🔻
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information	
📵 G3	Dissociation		Buffer				1X Kinetics Buffer	
📵 H3	Dissociation		Buffer				1X Kinetics Buffer	
🔘 A4	Association		Sample	10	150	66.67	1X Kinetics Buffer	
🔵 В4	Association		Sample	5	150	33.33	1X Kinetics Buffer	
🔵 C4	Association		Sample	2.5	150	16.67	1X Kinetics Buffer	
🔵 D4	Association		Sample	1.25	150	8.333	1X Kinetics Buffer	
🔵 E4	Association		Sample	0.625	150	4.167	1X Kinetics Buffer	
🔵 F4	Association		Reference				1X Kinetics Buffer	
🔵 G4	Association		Reference				1X Kinetics Buffer	
🔵 H4	Association		Reference				1X Kinetics Buffer	

Figure 9-11: Add Sample Annotations in the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE**The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

## **Replicate Groups**

**Replicate Groups** enable data to be organized into custom groups during data analysis (see Figure 9-12).

Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.	
20	x		C2	SA (Streptavidin)		3	C3	
21	x		C2	SA (Streptavidin)		3	C3	
22	x		D2	SA (Streptavidin)		4	D3	
23	x		D2	SA (Streptavidin)		4	D3	
24	x		E2	SA (Streptavidin)		5	E3	
25	x		E2	SA (Streptavidin)		5	E3	
26	x		F2	SA (Streptavidin)		6	F3	
27	x		F2	SA (Streptavidin)		6	F3	
28	x		G2	SA (Streptavidin)		6	G3	
29	x		G2	SA (Streptavidin)		6	G3	
30	x		H2	SA (Streptavidin)		6	H3	
31	x		H2	SA (Streptavidin)		6	H3	Ξ
32	x		A3	SA (Streptavidin)		1	A3	
33	x		A3	SA (Streptavidin)		1	A3	
34	x		B3	SA (Streptavidin)		2	B3	
35	x		B3	SA (Streptavidin)		2	B3	
36	x		C3	SA (Streptavidin)		3	C3	
37	x		C3	SA (Streptavidin)		3	C3	
38	x		D3	SA (Streptavidin)		4	D3	
39	v		צח	SA (Strantavidin)		4	D3	Ψ.
<							•	



NOTICE: Replicate Group information can also be entered in the software.

Assigning Replicate Groups in the Sample Plate Map

#### To assign **Replicate Groups** in the **Sample Plate Map**:

- 1. Select the samples you wish to group, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 9-13), enter a name in the Replicate Group box and click OK.

Well Information	Dilution Series		
Sample ID:	Apply to:	Concer	ntration
Analyte 1		🔵 Molar (	Concentration
Replicate Group:	Starting value	e (μg/ml): [1	0
Mall lafa marking	Series operat	ior: /	$\sim$
1x Kinetics Buffer	Series operar	nd: 2	
	Dilution orier	ntation	
		Right	Left
Well Data - Sample only		Down	OUp
Molecular Weight (kD): 150		ěě	
Molar Concentration (nM):			
Concentration (up/ml):			

Figure 9-13: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The Octet<sup>®</sup> BLI Analysis software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 9-14).



Figure 9-14: Replicate Groups in Sample Plate Map

		1					
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
🔵 A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
🔵 B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
🔵 C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
🔵 D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
🔵 E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
🔵 F4	Association	6	Reference				1X Kinetics Buffer
🔵 G4	Association	6	Reference				1X Kinetics Buffer
🔵 H4	Association	6	Reference				1X Kinetics Buffer
🔵 A5	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
🔵 B5	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
🔵 C5	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
🔵 D5	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
🔵 E5	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
🔵 F5	Association	6	Reference				1X Kinetics Buffer

The Sample Plate Table updates with the Replicate Group names entered (see Figure 9-15).

Figure 9-15: Replicate Groups in Sample Plate Table

## Assigning Replicate Groups in the Sample Plate Table

#### To assign Replicate Groups in the Sample Plate Table:

- 1. Double-click the desired cell in the **Replicate Group** table column.
- 2. Enter a group name (see Figure 9-16).

Sample	Plate Table				Ca	oncentration units:	µg/ml	•
					M	olar concentration un	its: nM	•
Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information	
🔵 A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffe	эr
🔵 B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffe	зr
🔵 C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffe	зr
🔵 D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffe	зr
🔵 E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffe	эr
🔵 F4	Association	6	Reference				1X Kinetics Buffe	эr
🔵 G4	Association	6	Reference				1X Kinetics Buffe	эr
🔴 H4	Association	6	Reference				1X Kinetics Buffe	эr

Figure 9-16: Add Replicate Group from the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software only recognizes and groups samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

## Editing the Sample Table

## Changing Sample Well Designations

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection.

								Concentration units:	µg/ml	
			0	Sample				Molar concentration units:	nM	
₩ell	Sample ID	Replicat	ŏ	Reference	.D]	Molar Conc (nM)	Information			
B) A1			õ	Control						
🖲 B1				Negative Control						
C1				Negative Control						
B) D1			C	Positive Control						
B) E1			₿	Buffer						
B) F1			(8)	Activation						
B) G1			0	Quench						
B) H1				Load						
L) A2			()	Wash						
L) B2			R	Regeneration						
L) C2			N	Neutralization						
L) D2			0		_					
L) E2				Set Well Data						
L) F2				Clear Data						
L) G2				Convite Clinhoard						
D H2			-	Copy to Cipboard						
A3	-		~	Extended Sample Types						
В3				Sample						
				Canal						

Figure 9-17: Sample Plate Table - Well Designation

## Editing Sample Information

To edit sample data in the Sample Plate Table, double-click a value and enter a new value.

Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information	
📵 H3	Dissociation		Buffer				1X Kinetics Buffer	
🔵 A4	Association	1	Sample	10	150	66.67	1Y Kinotice Buffor	
🔵 B4	Association	2	Sample	5	150	Jndo		
🔵 C4	Association	3	Sample	2.5	150	Cut		
🔵 D4	Association	4	Sample	1.25	150	-		
🔵 E4	Association	5	Sample	0.625	150	гору		
🔵 F4	Association	6	Reference			Paste		
🔵 G4	Association	6	Reference			Delete		
🔴 H4	Association	6	Reference			- I - I AU		
🔵 A5	Association	1	Sample	10	150	Select All		
🔵 B5	Association	2	Sample	5	150	Right to left Readir	na order	
🔵 C5	Association	3	Sample	2.5	150 ,	Show Unicode con	trol characters	
🔵 D5	Association	4	Sample	1.25	150			
🔵 E5	Association	5	Sample	0.625	150	nsert Unicode con	trol character	,
🔵 F5	Association	6	Reference			Open IME		
🔴 G5	Association	6	Reference			Reconversion		
🔴 H5	Association	6	Reference			(cconversion		

Figure 9-18: Sample Plate Table - Editing Sample Data

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the right-click menu used to designate sample types.

## Managing Sample Plate Definitions

NOTICE: After you define a sample plate, you can export and save the plate definition for future use.

## Exporting a Plate Definition

To export a plate definition:

### 1. In the Sample Plate Map, click Export.

Plate 1 (96 wells)
Unassigned Import Export Remove Print

Figure 9-19: Sample Plate Map-Export Button

2. In the **Export Plate Definition** window, select a folder, enter a name for the plate (.csv), and click **Save**.



Figure 9-20: Export Plate Definition Window

## Importing a Plate Definition

To import a plate definition:

1. In the Sample Plate Definition window (see Figure 9-19: on page 368), click **Import**.



Figure 9-21: Sample Plate Map- Import Button

2. In the Import Plate Definition window (see Figure 9-22), select the plate definition (.csv), and click Open.

🖽 Import Plate 1 Definition					×
🔶 🔿 🗠 🋧 📙 > Thi	s PC → Local Disk (C:) → data	~	ى بې Se	arch data	
Organize 🔻 New folde	r			== -	?
	Name	Date modified	Туре	Size	
Quick access	96 standard plate.csv	1/8/2021 10:14 AM	CSV File	1 KB	
<ul> <li>OneDrive</li> </ul>					
💻 This PC					
3D Objects					
E Desktop					
Documents					
🖶 Downloads					
Music					
Pictures					
Videos					
🏪 Local Disk (C:)					
C DVD Drive (D:) O					
· · · · · · · · · · · · · · · · · · ·					
File <u>n</u> a	96 standard plate.csv		<ul> <li>✓ CSV File</li> </ul>	es (*.csv)	~
			Or	oen Canc	el

Figure 9-22: Import Plate Definition Window

NOTICE: You can also create a .csv file for import. Figure 9-23 shows the appropriate column information layout.

	А	В	С	D	E	F	G	Н	-
1	PlateWells	96							
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information	=
3	A1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
4	B1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
5	C1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
6	D1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
7	E1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
8	F1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
9	G1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
10	H1	Kinetics Buffer		Buffer				<b>1X Kinetics Buffer</b>	
11	A2	Loading		Load				12.5 ug/ml ProA	
12	B2	Loading		Load				12.5 ug/ml ProA	Ŧ
14 -	( ) × × 96 s	tandard plate ⁄ 🖅							

Figure 9-23: Example Plate Definition File (.csv)

## Printing a Sample Plate Definition

To print a plate definition:

## 1. In the Sample Plate Map (see Figure 9-24), click Print.



Figure 9-24: Sample Plate Print Button

The associated Sample Plate Table information will print.

## Defining a Kinetic Assay

After the sample plate is defined, the assay must be defined. The steps to define a kinetic assay include

## Defining Step Types

	Step	See Page
1.	Define the step types.	371
2.	Build the assay by assigning a step type to a column(s) in the sample plate.	375
3.	Save the sample plate definition (optional).	368

Table 9-3 lists the example step types to define a kinetic assay. Use these examples as a starting point to create your own step types.

#### Table 9-3: Sample Step Types for Kinetic Assays

Step Type	Step Description
Association	Calculates the $k_{obs}$ and the $k_{a}$ . Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the $k_{d}$ . Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the base- line step directly precedes an association step, perform the baseline step at 1,000 rpm.
	<b>IMPORTANT:</b> An assay must include a baseline step followed by a set of association/dissocia- tion steps to be analyzed. The software recognizes the baseline/association/dissociation step series during processing. Data cannot be processed if this sequence is not included in the assay setup.
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor.
	<b>NOTICE:</b> This step may be performed offline (outside the $Octet^{\mathbb{B}}$ instrument).
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.
Regeneration	Used when employing a reagent to chemically regenerate biosensors and remove bound ana- lyte.
Custom	Can be used for an activity not included in any of the above step types.

### Creating Step Types

Click the **Assay Definition** tab, or click the  $\rightarrow$  arrow to access the Assay Definition window (Figure 9-25). The **Step Data List** shows the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay steps:

- 1. Click Add.
- 2. In Assay Step Definition dialog box (Figure 9-25), specify the step information:
  - a. Choose a step type.
  - b. Optional: Edit the step name.
  - c. Set the step time and shake speed (**Time** range: 2 to 48,000 seconds, **Shake speed**: Off 0 rpm or On (100 to 1,500 rpm).

1 Plate Definition 2 Assay	Definition <b>3</b> Sensor Assignment	nent	4	Review Experime	ent <b>5</b> Ru	in Experiment					
In this step, the assay steps will be assembled from the Step Data List. Select a group of sensors and append the currently selected step into the current assay with a double click, or right click for more options.											
Plate 1 (96 wells)				Sten Data List -				1	lîme in (s),	Shake speed	lin (npm)
			_	Add	Сору	Remove	Regen	eration Pa	arams	Threshold P	arams
				Name	Time Sha	ake speed T	уре	Thre	eshold		
BBLBO	$\mathbf{O}$			→ Baseline	600 1000	<u>ط</u> ر (	Baseline				_
		Add	l Step	Definitions						×	
	$\overline{\mathbf{O}}$				Name		Tin	e (s)	Shake	speed (rpm)	
			۲	Association	Associa	tion	60	)	1000	<b>•</b>	
FBLBOO			Δ.	Dissociation	Dissocia	ation	60	)	1000		
GBLBOO			<u></u>	Baseline	Baseline	•	60	)	1000	×	Table
HBLBOO			Ľ	Loading	Loading	1	60	)	1000		mmen
Assayed samples	O Unassigned samples		<u></u>	Activation	Activatio	on	60	)	1000		
			Ŧ	Quenching	Quench	ing	60		1000	-	



- 3. Apply a threshold to the step:
  - a. In the Step Data List, click the Threshold check box.

The Threshold Parameters dialog box appears (see Figure 9-26).

b. Set the threshold parameters (refer to Table 9-4 for the parameter definitions).



Figure 9-26: Setting Assay Step Threshold Parameters

NOTICE: If thresholds are applied, the step is terminated when either the step time elapses or the threshold termination criteria is reached.

Table 9-4: Threshold Parameters

ltem	Description
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step:
	The threshold is achieved on ALL channels
	The threshold is achieved on ANY ONE channel
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

Click **OK** to save the newly-defined step. The new step type appears in the **Step Data List**. 4.

Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 9-27). 5.

Step	Data List				
A	dd Copy		Remove Re	generation Params	hreshold Params
	Name	Time	Shake speed	Туре	Threshold
	Baseline	10	1000	🛌 Baseline	
	Loading	20	1000	🖌 Loading	
	Wash	15	1000	🙀 Custom	
	Association	30	1000	🞽 Association	
	Long Dissociation	2000	1000	📐 Dissociation	
•	Regeneration	24	1000	💈 Regeneration	
	Activation	25	1000	🛧 Activation	

Figure 9-27: Step Data List with Step Types

6. To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

### Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the **Step Data List**.

To define a step type by editing an existing one:

1. Double-click the cell in the step's **Name, Time** or **Shake speed** column and then enter a new value. Or, right-click the cell to display a shortcut menu of editing commands (see Figure 9-28, left).

**NOTICE:** Keyboard commands can also be used (Ctrl+x=cut, Ctrl+c=copy, Ctrl+v=paste, Ctrl+z=undo).

2. Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 9-28, right).

Step	) Data List					Step	Data List					
	Add Copy		Remove	Threshold Params			Add Copy		Remove Re	gener	ation Params	hreshold Params
_				1			Name	Time	Shake speed	Тур	e	Threshol J
	Name	Time	Shake speed	Туре	Threshold	>	Baseline	10	1000	<u>⊨</u> E	Baseline 🗸 👻	
	Equilibration	10	1000	蟇 Custom			Loading	20	1000		Association	
	ProA Immobilization	120	1200	V Loading			Wash	15	1000		Dissociation	
4	Bacolino	008		L. Baceline			Association	30	1000		Baseline	
~	Assessed	200	Undo				Long Dissociation	2000	1000		Loading	
	Association	300	Undo				Regeneration	24	1000		Activation	
	Dissociation	600	Cut				Activation	25	1000		Oursehine	
	Regeneration	900	Cut			Assa	y Steps List				Quenching	tep
	Neutralization	10	Сору			Ne	w Assay Move U	p_Mc	ove Down Ren		Regeneration	
	Equilibration?	10	Paste			As	ay Sample Plat	e Ste	p Name Step		Custom	ay Ti
	Equiliprotonic	10				1	A1 1	Bas	eline 👻 🛌 Ba		Dip	C 30
ss	av Steps List		Delete			_						
N	Movel		Select All									
1.4	ew Assay	P I	Select All									
As	say Sample Step	Nam	Right to lef	t Reading order								
			Right to let	t Reduing order								
			Show Unic	ode control chara	octers							
			Insert Unic	ode control chara	cter 🕨							
			and office	cae control chard								
			Open IME									
			Keconversi	on								

Figure 9-28: Editing a Step Value (left) or Step Type (right)

. . . . .

## Building an Assay

After creating the different step types that the assay will use, step types are assigned to columns in the Sample Plate or Reagent Plate maps.

To build an assay:

- 1. Select a step type in the **Step Data List**.
- 2. In the **Sample Plate Map**, double-click the column that is associated with the selected step type. For information about sample plate wells, mouse over a well to view a tool tip (see Figure 9-29).



Figure 9-29: Tool Tip of Well Information

The selected wells are marked with hatching (for example, 🍥) and the step appears in the **Assay Steps List** (see Figure 9-30) with an associated **Assay Time**.

1 Plate Definition 2 Assay D	Definition 3 Sensor Assignment 4	Revie	w Experiment	5 Ru	in Experiment		
In this step, the assay s Select a group of sens	steps will be assembled from the Step Da ors and append the currently selected st	ata List. ep into f	the current assa	y with a	a double click, or r	ight click for more optio	ins.
Plate 1 (96 wells)		- Step I	Data List			Time	in (s), Shake speed in (rpm)
		A	dd C	ору	Remove	Regeneration Param	s Threshold Params
			Name	Time	Shake speed	Туре	Threshold
		•	Baseline	600	1000	Baseline	
			Association	600	1000	Association	
			Dissociation	600	1000	L Dissociation	
			Regeneration	30	1000	Regeneration	
		Assay	Steps List				
		Nev	Assay Mov	ve Up	Move Down	Remove Replicate	Edit Step Info Table
Assayed samples	O Unassigned samples	1	1 1	Ba	seline 🔹 🛌 B	aseline SA (Streptavid	lin) + 0:10:30

Figure 9-30: Assigning a Step Type to a Column in the Sample Plate

3. Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 9-31).

New As	ssay M	ove Up Move Down	Remove	eplicate Edit	Step	
Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time	
1	1	Baseline	🛌 Baseline	SA (Streptavidin)		
1	2	Loading	🖌 Loading	SA (Streptavidin)		
1	7	Wash	🙀 Custom	SA (Streptavidin)		
1	3	Association	🞽 Association	SA (Streptavidin)		
1	8	Long Dissociation	📐 Dissociation	SA (Streptavidin)		
1	10	Regeneration	💈 Regeneration	SA (Streptavidin)	0:35:23	
2	1	Baseline	닖 Baseline	SA (Streptavidin)		
2	2	Loading	🖌 Loading	SA (Streptavidin)		Total Assav Tim
2	7	Wash	🙀 Custom	SA (Streptavidin)		To tail to bay min
2	4	Association	🞽 Association	SA (Streptavidin)		
2	8	Long Dissociation	📐 Dissociation	SA (Streptavidin)	0:35:15	
3	1	Baseline 🔹	🖵 Baseline	SA (Streptavidin) 💌		
3	2	Loading	🖌 Loading	SA (Streptavidin)		
3	7	Wash	🐺 Custom	SA (Streptavidin)		
3	5	Association	🞽 Association	SA (Streptavidin)		
3	8	Long Dissociation	📐 Dissociation	SA (Streptavidin)		
3	10	Regeneration	💈 Regeneration	SA (Streptavidin)	0:35:23	

Figure 9-31: Experiment and Assay Time Updates as Steps Are Added to the Assay

**IMPORTANT:** If you intend to analyze the data from a sample using the Inter-step correction feature in the Octet<sup>®</sup> BLI Discovery software, the assay must use the same well to perform baseline and dissociation for the sample.

#### Adding a Regeneration Step

1. In the Sample Plate Map, assign wells as Regeneration or Neutralization (Figure 9-32).



Figure 9-32: Regeneration Step

2. Click Add (Figure 9-33) to display the Add Step Definition dialog box (Figure 9-34).

Step Data List-

A	dd 🔪 🦲 C	ору	Remove	Regeneration Params	Threshold F	arams
	Name	Time	Shake speed	Туре	Threshold	
	Baseline	60	1000	🛌 Baseline		
•	Regeneration	30	400	💈 Regeneration		

Figure 9-33: Add Button

Add Step Definitions			$\times$
	Name	Time (s)	Shake speed (rpm)
Association	Association	600 🔹	1000 🚔
Dissociation	Dissociation	600 🔶	1000 🜲
🗌 📥 Baseline	Baseline	600 🔹	1000
🗌 🔟 Loading	Loading	600 🔹	1000 🜲
Activation	Activation	600 🗘	1000 ≑
Quenching	Quenching	600	1000
Regeneration	Regeneration	30 🌲	1000 ≑
🗆 🖬 Custom	Custom	600 🖨	1000
Dip 🗵	Dip	600 🜩	1000
	OK Ca	ncel	Defaults

Figure 9-34: Add Step Definition Dialog Box

- 3. Select **Regeneration** and click **OK**.
- 4. Click Regeneration Params (Figure 9-35).

Step [	Data List					
A	dd 🛛 🔂 📿 C	ору	Remove	Regeneration Params	Threshold	Params
	Name	Time	Shake speed	Туре	Threshold	
	Baseline	60	1000	🛌 Baseline		
•	Regeneration	30	400	💈 Regeneration		

Figure 9-35: Regeneration Params Button

The Regeneration Parameters dialog box (Figure 9-36) appears and you can edit Regeneration parameters.

Regeneration Parame	ters	×
Step Name:	Regeneration	
	Time (s)	Shake speed (rpm):
Regeneration:	5	1000
Neutralization:	5	1000
Regeneration cycles:	3	
Total step time:	30 s	OK Cancel

Figure 9-36: Regeneration Parameters Dialog Box

## Replicating Steps within an Assay

To copy steps and add them to an assay:

- 1. In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 9-37, step rows 1-4 are selected).
  - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
  - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
- 2. In the **Replicate Steps** dialog box (see Figure 9-37), click the **Append to current assay** option.
- 3. Click the **Offset steps** check box and set the options, as appropriate. (For more details on offset options, see Table 9-5.)



Figure 9-37: Replicating Assay Steps by Appending

4. Click OK. The step(s) appear at the end of the assay in the Assay Steps List.

Item	Description
Add as a new assay	Adds the replicate step(s) as a new assay to the <b>Assay Steps List</b> .
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
Sample steps only	Applies the offset to the sample plate only.
All steps	Applies the offset to the sample plate.

Table 9-5: Replicate Steps Options .

Table 9-5: Replicate Steps Options (Continued).

Item	Description
Sample steps will be adjusted hori- zontally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter <b>1</b> . Enter <b>0</b> to apply the step(s) to the same columns.

### Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensor column:

- 1. Select a column in the **Sample Plate Map**.
- 2. Right-click to view the shortcut menu and select Start New Assay (see Figure 9-38).
- 3. Add steps to the assay as described earlier.



Figure 9-38: Start New Assay

#### Inserting or Adding an Assay Step

To insert an assay step:

- 1. Select a step in the Step Data List.
- 2. In the Assay Steps List, select the row above where you want to insert the step.
- In the Sample Plate Map, right-click the column to which the step will be applied and select Insert Assay Step. The step is inserted into the Assay Steps List.

To add an assay step:

- 1. Select a step type in the **Step Data List**.
- In the Sample Plate Map, right-click the column to which the step will be applied, and select Add Assay Step. The step is added to the end of the Assay Steps List.

## Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow (**•**) for any step in the assay and select a sensor type from the drop-down list (Figure 9-39). The biosensor type will automatically update for every assay step.



Figure 9-39: Selecting an Assay Sensor Type

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.

## Editing an Assay

To edit the step type or the biosensor type:

#### 1. In the Assay Steps List:

- To change the step type, click the **Step Name** arrow (♥) and select a step name from the drop-down list (Figure 9-40, top).
- To change the biosensor type, click the Sensor Type arrow ( ) for any step in the assay and select a sensor type from the drop-down list (Figure 9-40, bottom). The biosensor type will automatically update for every assay step.

NOTICE: The Step Name drop-down list includes only the step types defined in the Step Data List.

New A:	ssay M	ove Up	Move Do	wn	Re	emove	R	eplicate		Edit 9	itep	J			
Assay	Sample	Step Name			Step	р Туре		Sensor Type			Assa	y Time	•		
1	1	Baseline		F	🛌 Baseline		SA (Strepta	avidi	in)						
1	2	Loading	)		🗾 Loading			SA (Streptavidin)							
1	7 W		/ash		豆 Custom		SA (Streptavidin)								
1	3 Association		<u>₹</u> [	🗶 Association		SA (Streptavidin) 👻									
	8	8 Baseline 10 Loading 1 Wash			n on		SA (Strepta	avidi	in)						
	10						on	SA (Strepta	reptavidin)		0:35	23			
2	1						SA (Streptavidin)								
2	2	Accociation					SA (Streptavidin)								
2	7	Long Dissocia Regeneration		5				SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)							
2	4			clatic	tion )										
!	8			on			0:35				15				
	1	Baseline		Ŀ	🔚 Baseline			SA (Streptavidin)							
J	2	Loading			🔟 Loading			SA (Streptavidin)							
J	7	Wash			🕁 Custom			SA (Streptavidin)							
1	5	Association		K	🗶 Association		on	SA (Strepta	avidi	in)					
3	8	Long Di	Long Dissociation		📐 Dissociation		SA (Streptavidin)								
ļ	10	Regene	Regeneration		Regeneration		SA (Streptavidin)		0:35	:23					
Assay	Sample	Step N	lame	9	Step	р Туре		Sensor T	уре	•	Assa	y Time	•		
	1	Baseline	•	- F	- E	aseline		SA (Strepta	avidi	inj )					
	2	Luduriy		¥ ∓			SA (Streptavidin)								
_	2	Association		掌				SA (Streptavidin)					-		-
	о О	Associa	uon	• K	_ P	Vissociati	ion	SA (Strepta	aviu	nj 🔻					_
	0	Percent	ration	4		legenera	iun	SA (	(Stre	ptavi	din)				
	10			- *			AHC (Anti-hl			lgG Fc Capture)					
-	2	Loading			L Loading		Anti-GST								
;	7	Wash		Ŧ	T Custom			Anti-Human			Fab-C	H1 (FA	B)		
-	4	Associa	tion				Anti-FLAG (F			LG)					
;	8	Long Dissociation		L N	P Association		APS (Aminor			propyls	(ane)				
	1	Paselina					AR	nine R	eactive	15					
) 2	2	Loading			Loading		AR2G (Amine Reactive 2nd Gen)								
	7	Wash		Ŧ	T Custom		SSA (Super Strentavidin)								
	5	Associa	sociation				JJA	SSA (Super Streptavidin)							
3	8	Long Dissociation		L	Dissociation		AM	AMC (Anti-mlgG Fc Capture)							
3	10	Regeneration			Begeneration			Ni-I	NIA	•					
		regene		•		genon		Cus	tom	ı					
								anti	-pe	nta hi	s				

Figure 9-40: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

To reorder or remove an assay step:

- 1. Select a step (row) in the Assay Steps List.
- 2. Click the **Move Up**, **Move Down**, or **Remove** button located above the list.

**IMPORTANT:** An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. The software recognizes the baseline/association/dissociation set of steps.

## Adding an Assay Through Replication

A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

- 1. In the Assay Steps List, select the steps to copy and click Replicate.
  - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
  - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
- 2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 9-41).



Figure 9-41: Adding a Replicate Assay to a Plate

- Click the Offset steps check box and set the options as appropriate (see Table 9-5 on page 378 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the Offset steps option. If the replicate assay uses a different sample column, select Offset steps and the appropriate options.
  - Sample steps only offsets the sample wells by the value specified under Sample steps will be adjusted. The offset will not be applied to reagent wells such as buffer, loading, regeneration, neutralization and detection.
  - All Steps offsets all wells in the assay, including sample and reagent wells, by the value specified under Sample steps will be adjusted.
- 4. Click **OK**. The new assay appears in the **Assay Steps List**.
- 5. Continue to add assay steps as needed.

## Assigning Biosensors to Samples

After you define the sample plate and assay(s), click the **Sensor Assignment** tab, or click the arrow  $\rightarrow$  to access the Sensor Assignment window. The color-coded Sensor Tray and Sample Plate Map show the locations of the biosensors associated with the samples Figure 9-42.

**NOTICE:** If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 9-42, the experiment includes three assays in the same wells. The use of those wells by three different biosensors is indicated by the pie chart colors.

**NOTICE:** The Sensor Type for the assay must be first be defined in the Assay Steps List on the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



Figure 9-42: Sensor Assignment Window

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 9-43).



Figure 9-43: Tool Tip of Well Information

## Replacing the Biosensors in the Biosensor Tray

After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 9-44).



Figure 9-44: Replace Sensors in Tray After Use Check Box

**NOTICE:** Biosensors can be regenerated up to a max of 11 times per experiment.

### Entering Biosensor Information

To enter information about a biosensor:

- Optional: Double-click in any cell in the Lot Number column to enter the biosensor lot number. All wells in the Lot Number column for that biosensor type will automatically populate with the lot number entered (see Figure 9-45).
- 2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 9-45).

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

Well	Sensor Type	Lot Number	Informatio	n
A1	SA (Streptavidin)	10102020	Default	
B1	SA (Streptavidin)	10102020		Undo
C1	SA (Streptavidin)	10102020		
D1	SA (Streptavidin)	10102020		Cut
E1	SA (Streptavidin)	10102020		Сору
F1	SA (Streptavidin)	10102020		Paste
G1	SA (Streptavidin)	10102020		Delete
H1	SA (Streptavidin)	10102020		
A2	SA (Streptavidin)	10102020		Select All
B2	SA (Streptavidin)	10102020		Diskton left Deselie e ander
C2	SA (Streptavidin)	10102020		Right to left Reading order
D2	SA (Streptavidin)	10102020		Show Unicode control characters
E2	SA (Streptavidin)	10102020		Insert Unicode control character
F2	SA (Streptavidin)	10102020		One INF
G2	SA (Streptavidin)	10102020		Open IME
H2	SA (Streptavidin)	10102020		Reconversion

Figure 9-45: Entering or Editing Biosensor Information

#### Changing the Biosensor Location

If you prefer to not use the default biosensor columns, you can select other column(s) to use. There are two ways to do this:

- Method 1—In the Sensor Tray Map, Remove the columns you do not want to use. The software automatically selects the next available column(s).
- Method 2-Remove all columns from the Sensor Tray Map, then select the columns you want to use.

#### Method 1

- In the Sensor Tray Map (see Figure 9-46), select the columns to not use and click Remove. Or, right-click the selection and select Remove (Figure 9-46 left). The software automatically selects the next available biosensor columns in the tray (Figure 9-46 right).
- 2. Click Fill Plate to return the Sensor Tray Map to the default layout.



Figure 9-46: Changing Biosensor Location (Method 1)

#### Method 2

- 1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 9-47 top left). Or, right-click the selection and select **Remove**. All columns will be shown as **Missing** (Figure 9-47 top right).
- 2. Select the column(s) to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 9-47 bottom left). The software fills the selected columns in the tray (Figure 9-47 bottom right).



Figure 9-47: Changing Biosensor Location (Method 2)

Click Fill Plate to return the Sensor Tray Map to the default layout.

### Using Heterogeneous Trays

If heterogeneous biosensor trays will be used, the column location of each biosensor type in the tray can be identified in the **Sensor Assignment Tab**. Assignment of biosensors that will not be used in the assay enables the software to auto-assign the biosensors that will be used in the assay by biosensor type.

There are two ways to change the biosensor type:

 Select a column in the Sensor Tray Map, right-click and select a biosensor type from the drop-down list (Figure 9-48 left). The associated wells in the Sensor Type column will automatically populate with the biosensor type selected.
Select a cell in the Sensor Type table column, click the down arrow and select a biosensor type from the dropdown list (Figure 9-48 right). All other wells in the same column of the Sensor Tray Map as the selected cell will automatically populate with the biosensor type selected.



Figure 9-48: Sensor Assignment Window-Changing the Biosensor Type

The biosensor types shown in the **Sensor Assignment** window were specified previously in the **Assay Definition** window, and default locations are assigned automatically. To assign biosensor types for heterogeneous trays:

1. Select the column location of the biosensor type (see Figure 9-49).



Figure 9-49: Selecting a Sensor Tray Column

Right-click in the Sensor Tray Map or click in a cell in the Sensor Type table column and select a biosensor type from the drop-down list. The biosensor type associated with the assay will shift location accordingly (see Figure 9-50). In the example shown, Streptavidin is the Sensor Type used for the current assay. Column 1 was reassigned as AHC according to the heterogeneous tray being used.



Figure 9-50: Assay Sensor Type Reassignment

3. Repeat the previous steps to assign locations for the remaining biosensor types in the tray.

**IMPORTANT:** Ensure that the biosensor types selected in the Assay Definition window have assigned column(s) in the Sensor Assignment window or the experiment cannot be run.

#### Using Partial Biosensor Trays

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 9-51) includes three assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 9-51 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 9-51 bottom). If necessary, change the location of these biosensors.

Sensor Tray	~	T		
Replace sensors in tray after use	Sensor	r Iray I ∨	of 2	
	Well	Sensor Type	Lot Number	Information
	A5	SA (Streptavidin) 👻		
	B5	SA (Streptavidin)		
	C5	SA (Streptavidin)		
	D5	SA (Streptavidin)		
	E5	SA (Streptavidin)		
	F5	SA (Streptavidin)		
	G5	SA (Streptavidin)		
	H5	SA (Streptavidin)		
	A6	SA (Streptavidin)		
	B6	SA (Streptavidin)		
Legend: Unassigned sensors 🕅 Missing sensors	C6	SA (Streptavidin)		
	D6	SA (Streptavidin)		
	E6	SA (Streptavidin)		
Sensor Trav				
Replace sensors in tray after use	Sensor	r Tray: Tray 2 🗸	of 2	
Replace sensors in tray after use           1         2         3         4         5         6         7         8         9         10         11         12	Sensor Well	Tray: Tray 2 V	of 2	Information
Application	Sensor Well	Tray: Tray 2 Sensor Tray 1 Tray 2 SA (Streptavidin) -	of 2 Lot Number	Information
A         A         5         6         7         9         10         11         12           A         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B	Sensor Well A5 B5	Tray: Tray 2 Sensor Tray 1 Tray 2 SA (Streptavidin) SA (Streptavidin)	of 2	Information
Image: Construction of the second	Sensor Well A5 B5 C5	Tray: Tray 2 Sensor Tray 1 Tray 2 SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)	of 2 Lot Number	Information
Image: Construction of the second	Sensor Well A5 B5 C5 D5	Tray: Tray 2 ∨ Sensor Tray 1 Tray 2 SA (Streptavidin) ▼ SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)	of 2 Lot Number	Information
Image: Construction of the second	Sensor Well A5 B5 C5 D5 E5	Tray: Tray 2 ✓ Sensor Tray 1 Tray 2 SA (Streptavidin) ✓ SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)	of 2	Information
Image: Construction of the second	Sensor Well A5 B5 C5 D5 E5 F5	Tray :     Tray 2       Sensor     Tray 1       Tray 2     Tray 2       SA (Streptavidin) *     SA (Streptavidin) *       SA (Streptavidin)     SA (Streptavidin)       SA (Streptavidin)     SA (Streptavidin)       SA (Streptavidin)     SA (Streptavidin)	of 2 Lot Number	Information
Image: Construction of the sector of the	Sensor Well A5 B5 C5 D5 E5 F5 G5	Tray 2     Tray 1       Sensor     Tray 1       Tray 2     Tray 2       SA (Streptavidin)     Tray 2       SA (Streptavidin)     SA (Streptavidin)	of 2 Lot Number	Information
Project sensors in tray after use         1       2       3       4       5       6       7       9       10       11       12         A       1       2       3       4       5       6       7       9       10       11       12         B       1       1       1       1       1       10       11       12         C       1       1       1       1       1       10       11       12         B       1       1       1       1       1       1       10       11       12         B       1       1       1       1       1       1       1       10       11       12         B       1       1       1       1       1       1       10       10       11       12         C       1       1       1       1       1       1       1       10       10       11       12         C       1       1       1       1       1       10       10       10       10       10       10       10       10       10       10       10       10       10 <td>Sensor <b>Well</b> A5 B5 C5 D5 E5 F5 G5 H5</td> <td>Tray 2     Tray 1       Sensor     Tray 1       Tray 2     Tray 2       SA (Streptavidin)     Tray 2       SA (Streptavidin)     SA (Streptavidin)       SA (Streptavidin)     SA (Streptavidin)</td> <td>of 2 Lot Number</td> <td>Information</td>	Sensor <b>Well</b> A5 B5 C5 D5 E5 F5 G5 H5	Tray 2     Tray 1       Sensor     Tray 1       Tray 2     Tray 2       SA (Streptavidin)     Tray 2       SA (Streptavidin)     SA (Streptavidin)	of 2 Lot Number	Information
Project sensors in tray after use         1       2       3       4       5       6       7       9       10       11       12         A       1       2       3       4       5       6       7       8       10       11       12         B       1       1       1       1       10       11       12         C       1       1       1       10       10       10       11       12         B       1       1       1       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       11       12       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10	Sensor <b>Well</b> B5 C5 D5 E5 F5 G5 H5 A6	Tray :     Tray 2       Sensor     Tray 1       Tray 2     Tray 2       SA (Streptavidin)     Tray 2       SA (Streptavidin)     SA (Streptavidin)	of 2 Lot Number	
Project sensors in tray after use         1       2       3       4       5       6       7       9       10       11       12         A       1       2       3       4       5       6       7       8       10       11       12         B       1       1       1       1       10       11       12         C       1       1       1       10       10       11       12         C       1       1       1       10       10       10       10       10         D       1       1       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10	Sensor Well B5 C5 D5 E5 F5 G5 H5 A6 B6	Tray :         Tray 2           Sensor         Tray 1           Tray 2         Tray 2           SA (Streptavidin)         Tray 2           SA (Streptavidin)         Tray 2           SA (Streptavidin)         SA (Streptavidin)	of 2 Lot Number	
Replace sensors in tray after use         1       2       3       5       6       7       9       10       11       12         A       1       2       3       4       5       6       7       8       10       11       12         B       1       1       1       10       11       12       10       11       12         B       1       1       10       10       11       12       10       11       12         B       1       1       10       10       10       11       12       10       11       12         B       1       1       10       10       10       11       12       10       11       12       10       10       10       10       10       10       11       12       12       10       10       10       10       10       10       10       10       10       10       11       12       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10	Sensor Well A5 C5 D5 E5 F5 G5 H5 A6 B6 C6	Tray :         Tray 2           Sensor         Tray 1           SA (Streptavidin)         Tray 2           SA (Streptavidin)         Tray 2           SA (Streptavidin)         Tray 2           SA (Streptavidin)         SA (Streptavidin)           SA (Streptavidin)         SA (Streptavidin)	of 2 Lot Number	
Propiace sensors in tray after use         1       2       3       5       6       7       9       10       11       12         A       1       2       3       4       5       6       7       8       10       11       12         B       1       1       1       10       11       12       10       11       12         B       1       1       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       11       12       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10       10 </td <td>Sensor Well A5 C5 D5 E5 F5 G5 H5 A6 B6 C6 D6</td> <td>Tray: Tray 2 Sensor Tray 1 Tray 2 SA (Streptavidin) SA (Streptavidin)</td> <td>of 2 Lot Number</td> <td></td>	Sensor Well A5 C5 D5 E5 F5 G5 H5 A6 B6 C6 D6	Tray: Tray 2 Sensor Tray 1 Tray 2 SA (Streptavidin) SA (Streptavidin)	of 2 Lot Number	
Image: Sensors in tray after use         Image: Sensor in	Sensor           Well           A5           B5           C5           D5           E5           F5           G5           H5           A6           B6           C6           D6           E6	Tray : Tray 2 ✓ Tray 1 Tray 1 Tray 2 SA (Streptavidin) ✓ SA (Streptavidin) ✓ SA (Streptavidin) SA (Streptavidin)	of 2 Lot Number	

Figure 9-51: Example Experiment Using Two Biosensor Trays

**NOTICE:** Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet<sup>®</sup> instrument.

#### **Reference Biosensors**

To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.

NOTICE: Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

#### Changing the Biosensor Type

The biosensor type used in the assay must be selected in the **Assay Definition** window. To change the biosensor type:

- 1. Click the Assay Definition Tab.
- 2. In the Assay Steps List, click the cell in the Sensor Type column to change.
- 3. Select from the drop-down list (see Figure 9-52).

**IMPORTANT:** Ensure that the same biosensor types are selected in both the Assay Definition and the Sensor Assignment windows or the experiment cannot be run.

New A:	ssay Mi	ove Up Move Dowr	Remove	eplicate Edit 9	Step	
Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time	
1	1	Baseline	🛌 Baseline	SA (Streptavidin)		
1	2	Loading	📝 Loading	SA (Streptavidin)		
1	7	Wash	₩ Custom	SA (Streptavidin)		
1	3	Association -	🖌 Association	SA (Streptavidin) 👻		
1	8	Long Dissociation	▶ Dissociation	SA (Streptavi)	din)	1
1	10	Regeneration	Regeneration	AHC (Anti-h	InG Ec Canture)	
2	1	Baseline	🔙 Baseline	Anti CST	igo i e cupture)	
2	2	Loading	📝 Loading	Anti-OST		
2	7	Wash	🛱 Custom	Anti-Human	Fab-CH1 (FAB)	
2	4	Association	C Association	Anti-FLAG (F	LG)	
2	8	Long Dissociation	N Dissociation	APS (Amino	propyls (ane)	

Figure 9-52: Assay Definition Window-Changing the Biosensor Type

## **Reviewing Experiments**

Before running an experiment, you can review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window (Figure 9-53), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the  $\leftarrow$  arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.



Figure 9-53: Review Experiment Window

# Saving Experiments

After an experiment is run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment:

1. Click Save Method File ( ), or on the main menu, click File > Save Method File.

If there is more than one open experiment and you want to save all of them, click Save All Methods Files 😤 .

2. In the Save dialog box, enter a name and location for the file, and click Save.

**NOTICE:** If you edit a saved experiment and want to save it without overwriting the original file, click File > Save Method File As and enter a new name for the experiment.

## Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment > Templates > Kinetics > Experiment Name** (Figure 9-54).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\Sartorius\OctetBLIDiscovery\TemplateFiles.

**IMPORTANT:** Do not change the location of the Template folder. If the Template folder is moved from the factoryset location, the software may not function properly.

Experiment Instrument Win	dow Help					
✓ New Experiment Wizard Edit Assay Parameters	Ctrl+N					
Edit Sensor Types						
Set Plate Temperature						
Templates	•	Kinetics	•	Biomolecule kinetics - AHC biosensor	•	Kinetic Characterization_8CH_96W.fmf
Skip Step		Quantitation	•	Biomolecule kinetics - AMC biosensor	•	Screening_8CH_96W.fmf
Stop				Biomolecule kinetics - AR biosensor	- 1	
		1		Biomolecule kinetics - SA biosensor		
				Small Molecule and Fragment Kinetics - SSA biosensor	•	

Figure 9-54: Saved Experiments in the Template Folder

## Running a Kinetics Experiment

**IMPORTANT:** Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.

## Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

- 1. Open the Octet<sup>®</sup> instrument door (lift the handle up).
- 2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 9-55).

3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 9-55).



Figure 9-55: Biosensor Stage (left) and Sample Stage (right)

**IMPORTANT:** Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.

- 4. Octet<sup>®</sup> RED96e and Octet<sup>®</sup> R8 only, optional. Cover the microplate with the evaporation cover to prevent evaporation from samples during analysis and lengthen the experiment time (only applies to RED96e and Octet<sup>®</sup> R8 instruments). For more information, see "Microplate Evaporation Cover" on page 64.
- 5. Close the Octet<sup>®</sup> instrument door.
- 6. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you're cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

## Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow ( $\rightarrow$ ) to access the Run Experiment window (see Figure 9-56).

Data File Location a	and Names			Prior to pres	sing "Go" confirm the Assay.
Kinetics data reposi	itory:	C:\data			
Experiment run nam	ne (sub directory):	Experiment_1	-	•	
Plate name/barcod	le (file prefix):	201103			
Auto-increment file	ID start:	1			
Data files will be sto	ored as follows:			Total experime	nt time:
C:\data\Experimen C:\data\Experimen C:\data\Experimen 	ıt_1∖201103_001.frd ıt_1∖201103_002.frd ıt_1∖201103_003.frd			2.07.30	
Run Settings					
Delayed experin Shake sample p	nent start Start after (s): 600 🚔 plate while waiting	✓ Open runtime charts au ✓ Automatically sav ✓ Set plate temperature (	utomatically e runtime chart °C): 25		
Delayed experim     Settings     Shake sample p     Advanced Settings	nent start Start after (s): 600 ▲	✓ Open runtime charts au ✓ Automatically sav ✓ Set plate temperature (	utomatically e runtime chart °C): 25 🐳		
Delayed experin     Shake sample p     Advanced Settings     Acquisition rate:	nent start Start after (s): 600 🚖 olate while waiting Standard kinetics	Open runtime charts au Automatically sav Set plate temperature ( 5.0 Hz)	utomatically e runtime chart "C): 25		
Advanced Settings	nent start Start after (s): 600 (\$ I and a standard kinetics Standard kinetics Warning: changing the J are unsure of how to	C Open runtime charts au Automatically sav Set plate temperature ( 5.0 Hz) se settings could affect assay signal to-no use these settings, please consult the Us	tomatically e runtime chart "C): 25 + Default vise. er Guide		
Advanced Settings Advanced Settings Acquisition rate:	nent start Start after (s): 600 (\$ Idate while waiting Standard kinetics Warning: changing the J are unsure of how to	C Open runtime charts au Automatically sav Set plate temperature ( 5.0 Hz) se settings could affect assay signal-to-no use these settings, please consult the Us	tomatically e runtime chart "C): 25 🐳 Default vise. er Guide		
Advanced Settings Advanced Settings Advanced Settings Acquisition rate: If you Seneral Information User name:	nent start Start after (s): 600 ( start after	C Open runtime charts au Automatically sav C Set plate temperature ( 5.0 Hz) Se settings could affect assay signal-to-nc use these settings, please consult the Us Machine name: DESKT	tomatically e runtime chart "C): 25 🔪 Default oise. er Guide		
Advanced Settings Advanced Settings Advanced Settings Acquisition rate: If you Seneral Information User name: Description:	nent start Start after (s): 600 ( Standard kinetics Standard kinetics Warning: changing the u are unsure of how to	C Open runtime charts au Automatically sav Set plate temperature ( 5.0 Hz) Se settings could affect assay signal-to-nc use these settings, please consult the Us Machine name: DESKT	tomatically e runtime chart "C): 25 🐳 Default oise. er Guide		

Figure 9-56: Run Experiment Window-Octet RED96

2. Confirm the default settings or enter new settings. See "Run Experiment Window Settings" on page 395 for more information on experimental settings.

**NOTICE:** If you delay the experiment start, you have the option to shake the plate until the experiment starts. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you're cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

- 3. Optional if you are using a microplate evaporation cover. Hold plate at temperature after run is pertinent when you are running very long experiments with the evaporation cover. If you are running a 10-12 hour assay and want to ensure that the plate temperature remains at the set plate temperature, then check Hold plate at temperature after run. If it is acceptable for the plate to go back to room temperature post-run, then leave that option unchecked.
- 4. To start the experiment, click **GO**.

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 9-57).

**NOTICE:** For more details about the Runtime Binding Chart, see "Managing the Runtime Binding Chart" on page 397.



Figure 9-57: Runtime Binding Chart

5. Optional: Click View > Instrument Status to view the log file (see Figure 9-58).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.

Instrume	ent Status			3
14:47:39	Sensor 7: Integration Time = 1.0 ms			
14:47:39	Sensor 8: Integration Time = 1.0 ms			
14:47:40	Picking sensors completed location A1			
14:47:40	Plate temperature = 30 C			
014:47:40	Ready to move to sample location A1			
014:47:40	Moving to sample location A1			
014:47:41	Arrived at sample location A1			
014:47:41	Waiting to start sample location A1			
14:47:41	Processing sample location A1			
014:47:51	Sample completed location A1			
-14:47:51	Waiting to start new step			
-14:47:51	Starting new step			
014:47:52	Ready to move to sample location A2			
014:47:52	Moving to sample location A2			=
014:47:53	Arrived at sample location A2			
014:47:53	Waiting to start sample location A2			
14:47:53	Processing sample location A2			
				Ψ.
•	III		•	
🔽 Auto scro	I to bottom	Sa	ve to Fi	e

Figure 9-58: Instrument Status Log



**WARNING:** Do not open the Octet<sup>®</sup> instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



**WARNING:** N'ouvrez pas la porte de l'instrument Octet<sup>®</sup> lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



**WARNING:** Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

## Run Experiment Window Settings

The following Data File Location and Name settings are available on the Run Experiment Tab:

#### Table 9-6: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Kinetics data reposi- tory	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click <b>Browse</b> to select another data location.
	<b>NOTICE:</b> Save the data to the local machine first, then transfer to a network drive.
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/bar- code	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

#### The following Run Settings are available on the Run Experiment Tab:

Table 9-7: Run Settings

ltem	Description
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click <b>GO</b> .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the <b>Runtime Binding Chart</b> for the current biosensor during data acquisition.

#### Table 9-7: Run Settings (Continued)

Item	Description			
Automatically save runtime chart	Saves an image (.jpg) of the <b>Runtime Binding Chart</b> . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.			
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in <b>File &gt; Options</b> . The factory set default temperature is 30 °C.			
	<b>NOTICE:</b> If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet <sup>®</sup> BLI Discovery software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.			

The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet<sup>®</sup> system per minute and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. The choice of a setting should be determined based upon consideration of the binding rate and the amount of signal generated in your assay, and some experimentation with the settings.

#### Table 9-8: Advanced Settings

Item	Description
Acquisition rate • Octet <sup>®</sup> QKe	<ul> <li>High sensitivity kinetics (0.3 Hz, averaging by 40) - The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds.</li> </ul>
	<ul> <li>Standard kinetics (0.6 Hz, averaging by 5) - The average of five data frames is reported as one data point. One data point is reported every 1.6 seconds.</li> </ul>
Acquisition rate <ul> <li>Octet<sup>®</sup> RED96,</li> <li>Octet<sup>®</sup> RED96e</li> </ul>	<ul> <li>High sensitivity kinetics (2 Hz, averaging by 50): - The average of 50 data frames is reported as one data point. Two data points are reported per second.</li> </ul>
Octet <sup>®</sup> R8	<ul> <li>Standard kinetics (5 Hz, averaging by 20 - The average of 20 data frames is reported as one data point.</li> </ul>
Sensor offset (mm) • Octet <sup>®</sup> QKe only	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets acquisition rate and sensor offset to the defaults.

## Stopping an Experiment

To stop an experiment in progress, click  $\bigotimes$  or click **Experiment** > **Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.

NOTICE: After the experiment is run, the software automatically saves the experiment method (.fmf).

# Managing the Runtime Binding Chart

If the **Open runtime charts automatically** check box is selected in the Run Experiment window (Figure 9-59), the Runtime Binding Charts are automatically displayed when data acquisition starts. The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active biosensor column is colorcoded (A=green, B=magenta, C=orange, D=purple, E=olive, F= black, G=red, H=blue) within the **Sensor Tray Map**. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each assay in the experiment is represented by **Assay X** in the **Current Binding Charts** box.

To display the data for a particular assay:

- 1. Click the corresponding Assay number.
- 2. Select a subset of sensors for a displayed column under Sensors to Chart box (see Figure 9-59).

**IMPORTANT:** Do not close the Runtime Binding Chart window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet<sup>®</sup> BLI Discovery software saves the Runtime Binding Chart as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.



Figure 9-59: Runtime Binding Chart Window

## Opening the Runtime Binding Chart

After an experiment is run, you can open and review the Runtime Binding Chart at any time:

- 1. Click File > Open Experiment.
- 2. In the dialog box that appears, select an experiment folder and click **Select**.

## Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the Sensor Assignment tab
- During acquisition in the Runtime Binding Chart Sensors to Chart box
- During analysis in the **Data Selection** tab

## Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the Sensors to Chart list or the Sensor Tray, right-click a biosensor and select Reference (see Figure 9-60).



Figure 9-60: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray (**see Figure 9-61).

2. Click the Subtract reference sensors check box (see Figure 9-61).



Figure 9-61: Subtract Reference Sensors check box in the Runtime Binding Chart

**NOTICE:** Subtracting reference data in the Runtime Binding Chart only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be repeated during data analysis if needed.

## Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 9-62). Uncheck the box to return to the default data display.



Figure 9-62: Data Inverted Using Flip Data Function

## Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 9-63), right-click a step and select **Align to Step <number>**.



#### To remove the step alignment, right-click the step and select **Unaligned**.

Figure 9-63: Runtime Binding Chart–Aligning the Data to a User-Selected Step

## Aligning Data to a Specific Time

1. To align the binding data to a specific time, in the **Runtime Binding Chart** (see Figure 9-64), right-click and select **Align at time**.



Figure 9-64: Runtime Binding Chart-Aligning the Data to a User-Specified Time

The Align at Time dialog box appears (Figure 9-65).



Figure 9-65: Align at Time Dialog Box

2. Enter the time point you want to align to and click **OK**. The binding chart will then align to the time point specified.

To remove the time alignment, right-click and select **Unaligned**.

## Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.

**NOTICE:** If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.

To extend the duration of the active step:

- 1. In the chart window, click the **Extend Current Step** button.
- 2. In the **Extend Current Step** dialog box (see Figure 9-66), enter the number of seconds to extend the step and click **OK**.

Extending this s Parallel Referen	tep will prevent da ce, Double Refere	ata analysis if ence, or Avera	these sensors are ige Reference sul	e to be used in btraction methods.
Assay 1, Step 2				
Duration (s):	600			

Figure 9-66: Extend Current Step Dialog Box

## Terminating a Step to Begin the Next Step

To terminate a step and begin the next step in the assay:

- 1. In the chart window, click the **Go to Next Step** button.
- 2. In the Data Acquisition dialog box, click OK.

## Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the area.

To undo the magnification, right-click the chart and select **Undo Zoom**.

## Scaling a Runtime Binding Chart

#### To scale the Runtime Binding Chart:

- 1. Right-click the chart and select Properties.
- 2. In the Runtime Graph Properties dialog box, select Fullscale or Autoscale.

## Adding a Runtime Binding Chart Title

#### To add a Runtime Binding Chart title:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, enter a graph title or subtitle.

## Selecting a Runtime Binding Chart Legend

To select a Runtime Binding Chart legend:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box (see Figure 9-67), select one of the following legends:
  - Sensor Location
  - Sample ID
  - Sensor Information
  - Concentration/Dilution

Runtime Graph Propert	ies 🔼
Title:	
Subtitle:	
Legend	
Sensor Location	Sensor Information
Sample ID	Concentration / Dilution
	~

Figure 9-67: Selecting a Runtime Binding Chart Legend

**NOTICE:** Text for Sample ID, Sensor Information, or Concentration/Dilution is taken from the Plate Definition and Sensor Assignment tabs, and must be entered before the experiment is started.

3. Click OK.

## Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click Window > New Window.

## Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

1. Right-click the chart and select **Export Data**.

2. In the **Exporting** dialog box (see Figure 9-68), select the export options and click **Export**.

porting						
Export EMF	© WMF	O BMP	© JPG	O PNG	⊚ Text / D	ata
Export D	estination					
ClipB	oard					
🔘 File		Browse				
Printe	er					
Export S	ize					
		Millimete	rs 🔘 Ir	iches 🔘 Po	pints	
	Width: 152.4	1 00	101.600	Millimeters		Export

Figure 9-68: Exporting Dialog Box

 Table 9-9: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	$\checkmark$		Click <b>File</b> > <b>Browse</b> to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Run- time Binding Chart to a graphic file		$\checkmark$	Click <b>File &gt; Browse</b> to select a folder and enter a file name.	Creates a graphic image.
Copy the Run- time Binding Chart		$\checkmark$	Clipboard	Copies the chart to the system clip- board
Print the Runtime Binding Chart		$\checkmark$	Printer	Opens the Print dialog box.

# Managing Experiment Method Files

After you run an experiment, the Octet<sup>®</sup> BLI Discovery software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. A read-only copy of the method used for an experiment is automatically saved in the experiment folder. Open a method (.fmf) and edit it if necessary.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Menu Bar Command/Toolbar Button	Description
File > Open Method File 📩	Enables you to select and open a method file (.fmf)
File > Save Method File 📩 or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

Table 9-10: Managing Experiment Method Files

# Epitope Binning

The goal of a typical epitope binning or cross-blocking experiment is to identify antibodies which bind to different or identical epitopes on the antigen. Antibodies are tested two at a time for competitive binding to one antigen. By competing antibodies against one another in a pairwise and combinatorial format, antibodies with distinct blocking behaviors can be discriminated and assigned to "bins". The end result is matrix of pairwise binders and blockers.

An epitope binning or cross-blocking experiment must be run as a kinetic experiment with repeating steps in the Octet<sup>®</sup> BLI Discovery software.

**NOTICE:** Sartorius highly recommends using the Loading, Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

After starting the Octet<sup>®</sup> system and the Octet<sup>®</sup> BLI Discovery software, follow the steps in Table 9-11 to set up and run an epitope binning experiment.

 Table 9-11: Octet<sup>®</sup> BLI Discovery Steps for Epitope Binning Assays

Octet Software		Functions			
Octet <sup>®</sup> BLI Discovery	1.	Select Epitope Binning under New Kinetics Experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf).			
	NC set me tio	<b>NOTICE:</b> In the Experiment Menu, the Templates command allows users to pick from a set of predefined method templates for Kinetic, Quantitation, or Epitope Binning experiments. Users may also modify existing method templates to suit their experimental conductions and save as a new method file and new method file name.			
	2.	Define a sample plate or open a sample plate definition.			
	3.	Specify assay steps.			
	4.	Assign biosensors to samples.			
	5.	Run the experiment.			

## Starting an Experiment

You can start a kinetics experiment using one of the following options:

- Launch the Experiment Wizard by clicking Experiment > New Experiment Wizard, and selecting New Kinetics Experiment and Epitope Binning.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click Experiment > Templates > Epitope Binning.
- 6. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

### Tab1(Plate Definition)

NOTICE: The Sample plate and the Reagent plate are now referred to as "Plate 1" and "Plate 2" in the software.

- 1. Designate layouts for the plate by selecting wells in the plate map and designating sample types. There are several ways to select sample wells in the plate map:
  - · Click a column header or select adjacent column headers by click-hold-drag.
  - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
  - Click a row header or select adjacent row headers by click-hold-drag.
  - Click a well or draw a box around a group of wells.
- 2. Designate well types by right-clicking on selected wells and assigning a sample type:





- 3. Enter sample information by selecting the table for the plate. There are several ways to enter sample information:
  - Select an individual well in the plate table.
  - · Click-drag-hold several wells in the plate table, right-click and choose Set Well Data.

**NOTICE:** Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.

**NOTICE:** More information on sample information and annotation can be found in "Entering Sample Information" on page 360.

#### Tab 2 (Assay Definition)

After completing the plate layout, an Epitope Binning Assay can be defined by building a kinetic assay.

- 1. Click on Tab 2 (Assay Definition).
- 2. Add assay step types in the Step Data List:
  - a. Click the **Add** button. The Add Step Definition box will display:

Add Step	Definitions			$\times$
	Association Dissociation	Name Association Dissociation	Time (s)	Shake speed (rpm) 1000
	Baseline Loading Activation	Baseline       Loading       Activation		
□ ¥ ₹	Quenching Regeneration Custom	Quenching Regeneration Custom	600 ÷ 30 ÷ 600 •	1000 🗭 1000 🗢 1000 🗢
	Dip	Dip OK Ca	600 🚖	1000 Cefaults

Figure 9-70: Add Step Definition Box

- b. Choose a step type.
- c. Optional: edit step name.
- d. Set the step time and shake speed.
- e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

Regeneration Paramete	ers		×
Step Name:	Regeneration		
	Time (s)	Shake speed (rpm):	
Regeneration:	5	1000	
Neutralization:	5	1000	
Regeneration cycles:	3		
Total step time:	30 s	ОК	Cancel

Figure 9-71: Regeneration Parameters Box

- f. Optional: assign a threshold. See "Creating Step Types" on page 372 for more information.
- 3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).

**NOTICE:** Sartorius highly recommends using the Associate or Dissociate assay steps instead of Custom for epitope binning and cross-blocking experiments.

- a. Select a step type in the Step Data List.
- b. In the plate map, double-click the columns that you want associated with that step type.
- c. The selected wells are marked with hatching, and the new step appears in the Assay Steps List:

Plate Definition 2 Assa In this step, the ass Select a group of se	ay steps will be assembled from the Step i ensors and append the currently selected	4 Revie Data List step into	the curre	ment <b>5</b> R ntassaywith	un Experir a double (	nent click, or	right click	for more option	15.	
Dista 1 (0C usella)		Cier.	Data List					Time	in (s), Shake speed	in (p
riate i (56 weis)		Step	Udia Usi	Conv	Rem	ove	Regen	eration Params	Threshold Pa	rams
1 2 3 4 5	6 7 8 9 10 11 12		Masa	000)	Trees	Chal	riogor	Trees	Thread-old	
A 🎯 🎯 🎯 🎯	} <b>@@@@@@@@</b>	-	Sensor	check	30	1000	e speeu	I Raseline	Threshold	9
B 🔕 🕲 🕲 🕲 🕲	300000000	-	Antigen	immobilization	300	1000				
ŇŇŇŇĬ	ààààààààà		Raceline		30	1000		Receipe		
			Saturati	- na Ah (1st Ah	600	1000		Associatio		
	? <b>````````````````````````````````</b> ``````		competi	ing Ab (2nd A	300	1000				
		Assa Ner	y Steps Li w Assay	st Move Up	Move I	Down	Remove	Replicate	Edit Step Info	o Tab
$\sqcap @ @ @ @ @ @ @ @ @ @ @ @ @ @ @ @ @ @ @$	2	Ass	ay No.	Sample S	ep Nam	е		Step Type	Sensor Type	
Assayed samples	<ul> <li>Unassigned samples</li> </ul>	1	1	1 Se	nsor che	*	•	Baseline	SA (Streptavidin)	-
		1	2	2 Ar	tigen imm	obilizati	ion 📘	🖊 Loading	SA (Streptavidin)	
		1	3	3 Ba	iseline		ŀ	Baseline	SA (Streptavidin)	
		1	4	4 Sa	turating A	b (1st /	Ab)	Association	SA (Streptavidin)	
		1	5	5 Ba	iseline		ł	Baseline	SA (Streptavidin)	
		1	6	6 cc	mpeting /	Vb (2nd	Ab) 👔	Association	SA (Streptavidin)	
		2	1	1 Se	nsor che	*	L	Baseline	SA (Streptavidin)	
		2	2	2 Ar	tigen imm	obilizati	ion 🚦	🖌 Loading	SA (Streptavidin)	
		2	3	3 Ba	iseline		1	Baseline	SA (Streptavidin)	
		2	4	4 Sa	turating A	b (1st /	4b)	Association	SA (Streptavidin)	
		2	5	5 B:	seline		1	Pasalina	CA (Street suidie)	

Figure 9-72: Assay Steps List

- d. Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition).
- e. Repeat the previous steps to define other steps in the assay.
- f. New assays may be added by clicking the **New Assay** button in the Assay Steps List:

	ready of	epsition								
(	New A:	ssay M	ove Up	Move Dow	Remove	Replicate	Edit Step			
	Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time	
	1	A14	1	Baseline	🛌 Baseline	SA (Streptavidin)	32 channels	no	0:01:20	
	2	A14	1	Baseline	🛏 Baseline	SA (Streptavidin)	32 channels	no	0:01:20	
	3	A14	1	Baseline 🔹	🔙 Baseline	SA (Streptavidin) 💌	32 channels	no	0:01:20	

Figure 9-73: New Assay Button

**NOTICE:** More information on assay step editing in Tab 2 (Assay Definition) can be found in "Creating Step Types" on page 372.

Tab 3 (Sensor Assignment):

After completing the assay definition, click on Tab 3 (Sensor Assignment) to verify sensor type(s) for the epitope binning experiment.

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.

**NOTICE** Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in "Assigning Biosensors to Samples" on page 383.

Replacing Biosensors in the Biosensor Tray. Return biosensors to the biosensor tray or eject them through the chute. To return them to the tray, click the Replace sensors in tray after use check box:



Figure 9-74: Replace Sensors in Tray After Use Check Box

#### Tab 4 (Review Experiment)

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:



Figure 9-75: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

#### Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

- 1. Select File > Save Method File.
- 2. In the Save dialog box, enter a name and location for the file, and click Save.

#### Loading the Biosensor Tray and Sample Plates

To load the biosensor tray and sample plate:

- 1. Open the Octet<sup>®</sup> instrument door (lift the handle up).
- 2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 9-55).
- 3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 9-55).



Figure 9-76: Biosensor Stage (left) and Sample Stage (right)

**IMPORTANT:** Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.

- 4. Octet<sup>®</sup> RED96e and Octet<sup>®</sup> R8 only, optional. Cover the microplate with the evaporation cover to prevent evaporation from samples during analysis and lengthen the experiment time (only applies to Octet<sup>®</sup> RED96e and Octet<sup>®</sup> R8 instruments). For more information, see "Microplate Evaporation Cover" on page 64.
- 5. Close the Octet<sup>®</sup> instrument door.
- 6. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you're cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

#### Tab 5 (Run Experiment)

- 1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
- 2. To start the experiment, click the **GO** button:

Data File Location and Names			Prior to pressing "Go" confirm the Assa
Kinetics data repository:	C:\data		
Experiment run name (sub directory):	Experiment_1	<b>→</b>	
Plate name/barcode (file prefix):	201103		
Auto-increment file ID start:	1		
Data files will be stored as follows:			Total experiment time:
C:\data\Experiment_1\201103_001.frd C:\data\Experiment_1\201103_002.frd C:\data\Experiment_1\201103_003.frd 			2:07:30
Run Settings			
Delayed experiment start	Open runtime charts automatic	ally	
Start after (s): 600	Automatically save runtim	ne chart	
Shake sample plate while waiting	Set plate temperature (°C):	25	
Advanced Settings	- (5.0.1)	Default	
Advanced Settings Acquisition rate: Standard kinetics	s (5.0 Hz) ~	Default	
Advanced Settings Acquisition rate: Standard kinetics Warning: changing th If you are unsure of how to	s (5.0 Hz) ese settings could affect assay signal-to-noise. o use these settings, please consult the User Guide	Default	
Advanced Settings Acquisition rate: Standard kinetics Warning: changing th If you are unsure of how to Seneral Information	s (5.0 Hz)  v Hese settings could affect assay signal-to-noise. o use these settings, please consult the User Guide	Default	
Advanced Settings Acquisition rate: Standard kinetic: Warning: changing th If you are unsure of how to Seneral Information User name:	s (5.0 Hz)  v nese settings could affect assay signal-to-noise. o use these settings, please consult the User Guide Machine name: DESKTOP-0E#	Default e HTC34	
Advanced Settings Acquisition rate: Standard kinetic: Warning: changing th If you are unsure of how to General Information User name: Description:	s (5.0 Hz)  v rese settings could affect assay signal-to-noise. o use these settings, please consult the User Guide Machine name: DESKTOP-0E	Default e HTC34	

Figure 9-77: GO Button

## Chapter 10:

# Kinetics Experiments: Octet QK<sup>®</sup> 384, Octet RH<sup>®</sup> 96 and Octet RH<sup>®</sup> 16

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# Introduction

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet<sup>®</sup> system hardware and the Octet<sup>®</sup> BLI Discovery software, follow the steps (in Table 10-1) to set up and analyze a quantitation experiment.

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

Octet <sup>®</sup> Software		Step	See
BLI Discovery	1.	Select a kinetics experiment in the <b>Experi-</b> <b>ment Wizard</b> or open a method file (.fmf).	"Starting a Basic Kinetics Experiment: Octet <sup>®</sup> RH16 and Octet <sup>®</sup> QK384″ on page 415
	2.	Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 416
	3.	Define a or import a reagent plate (optional).	"Printing a Sample Plate Definition" on page 433
	4.	Specify assay steps.	"Defining a Kinetic Assay" on page 435
	5.	Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 447
	6.	Run the experiment.	"Running a Kinetics Experiment" on page 472
Analysis Studio	7.	View and process the raw data.	Octet <sup>®</sup> Analysis Studio Software User Guide
Octet Analysis Studio 12.2	8.	Analyze the data.	

Table 10-1: Setting Up and Analyzing a Kinetic Experiment

**NOTICE:** Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet<sup>®</sup> BLI Discovery software is closed, the plate temperature will reset to the default startup value specified in the Options window when the software is relaunched.

# Starting a Basic Kinetics Experiment: Octet<sup>®</sup> RH16 and Octet<sup>®</sup> QK384

You can start a kinetics experiment using one of the following options:

- Launch the Experiment Wizard.
- Open a method file (.fmf) by clicking File > Open Method File. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run. For more details on method files see "Managing Experiment Method Files" on page 487.
- On the menu bar, click Experiment > Templates > Kinetics.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

## Starting an Experiment Using the Experiment Wizard

To start an experiment from the **Experiment Wizard**:

- 1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button (A), or click **Experiment > New Experiment Wizard** (**Ctrl+N**) from the **Main Menu**.
- 2. In the Experiment Wizard, click New Kinetics Experiment (see Figure 10-1, left).
- 3. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.
- 4. Click the arrow button ( $\rightarrow$ ). The **Basic Kinetics Experiment** window displays (Figure 10-1, right).



Figure 10-1: Starting a Kinetics Experiment with the Experiment Wizard

## Defining the Sample Plate

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher (Figure 10-2).



Figure 10-2: Sample Plate Renamed Plate 1 in Software Versions 8.0 and Higher

The steps to define a sample plate include:

	Step	See Page
1.	Select the instrument read head configuration (8 or 16 channels).	417
2.	Select the sample plate format (96 or 384 wells).	418
3.	Designate the samples.	418
4.	Save the sample plate definition (optional).	430

#### Read Head Configuration and Plate Layout

The Octet<sup>®</sup> read head contains the collection optics. If the read head is set to 8 channels, one column of 8 biosensors interrogate 8 plate wells. If the read head is set to 16 channels, two columns of biosensors interrogate 16 wells (see Figure 10-3). The read head configuration and the plate format (96 or 384 wells) determine the plate layout (see example Figure 10-4).



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so o

Figure 10-3: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

8 Channel Read Head

16 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.



**NOTICE:** Keep the read head configuration in mind when laying out the sample plate. While reading a 384-well sample plate, both the 8 channel and 16 channel read heads can freely step through the plate by either moving left or right to step across columns or step one row up or down.

Changing the Sample Plate Format

#### NOTICE:

- Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.
- The default plate format can be changed from 96-well plate to 384-well plate by selecting File > Options and Default Sample Plate(s).

To change the sample plate format:

- 1. Click **Modify** (above the plate map).
- 2. In the Modify Plates dialog box, select 96 Well or 384 Well format.



Figure 10-5: Changing the Sample Plate Format

## Designating Samples

**NOTICE:** It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 10-2 will be included in the assay.

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

Table 10-2 displays the well types that can be assigned to a plate map.

Table 10-2: Types of Sample Wells

lcon	Description
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for sys- tem drift.
Controls	<ul> <li>A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis.</li> <li>Positive Control: A control sample that contains analyte of known concentration</li> <li>Negative Control: A control sample known not to contain analyte</li> </ul>
Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
(Activation	Activation reagent. Makes the biosensor competent for binding.
Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
Load	Ligand to be immobilized (loaded) on the biosensor surface.
🛞 Wash	Wash buffer.
Regeneration	Regeneration reagents dissociate the analyte from the ligand.
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

#### Selecting Wells in the Sample Plate Map

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

There are several ways to select wells in the Sample Plate Map:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 10-6 left). To select nonadjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 10-6, center).
- Click a well or draw a box around a group of wells(Figure 10-6, right).



Figure 10-6: Selecting Wells in the Sample Plate Map

**NOTICE:** Shift-clicking in the Sample Plate Map mimics the head of the instrument during the selection.

#### Designating Well Types

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

In the Sample Plate Map, select the wells, right-click and select a sample type. (Figure 10-7).

Plate 1 (3	34 wells)	
123	4 5 6 7 8 9 1011121314151	161718192021222324
BO	Sample	000000
6 D	Reference	0000000
5 O	Control	000000
GC Ň	Negative Control	000000
i c 🖻	Positive Control	000000
K 🛛 🕒	Buffer	000000
MC (A)	Activation	000000
N Q	Quench	000000
PČ 🛈	Load	000000
$\odot$	Wash	emove Print
Plate R	Regeneration	
N	Neutralization	
	Set Well Data	192021222324
вĞ	Clear Data	000000
C		000000

Figure 10-7: Designating a Well Type in the Plate Definition Window

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 10-8).



Figure 10-8: Clearing Sample Data from a Sample Plate

### Entering Sample Information

**NOTICE:** You must specify sample (analyte) concentration and molecular weight; otherwise, the Octet<sup>®</sup> BLI Discovery software cannot compute a K<sub>D</sub> value. If the sample concentration is not specified, only k<sub>d</sub> and k<sub>obs</sub> are calculated. You can also annotate any well with Sample ID or Well Information, and assign Replicate Groups.

#### Assigning Molecular Weight and Molar Concentration

- 1. In the Sample Plate Map, select the sample wells, right-click and select Set Well Data.
- 2. In the **Set Well Data** dialog box, enter the analyte molecular and molar concentration (Figure 10-9).

Set Well Data			×
Well Information		Dilution Series	
Sample ID:		Apply to:	Concentration
			Molar Concentration
Replicate Group:		Starting value (µg/ml):	1
Wall Information :		Series operator:	/ ~
		Series operand:	2
		Dilution orientation	
		Right	CLeft
Well Data - Sample only		Down	
Molecular Weight (kD):	150	88	••
Molar Concentration (nM):	66.67		
Concentration (ug/ml):			
		0	K Cancel

Figure 10-9: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

The information appears in the Sample Plate Table (see Figure 10-10).

3. In the **Sample Plate Table**, select the sample concentration units and the molar concentration units.

Sample Plate				Concentration units:		μg/ml		
C Reag	ent Plate	Paplicata Crown	Tuno	Cono (ug/ml)		Molor Cone (aM)	Information	Г
	Directoin A	Replicate Group	Load	12.5	MW (KD)	MUIAI CUIIC (IIM)	mormauon	
	Protein A		Load	12.5				
	Protein A		Load	12.5				
	1X Kinetics Buffer		Buffer	12.0				
0 C9	1X Kinetics Buffer		Buffer					
B F9	1X Kinetics Buffer		Buffer					
G G9	1X Kinetics Buffer		Buffer					
(B) 19	1X Kinetics Buffer		Buffer					
ю К9	1X Kinetics Buffer		Buffer					
B M9	1X Kinetics Buffer		Buffer					
B 09	1X Kinetics Buffer		Buffer					
A11	1X Kinetics Buffer		Buffer					
B C11	1X Kinetics Buffer		Buffer					
B E11	1X Kinetics Buffer		Buffer					
🖲 G11	1X Kinetics Buffer		Buffer					
B 111	1X Kinetics Buffer		Buffer					1
8 K11	1X Kinetics Buffer		Buffer					
B M11	1X Kinetics Buffer		Buffer					
B 011	1X Kinetics Buffer		Buffer					
🔵 A13	human IgG		Sample	40	150	266.7		
🔵 C13	human IgG		Sample	20	150	133.3		
🔵 E13	human IgG		Sample	10	150	66.67		
🔵 G13	human IgG		Sample	5	150	33.33		
🔵 113	human IgG		Sample	2.5	150	16.67		
🔵 K13	human IgG		Sample	1.25	150	8.333		
🔵 M13	human IgG		Sample	0.625	150	4.167		
013	1X Kinetics Buffer		Reference					

Figure 10-10: Entering Molecular Weight and Molar Concentration from the Plate Table

#### Assigning User-Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. For 384-well plates, right-click the **Well** heading in the sample table to sort the plate based on rows, columns, quadrant-rows and quadrant-columns. Then, in the **Sample Plate Map**, select the desired wells, right-click and select **Set Well Data**.

**NOTICE:** A range of wells can be selected clicking and dragging, holding the Shift key and using the arrow keys to select sections of the plate, or holding the Ctrl key to select specific wells.

The Set Well Data dialog box displays (see Figure 10-11).

2. Select the **By value** option and enter the starting concentration value. If a range of cells was selected, all cells will update with the specified value.
| Set Well Data                   |                        | ×                     |
|---------------------------------|------------------------|-----------------------|
| Well Information                | Dilution Series        |                       |
| Sample ID:                      | Apply to:              | Concentration         |
|                                 |                        | O Molar Concentration |
| Replicate Group:                | Starting value (µg/ml) | 1                     |
|                                 | Series operator:       | / ~                   |
|                                 | Series operand:        | 2                     |
|                                 | Dilution orientation   |                       |
|                                 | Right                  | Cleft                 |
| Well Data - Sample only         | Down                   |                       |
| Molecular Weight (kD): 150      | 00                     | ••                    |
| Molar Concentration (nM): 66.67 |                        |                       |
| Concentration (µg/ml):          | (                      | OK Cancel             |

Figure 10-11: Sample Plate Map-Assigning Sample Concentrations by Value

3. Click OK. The Sample Plate Table will display the entered concentration.

#### Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

- In the Sample Plate Map, select the wells, right-click, and select Set Well Data. The Set Well Data dialog box displays (see Figure 10-12)
- 2. Select the **Dilution Series** option and enter the starting concentration value.

et Well Data	×
Well Information Sample ID:	Dilution Series Apply to:   Concentration
Replicate Group:	Molar Concentration Starting value (μg/ml): 10
Well Information:	Series operator: /
	Dilution orientation
Well Data - Sample only	O Down     O Up
Molecular Weight (KD): 150 Molar Concentration (nM):	
Concentration (µg/ml):	OK Cancel

Figure 10-12: Sample Plate Map-Assigning Sample Concentrations Using Dilution Series

3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 10-13).



Figure 10-13: Concentration Representation in Dilution Series:

4. Click OK.

The Sample Plate Table displays the standard concentrations.

#### **Annotating Samples**

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

#### Annotating Wells in the Sample Plate Map

To annotate one or more wells:

- 1. In the Sample Plate Map, select the samples to annotate, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 10-14), enter the Sample ID and/or Well Information and click OK.

et Well Data		×
Well Information	Dilution Series	
Analyte 1		Molar Concentration
Replicate Group:	Starting value (µg/ml):	10
Well Information:	Series operator:	/ ~
1x Kinetics Buffer	Series operand:	2
	Dilution orientation	
	Right	
Well Data - Sample only	Down	
Molecular Weight (kD): 150	lõõ	ēē
Molar Concentration (nM):		
Concentration (µg/ml):	OK	Cancel



#### Annotating Wells in the Sample Plate Table

To annotate an individual well in the Sample Plate Table:

- 1. Double-click the table cell for **Sample ID** or **Well Information**.
- 2. Enter the desired information in the respective field (see Figure 10-15).

**NOTICE:** A series of Sample IDs may also be assembled in Excel and pasted into the Sample Plate Table.

ſ	Sample F Concent	Plate Table ration units:	µg/ml  ▼	Export	. Import			
	Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	<b>Dilution Factor</b>	Information	
	🔵 A1	hlgG		Standard	200	n/a	human IgG	
	🔵 C1			Standard	100	n/a		
	🔵 E1			Standard	50	n/a		
	🔵 G1			Standard	25	n/a		
	🔵 l1			Standard	10	n/a		
	🔵 К1			Standard	5	n/a		

Figure 10-15: Add Sample Annotations in the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

#### Replicate Groups

Replicate Groups enable data to be organized into custom groups during data analysis (see Figure 10-16).

Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.	
20	x		C2	SA (Streptavidin)		3	C3	
21	x		C2	SA (Streptavidin)		3	C3	
22	x		D2	SA (Streptavidin)		4	D3	
23	x		D2	SA (Streptavidin)		4	D3	
24	x		E2	SA (Streptavidin)		5	E3	
25	x		E2	SA (Streptavidin)		5	E3	
26	x		F2	SA (Streptavidin)		6	F3	
27	x		F2	SA (Streptavidin)		6	F3	
28	x		G2	SA (Streptavidin)		6	G3	
29	x		G2	SA (Streptavidin)		6	G3	
30	x		H2	SA (Streptavidin)		6	H3	
31	x		H2	SA (Streptavidin)		6	H3	Ξ
32	x		A3	SA (Streptavidin)		1	A3	
33	x		A3	SA (Streptavidin)		1	A3	
34	x		B3	SA (Streptavidin)		2	B3	
35	x		B3	SA (Streptavidin)		2	B3	
36	x		C3	SA (Streptavidin)		3	C3	
37	x		C3	SA (Streptavidin)		3	C3	
38	x		D3	SA (Streptavidin)		4	D3	
20	v		צח	SA (Strentsvidin)		4	D3	Ψ.
•	111						4	

Figure 10-16: Replicate Group Color-Coding

**NOTICE:** Replicate Group information can also be entered in the software.

#### Assigning Replicate Groups in the Sample Plate Map

#### To assign Replicate Groups in the Sample Plate Map:

- 1. Select the samples you wish to group, right-click and select Set Well Data.
- 2. In the Set Well Data dialog box (see Figure 10-17), enter a name in the Replicate Group box and click OK.

Well Information	Dilution Series		[
Sample ID:	Apply to:	Concentration	ation
Analyte 1		O Molar Cor	ncentration
Replicate Group:	Starting value	e (μg/ml): <b>10</b>	
1 M-II I-f	Series operat	tor: /	$\sim$
1x Kinetics Buffer	Series operar	nd: 2	
	Dilution orier	ntation	
		Right 888	OLeft
Well Data - Sample only		Down	⊖ Up
Molecular Weight (kD): 150		ěě	
Molar Concentration (nM):			
Concentration (ug/ml):			

Figure 10-17: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 10-18).

Plate	1 (3	384	we	ells)																			
_																							
	2	3	4	5	6	7	8	9	10	11	12	13	14	115	16	17	18	31	92	0.2	1 22	223	24
A®	0	₿	0	U	0	0	0	₿	0	B	0	0	C	0	O	0	C	)(		C	)(	0	C
BO	0	Õ		Õ		Q		Õ	0			Õ	С	)Q		0							C
CB	0	₿	0	0	0	0	0	₿	0	B	0	0	C	0	0	0	C	)(		DC	) (	0	C
DO												0		0									
E®	0	8		0		0		8	0	B		0		0	0								
FO	0	0		0					0			0											
G®	0	ß		0		0		6	0	B		0		0	0								
HO	0	0	0	0	0	0	0	0	0		0	0		0	0							0	
10	0	₿		0		0		8	0	B		0		0	0								
JO	0			0																			
K®	Ō	<b>B</b>		Ū				<b>B</b>	Ō	B		Ó		0	Ō								
LŌ												Õ		)Õ									
M®	Ō	ß		Ō		Ō		B	Ō	B		Ō		Ō	Ō								
NÕ	Õ	Ő		Õ		Õ		Ő		õ		Õ		õ									
OB	Õ	ø		õ	õ	õ		ø	õ	B		Ő		Õ	Õ								
PÕ	õ	õ	õ	õ	õ	õ	õ	õ	õ	ñ	õ	Õ		0		õ						õ	

Figure 10-18: Replicate Groups Displayed in Sample Plate Map

💿 Samp	ole Plate		Concer		µg/ml			
O Reag	jent Plate		Molarc	concentration unit	s:	nM		Ŧ
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µq/ml)	MW (kD		Molar	
🛈 M7	Protein A		Load	12.5		• •		
07	Protein A		Load	12.5				
B A9	1X Kinetics Buffer		Buffer					
B C9	1X Kinetics Buffer		Buffer					
🖲 E9	1X Kinetics Buffer		Buffer					
📵 G9	1X Kinetics Buffer		Buffer					
B 19	1X Kinetics Buffer		Buffer					
📵 K9	1X Kinetics Buffer		Buffer					
📵 M9	1X Kinetics Buffer		Buffer					
B 09	1X Kinetics Buffer		Buffer					
🖲 A11	1X Kinetics Buffer		Buffer					
📵 C11	1X Kinetics Buffer		Buffer					
🖲 E11	1X Kinetics Buffer		Buffer					
📵 G11	1X Kinetics Buffer		Buffer					
B  11	1X Kinetics Buffer		Buffer					
📵 K11	1X Kinetics Buffer		Buffer					
📵 M11	1X Kinetics Buffer		Buffer					
B 011	1X Kinetics Buffer		Buffer					
🔵 A13	human IgG	Group 1	Sample	40				
🔵 C13	human IgG	Group 2	Sample	20				
🔵 E13	human IgG	Group 3	Sample	10				
🔵 G13	human IgG	Group 4	Sample	5				
🔵 l13	human IgG	Group 5	Sample	2.5				
🔵 K13	human IgG	Group 6	Sample	1.25				
🔵 М13	human IgG	Group 7	Sample	0.625				Ξ
013	1X Kinetics Buffer		Reference					
🔵 A15	human IgG	Group 1	Sample	40				
🔵 C15	human IgG	Group 2	Sample	20				
🔵 E15	human IgG	Group 3	Sample	10				
🔵 G15	human IgG	Group 4	Sample	5				
🔘 l15	human IgG	Group 5	Sample	2.5				
🔵 K15	human IgG	Group 6	Sample	1.25				
M15	human lqG	Group 7	Sample	0.625				

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 10-19)

Figure 10-19: Replicate Groups in Sample Plate Table

### Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

- 1. Double-click the desired cell in the **Replicate Group** table column.
- 2. Enter a group name (see Figure 10-20).

Samp	ole Plate		Conce	ntration units:	μg	/ml	Ŧ
Reag	ent Plate		Molar	concentration unit	is: nN	1	•
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml)	MW (k	)) Mola	
🕒 M7	Protein A		Load	12.5			
07	Protein A		Load	12.5			
📵 A9	1X Kinetics Buffer		Buffer				
📵 C9	1X Kinetics Buffer		Buffer				
📵 E9	1X Kinetics Buffer		Buffer				
📵 G9	1X Kinetics Buffer		Buffer				
📵 19	1X Kinetics Buffer		Buffer				
📵 K9	1X Kinetics Buffer		Buffer				
📵 M9	1×Kinetics Buffer		Buffer				
B 09	1X Kinetics Buffer		Buffer				
🖲 A11	1X Kinetics Buffer		Buffer				
🖲 C11	1×Kinetics Buffer		Buffer				
🖲 E11	1X Kinetics Buffer		Buffer				
📵 G11	1×Kinetics Buffer		Buffer				
B  11	1X Kinetics Buffer		Buffer				
📵 K11	1X Kinetics Buffer		Buffer				
📵 M11	1X Kinetics Buffer		Buffer				
B 011	1X Kinetics Buffer		Buffer				
🔵 A13	human IgG	Group 1	Sample	40			
🔿 C13	human IgG	Group 2	Sample	20			
🔵 E13	human IgG	Group 3	Sample	10			

Figure 10-20: Add Replicate Group from the Sample Plate Table

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the Sample Plate Map menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.

**IMPORTANT:** The software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group 2 are treated as two different groups.

#### Editing the Sample Table

#### **Changing Sample Well Designations**

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection (see Figure 10-21).



Figure 10-21: Sample Plate Table–Well Designation

#### **Editing Sample Information**

To edit sample data in the Sample Plate Table, double-click a value and enter a new value (see Figure 10-22).

💿 Sam	ole Plate		Conc	centration units:
🔘 Reag	gent Plate		Molar	ar concentration units: nM 🗸
Well	Sample ID	<b>Replicate Group</b>	Туре	Conc (µg/ml) MW (kD) Molar 🔺
🕒 M7	Protein A		Load	12.5
07	Protein A		Load	12.5 <sup>1</sup>
📵 A9	1X Kinetics Buffer		Buffer	Undo
📵 C9	1×Kinetics Buffer		Buffer	Cut
🖲 E9	1X Kinetics Buffer		Buffer	cut
📵 G9	1×Kinetics Buffer		Buffer	Сору
(B) 19	1×Kinetics Buffer		Buffer	Paste
📵 K9	1X Kinetics Buffer		Buffer	Delete
📵 M9	1×Kinetics Buffer		Buffer	Colore All
(B) O9	1X Kinetics Buffer		Buffer	Select All
🖲 A11	1X Kinetics Buffer		Buffer	Right to left Reading order
🖲 C11	1×Kinetics Buffer		Buffer	Show Unicode control characters
B E11	1X Kinetics Buffer		Buffer	show oncode control characters
📵 G11	1X Kinetics Buffer		Buffer	Insert Unicode control character
(B)  11	1XKinetics Buffer		Buffer	Open IME
🖲 K11	1X Kinetics Buffer		Buffer	Reconversion
(B) M11	1X Kinetics Buffer		Buffer	Reconversion

Figure 10-22: Sample Plate Table–Editing Sample Data

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the Sample Plate Table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

**NOTICE:** The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the right-click menu used to designate sample types.

## Managing Sample Plate Definitions

NOTICE: After you define a sample plate, you can export and save the plate definition for future use.

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher.

Exporting a Plate Definition

To export a plate definition:

### 1. In the Sample Plate Map, click Export (see Figure 10-23).



Figure 10-23: Sample Plate Map – Export Button

🖽 Export Plate 1 Definition × ← → ✓ ↑ 📙 → This PC → Local Disk (C:) → data ・ C Search data Organize 👻 New folder -? Name Date modified Туре Size lesson and the contract of the No items match your search. 💻 This PC 🧊 3D Objects 📃 Desktop Documents 🕹 Downloads 👌 Music Pictures 📕 Videos 🏪 Local Disk (C:) 🛄 DVD Drive (D:) O File name: 384 standard plate Save as type: CSV Files (\*.csv) Cancel <u>S</u>ave ∧ Hide Folders

2. In the **Export Plate Definition** window (see Figure 10-24), select a folder, enter a name for the plate (.csv), and click **Save**.

Figure 10-24: Export Plate Definition Window

#### Importing a Plate Definition

To import a plate definition:

1. In the Plate Definition window (see Figure 10-23: on page 430), click Import.



Figure 10-25: Sample Plate Map- Import Button

🖽 Import Plate I Definition					~
$\leftarrow \rightarrow \cdot \uparrow$ his	PC → Local Disk (C:) → data	~	ල 🔎 Searc	h data	
Organize 🔻 New folder					?
A	Name	Date modified	Туре	Size	
Culck access	384 standard plate.csv	1/8/2021 10:14 AM	CSV File	1 KB	
le OneDrive					
💻 This PC					
3D Objects					
📃 Desktop					
Documents					
🖶 Downloads					
b Music					
Pictures					
🛃 Videos					
🏪 Local Disk (C:)					
🔲 DVD Drive (D:) O					
· · · · · · · · · · · · · · · · · · ·					
File <u>n</u> ar	ne: 384 standard plate.csv		✓ CSV Files (	*.csv)	$\sim$
			<u>O</u> pen	Cancel	

2. In the **Import Plate Definition** window (see Figure 10-26), select the plate definition (.csv), and click **Open**.

Figure 10-26: Import Plate Definition Window

NOTICE: You can also create a .csv file for import. Figure 10-27 shows the appropriate column information layout.

	А	В	С	D	E	F	G	Н	
1	PlateWells	384							
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information	
3	A1	<b>1X Kinetics Buffer</b>		Buffer					
4	C1	<b>1X Kinetics Buffer</b>		Buffer					=
5	E1	<b>1X Kinetics Buffer</b>		Buffer					
6	G1	<b>1X Kinetics Buffer</b>		Buffer					
7	11	<b>1X Kinetics Buffer</b>		Buffer					
8	K1	<b>1X Kinetics Buffer</b>		Buffer					
9	M1	<b>1X Kinetics Buffer</b>		Buffer					
10	01	<b>1X Kinetics Buffer</b>		Buffer					
11	A3	<b>1X Kinetics Buffer</b>		Buffer					
12	C3	<b>1X Kinetics Buffer</b>		Buffer					
13	E3	<b>1X Kinetics Buffer</b>		Buffer					
14	G3	<b>1X Kinetics Buffer</b>		Buffer					
15	13	<b>1X Kinetics Buffer</b>		Buffer					
16	КЗ	<b>1X Kinetics Buffer</b>		Buffer					
17	M3	<b>1X Kinetics Buffer</b>		Buffer					
18	03	<b>1X Kinetics Buffer</b>		Buffer					
19	A5	Protein A		Load	12.5				
20	C5	Protein A		Load	12.5				Ŧ
14 -	▶ ▶  384	K method 4 expo	rt /如/			11			

Figure 10-27: Example Plate Definition File (.csv)

#### Printing a Sample Plate Definition

To print a plate definition:

1. In the Sample Plate/Plate 1 Map (see Figure 10-28), click Print.

Plate 1 (384 wells)				
_				
12345	67891	0 11 12 13 1	4 <u>15 16 17 18 1</u>	9 20 21 22 23 24
		0000	<b>0</b>	000000
BOOOOO				000000
		<b>0</b>	00000	000000
00000				000000
EBOBOD	0008	0000	00000	000000
F00000	00000	00000	00000	000000
GOOOO	OOOB (	000	00000	000000
HÕÕÕÕÕ	00000	0000	00000	000000
li@`@`@	ŎŨŎŨ	) O O O	ŐŐŐŐŐ	000000
100000	õõõõ			000000
Kačača	čečeč	Sece		
P00000	00000	00000	00000	000000
	Incode	Email	Demons	Direct
	Import	Export	Remove	Print

Figure 10-28: Sample Plate/Plate 1 Print Button

The associated **Sample Plate Table** information will print.

## Working with a Reagent Plate

**NOTICE:** Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet<sup>®</sup> BLI Discovery software versions 8.0 and higher (Figure 10-29).



Figure 10-29: Reagent Plate Renamed Plate 2 in Software Versions 8.0 and Higher

You can include an optional reagent plate in a Basic Kinetics experiment. Using a reagent plate enables higher sample throughput since no reagents are included in the sample plate. An experiment can include any combination of sample and reagent plate formats (96- or 384-well). The reagent plate can be used for reagents but not samples, references or controls.

**NOTICE:** Reagent plates can only contain reagents. Samples, references and controls must be assigned to the sample plate.

**NOTICE:** The reagent plate format (96- or 384-well) and the read head configuration (8 or 16 channels) determine the reagent plate layout. For more details, see "Read Head Configuration and Plate Layout" on page 417.

To modify a reagent plate:

2. Click Modify Plates above the Sample Plate Map. The Modify Plates dialog box displays (see Figure 10-30).

1 Plate Definitio	n 2 Assay Defi	nition <b>3</b> Sense	or Assignment	4 Review Experiment
Lin this Highlig	step, all the informa ght one or more well	ation about the san Is on the sample pl	nple plate and i ate, and right-c	ts wells will be entered. lick to enter/modify well da
Read Head: 10	6 channels	~	Modify Plates	Plate 1 Table
Plate 1 (384 wel	ls)			Plate 1 Table     Plate 2 Table
1 2 3 4 5 A 00000	Modify Plates		×	Well Sample ID
B 00000 C 00000 D 00000	Plate 1	384 Wells	~	
E 00000 F 00000 G 00000	Plate 2	384 Wells 96 Wells	~	
H00000		384 Wells	2	
к00000 L00000 M00000	ОК	Cance	el	
N 00000 0000000 P 000000	000000000000000000000000000000000000000	5555556 5000000	00000	
Unassigned	d Import E	Export Remo	ve Print	

Figure 10-30: Modifying the Reagent Plate

- 3. Select a reagent plate format (96 Well or 384 Well) and click OK.
- 4. Select the Reagent Plate radio button above the plate table. This will display the Reagent Plate Table.
- In the Reagent Plate Map, right-click a column to use and select Buffer, Activation, Quench, Load, Wash, or Regeneration from the shortcut menu (see Figure 10-31). The well designations appear in the Reagent Plate Table. Repeat this step to define other wells in the reagent plate.

Plate	2 (38	4 wells
-------	-------	---------



Figure 10-31: Defining Wells in the Reagent Plate

6. Optional: Enter well data or reagent information in the **Reagent Plate Table**.

To remove well designations, select the column(s) and click **Remove**, or right-click and choose **Clear Data**.

#### Saving a Reagent Plate Definition

Exporting and saving reagent plate definition is done in the same manner as you would for sample plates. For details "Managing Sample Plate Definitions" on page 430.

#### Printing a Reagent Plate Definition

To print a plate definition:

1. In the **Reagent Plate/Plate 2 Map** (see Figure 10-32), click **Print**.

Plate	2 (3	384 \	wells	)—																	
1	2	3	45	6	7	89	10	11	12	13	14	15	16	17	18	19	202	21 3	222	23 2	4
A	00	00	00	0	00	DO	0	0	0	0	0	0	0	$\bigcirc$	0	0	0	0	00	00	0
BO																					2
CO																					2
DO																					2
E																					2
FO																					2
G																					2
HO																					2
10																					2
JO																					2
KO																					2
LC																					2
MC																					2
NO																					2
0																					2
P	)0	00	00	0	O(	)0	$(\bigcirc$	0	0	0	0	0	0	$\bigcirc$	$\bigcirc$	0	0	$\bigcirc$	$\mathbf{O}($	)(	$\supset$
0	Jnas	ssign	ed		Impo	ort		Б	por	t		R	em	ove	;	F	nint				l

Figure 10-32: Reagent Plate/Plate 2 Print Button

The associated **Reagent Plate Table** information will print.

## Defining a Kinetic Assay

After the sample plate is defined, the assay must be defined. The steps to define a kinetic assay include:

	Step	See Page
1.	Define the step types.	436
2.	Build the assay by assigning a step type to a column(s) in the sample plate.	439
3.	Save the sample plate definition (optional).	430

## Defining Step Types

Table 10-3 lists the example step types to define a kinetic assay. Use these examples as a starting point to create your own step types.

Table 10-3: Sample Step Types for Kinetic Assays .

Step Type	Step Description
Association	Calculates the <i>k<sub>obs</sub></i> . Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the $k_{\rm d}$ . Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm.
	<b>IMPORTANT:</b> An assay must include a baseline step followed by a set of association/dissociation steps to be analyzed. The software recognizes the baseline/association/dissociation step series during processing. Data cannot be processed if this sequence is not included in the assay setup.
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor.
	<b>NOTICE:</b> This step may be performed offline (outside the Octet <sup>®</sup> instrument).
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.
Regeneration	Used when employing a reagent to chemically regenerate biosensors and remove bound analyte.
Custom	Can be used for an activity not included in any of the above step types.

#### **Creating Step Types**

Click the Assay Definition tab, or click the  $\rightarrow$  arrow to access the Assay Definition window (Figure 10-33). The Step Data List shows the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay step:

- 1. Click Add.
- 2. In Assay Step Definition dialog box (Figure 10-33), specify the step information:
  - a. Choose a step type.
  - b. Optional: Edit the step name.
  - c. Set the step time and shake speed (**Time** range: 2 to 48,000 seconds, **Shake speed** Off 0 rpm or On range: 100 to 1,500 rpm).

Add Step	Definitions			×
	Association Dissociation Baseline Loading Activation Quenching	Name Association Dissociation Baseline Loading Activation Quenching	Time (s) 600 + 600 + 600 + 600 + 600 + 600 + 600 +	Shake speed (pm) 1000 * 1000 * 1000 * 1000 * 1000 * 1000 *
<b>R</b> 2	Regeneration	Regeneration	30 🌲	1000 🚔
	Custom Dip	Custom	600 ÷	1000 💌 1000 🖛
		OK Ca	ncel	Defaults

Figure 10-33: Creating an Assay Step Type

- 3. Apply a threshold to the step:
  - a. In the **Step Data List**, click the **Threshold** check box.

The Threshold Parameters dialog box appears (see Figure 10-34).

b. Set the threshold parameters (refer to Table 10-4 for the parameter definitions).

Plate 1 (384 wells)	Step Data List					
	Add	Сору	Remove	Regeneration Par	rams Thre	shold Params
A B O B O O O B O O O O O O O O O O O O	Name	Time	Shake speed	Туре	Threshold	
	Baseline	60	1000	💑 Baseline		
	Association	600	1000	Association		
F 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Dissociation	600	1000	Dissociation		
	→ Loading	600	1000	🖌 Loading		
I B B C C B B Threshold Parameters					×	
L       Assay steps designated as "Thre criteria is reached.         M®       O       O       Assay steps designated as "Thre criteria is reached.         N       O       O       O       Assay steps designated as "Thre criteria is reached.         N       O       O       O       Assay steps designated as "Thre criteria is reached.         Assay steps designated as "Thre criteria is reached.       Active Channels:       Set Al         Main and the comparison of the criteria is reached.       Main and the criteria is reached.       Active Channel 1         Main and the comparison of the criteria is reached.       Main and the criteria is reached.       Main and the criteria is reached.         Main and the criteria is reached.       Main and the criteria is reached.       Main and the criteria is reached.         Main and the criteria is reached.       Main and the criteria is reached.       Main and the criteria is reached.         Main and the criteria is reached.       Main and the criteria is reached.       Main and the criteria is reached.         Main and the criteria is reached.       Main and the criteria is reached.       Main and the criteria is reached.         Main and the criteria is reached.       Main and the criteria is reached.       Main and the criteria is reached.         Main and the criteria is reached.       Main and the criteria is reached.       Main and the criteria is reached.	shold" will terminate whe Clear All annel 10 annel 11 annel 12 annel 13 annel 14	en either ✓ Signa Thresh The thr ● Bir ○ Bir	the step time ela al Change old (nm): eshold is achieve nding ascends by nding descends b	not the second s	termination start 9 start	Info Table
M         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q         Q	annel 15 annel 16	<b>Grad</b> Grad	ient old (nm/min): [ n (min): [	0.10		
F         O         O           G         Ø         O         O           H         O         O         O           I         Ø         O         O		The th stays b	reshold is achiev elow 'threshold' f	ed when the binding g or the given duration.	gradient	
the threshold is achieved on /	ALL channels	🗹 Filter	ng			
	ANY ONE channel	Filtering	is applied before	e the threshold is asse	essed.	
N 000000000000000000000000000000000000	ancel	Filter w	idth (s):	10.0		

Figure 10-34: Setting Assay Step Threshold Parameters

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**NOTICE:** If thresholds are applied, the step is terminated when either the step time elapses or the threshold termination criteria is reached.

Table 10-4: Threshold Parameters

Item	Description
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step:
	<ul> <li>The threshold is achieved on ALL channels</li> </ul>
	The threshold is achieved on ANY ONE channel
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

- 4. Click OK to save the newly-defined step. The new step type appears in the Step Data List.
- 5. Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 10-35).

Step	Data List					
	Add	Сору	Remove		Threshold Par	ams
	Name	Time	Shake speed	Тур	)e	Threshold
<b>→</b>	equilibration	120	1000	羣C	Sustom	
	Loading	300	1000	ĽL	oading	
	Baseline	300	1000	🛌 B	Baseline	
	Association	300	1000	K A	ssociation	
	Dissociation	600	1000	L D	)issociation	
	Regeneration	20	1000	₩C	Sustom	
	Neutralization	20	1000	₩C	Justom	

Figure 10-35: Step Data List-Displaying Step Types

6. To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

### Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the Step Data List.

To define a step type by editing an existing one:

1. Double-click the cell in the step's **Name**, **Time**, or **Shake speed** column and then enter a new value. Or, rightclick the cell to display a shortcut menu of editing commands (see Figure 10-36, left).

**NOTICE:** Keyboard commands can also be used (Ctrl+x=cut, Ctrl+c=copy, Ctrl+v=paste, Ctrl+z=undo).

2. Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 10-36, right).

	Name	Time	Shake speed	Туре	Thresho	bld		Name	Time	Shake speed	Тур	e	Thresho	ld
E	Baseline	10	1000	🛌 Baseline			•	Baseline	10	1000	han B	Baseline 👻	Г	
L	Loading	20	1000	📝 Loading				Loading	20	1000		Annestation		
N	Wash	15	1000	E Custom				Wash	15	1000		Association		
4	Association	30	1000	🞽 Association				Association	20	1000		Dissociation		
L	Long Dissociati	ion 2000	1000	L Dissociation		_		Association	30	1000	•	Baseline		
► F	Regeneration	24	1000	Recentation				Long Dissociation	2000	1000		Loading		- 1
_		_	Undo					Regeneration	24	1000	-	13		
isay 5 √ew A	Assay Move	eUp	Cut			itep		Activation	25	1000		Quenching		
	Sample S	ten k	Сору			Assau Time	issa	y Steps List				Quenening		ten.
aauj	1 B	aseline	Pastel			Have I Into	Ne	w Assay Move U	p Mo	ve Down Ren		Regeneration		
_	2 Lo	oadinc	Delete				۵	au Sample Plai	e Ste	n Name Sten		Custom		au T
	7 W	/ash					1		Page	line -   Pr		Dip		0.20
	3 As	ssocia	Select All					81 1	Dase					0.30
	8 Lo	ona Di	Right to left F	Reading order										
	10 B	egene	Show Unicod	e control characters		0:35:23								
	1 B	aceline	Insert Unicod	e control character										
	2 Lo	oadinc	and the officer	e control chorocter										
	7 W	/ash	Open IME											
			D (											

Step Data List

Figure 10-36: Editing a Step Value (left) or Step Type (right)

#### Building an Assay

After creating the different step types that the assay will use, step types are assigned to columns in the Sample Plate or Reagent Plate maps.

To build an assay:

- 1. Select a step type in the Step Data List.
- 2. In the **Sample Plate** or **Reagent Plate Map**, double-click the column that is associated with the selected step type. For information about sample or reagent plate wells, mouse over a well to view a tool tip (see Figure 10-37).

Plate 1 (384 wells)	
1       2       3       4       5       6       7       8       9       1011112         A       B       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O       O	131415161718192021222324
K     B     C     C     C     B     C       M     B     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C       N     C     C     C     C     C     C <t< th=""><th>Unassigned samples</th></t<>	Unassigned samples

Figure 10-37: Tool Tip of Well Information

The selected wells are marked with hatching (for example, 🍘) and the step appears in the **Assay Steps List** (see Figure 10-38) with an associated **Assay Time**.

NOTICE: In the Assay Steps List, Plate 1 is the Sample Plate and Plate 2 is the Reagent Plate.



Figure 10-38: Assigning a Step Type to a Column in the Sample Plate

3. Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 10-39).



Figure 10-39: Experiment and Assay Time Updates as Steps Are Added to the Assay

**IMPORTANT:** If you intend to analyze the data from a sample using the Inter-step correction feature in the Octet<sup>®</sup> BLI Discovery software, the assay must use the same well to perform baseline and dissociation for the sample.

#### Adding a Regeneration Step

1. In the Sample Plate Map, assign wells as Regeneration or Neutralization (Figure 10-40).



Figure 10-40: Regeneration Step

2. Click **Add t**o display the Add Step Definition dialog box (Figure 10-41).

Step	Data List					
A	.dd 🔪 🚾 C	Сору	Remove	Regeneration Params	Threshold	Params
	Name	Time	Shake speed	Туре	Threshold	
	Baseline	60	1000	🛌 Baseline		
•	Regeneration	30	400	💈 Regeneration		
		4				

Figure 10-41: Add Button

Add Step	Definitions			×
		Name	Time (s)	Shake speed (rpm)
	Association	Association	600 🚔	1000 🖨
	Dissociation	Dissociation	600 🔶	1000 🚔
	Baseline	Baseline	600	1000 🚔
	Loading	Loading	600	1000 🚔
□ 1	Activation	Activation	600	1000
⊡ <u></u>	Quenching	Quenching	600	1000 🚔
R. *	Regeneration	Regeneration	30 🔹	1000 🚔
	Custom	Custom	600 🖨	1000 🚔
	Dip	Dip	600	1000 🚔
		OK Car	ncel	Defaults

Figure 10-42: Add Step Definition Dialog Box

- 3. Select Regeneration and click OK.
- 4. Click Regeneration Params (Figure 10-43).

- S	tep [	Data List				1
	A	dd C	ору	Remove	Regeneration Params	Threshold Params
		Name	Time	Shake speed	Туре	Threshold
		Baseline	60	1000	🛌 Baseline	
•	•	Regeneration	30	400	💈 Regeneration	

Figure 10-43: Regeneration Params Button

The Regeneration Parameters dialog box appears, and you can edit Regeneration parameters as necessary.

Regeneration Parame	ters	×	<
Step Name:	Regeneration		
	Time (s)	Shake speed (rpm):	
Regeneration:	5	1000	
Neutralization:	5	1000	
Regeneration cycles:	3		
Total step time:	30 s	OK Cancel	

Figure 10-44: Regeneration Parameters Dialog Box

#### **Replicating Steps Within an Assay**

To copy steps and add them to an assay:

- 1. In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 10-45, step rows 1-4 are selected).
  - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
  - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
- 2. In the **Replicate Steps** dialog box (see Figure 10-45), click the **Append to current assay** option.
- 3. Click the **Offset steps** check box and set the options, as appropriate. (For more details on offset options, see Table 10-5.)

New A:	ssay 🕴	vlove Up	Move Down	Remove	Replicate	0:29:14	
Assay	Sample	Plate	Step Name	Step Type	Sensor	Гуре	Assay Time
1	A1	1	equilibration 🔹	」	AHC (Anti	-hlgG Fc Capture) 💌	
1	A5	1	Loading	🗹 Loading	AHC (Anti	-hlgG Fc Capture)	
	A9	1	Baseline	🛌 Baseline	AHC (Anti	-hlgG Fc Capture)	
	A13	1	Association	🔀 Association	AHC (Anti	-hlgG Fc Capture)	
I	A9	1	Dissociation	L Dissociation	AHC (Anti	-hlgG Fc Capture)	
	A1 A5	Rep	licate Steps			X	0:29:00
<ul> <li>Add as a new assay</li> <li>Append to current assay</li> <li>Offset steps</li> </ul>				Cancel			
		1	<ul> <li>Sample steps of</li> <li>All Steps</li> </ul>	only			L
			Sample steps will Horizontally Vertically	be adjusted; by 1 🗼 col by one row	umns		

Figure 10-45: Replicating Assay Steps by Appending

#### 4. Click **OK**. The step(s) appear at the end of the assay in the **Assay Steps List**.

 Table 10-5: Replicate Steps Options .

Item	Description
Add as a new assay	Adds the replicate step(s) as a new assay to the <b>Assay Steps List.</b>
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
Sample steps only	Applies the offset to the sample plate only.
All steps	Applies the offset to the sample plate and reagent plate.
Sample steps are adjusted horizon- tally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter <b>1</b> . Enter <b>0</b> to apply the step(s) to the same columns.
Sample steps are adjusted vertically by one row	Choose this option to put the replicate step in the same column, but the next row.

#### Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensor column:

- 1. Select a column in the **Sample Plate Map**.
- 2. Right-click to view the shortcut menu and select **Start New Assay** (see Figure 10-46).
- 3. Add steps to the assay as described earlier.

Plate 1 (384 wells)	Ste
A 2 3 4 5 6 7 8 9 1011 121 A 2 0 0 0 0 0 0 0 0 B 0 0 0 0 0 0 0 0 0 C 0 0 0 0 0 0 0 0 0	31415161718192021222324 OOOOOOOOOOOOO
D 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Add Assay Step
E 월 - 월 - U - U - B - B - € F	Insert Assay Step
H	Start New Assay
J00000000000000000000000000000000000	Show Sample Types
L 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Show Pie Charts
N 000000000000000000000000000000000000	Copy to Clipboard

Figure 10-46: Start New Assay

#### Inserting or Adding an Assay Step

To insert an assay step:

- 1. Select a step in the Step Data List.
- 2. In the Assay Steps List, select the row above where you want to insert the step.
- 3. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Insert Assay Step**.

The step is inserted into the **Assay Steps List**.

To add an assay step:

- 1. Select a step type in the Step Data List.
- 2. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Add Assay Step**.

The step is added to the end of the Assay Steps List.

#### Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow (•) or any step in the assay and select a sensor type from the drop-down list (Figure 10-47). The biosensor type will automatically update for every assay step.

Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time
1	1	Baseline	🛌 Baseline	SA (Streptavidin)	
1	2	Loading	🖌 Loading	SA (Streptavidin)	
1	7	Wash	🕁 Custom	SA (Streptavidin)	
1	3	Association	<ul> <li>C Association</li> </ul>	SA (Streptavidin)	-
1	8	Long Dissociation	▶ Dissociation	SA (Strepta)	vidin)
1	10	Regeneration	Regeneration	AHC (Anti-	hlaG Ec Canture)
2	1	Baseline	占 Baseline	Anti GST	ingo i e cupture,
2	2	Loading	🖌 Loading	Anti-OST	
2	7	Wash	🙀 Custom	Anti-Huma	n Fab-CHI (FAB)
2	4	Association	🗶 Association	Anti-FLAG	(FLG)
2	8	Long Dissociation	📐 Dissociation	APS (Amin	opropyls (ane)
3	1	Baseline	🖿 Baseline	AR (Amine	Reactive)
3	2	Loading	🖌 Loading	AR2G (Ami	ne Reactive 2nd G
3	7	Wash	₩ Custom	SSA (Super Streptavidin)	
3	5	Association	C Association	AMC (Anti-	mIgG Fc Capture
3	8	Long Dissociation	L Dissociation		

Figure 10-47: Selecting an Assay Sensor Type

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.

#### Editing an Assay

To edit the step type or the biosensor type:

#### In the Assay Steps List:

- To change the step type, click the **Step Name** arrow (♥) and select a step name from the drop-down list (Figure 10-48, top).
- To change the biosensor type, click the Sensor Type arrow (
   for any step in the assay and select a sensor type from the drop-down list (Figure 10-48, bottom). The biosensor type will automatically update for every assay step.

**NOTICE:** The Step Name drop-down list includes only the step types defined in the Step Data List.

ssay	Sample	Step Name		Step Type	Sensor Type	Assay Time
	1	Baseline		🛌 Baseline	SA (Streptavidin)	
	2	Loading		🖌 Loading	SA (Streptavidin)	
	7	Wash		🙀 Custom	SA (Streptavidin)	
	3	Association		🖌 Association	SA (Streptavidin) 👻	•
	8	Baseline		n	SA (Streptavidin)	
	10	Loading		pn	SA (Streptavidin)	0:35:23
	1	West			SA (Streptavidin)	
	2	vvasn			SA (Streptavidin)	
	7	Association	2		SA (Streptavidin)	
	4	Long Diss	ocia	tion	SA (Streptavidin)	
	8	Regenera	tion		SA (Streptavidin)	0:35:15
	1	Baseline		🔚 Baseline	SA (Streptavidin)	
	2	Loading		🖌 Loading	SA (Streptavidin)	
	7	Wash		🙀 Custom	SA (Streptavidin)	
	5	Association		K Association	SA (Streptavidin)	
	8	Long Dissociation	n i	L Dissociation	SA (Streptavidin)	
	10	Regeneration		Regeneration	SA (Streptavidin)	0:35:23
ssay	Sample 1	Step Name Baseline		Step Type	Sensor Type	Assay Time
-	1	Baseline		Saseline	SA (Streptavidin)	
	2	Loading		🖌 Loading	SA (Streptavidin)	
	7	Wash		🙀 Custom	SA (Streptavidin)	
	3	Association	-	🞽 Association	SA (Streptavidin) 👻	
	8	Long Dissociation	1	L Dissociation	SA (Streptay	vidin)
	10	Regeneration		Regeneration	AHC (Anti-H	hIgG Fc Capture)
	1	Baseline		ե Baseline	Anti-GST	
	2	Loading		🖌 Loading	Anti-Huma	n Fah-CH1 (FAR
	7	Wash		🙀 Custom	Anti-ELAC	
	4	Association		Association	ANU-FLAG (	(FLG)
	8	Long Dissociation	1	Dissociation	APS (Aminopropyls( ane)	
	1	Baseline		🔚 Baseline	AR (Amine Reactive)	
	2	Loading		🖌 Loading	AR2G (Amine Reactive 2nd Gen)	
	7	Wash		₩ Custom	SSA (Super	Streptavidin)
	5	Association		Association	AMC (Anti-	mIgG Fc Capture
		- D' ' '		Discontinu		
	8	Long Dissociation	1	N Dissociation	NI-NIA	
	8 10	Regeneration	1	<ul> <li>Dissociation</li> <li>Regeneration</li> </ul>	NI-NTA Custom	
	8 10	Regeneration	1	<ul> <li>Dissociation</li> <li>Regeneration</li> </ul>	NI-NTA Custom anti-penta k	nis

Figure 10-48: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

To reorder or remove an assay step:

- 1. Select a step (row) in the Assay Steps List.
- 2. Click the Move Up, Move Down, or Remove button located above the list.

**IMPORTANT:** An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. The software recognizes the baseline/association/dissociation set of steps.

#### Adding an Assay Through Replication

.A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

- 1. In the Assay Steps List, select the steps to copy and click Replicate.
  - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
  - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
- 2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 10-49).



Figure 10-49: Adding a Replicate Assay to a Plate

- Click the Offset steps check box and set the options as appropriate (see Table 10-5 on page 443 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the Offset steps option. If the replicate assay uses a different sample column, select Offset steps and the appropriate options.
  - Sample steps only offsets the sample wells by the value specified under Sample steps will be adjusted. The offset will not be applied to reagent wells such as buffer, loading, regeneration, neutralization and detection.

- All Steps offsets all wells in the assay, including sample and reagent wells, by the value specified under Sample steps will be adjusted.
- 4. Click OK. The new assay appears in the Assay Steps List.
- 5. Continue to add assay steps as needed.

## Assigning Biosensors to Samples

After you define the sample plate and assay(s), click the Sensor Assignment tab or click the arrow  $\rightarrow$  to access the Sensor Assignment window. The color-coded Sensor Tray and Sample Plate Map show the locations of the biosensors associated with the samples Figure 10-50.

**NOTICE:** When using a 96-well plate with the 8 channel read head, do not put biosensors in columns 2, 4, 6, 8, 10, and 12 if the biosensors will be returned to the biosensor tray and not discarded. If the biosensors will be ejected, biosensors can be placed in all columns.

**NOTICE:** If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 10-50, the experiment includes two assays in the same wells. The use of those wells by two different biosensors is indicated by the pie chart colors.

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



Figure 10-50: Sensor Assignment Window

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 10-51).



Figure 10-51: Tool Tip of Well Information

#### Replacing the Biosensors in the Biosensor Tray

After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 10-52).



Figure 10-52: Replace Sensors in Tray After Use Check Box

**NOTICE:** Biosensors can be regenerated up to a max of 11 times per experiment.

#### **Entering Biosensor Information**

To enter information about a biosensor:

- Optional: Double-click in any cell in the Lot Number column to enter the biosensor lot number. All wells in the Lot Number column for that biosensor type will automatically populate with the lot number entered (see Figure 10-53).
- 2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 10-53).

**NOTICE:** Edit commands (Cut, Copy, Paste, Delete) and shortcut keys (Cut - Ctrl+x, Copy - Ctrl+c, Paste - Ctrl+v, Undo - Ctrl+z) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

Well	Sensor Type	Lot Number	Information			
A1	AHC (Anti-hIgG Fc Capture)	10102020	Default Biosensor			
B1	AHC (Anti-hIgG Fc Capture)	10102020		Undo		
C1	AHC (Anti-hIgG Fc Capture)	10102020		Cut		
D1	AHC (Anti-hIgG Fc Capture)	10102020		cut		
E1	AHC (Anti-hIgG Fc Capture)	10102020		Сору		
F1	AHC (Anti-hIgG Fc Capture)	10102020		Paste		
G1	AHC (Anti-hIgG Fc Capture)	10102020		Delete		
H1	AHC (Anti-hIgG Fc Capture)	10102020				
A2	AHC (Anti-hIgG Fc Capture)	10102020		Select All		
B2	AHC (Anti-hIgG Fc Capture)	10102020		Right to left Reading order		
C2	AHC (Anti-hIgG Fc Capture)	10102020		Chow Unicode control characters		
D2	AHC (Anti-hIgG Fc Capture)	10102020		Show Unicode control characters		
E2	AHC (Anti-hIgG Fc Capture)	10102020		Insert Unicode control character		
F2	AHC (Anti-hIgG Fc Capture)	10102020		Open IMF		
G2	AHC (Anti-hIgG Fc Capture)	10102020		Reconversion		
H2	AHC (Anti-hIqG Fc Capture)	10102020		Reconversion		

Figure 10-53: Entering or Editing Biosensor Information

#### Changing the Biosensor Location

If you do not want to use the default biosensor columns, you can select other column(s) to use. There are two ways to do this:

- Method 1—In the Sensor Tray Map, Remove the columns you do not want to use. The software automatically selects the next available column(s).
- Method 2-Remove all columns from the Sensor Tray Map, then select the columns you want to use.

Method 1

In the **Sensor Tray Map**, select the columns to not use and click **Remove**. Or, right-click the selection and select **Remove** (Figure 10-54 left). The software automatically selects the next available biosensor columns in the tray (Figure 10-54 right).



Click Fill Plate to return the Sensor Tray Map to the default layout.

Figure 10-54: Changing Biosensor Location (Method 1)

#### Method 2

- 1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 10-55 top left). Or, right-click the selection and select **Remove**. All columns will be shown as **Missing** (Figure 10-55 top right).
- 2. Select the column(s) to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 10-55 bottom left). The software fills the selected columns in the tray (Figure 10-55 bottom right).

#### Sensor Tray Replace sensors in tray after use Replace sensors in tray after use A A В В С С D D Ε E F F G G Η Ηľ Legend: Unassigned sensors Missing se Legend: Missing sensors Una Fill Plate Remove Print. Remove Fill Plate Print... Fill Fill Sensor Tray Sensor Tray Replace sensors in tray after use Replace sensors in tray after use А A В В С С D D Е Е F F G G ΗK ΗK Legend: Unassigned sensors Missing ser Legend: Unassigned sensors Missing sensors Fill Fill Plate Remove Print... Remove Fill Fill Plate Print...

nsor Tray

Figure 10-55: Changing Biosensor Location (Method 2)

## Click Fill Plate to return the Sensor Tray Map to the default layout.

## Using Heterogeneous Trays

Sensor Tray

If heterogeneous biosensor trays will be used, the column location of each biosensor type in the tray can be identified in the Sensor Assignment Tab. Assignment of biosensors that will not be used in the assay enables the software to auto-assign the biosensors that will be used in the assay by biosensor type.

There are two ways to change the biosensor type:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (Figure 10-56 left). The associated wells in the **Sensor Type** column will automatically populate with the biosensor type selected.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-• down list (Figure 10-56 right). All other wells in the same column of the Sensor Tray Map as the selected cell will automatically populate with the biosensor type selected.

Replac	e sensors in trav after use		Well	Sensor Type	Lot Number	Information
	(auto-assign) SA (Streptavidin) AHC (Anti-hIgG Fc Capture) APS (AminopropyIsilane) AR (Amine Reactive) SSA (Super Streptavidin) Custom AHC Reta 1		A1 B1 C1 D1 E1 F1 G1 H1 A2 B2	AHC (Anti-higG Fc Capture) • A (auto-assign) A SA (Streptavidin) AHC (Anti-higG Fc Capture) APS (AminopropyIslane) AR (Amine Reactive) AR (Amine Reactive) ASA (Super Streptavidin) A Custom A AHC Beta 1 A		
F L G L H L Legenc	Reference Positive Control Negative Control Remove Fill	ng sensors				
Rei	Set Sensor Data Copy to Clipboard					

Figure 10-56: Sensor Assignment Window-Changing the Biosensor Type

The biosensor types in the **Sensor Assignment** window were specified previously in the **Assay Definition** window, and default locations are assigned automatically. To assign biosensor types for heterogeneous trays:

1. Select the column location of the biosensor type (see Figure 10-57).



Figure 10-57: Selecting a Sensor Tray Column

 Right-click in the Sensor Tray Map or click in a cell in the Sensor Type table column and select a biosensor type from the drop-down list. The biosensor type associated with the assay will shift location accordingly (see Figure 10-58). In the example shown, AHC is the Sensor Type used for the current assay. Columns 1 and 2 were reassigned as Streptavidin according to the heterogeneous tray being used.

V 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24	AS	And (Anithiga re Capitile)
AOOOOOOOOOOOOOOOOOO	B3	AHC (Anti-hlgG Fc Capture)
	C3	AHC (Anti-hlgG Fc Capture)
	D3	AHC (Anti-hlgG Fc Capture)
	E3	AHC (Anti-hlgG Fc Capture)
F0000000000000000000000000000000000000	F3	AHC (Anti-hlgG Fc Capture)
	G3	AHC (Anti-hlgG Fc Capture)
ÏŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎ	H3	AHC (Anti-hlgG Fc Capture)
100000000000000000000000000000000000000	A4	AHC (Anti-hlgG Fc Capture)
	B4	AHC (Anti-hlgG Fc Capture)
	C4	AHC (Anti-hlgG Fc Capture)
N 000000000000000000000000000000000000	D4	AHC (Anti-hlgG Fc Capture)
	E4	AHC (Anti-hlgG Fc Capture)
	F4	AHC (Anti-hlgG Fc Capture)
Legend: Unassigned samples	G4	AHC (Anti-hlgG Fc Capture)

Figure 10-58: Assay Sensor Type Reassignment

 $\mathbb{X}$ 

Missing sensors

Print...

3. Repeat the previous steps to assign locations for the remaining biosensor types in the tray.

Well Sensor Type

SA (Streptavidin)

SA (Streptavidin)

SA (Streptavidin) SA (Streptavidin)

SA (Streptavidin)

SA (Streptavidin)

A (Streptavidin)

SA (Streptavidin) SA (Streptavidin)

SA (Streptavidin)

SA (Streptavidin) <u>SA (</u>Streptavidin)

SA (Streptavidin)

SA (Streptavidir

H2

Lot Number Information

**IMPORTANT:** Ensure that the biosensor types selected in the Assay Definition window have assigned column(s) in the Sensor Assignment window or the experiment cannot be run.

## Using Partial Biosensor Trays

Sensor Tray Replace sensors in tray after use

Legend: Unassigned sensors

Remove Fill Fill Plate

A

B

С

D

E

F

G

Н

Plate 1 (384 wells)

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 10-59) includes two assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 10-59 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 10-59 bottom). If necessary, change the location of these biosensors.

Sensor Tray Replace sensors in tray after use	Sensor	Tray: Tray1 ∨ of 2		
1 2 3 4 5 6 7 8 9 10 11 12	Well	Sensor Type	Lot Number	Information
	A11	AHC (Anti-hlgG Fc Capture)		
	B11	AHC (Anti-hlgG Fc Capture)		
	C11	AHC (Anti-hlgG Fc Capture)		
	D11	AHC (Anti-hlgG Fc Capture)		
	E11	AHC (Anti-hlgG Fc Capture)		
	F11	AHC (Anti-hlgG Fc Capture)		
	G11	AHC (Anti-hlgG Fc Capture)		
	H11	AHC (Anti-hlgG Fc Capture)		
	A12	AHC (Anti-hlgG Fc Capture)		
	B12	AHC (Anti-hlgG Fc Capture)		
	C12	AHC (Anti-hlgG Fc Capture)		
[XX3 KXX] KX3 KXX] KXX KXX] KXX KXX] KXX KXX	D12	AHC (Anti-hlgG Fc Capture)		
Legend: Unassigned sensors 🕅 Missing sensors	E12	AHC (Anti-hlgG Fc Capture)		
Remove Fill Fill Plate Print	F12	AHC (Anti-hlgG Fc Capture)		
	G12	AHC (Anti-hlgG Fc Capture)		
Plate 1 (384 wells)	H12	AHC (Anti-hlgG Fc Capture)		
Sensor Tray				
Replace sensors in tray after use	Sensor	Tray: Tray 2 V of 2		
1 2 3 4 5 6 7 8 9 10 11 12	Well	Sensor Tray 2	Lot Number	Information
	A11	AHC (Anti-higG Fc Capture)		
	B11	AHC (Anti-hlgG Fc Capture)		
	C11	AHC (Anti-hlgG Fc Capture)		
	D11	AHC (Anti-hlgG Fc Capture)		
	E11	AHC (Anti-hlgG Fc Capture)		
	F11	AHC (Anti-hlgG Fc Capture)		
	G11	AHC (Anti-hlgG Fc Capture)		
	H11	AHC (Anti-hlgG Fc Capture)		
	A12	AHC (Anti-hlgG Fc Capture)		
	B12	AHC (Anti-hlgG Fc Capture)		
	C12	AHC (Anti-hlgG Fc Capture)		
	D12	AHC (Anti-hlgG Fc Capture)		
Legend: Unassigned sensors 🕅 Missing sensors	E12	AHC (Anti-hlgG Fc Capture)		
Remove Fill Fill Plate Print		AHC (Anti-bloG Ec Capture)		
Remove Fill Fill Plate Prof	112	(in the () that high is to capitally)		
Remove Fill Fill Flate Print	G12	AHC (Anti-hlgG Fc Capture)		

Figure 10-59: Example Experiment Using Two Biosensor Trays

**NOTICE:** Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet<sup>®</sup> instrument.

#### **Reference Biosensors**

To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.

**NOTICE:** Reference biosensors may also be designated in the Runtime Binding Chart during acquisition.

#### Changing the Biosensor Type

The biosensor type used in the assay must be selected in the **Assay Definition** window. To change the biosensor type:

- 1. Click the Assay Definition Tab.
- 2. In the Assay Steps List, click the cell in the Sensor Type column to change.
- 3. Select from the drop-down list (see Figure 10-60).

**IMPORTANT:** Ensure that the same biosensor types are selected in both the Assay Definition and the Sensor Assignment windows or the experiment cannot be run.

New Assay         Move Up         Move Down         Remove         Replicate         Exp. time: 0:29:14						
Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Assay Time
1	A1	1	equilibration 🔹	₩ Custom	AHC (Anti-hlgG Fc Capture) 🔹	
1	A5	1	Loading	🖌 Loading	ASA (Streptavidin)	
1	A9	1	Baseline	🛌 Baseline	AHC (Anti-hlgG Fc Capture)	
1	A13	1	Association	🞽 Association	APS (AminopropyIsilane)	
1	A9	1	Dissociation	📐 Dissociation	ASSA (Super Streptavidin)	
1	A1	2	Regeneration	🐺 Custom	ACustom	
1	A5	2	Neutralization	🐺 Custom	AHC Beta 1	0:29:00
						-

Figure 10-60: Assay Definition Window-Changing the Biosensor Type

# Starting a Basic Kinetics Experiment: Octet<sup>®</sup> RH96

The user-selectable Read Head can be used for kinetic experiments and provides the flexibility to choose multiple configurations in a single experiment, or given Method file (\*.fmf). After starting the Octet<sup>®</sup> RH96 system and the Octet<sup>®</sup> BLI Discovery software, follow the steps below to set up and run a kinetic experiment with multiple Read Head configurations

Table 10-6:  $Octet^{(\!\!R\!)}$  BLI Discovery Steps for Kinetic Assays

Octet <sup>®</sup> Software	Functions					
BLI Discovery	1. Select a kinetics experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf).					
	<b>NOTICE:</b> In the Experiment Menu, the Templates command allows users to pick from a set of predefined method templates for Kinetic, Quantitation, or Epitope Binning experiments. Users may also modify existing method templates to suit their experimental conditions and save as a new method file and new method file name.					
	2. Define a sample plate or open a sample plate definition.					
	3. Specify assay steps.					
	4. Assign biosensors to samples.					
	5. Run the experiment.					

## Starting an Experiment

You can start a kinetics experiment using one of the following options:

- Launch the Experiment Wizard by clicking Experiment > New Experiment Wizard, and selecting New Kinetics Experiment.
- Open an existing method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click Experiment > Templates > Kinetics.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

## Read Head Configuration and Plate Layout

The Octet<sup>®</sup> RH96 has a user-selectable Read Head for monitoring 8, 16, 32, 48, or 96 wells in parallel so you can tailor your assay design to maximize either throughput or detection sensitivity.

The 96 biosensor mode uses multiplexer switching to read 96 wells simultaneously either in a 96- or 384-well plate, with similar sensitivity as the Octet<sup>®</sup> QK384 system. Large sample sets are analyzed in the shortest amount of time using this Read Head setting, which is also ideal for rapid, whole plate analysis and biosensor loading in multi-step assays.

Figure 10-61 shows the biosensor layout in a 96- and 384-well plate with the 96-channels Read Head setting. Biosensors interrogate 96 wells in 12 columns at the same time.



Figure 10-61: Biosensor Layout in 96- and 384-well Plates Using 96-channels Read Head Setting.

**NOTICE:** A column of 16 wells is read in two sets of interrogations. Biosensors interrogate 8 wells in a column at a time: rows A, C, E, G, I, K, M and O are read first followed by rows B, D, F, H, J, L, N and P.

The 32 and 48 biosensor modes also use multiplexer switching to read 32 and 48 wells in parallel, with sensitivity equivalent to the Octet<sup>®</sup> QK384 system. Cross-blocking experiments as large as 32 x 32 or larger may be accomplished with the 32 or 48 biosensor modes combined with 384-well tilted-bottom plates in a shorter amount of time compared to other Octet<sup>®</sup> systems.

In Figure 10-62, biosensors interrogate 32 wells in 4 columns at a time or 48 wells in 6 columns at a time. Columns 1, 3, 5 and 7 are interrogated at the same time, and so on for the 32-channels setting. Columns 1, 3, 5, 7, 9 and 11 are interrogated at the same time, and so on for the 48-channel setting:



Figure 10-62: Biosensor Layout in 384-well Plates Using 32 (left) and 48 (right) Channels Read Head Setting.

The 8 and 16 biosensor modes provide high sensitivity for measuring small molecule binding interactions and protein quantitation down to 50 ng/mL, similar to the Octet<sup>®</sup> RED96e and Octet<sup>®</sup> RH16 systems. These two modes are best for assays requiring a wide dynamic range or fine signal resolution, and may be combined with the other Read Head options in a single experiment



Figure 10-63: Zoomed View of Closely Overlaid Traces Shows Fine Signal Resolution for Human IgG Quantitation Assay with Protein A Biosensors

#### 8 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

#### 16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so

Figure 10-64: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head
#### 8 Channel Read Head

16 Channel Read Head





Biosensors interrogate 8 wells in a column, one column is interrogated at a time. Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so

Figure 10-65: Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head

### Tab1(Plate Definition)

1. Choose the number of simultaneous wells to be read from the **Read Head** drop down list:



Figure 10-66: Select Wells to be Read

**NOTICE:** A column of 16 wells is read in two sets of interrogations. Biosensors interrogate 8 wells in a column at a time: rows A, C, E, G, I, K, M and O are read first followed by rows B, D, F, H, J, L, N and P.

2. Choose a plate format for Plate 1 and Plate 2 by clicking **Modify Plates**. Select either the 96- or 384 well format for each plate:

**NOTICE:** The default plate format can be changed from 96-well plate to 384-well plate by selecting File > Options and Default Sample Plate(s).

1 Plate Definition 2	Assay Definition	3 Sensor Assignment	4	Review Experiment
In this step, all t Highlight one o	the information abour r more wells on the s	ut the sample plate and sample plate, and right-	its we click t	ells will be entered. to enter/modify well d
Read Head: 96 channe	ls (high throughput)	✓ Modify Plate	s	Plate 1 Table
Plate 1 (96 wells)				O Plate 2 Table
A O O O	Modify Plates		×	Well Sample II
BOOOD	Plate 1	96 Wells	$\sim$	
	Plate 2	384 Wells	$\sim$	
DÕÕÕ				
EÓÓÓÓ				
FOOOC	ОК	Cancel	]	

Figure 10-67: Select Plate 1 and Plate 2 Formats

3. Designate plate layouts for Plate 1 and Plate 2 by selecting wells in the plate maps and designating sample types.

**NOTICE:** It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 10-2 will be included in the assay.

lcon	Description
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.
Controls	A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis. • Positive Control: A control sample that contains analyte of known concentration
	<ul> <li>Negative Control: A control sample known not to contain analyte</li> </ul>
Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
Activation	Activation reagent. Makes the biosensor competent for binding.
(Q) Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
Load	Ligand to be immobilized (loaded) on the biosensor surface.
₩wash	Wash buffer.
Regeneration	Regeneration reagents dissociate the analyte from the ligand.
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

Table 10-7: Types of Sample Wells

There are several ways to select sample wells in either plate map:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 10-68, top left).
- To select non-adjacent columns, hold the **Ctrl key** and click the column header (Figure 10-68, top right).

- Click a row header or select adjacent row headers by click-hold-drag (Figure 10-68, bottom left).
- Click a well or draw a box around a group of wells (Figure 10-68, bottom right).



Figure 10-68: Selecting Sample Wells in a Plate Map

4. Designate well types by right-clicking on selected wells and assigning a sample type:



Figure 10-69: Designating Well Types

5. To remove a well designation in either plate map, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data**:

ate 1 (384 wells)	Plate 1 (384 wells)	
1 2 3 4 5 6 7 8 9 101112131415161718192021222324	A Sample	2021222324
	C     Reference       C     Control	
	G N Negative Control	
	K B Buffer	
$\begin{array}{c} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 $	N € @ Quench OC PC ① Load	
Unassigned Import Export Remove Print	W         Wash         emo           Plate         R         Regeneration	ve Print
	Neutralization     Set Well Data	10 11 12
	B Clear Data	
	Extended Sample Types	

Figure 10-70: Clearing Sample Well Designations from the Plate Map

**NOTICE:** Shift-clicking in the plate map simultaneously selects a set of wells equal to the number of channels chosen in the read head option.

**NOTICE:** All sample types can be placed in either plate position 1 or 2, or both.

6. Enter sample information.

**NOTICE:** You must specify sample (analyte) concentration and molecular weight to allow the software to compute a  $K_D$  value. If the sample concentration is not specified, only  $k_d$  and  $k_{obs}$  are calculated. You can also annotate any well with Sample ID or Well Information, and assign Replicate Groups.

Select the table for either Plate 1 or Plate 2. There are several ways to enter sample information:

- Select an individual well in the plate table and enter information per well.
- Click-drag-hold several wells in the plate table, right-click and choose **Set Well Data**:

Set Well Data			×
Well Information		Dilution Series	
Sample ID:		Apply to:	<ul> <li>Concentration</li> </ul>
			O Molar Concentration
Replicate Group:		Starting value (µg/ml)	: 1
Well Information:		Series operator:	/ ~
		Series operand:	2
		Dilution orientation	
		Right	Cleft
Well Data - Sample only		Down	
Molecular Weight (kD):	150 🗹	ŏŏ	ěě
Molar Concentration (nM):	66.67		
Concentration (µg/ml):	1		OK Cancel

Figure 10-71: Entering Molecular Weight and Molar Concentration

**NOTICE:** More information on sample information and annotation can be found in "Entering Sample Information" on page 421.

### Tab 2 (Assay Definition)

After completing the plate layout(s), a Kinetic Assay can be defined:

- 1. Click on Tab 2 (Assay Definition).
- 2. Add assay step types in the Step Data List:
  - a. Click Add. The Add Step Definition box will display:

Add Step	Definitions			$\times$
	Association Dissociation	Name Association Dissociation	Time (s) 600 ♀ 600 ♀	Shake speed (rpm)
	Baseline Loading	Baseline	600	1000
	Activation	Activation	600 ÷	
	Regeneration	Regeneration	30	
	Dip	Dip	600	1000 -
		OK Ca	ancel	Defaults

Figure 10-72: Add Step Definition Box

- b. Choose a step type.
- c. Optional: Edit step name.
- d. Set the step time and shake speed.

e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

A	vdd C	ору	Remove	Regeneration Params	Threshold P	aram
	Name	Time	Shake speed	Туре	Threshold	
	Baseline	60	1000	🛌 Baseline		
•	Regeneration	30	1000	💈 Regeneration		
:	Step Name:		Regeneration			
:	Step Name:		Regeneration Time (s)	Shake speed (rpm):		
9	Step Name: Regeneration:		Regeneration       Time (s)       5	Shake speed (rpm):		
:	Step Name: Regeneration: Neutralization:		Regeneration Time (s) 5 v 5	Shake speed (rpm): 1000 🖕 1000 🖕		
9 1 1 1	Step Name: Regeneration: Neutralization: Regeneration c	ydes:	Regeneration       Time (s)       5       5       3	Shake speed (rpm):		

Figure 10-73: Regeneration Parameters Box

- f. Optional: Assign a threshold. See "Creating Step Types" on page 436 for more information.
- 3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).

**NOTICE:** Each assay color group must use the same Read Head setting for each of their steps, as listed in the Sensors column.

NOTICE: Individual assays are differentiated by color in the Assay column.

NOTICE: Individual assays may have different Read Head settings.

Select a step type in the Step Data List.

- g. In the Plate 1 or Plate 2 map, double-click the columns that you want associated with that step type.
- h. The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

1 Plate Definition 2 Assay Definition	3 Sensor Assignment	4 Review Experiment	5 R	un Experimer	t						
In this step, the assay steps will Select a group of sensors and a	be assembled from the Step append the currently selecte	Data List. d step into the current ass	ay with	a double clic	k, or rigł	nt click for more o	ptions.				
Plate 1 (384 wells)			Step D	)ata List					Time in	s), Shake spee	d in (rpm)
	4 40 40 44 45 46 47 49	10 20 24 22 22 24	Ac	id	Сору	Remove	Regeneration Par	ams	Threshold Params		
		R @ R O O O		Name	Time	Shake speed	Туре	Thresho	ld		
B 000000000000000000000000000000000000		NONCO		Baseline	60	1000	🔜 Baseline				
000000000000000000000000000000000000000	) • • Ø R Ø R Ø • •	RØROOO		Association	600	1000	Association				
		NBNOOO	•	Dissociation	600	1000	📐 Dissociation				
H 000000000000		NONCO									
000000000000000000000000000000000000000	) • • Ø 8 Ø 8 Ø • •	8 <b>000</b>	A	One list							
100000000000000000000000000000000000000		NBNOOO	New	Assav M	ove Un	Move Down	Remove Rer In	fo Table	Sten		
				No Con	and a D	Nata Chas Nas	Chan Turne	C	- Trans Con		
		R R R R R R R R R R R R R R R R R R R	ASSo 1	1 A14	npie r		ne Step Type	Senso	r Type Sei	hannolo no	
NŎŎŎŎŎŎŎŎŎŎ		NBNOOO		1 /14		Dissociatio		1 SA (Sue	spravidiri) • 32 c	nanneis no	0.10
000000000000000000000000000000000000000		8 <b>0</b> 8000									
P0000000000000000000000000000000000000											
Assayed samples	Unassigned samples										

Figure 10-74: Assay Steps List

- i. Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition). The number of biosensors listed must remain the same for that assay color group.
- j. Repeat the previous steps to define other steps in the assay.
- k. New assays may be added by clicking the **New Assay** button in the Assay Steps List:

ssay St	eps ist							
New A	ssay 📕 M	ove Up	Move Down	Remove	Replicate Edit SI	:ep		
Assuy	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time
1	A14	1	Baseline	🛌 Baseline	SA (Streptavidin)	32 channels	no	
1	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channels	no	
1	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channels	no	0:22:00
2	A14	1	Baseline	🔙 Baseline	SA (Streptavidin)	32 channels	no	
2	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channels	no	
2	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channels	no	0:22:00
3	A14	1	Baseline	🔙 Baseline	SA (Streptavidin)	32 channels	no	
3	B14	1	Association 👻	🞽 Association	SA (Streptavidin) 👻	32 channels	no	
3	A15	1	Dissociation	L Dissociation	SA (Streptavidin)	32 channels	no	0:22:00

Figure 10-75: New Assay Button

4. Change the Read Head setting for an individual assay by clicking the **Edit Step** button to bring up the edit step dialogue:

Assay St New A:	eps List ssay M	ove Up	Move Down	Remove	Replicate Edit St	ep				
Assay	Sample	Plate	Step Name	Step Type	Sensor Typ	Sensors	Reuse	Assay Time		
1	A14	1	Baseline	🛌 Baseline	SA (Streptavidin)	32 channels	no			
1	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channels	no			
1	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channels	no	0:22:00		
2	A14	1	Baseline 🔹	🖵 Baseline	SA (Streptavidin) 💌	32 channels	no			
2	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channels	no			
2	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channels	no	0:22:00		
3	A14	1	Baseline	🖵 Baseline	SA (Streptavidin)	32 channels	no			
3	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channels	no			
3	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channels	no	0:22:00		
										1.00.00
									Exp. time:	1:06:00

Figure 10-76: Edit Step Button

5. Choose a new setting from the Read Head drop-down list, then click **OK**:

Assay Sa	ample Plate Step Name Ste	ер Туре	Sensor Type	Sensors	Reuse	Assay Time
Step	A PARTY IN			32 channels	no	
				32 channels	no	
Assay Parameters	2		ОК	32 channels	no	0:22:00
Assay:	2	1		32 channels	no	
Read head:	32 sensors		Cancel	32 channels	no	
	48 sensors			32 channels	no	0:22:00
	32 sensors			32 channels	no	
Step Parameters	8 sensors			32 channels	no	
Step Name:	Baseline			32 channels	no	0:22:00
Step Type:	Baseline					
Sensor Type:	SA (Streptavidin)	•				
	Plate 1 Sample A14 Char					

Figure 10-77: Setting Read Head Sensors

6. Repeat for new Read Head settings for other assays in the experiment:



Figure 10-78: Setting Read Head Sensors for Experiment Assays

7. Edit or change the columns associated with a step type by selecting the individual step and clicking the **Edit Step** button:

Assay St	eps List ssau M	ovelln	Move Down	Bemove	Benlicate	en		
Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Csors	Reuse	Assay Time
1	A14	1	Baseline	🛌 Baseline	SA (Streptavidin)	32 channels	no	
1	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channels	no	
1	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channels	no	0:22:00
2	A14	1	Baseline 🔹	😓 Baseline	SA (Streptavidin) 💌	16 channels	no	
2	B14	1	Association	🞽 Association	SA (Streptavidin)	16 channels	no	
2	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	16 channels	no	0:22:00
3	A14	1	Baseline	📙 Baseline	SA (Streptavidin)	8 channels	no	
3	B14	1	Association	🞽 Association	SA (Streptavidin)	8 channels	no	
3	Δ15	1	Dissociation	Dissociation	SA (Strentavidin)	8 channels	no	0.22.00

Figure 10-79: Edit Step Button

8. Click the **Change** button, then click **OK**.



Figure 10-80: Edit Step Change Button

9. This will bring up the Set Position plate map. Click on the new column(s) associated with that step:



Figure 10-81: Set Position Plate Map

10. You can use new biosensors or reuse the same biosensors for the next color assay group. The default **Reuse** selection is no, which will use new biosensors:

Assay	oteps List							
New	Assay M	ove Up	Move Down	Remove	Replicate Edit St	ep		
Assa	y Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time
1	A14	1	Baseline	🛌 Baseline	SA (Streptavidin)	32 channe <mark>s</mark>	no	
1	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channe <mark>s</mark>	no	
1	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channe <mark>s</mark>	no	0:22:00
2	A17	1	Baseline 🔹	🔙 Baseline	SA (Streptavidin) 💌	16 channe <mark>s</mark>	no	
2	A17 B14	1	Baseline <ul><li>Association</li></ul>	Baseline	SA (Streptavidin) 🔻 SA (Streptavidin)	16 channes 16 channes	no no	
2 2 2	A17 B14 A15	1 1 1	Baseline▼AssociationDissociation	<ul> <li>Baseline</li> <li>Association</li> <li>Dissociation</li> </ul>	SA (Streptavidin) 💌 SA (Streptavidin) SA (Streptavidin)	16 channes 16 channes 16 channes	no no no	0:22:00
2 2 2 3	A17 B14 A15 A14	1 1 1 1	Baseline  Association Dissociation Baseline	Baseline Association Dissociation Baseline	SA (Streptavidin)  SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)	16 channes 16 channes 16 channes 8 channels	no no no no	0:22:00
2 2 3 3	A17 B14 A15 A14 B14	1 1 1 1 1	Baseline•Association•Dissociation•Baseline•Association•	➡         Baseline           ✓         Association           ▲         Dissociation           ➡         Baseline           ✓         Association	SA (Streptavidin)  SA (Streptavidin) SA (Streptavidin) SA (Streptavidin) SA (Streptavidin)	16 channes 16 channes 16 channes 8 channels 8 channels	no no no no no	0:22:00

Figure 10-82: Default Biosensor Reuse Selection

**NOTICE:** The Reuse option is only available for the Octet<sup>®</sup> RH96 system at this time.

**NOTICE:** Sartorius recommends adding Regeneration steps at the end of the current assay before reusing the same biosensors on the next color assay group.

To reuse the biosensors from the current assay color group for the next color assay group, select a step in the current assay and click the **Edit Step** button. Select the **Reuse sensors** box and click **OK**. The **Reuse** selection will now be set to yes.

Assay St New A:	New Assay Move Up Move Down Remove Replicate Edit Step								
Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time	
1	B1	1	Baseline 👻	🛌 Baseline	SA (Streptavidin) 👻	96 channels	yes		
1	A1	1	Association	🞽 Association	SA (Streptavidin)	96 channels	yes		
1	B1	1	Dissociation	📐 Dissociation	SA (Streptavidin)	96 channels	yes		
1	A2	1	Regeneration	💈 Regeneration	SA (Streptavidin)	96 channels	yes	0:03:55	
				Edit Step Assay Param Assay: Read head: Step Paramet Step Name: Step Type: Sensor Type Position:	eters 1 96 sensors V Reuse senso ters Baseline Baseline :: SA (Streptavidi Plate 1, Sample	n) B1	Change.	<ul> <li>OK</li> <li>Cance</li> <li>▼</li> <li>▼</li> </ul>	

Figure 10-83: Changing the Biosensor Reuse Selection

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### Tab 3 (Sensor Assignment)

After completing the assay definition, click on Tab 3 (Sensor Assignment) to assign sensor type(s) for the kinetic experiment.

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.

**NOTICE:** Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in "Assigning Biosensors to Samples" on page 447.

Replacing Biosensors in the Biosensor Tray

After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the **Replace sensors in tray after use** check box:



Figure 10-84: Replace Sensors in Tray After Use Check Box

# **Reviewing Experiments**

Before running an experiment, you can review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window (Figure 10-85), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the  $\leftarrow$  arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.



Figure 10-85: Review Experiment Window

# Saving Experiments

After an experiment is run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method

To manually save an experiment:

#### 1. Click Save Method File ( ), or on the main menu, click File > Save Method File.

If there is more than one open experiment and you want to save all of them, click Save All Methods Files 🐴 .

2. In the Save dialog box, enter a name and location for the file, and click Save.

**NOTICE:** If you edit a saved experiment and want to save it without overwriting the original file, click File > Save Method File As and enter a new name for the experiment.

### Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment > Templates > Kinetics > Experiment Name** (see Figure 10-86).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\Sartorius\OctetBLIDiscovery\TemplateFiles.

**IMPORTANT:** Do not change the location of the Template folder. If the Template folder is moved from the factoryset location, the software may not function properly.

Ex	periment Instrument Windo	w Help					
<ul> <li>✓</li> </ul>	New Experiment Wizard Edit Assay Parameters	Ctrl+N					
	Edit Sensor Types						
	Set Plate Temperature						
	Templates	•	Kinetics	•	Biomolecule kinetics - AHC biosensor	•	Kinetic Characterization_16CH_96W.fmf
	Skip Step		Quantitation	•	Biomolecule kinetics - AMC biosensor	•	Kinetic Characterization_8CH_96W.fmf
	Stop				Biomolecule kinetics - AR biosensor	+	Screening_16CH_384W.fmf
					Biomolecule kinetics - SA biosensor	•	Screening_8CH_96W.fmf
					Small Molecule and Fragment Kinetics - SSA biosensor	<u> </u>	

Figure 10-86: Saved Experiments in the Template Folder

# Running a Kinetics Experiment

**IMPORTANT:** Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.

### Loading the Biosensor Tray, Sample, and Reagent Plates

To load the biosensor tray, sample plate, and reagent plate:

- 1. Open the Octet<sup>®</sup> instrument door (lift the handle up) and present the instrument stage (click the **Present Stage** button, ▲ ).
- 2. Place the biosensor tray, sample plate, and reagent plate on the appropriate stage so that well A1 is located at the upper right corner (see Figure 10-87):
  - a. Place the rehydration plate and biosensor tray on the biosensor stage (left platform).
  - b. Place the sample plate on the sample stage (middle platform).
  - c. Place the reagent plate on the reagent stage (right platform).



Figure 10-87: Octet<sup>®</sup> Instrument Stage Platform

**IMPORTANT:** Ensure that the bottom of the sample plate, reagent plate and biosensor tray are flat on the stages.

- 3. Click  $\blacktriangle$  to close the Octet<sup>®</sup> instrument door.
- 4. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

### Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow  $(\rightarrow)$  to access the Run Experiment window (see Figure 10-88).

C:\data Experiment_1 201111 1		····	Prior to pressing "Go" confirm t
C:\data Experiment_1 201111 1		···	
Experiment_1 201111 1		<b>→</b>	
201111			
1			
1			
			Total experiment time: 1:12:10
			1.12.10
🗹 Open ru	ntime charts automatica	ally	
🗧 🖂 Au	tomatically save runtime	e chart	
Set plate	e temperature (°C):	30 🚔	
distance to sensor tip from	bottom of well	Default	
s (5.0 Hz)	~		
hese settings could affect as	sav signal to poise		
to use these settings, please	consult the User Guide		
Machine name	DESKTOP-0EH	TC34	
		$\sim$	
	Open ru     Au     Set plat     distance to sensor tip from     s (5.0 Hz) hese settings could affect as: to use these settings, please     Machine name	Open runtime charts automatically save runtime     Automatically save runtime     Set plate temperature ('C):      distance to sensor tip from bottom of well     s (5.0 Hz)      hese settings could affect assay signal-to-noise.     to use these settings, please consult the User Guide     Machine name: DESKTOP-0EH	

Figure 10-88: Run Experiment Tab-Octet®16

2. Confirm the default settings or enter new settings. See "Run Experiment Window Settings" on page 477 for more information on experimental settings.

**NOTICE: I**f you delay the experiment start, you have the option to shake the plate until the experiment starts.

3. To start the experiment, click **GO**.

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 10-89).

**NOTICE:** For more details about the Runtime Binding Chart, see "Managing the Runtime Binding Chart" on page 479.



Figure 10-89: Runtime Binding Chart

4. Optional: Click View > Instrument Status to view the log file (see Figure 10-90).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



Figure 10-90: Instrument Status Log



**WARNING:** Do not open the Octet <sup>®</sup> instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



**WARNING:** N'ouvrez pas la porte de l'instrument Octet<sup>®</sup> lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



**WARNING:** Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

### Run Experiment Window Settings

### The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

#### Table 10-8: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Kinetics data repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click <b>Browse</b> to select another data location.
	<b>NOTICE:</b> Save the data to the local machine first, then transfer to a network drive.
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/bar- code	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

#### The following **Run Settings** are available on the **Run Experiment** Tab:

#### Table 10-9: Run Settings

Item	Description
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click <b>co</b> .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the <b>Runtime Binding Chart</b> for the current biosensor during data acquisition.
Automatically save run- time chart	Saves an image (.jpg) of the <b>Runtime Binding Chart</b> . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in <b>File &gt; Options</b> . The factory set default temperature is 30 °C.
	<b>NOTICE:</b> If the actual plate temperature is not equal to the set plate temperature, a warn- ing displays and the Octet <sup>®</sup> BLI Discovery software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.

The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet<sup>®</sup> system per second and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

The following **Advanced Settings** are available for the Octet<sup>®</sup> RH16 system:

Table 10-10: Advanced	d Settings	Octet <sup>®</sup>	RH16
-----------------------	------------	--------------------	------

Item	Description
Acquisition rate	<ul> <li>High sensitivity kinetics (2.0 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second.</li> </ul>
	<ul> <li>Standard kinetics (5.0 Hz, averaging by 20)—The average of 50 data frames is reported as one data point. Five data points are reported per second.</li> </ul>
	<ul> <li>Fast kinetics (10.0 Hz, averaging by 5)- The average of 5 data frames is reported as one data point. Ten data points are reported per second.</li> </ul>
Sensor off set (mm)	Recommended sensor offset: Large molecule kinetics-4 mm
Default Sets the acquisition speed and sensor offset at the default settings.	

#### The following **Advanced Settings** are available for the Octet<sup>®</sup> QK384 system:

#### Table 10-11: Advanced Settings Octet QK384

ltem	Description
Acquisition rate	<ul> <li>High sensitivity kinetics (0.3 Hz, averaging by 40) - The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds.</li> </ul>
	<ul> <li>Standard kinetics (0.6 Hz, averaging by 5) - The average of 5 data frames is reported as one data point. One data point is reported every 1.6 seconds.</li> </ul>
Sensor off set (mm)	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

#### The following General Settings are available on the Run Experiment Tab:

#### Table 10-12: General Settings

Item	Description
Machine name	The computer name that controls the $Octet^{(\!\!\!\mathrm{I}\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$
User name	The user logon name.
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

### Stopping an Experiment

To stop an experiment in progress, click  $\bigotimes$  or click **Experiment** > **Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.

NOTICE: After the experiment is run, the software automatically saves the experiment method (.fmf).

# Managing the Runtime Binding Chart

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 10-91). The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active biosensor column is colorcoded (A=green, B=magenta, C=orange, D=purple, E=olive, F= black, G=red, H=blue) within the **Sensor Tray Map**. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each assay in the experiment is represented by **Assay X** in the **Current Binding Charts** box.

To selectively display data for particular assay:

- 1. Click the corresponding Assay number.
- 2. Select a subset of sensors for a displayed column under Sensors to Chart box (see Figure 10-91).

**IMPORTANT:** Do not close the Runtime Binding Chart window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet<sup>®</sup> BLI Discovery software saves the Runtime Binding Chart as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.



Figure 10-91: Runtime Binding Chart Window

### Opening the Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

- 1. Click File > Open Experiment.
- 2. In the dialog box that appears, select an experiment folder and click **Select**.

### Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the Sensor Assignment tab
- During acquisition in the Runtime Binding Chart **Sensors to Chart** box
- During analysis in the **Data Selection** tab

#### Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 10-92).



Figure 10-92: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray (**see Figure 10-93).



#### 2. Click the **Subtract reference sensors** check box (see Figure 10-93).

Figure 10-93: Subtract Reference Sensors check box in the Runtime Binding Chart

**NOTICE:** Subtracting reference data in the Runtime Binding Chart only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be repeated during data analysis if needed.

### Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 10-94). Uncheck the box to return to the default data display.



Figure 10-94: Data Inverted Using Flip Data Function

### Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 10-95), right-click a step and select **Align to Step** <*number>*.

To remove the step alignment, right-click the step and select **Unaligned**.



Figure 10-95: Runtime Binding Chart-Aligning the Data to a User-Selected Step

### Aligning Data to a Specific Time

1. To align the binding data to a specific time, in the **Runtime Binding Chart** (see Figure 10-96), right-click and select **Align at time**.



Figure 10-96: Runtime Binding Chart–Aligning the Data to a User-Specified Time

The Align at Time dialog box displays (Figure 10-97).

Align at Time	×
Timer (s):	320
ОК	Cancel

Figure 10-97: Align at Time Dialog Box

2. Enter the time point you want to align to and click **OK**. The binding chart will then align to the time point specified.

To remove the time alignment, right-click and select **Unaligned**.

### Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.

**NOTICE:** If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.

To extend the duration of the active step:

- 1. In the chart window, click the **Extend Current Step** button.
- 2. In the **Extend Current Step** dialog box (see Figure 10-98), enter the number of seconds to extend the step and click **OK**.

Extending this a	tan will provent data	analysis if these		he used in
Parallel Referen	ce, Double Reference	e, or Average F	Reference subtra	ction methods.
Assay 1, Step 2				
Duration (s):	600			
			-	

Figure 10-98: Extend Current Step Dialog Box

To terminate a step and begin the next step in the assay:

- 1. In the chart window, click the **Go to Next Step** button.
- 2. In the **Data Acquisition** dialog box, click **OK**.

### Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

### Scaling a Runtime Binding Chart

To scale the Runtime Binding Chart:

- 1. Right-click the Runtime Binding Chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, select Fullscale or Autoscale.

### Adding a Runtime Binding Chart Title

To add a Runtime Binding Chart title:

- 1. Right-click the chart and select **Properties**.
- 2. In the **Runtime Graph Properties** dialog box, enter a graph title or subtitle.

### Selecting a Runtime Binding Chart Legend

To select a Runtime Binding Chart legend:

- 1. Right-click the chart and select **Properties**.
- 2. In the Runtime Graph Properties dialog box, select one of the following legends:
  - Sensor Location
  - Sample ID
  - Sensor Information
  - Concentration/Dilution

Runtime Graph Properties				
Title:				
Subtitle:				
Logond				
<ul> <li>Sensor Location</li> </ul>	© Sensor Information			
Sample ID	Concentration / Dilution			
	OK Cancel			

Figure 10-99: Selecting a Runtime Binding Chart Legend

**NOTICE:** Text for Sample ID, Sensor Information, or Concentration/Dilution is taken from the Plate Definition and Sensor Assignment tabs, and must be entered before the experiment is started.

3. Click OK.

### Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window** > **New Window**.

### Exporting or Printing the Runtime Binding Chart

To export the Runtime Binding Chart as a graphic or data file:

- 1. Right-click the chart and select **Export Data**.
- 2. In the **Exporting** dialog box (see Figure 10-100), select the export options and click **Export**.

Export					
EMF	© ₩MF	BMP	◎ JPG	O PNG	🔘 Text / Data
Export D	estination				
ClipBo	pard				
) File		Browse			
Printe	r				
O Printe Export Si	r ze				
Printe Export Si	ze	<ul> <li>Millimete</li> </ul>	rs 🔘 Ir	iches 🔘 Points	3
Printe Export Si	r ze Width: 152.4	Millimete	rs O Ir 101.600	nches ⑦ Points Millimeters	Export

Figure 10-100: Exporting Dialog Box

Table 10-13: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	$\checkmark$		Click <b>File</b> > <b>Browse</b> to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Run- time Binding Chart to a graphic file		✓	Click <b>File &gt; Browse</b> to select a folder and enter a file name.	Creates a graphic image.
Copy the Run- time Binding Chart		$\checkmark$	Clipboard	Copies the chart to the system clip- board

able 10-13: Runtime Binding Chart Export Options (Continued)					
Task	Export	Option	Export Destination	Result	
Print the Runtime Binding Chart	Ň	/	Printer	Opens the Print dialog box.	

# Managing Experiment Method Files

After you run an experiment, the Octet<sup>®</sup> BLI Discovery software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. A read-only copy of the method used for an experiment is automatically saved in the experiment folder. Open a method (.fmf) and edit it as needed.

**NOTICE:** When using the 21 CFR Part 11 version of the Octet<sup>®</sup> BLI Discovery software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indica4ting this will be presented.

Table 10-14: Managing Experiment Method Files

Menu Bar Command/Toolbar Button	Description		
File > Open Method File 🗂	Enables you to select and open a method file (.fmf)		
File > Save Method File 📩 or	Saves one method file or all method files. Saves a method file before the experiment is run.		
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.		

# Epitope Binning

The goal of a typical epitope binning or cross-blocking experiment is to identify antibodies which bind to different or identical epitopes on the antigen. Antibodies are tested two at a time for competitive binding to one antigen. By competing antibodies against one another in a pairwise and combinatorial format, antibodies with distinct blocking behaviors can be discriminated and assigned to "bins". The end result is matrix of pairwise binders and blockers.

An epitope binning or cross-blocking experiment must be run as a kinetic experiment with repeating steps in the Octet<sup>®</sup> BLI Discovery software.

**NOTICE:** Sartorius highly recommends using the Loading, Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

After starting the Octet<sup>®</sup> system and the Octet<sup>®</sup> BLI Discovery software, follow the steps in Table 10-15 to set up and run an epitope binning experiment.

Table 10-15: Octet<sup>®</sup> BLI Discovery Steps for Epitope Binning Assays

Octet <sup>®</sup> Software	Functions				
	<ol> <li>Select Epitope Binning under New Kinetics Experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf).</li> </ol>				
Octet BLI Discovery 12.2	<b>NOTICE:</b> In the Experiment Menu, the Templates command allows users to pick from a set of predefined method templates for Kinetic, Quantitation, or Epitope Binning experi- ments. Users may also modify existing method templates to suit their experimental condi- cions and save as a new method file and new method file name.				
	2. Define a sample plate or open a sample plate definition.				
	3. Specify assay steps.				
	4. Assign biosensors to samples.				
	5. Run the experiment.				

## Starting an Experiment: Octet<sup>®</sup> RH16 or Octet<sup>®</sup> QK384

You can start a kinetics experiment using one of the following options:

- Launch the Experiment Wizard by clicking Experiment > New Experiment Wizard, and selecting New Kinetics Experiment and Epitope Binning.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment** > **Templates** > **Epitope Binning**.
- Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

#### Tab1(Plate Definition)

NOTICE: The Sample plate and the Reagent plate are now referred to as "Plate 1" and "Plate 2" in the software.

1. For Octet<sup>®</sup> QK384 and Octet<sup>®</sup> RH16, choose a plate format for both plate positions by clicking **Modify Plates**. Select either the 96- or 384 well format for each plate:



Figure 10-101: Select Plate Formats

- 2. Designate layouts for both plates by selecting wells in the plate maps and designating sample types. There are several ways to select sample wells in either plate map:
  - · Click a column header or select adjacent column headers by click-hold-drag.
  - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
  - · Click a row header or select adjacent row headers by click-hold-drag.
  - · Click a well or draw a box around a group of wells.

3. Designate well types by right-clicking on selected wells and assigning a sample type:\

Plate 1 (3	84 wells)		
123	4 5 6 7 8 9 1011121314151617	18192021	222324
в	Şample	0000	000
60	Réference	0000	000
F O	Control	888	
GC Ň	Negative Control	000	000
i 🕻 🕑	Positive Control	000	000
KC B	Buffer	0000	0000
MC (A)	Activation	0000	000
N ( @	Quench	888	
PL D	Load	000	000
$\odot$ $\otimes$	Wash	emove	Print
Plate R	Regeneration		
<b>N</b>	Neutralization	L	
	Set Well Data	000	000
BCC	Clear Data	0000	

Figure 10-102: Designating Well Types

- 4. Enter sample information by selecting the table for either plate. There are several ways to enter sample information:
  - Select an individual well in the plate table.
  - Click-drag-hold several wells in the plate table, right-click and choose Set Well Data.

**NOTICE:** Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.

**NOTICE:** More information on sample information and annotation can be found in "Entering Sample Information" on page 421.

#### Tab 2 (Assay Definition)

After completing the plate layout(s), an Epitope Binning Assay can be defined by building a kinetic assay.

- 1. Click on Tab 2 (Assay Definition).
- 2. Add assay step types in the Step Data List:
  - a. Click the **Add** button. The Add Step Definition box will display:

Add Step Definitions			×
Association	Name Association Dissociation Baseline	Time (s) 600 • 600 • 600 •	Shake speed (rpm)
Loading     Activation     Junching	Loading Activation Quenching		
Regeneration	Custom Dip	30 × 600 × 600 ×	1000 ¢ 1000 ¢ 1000 ¢
	ОК	Cancel	Defaults

Figure 10-103: Add Step Definition Box

- b. Choose a step type.
- c. Optional: edit step name.
- d. Set the step time and shake speed.
- e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

Regeneration Paramet	ters		×
Step Name:	Regeneration		
	Time (s)	Shake speed (rpm):	
Regeneration:	5 🜲	1000	
Neutralization:	5	1000	
Regeneration cycles:	3		
Total step time:	30 s	OK Cancel	

Figure 10-104: Regeneration Parameters Box

- f. Optional: assign a threshold. See "Creating Step Types" on page 436 for more information.
- 3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).

**NOTICE:** We highly recommend using the Associate or Dissociate assay steps instead of Custom for epitope binning and cross-blocking experiments.

a. Select a step type in the Step Data List.

- b. In the plate map, double-click the columns that you want associated with that step type.
- c. The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:



Figure 10-105: Assay Steps List

- d. Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition).
- e. Repeat the previous steps to define other steps in the assay.
- f. New assays may be added by clicking the **New Assay** button in the Assay Steps List:

New A	ssay M	ove Up	Move Dowr	n Remove	Replicate	Edit Step		
Assay	sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time
1	A14	1	Baseline	⊾ Baseline	SA (Streptavidin)	32 channels	no	0:01:20
2	A14	1	Baseline	🛏 Baseline	SA (Streptavidin)	32 channels	no	0:01:20
3	A14	1	Baseline 🔹	🔙 Baseline	SA (Streptavidin) 💌	32 channels	no	0:01:20

Figure 10-106: New Assay Button

. . .

**NOTICE:** More information on assay step editing in Tab 2 (Assay Definition) can be found in "Creating Step Types" on page 436.

#### Tab 3 (Sensor Assignment)

After completing the assay definition, click on Tab 3 (Sensor Assignment) to verify sensor type(s) for the epitope binning experiment.

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.

**NOTICE:** Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in "Assigning Biosensors to Samples" on page 447.

Replacing Biosensors in the Biosensor Tray. After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the **Replace sensors in tray after use** check box:



Figure 10-107: Replace Sensors in Tray After Use Check Box

#### Tab 4 (Review Experiment)

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:



Figure 10-108: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

#### Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

- 1. Select File > Save Method File.
- 2. In the Save dialog box, enter a name and location for the file, and click **Save**.
### Loading the Biosensor Tray and Sample Plates

To load the biosensor tray and plate positions 1 and 2:

1. Click **Instrument > Present Stage** to open the door and present the stage. Alternatively, click the **Present Stage** button:

 Image: Second system
 Image: Second system

 Image: Second

- 2. Place the biosensor tray, biosensor wetting plate, Plate 1, and Plate 2 on the appropriate stage so that well A1 is located at the upper right corner.
- 3. Close the stage and door by clicking the **Present Stage** button again.

#### Tab 5 (Run Experiment)

- 1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
- 2. To start the experiment, click the **GO** button:

Data File Location and	Names			Prior to pressing "Go" confirm the Assay.	$\leftarrow$
Kinetics data repository	e.	C:\data			
Experiment run name (	sub directory):	Experiment_1	<b>→</b>		
Plate name/barcode (f	le prefix):	201111			
and Plate name/barco	de:				
Auto-increment file ID :	tart:	1		Total experiment time: 1:12:10	
Data files will be stored	as follows:				
C:\data\Experiment_1 C:\data\Experiment_1 C:\data\Experiment_1	201111_001 frd 201111_002 frd 201111_003 frd				
van Seungs ☐ Delayed experimen Star ☐ Shake sample plate ☑ Present stage at en	start after (s): 600 🔹 while waiting d of experiment	Open runtime charts automatically Automatically save runtime c Set plate temperature ('C):	hart 30		
Advanced Settings					
Sensor offset (mm):	3 ~	distance to sensor tip from bottom of well	Default		
Acquisition rate:	Standard kinetics (	(5.0 Hz) ~			
Wa	ning: changing the e unsure of how to i	se settings could affect assay signal-to-noise. use these settings, please consult the User Guide			
ir you an					
ieneral Information			34		
ieneral Information Jser name:		Machine name: DESKTOP-0EHT0			
Even and the second sec		Machine name: DESKTOP-DEHTC	<u>^</u>		

Figure 10-110: GO Button

### Starting an Experiment: Octet<sup>®</sup> RH96

You can start a kinetics experiment using one of the following options:

- Launch the Experiment Wizard by clicking Experiment > New Experiment Wizard, and selecting New Kinetics Experiment.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click Experiment > Templates > Epitope Binning.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

### Tab1(Plate Definition)

1. Choose the number of simultaneous wells to be read from the **Read Head** drop down list:



Figure 10-111: Select Wells to be Read

See "Read Head Configuration and Plate Layout" on page 456 for biosensor configurations.

2. Choose a plate format for Plate 1 and Plate 2 by clicking **Modify Plates**. Select either the 96- or 384 well format for each plate:



Figure 10-112: Select Plate 1 and Plate 2 Formats

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- 3. Designate plate layouts for Plate 1 and Plate 2 by selecting wells in the plate maps and designating sample types. There are several ways to select sample wells in either plate map:
  - Click a column header or select adjacent column headers by click-hold-drag.
  - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
  - Click a row header or select adjacent row headers by click-hold-drag.
  - Click a well or draw a box around a group of wells.
- 4. Designate well types by right-clicking on selected wells and assigning a sample type:

Plate 1 (38	34 wells)		
123	4 5 6 7 8 9 1011121314151617	18192021	222324
Ê	Sample	000	000
	Reference	000	000
Ę 🖌 🔘	Control	000	000
GC N	Negative Control	000	000
Î 🕻 🕑	Positive Control	0000	000
K 🖪	Buffer	-000	000
MC (A)	Activation	0000	000
N <b>( ( ( ( ( ( ( ( ( (</b>	Quench	888	
PC O	Load	000	000
$\odot$	Wash	emove	Print
Plate ®	Regeneration		
N	Neutralization	400004	2222.24
A	Set Well Data	000	000
BCC	Clear Data		

Figure 10-113: Designating Well Types

- 5. Enter sample information by selecting the table for either Plate 1 or Plate 2. There are several ways to enter sample information:
  - Select an individual well in the plate table.
  - Click-drag-hold several wells in the plate table, right-click and choose Set Well Data.

**NOTICE:** Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.

**NOTICE:** More information on sample information and annotation can be found in "Entering Sample Information" on page 421.

#### Tab 2 (Assay Definition)

After completing the plate layout(s), an Epitope Binning Assay can be defined by building a kinetic assay.

- 1. Click on Tab 2 (Assay Definition).
- 2. Add assay step types in the Step Data List:
  - a. Click the **Add** button. The Add Step Definition box will display:



Figure 10-114: Add Step Definition Box

- b. Choose a step type.
- c. Optional: edit step name.
- d. Set the step time and shake speed.
- e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

Regeneration Paramet	Regeneration Parameters ×										
Step Name:	Regeneration										
	Time (s)	Shake speed (rpm):									
Regeneration:	5	1000									
Neutralization:	5	1000									
Regeneration cycles:	3										
Total step time:	30 s	OK Cancel									

Figure 10-115: Regeneration Parameters Box

- f. Optional: assign a threshold. See "Creating Step Types" on page 436 for more information.
- 3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).

**NOTICE:** We highly recommend using the Associate or Dissociate assay steps instead of Custom for epitope binning and cross-blocking experiments.

- a. Select a step type in the Step Data List.
- b. In the Plate 1 or Plate 2 map, double-click the columns that you want associated with that step type.
- c. The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

1 Plate Definition 2 Assay Definition 3 Sensor Assignment 4 Review Experiment	5 Run Experiment						
In this step, the assay steps will be assembled from the Step Data List. Select a group of sensors and append the currently selected step into the current as	say with a double click, or	right click for more o	options.				
Plate 1 (384 wells)	Step Data List				Time in (s), Shake	speed in (	(rpm)
	Add Cop	Remove	Regeneration Pa	rams Thresh	hold Params		
	Name Tir	e Shake speed	Туре	Threshold			
	Baseline 60	1000	🛌 Baseline				
	Association 600	1000	🞽 Association				
	→ Dissociation 600	1000	L Dissociation				
	Assay Steps List				-		
ĸ <b>ŎŎŎŎŎŎŎŎŎŎŎŎŎŎŎ</b> خخخŎŎŎŎ	New Assay Move	Jp Move Down	Remove Rep I	nfo Table t Step			
	Assay No. Sample	Plate Step Na	me Step Type	Sensor Typ	e Sensors	Reuse	Assa
	1 1 A14	1 Dissociatio	on 👻 📐 Dissociatio	n SA (Streptavio	din) 👻 32 channels	no	0:10
Assayed samples O Unassigned samples							

Figure 10-116: Assay Steps List

- d. Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition). The number of biosensors listed must remain the same for that assay color group.
- e. Repeat the previous steps to define other steps in the assay.
- f. New assays may be added by clicking the **New Assay** button in the Assay Steps List:

New Assay Move Up Move Down Remove Replicate Edit Step										
Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time		
1	A14	1	Baseline	🔙 Baseline	SA (Streptavidin)	32 channels	no	0:01:20		
2	A14	1	Baseline	📙 Baseline	SA (Streptavidin)	32 channels	no	0:01:20		
3	A14	1	Baseline 🔹	🔙 Baseline	SA (Streptavidin) 💌	32 channels	no	0:01:20		

Figure 10-117: New Assay Button

4. You can use new biosensors or reuse the same biosensors for the next color assay group. The default **Reuse** selection is no, which will use new biosensors:

Assay Steps List New Assay Move Up Move Down Remove Replicate Edit Step												
Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time				
1	A14	1	Baseline	🛌 Baseline	SA (Streptavidin)	32 channe <mark>s</mark>	no					
1	B14	1	Association	🞽 Association	SA (Streptavidin)	32 channe <mark>s</mark>	no					
1	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	32 channe <mark>s</mark>	no	0:22:00				
2	A17	1	Baseline 🔹	🖵 Baseline	SA (Streptavidin) 💌	16 channes	no					
2	B14	1	Association	🞽 Association	SA (Streptavidin)	16 channes	no					
2	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	16 channe <mark>s</mark>	no	0:22:00				
3	A14	1	Baseline	🔙 Baseline	SA (Streptavidin)	8 channels	no					
3	B14	1	Association	🞽 Association	SA (Streptavidin)	8 channels	no					
3	A15	1	Dissociation	📐 Dissociation	SA (Streptavidin)	8 channels	no	0:22:00				

Figure 10-118: Default Biosensor Reuse Selection

**NOTICE:** The Reuse option is available only for the Octet<sup>®</sup> RH96 system.

**NOTICE:** We recommend adding Regeneration steps at the end of the current assay before reusing the same biosensors on the next color assay group.

**NOTICE:** Do not using biosensors ('no' in Reuse column) with epitope binning experiments. Regeneration is recommended within an individual color group assay, but start the next assay color group with next set of biosensors.

To reuse the biosensors from the current assay color group for the next color assay group, select a step in the current assay and click the **Edit Step** button. Select the **Reuse sensors** box and click **OK**. The **Reuse** selection will now be set to yes.

Assay S	teps List								
New A	.ssay M	ove Up	Move Down	Remove	plicate Edit Step				
Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time	
1	B1	1	Baseline 👻	🛌 Baseline	SA (Streptavidin) 👻	96 channels	yes		
1	A1	1	Association	🞽 Association	SA (Streptavidin)	96 channels	yes		
1	B1	1	Dissociation	📐 Dissociation	SA (Streptavidin)	96 channels	yes		
1	A2	1	Regeneration	💈 Regeneration	SA (Streptavidin)	96 channels	yes	0:03:55	
				Edit Step Assay Paran Assay: Read head: Step Parame Step Name: Step Type: Sensor Type Position:	Inters 1 96 sensors V Reuse sensor ters Baseline Baseline Easeline SA (Streptavid Plate 1, Sample	in) B1	▼ Change.	•	OK ancel

Figure 10-119: Changing the Biosensor Reuse Selection

**NOTICE:** More information on assay step editing in Tab 2 (Assay Definition) can be found in "Creating Step Types" on page 436.

#### Tab 3 (Sensor Assignment)

After completing the assay definition, click on Tab 3 (Sensor Assignment) to assign sensor type(s) for the epitope binning experiment.

**NOTICE:** The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.

**NOTICE:** Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in "Assigning Biosensors to Samples" on page 447.

Replacing Biosensors in the Biosensor Tray. After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the **Replace sensors in tray after use** check box:



Figure 10-120: Replace Sensors in Tray After Use Check Box

### Tab 4 (Review Experiment)

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:



Figure 10-121: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

#### Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf)

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

- 1. Select File > Save Method File.
- 2. In the Save dialog box, enter a name and location for the file, and click Save.

Loading the Biosensor Tray and Sample Plates

To load the biosensor tray and plate positions 1 and 2:

1. Click **Instrument > Present Stage** to open the door and present the stage. Alternatively, click the **Present Stage** button:



Figure 10-122: Present Stage Button

- 2. Place the biosensor tray, biosensor wetting plate, Plate 1, and Plate 2 on the appropriate stage so that well A1 is located at the upper right corner.
- 3. Close the stage and door by clicking the **Present Stage** button again.

#### Tab 5 (Run Experiment)

- 1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
- 2. To start the experiment, click the **GO** button:

Plate Definition 2 Assay Definition 3 Sensor Assignment 4 Review Experiment   Data File Location and Names   Knetics data repository:   C'data   Experiment run name (sub directory):   Experiment run name:   Obts file sub best as a follows:   C'ddas Experiment run runne (run name:   C'ddas Experiment run runne (run name:   Default   C'das Statings   Sensor diret (rm):   3   General information   Warning: changing these estings could affect assay signal-to-noise.   F'y ou are unsure of how to use these settings. please consult the User Guide   General information   Warning: changing these settings. please consult th			_		
Data File Location and Names       Prior to pressing "Go" confirm the Assay.         Kinetics data repository:       C_vdata         Experiment run name (sub directory):       Experiment_1         Pate name/barcode:	Plate Definition 2 Assay Definition	3 Sensor Assignment 4 Review Experiment	5 Run Expe	riment	
Kinetics data repository: C:\data   Experiment run name (sub directory): Experiment_1   Plate name/barcode Image: Second file perify:   Auto-increment file ID stat: Image: Second Se	Data File Location and Names			Prior to pressing "Go" confirm the Assay.	← GO
Kinetics data repository:       C:\data         Experiment run name (abd directory):       Experiment_1         Plate name/barcode:       Image: Comparison of the Distant:         Auch-increment file ID start:       Image: Comparison of the Distant:         C:\data/Experiment_1201111_001/rd       C:\data/Experiment_1201111_002/rd         C:\data/Experiment_1201111_002/rd       Image: Comparison of the Distant:         Played experiment_1201111_002/rd       Image: Comparison of the Distant:         Played experiment_1201111_002/rd       Image: Comparison of the Distant:         Played experiment_1201111_002/rd       Image: Comparison of the Distant:         Shake sample plate white wating       Image: Set plate temperature (C):       Image: Comparison of the Distant image: Comparison of the Di					
Experiment run name (sub directory): Experiment_1 Plate name/barcode file prefx): 201111 Chate name file 10 stat: 1 Chate Namement_1201111_001rd Chate Namement_12011111_001rd Chate Namement_1201111_001rd Chate Namem	Kinetics data repository:	C:\data			
Plate name/barcode (ile prefix):       201111         2nd Plate name/barcode;	Experiment run name (sub directory):	Experiment_1	<b>→</b>		
2nd Plate name/barcode:   Auto-increment file ID stat:   Data files will be stored as follows:   C:\data\Experiment1\201111_001frd   C:\data\Experiment1\201111_002frd   C:\data\Experiment1\201111_003frd   Plate stater (a): [600  Image: Im	Plate name/barcode (file prefix):	201111			
Auto-increment file ID start:       1         Data files will be stored as follows:       1:12:10         C:\data \Experiment_1\201111_001frd       1:12:10         C:\data \Experiment_1\201111_002rd       1:12:10         Run Settings       Image: Comparison of the store of the st	2nd Plate name/barcode:				
Data files will be stored as follows:         C:\data \Experiment_1201111_001 frd         C:\data \Experiment_1201111_003 frd         Run Settings         Delayed experiment_1v201111_003 frd         Stat after (s): 600 •         Automatically save nuntime charts automatically         Stat after (s): 600 •         Automatically save nuntime chart         Shake sample plate while waiting         Set plate temperature (°C):         30 •         Present stage at end of experiment         Advanced Settings         Sensor offset (mm):         3       distance to sensor tip from bottom of well         Acquisition rate:       Standard kinetics (5.0 Hz)         Warning: changing these settings could affect assay signal to noise.         If you are unsure of how to use these settings, please consult the User Guide         General Information         User name:       Machine name:         DESKTOP-0EHTC34         Description:         I	Auto-increment file ID start:	1		Total experiment time: 1:12:10	
C:\data \Experiment_1\201111_001frd         C:\data \Experiment_1\201111_002frd         C:\data \Experiment_1\201111_003frd         Pleayed experiment_stat       Open runtime charts automatically         Stat after (s):       60 •         Shake sample plate while waiting       Set plate temperature (*C):         Present stage at end of experiment         Advanced Settings         Sensor offset (mm):       3         distance to sensor tip from bottom of well         Default         Acquisition rate:       Standard kinetics (5.0 Hz)         Warning: changing these settings could affect assay signal to noise.         if you are unsure of how to use these settings.please consult the User Guide         General Information         User name:       Machine name:         DESKTOP-0EHTC34	Data files will be stored as follows:				
Run Settings       Open runtime charts automatically         Start after (s): 600 •       Automatically save runtime chart         Shake sample plate while waiting       Set plate temperature (°C): 30 •         Present stage at end of experiment         Advanced Settings         Sensor offset (mm):       3 •         distance to sensor tip from bottom of well         Default         Acquisition rate:       Standard kinetics (5.0 Hz)         Warning: changing these settings could affect assay signal to noise.         if you are unsure of how to use these settings, please consult the User Guide         General Information         User name:       Machine name:         DESKTOP-0EHTC34	C:\data\Experiment_1\201111_001.frd C:\data\Experiment_1\201111_002.frd C:\data\Experiment_1\201111_003.frd				
☑ Delayed experiment stat       ☑ Open nurtime charts automatically         Stat after (s): 600 ○       ☑ Automatically save nurtime chart         ☐ Shake sample plate while waiting       ☑ Set plate temperature (°C): 30 ○         ☑ Present stage at end of experiment       ☑ Open nurtime charts         Advanced Settings       ☑         Sensor offset (mm):       3 ○         ☑ Maring: changing these settings could affect assay signal to noise.         If you are unsure of how to use these settings, please consult the User Guide         General Information         User name:       Machine name:         DESKTOP-0EHTC34	Run Settings				
Stat after (s): 600  Automatically save runtime chat  Shake sample plate while waiting Step late temperature (*C): 30  Present stage at end of experiment  Advanced Settings  Advanced Settings Sensor offset (mm): 3  distance to sensor tip from bottom of well Default Acquisition rate: Standard kinetics (5.0 Hz)  Warning: changing these settings could affect assay signal to noise. If you are unsure of how to use these settings, please consult the User Guide  General Information User name: Machine name: DESKTOP-0EHTC34 Description:	Delayed experiment start	Open runtime charts automatical	ly		
Shake sample plate while waiting       Set plate temperature (°C):       30 €         Present stage at end of experiment         Advanced Settings         Sensor offset (mm):       3 ✓ distance to sensor tip from bottom of well         Default         Acquisition rate:       Standard kinetics (5.0 Hz)         Warning: changing these settings could affect assay signal to noise.         If you are unsure of how to use these settings, please consult the User Guide         General Information         User name:       Machine name:         Description:         I	Start after (s): 600	Automatically save runtime	chart		
Advanced Settings         Sensor offset (mm):       3       distance to sensor tip from bottom of well         Default       Acquisition rate:       Standard kinetics (5.0 Hz)         Warning: changing these settings could affect assay signal to noise.       if you are unsure of how to use these settings, please consult the User Guide         General Information       User name:       DESKTOP-0EHTC34         Description: <ul> <li></li></ul>	Shake sample plate while waiting	Set plate temperature (°C):	30		
Advanced Settings Sensor offset (mm): 3 v distance to sensor tip from bottom of well Default Acquisition rate: Standard kinetics (5.0 Hz) v Warning: changing these settings could affect assay signal-to-noise. If you are unsure of how to use these settings, please consult the User Guide General Information User name: Machine name: DESKTOP-0EHTC34 Description:	Present stage at end of experiment				
Sensor offset (mm): 3 v distance to sensor tip from bottom of well Acquisition rate: Standard kinetics (5.0 Hz) Warning: changing these settings could affect assay signal to noise. If you are unsure of how to use these settings, please consult the User Guide General Information User name: Machine name: DESKTOP-0EHTC34 Description: I	Advanced Settings				
Acquisition rate: Standard kinetics (5.0 Hz)	Sensor offset (mm): 3 ~	distance to sensor tip from bottom of well	Default		
Warning: changing these settings could affect assay signal to noise.         If you are unsure of how to use these settings, please consult the User Guide         General Information         User name:       Machine name:         Description:         I	Acquisition rate: Standard kinetic:	s (5.0 Hz) ~			
If you are unsure of how to use these settings, please consult the User Guide       General Information       User name:     Machine name:       Description:       I	Warning: changing th	nese settings could affect assay signal-to-noise.			
General Information User name: DESKTOP-0EHTC34 Description: I	If you are unsure of how t	o use these settings, please consult the User Guide			
User name: DESKTOP-0EHTC34 Description:	General Information				
Lescaption:	User name:	Machine name: DESKTOP-0EHT	C34		
×	Description:		~		
			$\sim$		

Figure 10-123: GO Button

# Chapter 11: Dose Response Experiments

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## Dose Response Analysis Overview

In an Octet<sup>®</sup> dose response experiment, what is typically measured is the binding response curve upon binding of an analyte molecule to a ligand or a binding complex at various concentrations. The binding response will be minimal at the lowest concentrations where there is no signal above the baseline which will generate the lower asymptote. At higher concentrations the response is saturated, and signals become insensitive to small concentration changes which will form the upper asymptote. In between these regions there is a large change in response. Measuring the response over a wide range of concentrations typically produces a sigmoidal plot as shown in Figure 11-1. If the response increases with concentration, the mid-point is the half-maximal effective concentration ( $EC_{50}$ ). Likewise, if the complex of interest causes the response to decrease with increasing concentration, the mid-point is the half-maximal inhibitory concentration ( $IC_{50}$ ).



Figure 11-1: Determining  $\text{EC}_{50}$  from the Dose Response Curve

The preferred method to set up a dose response experiment in the software is to use one of the included, two or three step templates found in the wizard. These templates are based upon quantitation assays, so we recommend you first become familiar with the basic quantitation procedures described in this user guide.

Alternatively, you may design a custom dose response experiment using one of the custom dose response templates as a starting point. The customization options are the same as the kinetic assay options, so we recommend you first become familiar with kinetic experiments described in this user guide.

**NOTICE:** The following information and experiment examples are performed on an Octet<sup>®</sup> RH16 system, but the analysis remains largely the same regardless of the Octet<sup>®</sup> instrument used to collect the data.

## Starting a Dose Response Experiment

You can start a dose response experiment using one of the following options:

- Via the menu or icon Start the wizard in the menu by selecting Experiment | New Experiment Wizard or by clicking the Experiment Wizard icon in the toolbar (Figure 11-2). Select Dose Response in the Experiment Wizard (Figure 11-3).
- Via a method file Open a method file (.fmf) by clicking File > Open Method File. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- Via the menu bar On the menu bar, click Experiment > Templates > Dose Response.
- Via Recent Methods You can also click Recent Methods to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.



Figure 11-2: Starting the Experiment Wizard



Figure 11-3: Experiment Wizard

### Tab 1: Plate Definition

At the top left is the default Read Head setting of 16 channels, meaning 16 biosensors will be used simultaneously to measure the binding response of 16 wells at a time. The default acquisition rate is 5.0 Hz, which produces a binding curve data point approximately every 0.2 seconds. You can also choose the slower, high-sensitivity mode of 2.0 Hz. For strong signal where the initial binding curve change is very fast, you can choose the fast high concentration mode of 10.0 Hz.

In the Assay Settings box is a description of the assay (Figure 11-4). In this example, the assay consists of four steps: Buffer, Sample, Buffer, and Detection. To carry out a dose response analysis, you need an analyte of several concentrations and a binding response signal that is proportional to these analyte concentrations. Octet<sup>®</sup> Analysis Studio software will automatically use the analyte concentrations of the sample wells and the binding response of the detection wells to perform the dose response curve fit.

Dose Response Exp	eriment - 2step_16CH_96W_1Te	stAndStandard_14	lconc							
Plate Definition	2 Sensor Assignment 3 Revie	w Experiment 4	Run E	kperiment						
In this step	all the information about the sampl	e plate and its wells	will be e	ntered						$\leftarrow$
First, check	the assay settings. Then highlight	one or more wells o	n the sar	mple plate, and	right-click to enter/r	nodif	y well data.			$\mathbf{i}$
Deed Used:	10 1 1		- Plate 1	Table (96 well	s)					
head head.	16 channels	~	Conce	ntration units:	ua/ml v		Export	Import	Print	
Acquisition Rate:	Standard (5.0 Hz)	$\sim$	Wall	Sample ID	Replicate Group	Ти		Conc (up/ml)	Dilution Fact	•
ssay Settings			D3	standard	Replicate Group		Standard	cone (µg/mi)	Dilution Fact	<u>^</u>
ssay: Do	se Response andard Assau	Modify	E3	etandard			Standard		n/a	
Sin	igle analyte		E3	etandard			Standard		n/a	
Tin 10	ne (s): Shake speed (rpm):		63	etandard			Standard		n/a	
mple 30	0 1000		нз	standard			Reference	n/a	n/a	
ffer 24	0 1000		A4	standard			Standard	17.4	n/a	
election 30	0 1000		R/	etandard			Standard		n/a	
			C4	etandard			Standard		n/a	
			DA	etandard			Standard		n/a	
			E4	etandard			Standard		n/a	
			E4	atandard			Standard		n/a	
			GA	etandard			Standard		n/a	
			ни	etandard			Reference	n/a	n/a	
'late 1 (96 wells)		✓ Modify	45	teet cample			Sample	170	n/a	
1 2 3	4 5 6 7 8 9	10 11 12	R5	teet eample			Sample		n/a	
A (B (B (	$\bigcirc \bigcirc $		C5	test sample			Sample		n/a	
3 6 6 0			D5	test sample			Sample		n/a	
		- Co	E5	test comple			Sample		n/a	
			E5	test sample			Sample		n/a	
			65	test sample			Sample		n/a	
EBBO			ЦБ	test sample			Deference	2/2	n/a	
FÃÃ		õõ	AC	test sample			Sample	in d	n/a	
			PC No	test sample			Sample		n/a	
		$\bigcirc$ $\bigcirc$ $\bigcirc$ $\bigcirc$	00	test sample			Sample		n/d	
-  🖪 🕒			DC	test sample		0	Sample		n/a	
			00	test sample			Sample		n/d	
Standard		gnea	E6	test sample		0	Sample		n/a	×
Sample	Reference Reserv	red	S.						>	

Figure 11-4: Plate Definition Tab Read Head and Assay Settings

In the plate view there are 16 buffer wells, 14 standards wells, 14 sample wells, 16 detection wells, and 4 reference wells. Reference wells only contain buffer or the sample matrix for non-specific binding reference subtraction.

Standard wells provide the data to generate the standard or the reference response curve. Sample wells provide the data of an unknown dose response relationship that will be compared to the standards. Both sets of wells require known concentrations of analyte. Before starting the experiment, these concentrations need to be entered into the table on the right, or by selecting one or more wells and right clicking to access the Set Well Data. There you can set individual well concentrations or a series dilution for a group of wells (Figure 11-5).

Dose Response Exp	periment - 2step_16CH_96W_1TestAndStan	dard_14cc	onc								
Plate Definition	2 Sensor Assignment 3 Review Experim	nt <b>4</b> F	Run Ex	periment							
In this step First, chec	<ul> <li>all the information about the sample plate and k the assay settings. Then highlight one or more</li> </ul>	its wells wi wells on ti	ill be er he san	ntered. nple plate, and	right-click to	enter/m	odify well o	ata.		$\leftarrow$	$\rightarrow$
Read Head:	16 channels	~ P	late 1	Table (96 well	s)						
Acquisition Rate:	Standard (5.0 Hz)	~	Concer	ntration units:	µg/ml	$\sim$	Export.	. Import	Print		
Assay Settings			Well	Sample ID	Replicate	Group	Туре	Conc (µg/m	I) Dilution Fact	^	
Assay: D	ose Response Moo	fv	A3	standard		(	Stand	rd 100	n/a		
Si	andard Assay		B3	standard		(	Stand	ird 50	n/a		
Ti	me (s): Shake speed (rpm):	1	C3	standard		(	Stand	rd 25	n/a		
Buffer 18 Sample 20	30 1000 10 1000		D3	standard		(	Stand	rd 12.5	n/a		
Buffer 24	40 1000		E3	standard		(	Stand	rd 6.25	n/a		
Detection 3	00 1000		F3	standard		(	Stand	rd 3.125	n/a		
			G3	standard		(	Stand	rd 1.563	n/a		
			H3	standard		(	Refere	nce n/a	n/a		
			A4	standard		(	Stand	rd 0.7815	n/a		
			B4	standard		(	Stand	rd 0.3907	n/a		
			C4	standard		(	Stand	rd 0.1954	n/a		
			D4	standard		(	Stand	rd 0.09769	n/a		
Plate 1 (96 wells)	~ Moo	fy	E4	standard		(	Stand	rd 0.04884	n/a		
1 2 3	4 5 6 7 8 9 10 11	2	F4	standard		(	Stand	rd 0.02442	n/a		
ABB			G4	standard		(	Stand	rd 0.01221	n/a		
		5	H4	standard		(	Refere	nce n/a	n/a		
			A5	test sample		(	Sampl	e 100	n/a		
			B5	test sample		(	Sampl	50	n/a		
			C5	test sample		(	Sampl	25	n/a		
F		5	D5	test sample		(	Sampl	12.5	n/a		
			E5	test sample		(	Sampl	6.25	n/a		
	$\bigcirc \bigcirc $		F5	test sample		(	Sampl	3.125	n/a		
GBBC			G5	test sample		(	Sampl	9 1.563	n/a		
H			H5	test sample		(	Refere	nce n/a	n/a		
			A6	test sample		(	Sample	0.7815	n/a		
Standard	Control Unassigned		B6	test sample		(	Sampl	0.3907	n/a	¥	
Camala	Reference     Reserved		<						>		

Figure 11-5: Plate Definition Tab Concentration

In addition to adding concentrations, you can change Sample IDs to labels that will aid in record-keeping and analysis. Typically, the Sample ID will contain a label describing the chemical of interest or lot number of a collection of wells. For example, the sample wells might all have a Sample ID of Sample TNFa Lot 47. Using this, Octet<sup>®</sup> Analysis Studio Software can group all the related samples' data into one dose response curve fit. You should not put wellspecific identifiers into the Sample ID or this will make it more difficult to group your related data for analysis. For example, IDs like TNFa 100 µg/mL, TNFa 50 µg/mL, TNFa 25 µg/mL, etc. add more work to the analysis because Octet<sup>®</sup> Analysis Studio Software will treat those samples as independent by default. If you wish to additional wellspecific information, enter it into the table's **Information** column on the far right. Please see the Octet<sup>®</sup> Analysis Studio User Guide for additional information.

### Tab 2: Sensor Assignment

The Sensor Assignment tab shows the default layout of the biosensors used in this experiment. The Sensor tray (Figure 11-6, top left), shows 16 purple biosensors and 16 biosensors in yellow. The first set are used to measure the standards, the second set measures the samples. If there are any biosensors in the remaining tray locations, they are not used.

If the checkbox **Replace sensors in tray after use** is checked, after an assay is completed, the used biosensors are returned to their starting position. This can be useful if the current assay is preparing the biosensors for a larger experiment where the biosensor preparation wells cannot fit on the main experiment's sample plate, or you are running tandem experiments with biosensor regeneration. If the box is unchecked, biosensors are dropped into the ejector chute and should not be reused.

Dose Response Experiment - 2step_16CH_96W_1TestAndStandard_	14conc				
1 Plate Definition 2 Sensor Assignment 3 Review Experiment	4 Run	Experiment			
In this step, sensors are assigned to samples, If you have a partial sensor tray it can be accomodated by select Only the first sensor tray can be a partial plate.	ting the i	nissing sensors and clicking 'Remove'.			$\leftarrow \rightarrow$
	Well	Sensor Type	Lot Number	Information ^	-
	A1	SAX2 (High Precision Streptavidin 2.0)			
	B1	SAX2 (High Precision Streptavidin 2.0)			
	C1	SAX2 (High Precision Streptavidin 2.0)			
	D1	SAX2 (High Precision Streptavidin 2.0)			
	E1	SAX2 (High Precision Streptavidin 2.0)			
	F1	SAX2 (High Precision Streptavidin 2.0)			
	G1	SAX2 (High Precision Streptavidin 2.0)			
	H1	SAX2 (High Precision Streptavidin 2.0)			
	A2	SAX2 (High Precision Streptavidin 2.0)			
	B2	SAX2 (High Precision Streptavidin 2.0)			
Land, Ulandara XX Minima and	C2	SAX2 (High Precision Streptavidin 2.0)			
	D2	SAX2 (High Precision Streptavidin 2.0)			
Remove Fill Fill Plate Print	E2	SAX2 (High Precision Streptavidin 2.0)			
	F2	SAX2 (High Precision Streptavidin 2.0)			
Plate 1 (96 wells)	G2	SAX2 (High Precision Streptavidin 2.0)			
	H2	SAX2 (High Precision Streptavidin 2.0)			
	A3	SAX2 (High Precision Streptavidin 2.0)			
	B3	SAX2 (High Precision Streptavidin 2.0)			
	C3	SAX2 (High Precision Streptavidin 2.0)			
	D3	SAX2 (High Precision Streptavidin 2.0)			
	E3	SAX2 (High Precision Streptavidin 2.0)			
	F3	SAX2 (High Precision Streptavidin 2.0)			
	G3	SAX2 (High Precision Streptavidin 2.0)			
	H3	SAX2 (High Precision Streptavidin 2.0)			
	A4	SAA2 (High Precision Streptavidin 2.0)			
	84	SAA2 (High Precision Streptavidin 2.0)			
Legend: Unassigned samples	C4	SAA2 (nigh Precision Streptavidin 2.0)			
	D4	SAA2 (High Precision Streptavidin 2.0)		•	

Figure 11-6: Selecting Biosensors for Reuse

Biosensor type and purpose can be set by selecting one or more biosensors and right clicking to access the menu options (Figure 11-7). The Set Sensor Data submenu lets you set the biosensor type, lot number, and additional information. The same information can also be entered directly into the table on the right.

Dose Response Exper	riment - 2step_16CH_96W_1TestAndStandard_14	Aconc			
In this step, s if you have a Only the first Sensor Tray	ensors are assigned to samples. partial sensor tray it can be accomodated by selectin sensor tray can be a partial plate.	ng the missing sensors and clicking 'Remove'.			$\leftarrow \rightarrow$
1 2 3	4 5 6 7 8 9 10 11 12	Well Sensor Type	Lot Number	Information	^
A       B         B       C         C       C         D       C         E       C         F       C         G       C         H       C         H       C         Plate 1 (96 wells)         1       2         A       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C       C         C	(auto-assign) Anti-Human IgG Fc AHC2 (Anti-hIgG Fc Capture 2.0) Anti-Mouse IgG Fv Anti-GST FAB2G (Anti-Fab 2nd generation) HIS2 (Anti-FIS) Anti-CHO HCP Protein A Protein G Protein L GlyS (Sialic Acid) GlyM (Mannose) SA (Streptavidin) SAX2 (High Precision Streptavidin) SAX2 (High Precision Streptavidin) SAX2 (High Precision Streptavidin) SAX2 (High Precision Streptavidin 2.0) Residual Protein A HIS1K (Anti-Penta-HIS) Ni-NTA Custom Reference Positive Control	<ul> <li>AX2 (High Precision Streptavidin 2.0)</li> </ul>			
Legend: Unass	Remove Fill	AX2 (High Precision Streptavidin 2.0) AX2 (High Precision Streptavidin 2.0)			v
	Set Sensor Data 🔓				

Figure 11-7: Setting Sensor Data

In the lower left plate view, the color-coded wells show which biosensors are dipped in each well. For example, both biosensors C2 and C4 are dipped in sample well C12. This color-coding scheme is useful to spot wells that are reused too often, potentially leading to cross contamination or dilution effects.

### Tab 3: Review Experiment

In this view you can review the order in which biosensors are used and the sample wells they are dipped into. Active biosensors are highlighted with a black border. Figure 11-8 shows a view of the first step of the experiment. In assay 1, step 1, the first two columns of biosensors (purple squares) are picked up and dipped into the buffer wells (grey circles labeled **B**).



Figure 11-8: Review Experiment Tab

Clicking the left and right arrow buttons at the top of the screen moves to the next or previous step in the assay. The time label to the right of the arrow buttons shows the estimated elapsed time of the current step being viewed and the estimated total experiment time.

The sequence in this experiment is Assay 1: buffer, standard, buffer, detection and Assay 2: buffer, sample, buffer, detection.

### Tab 4: Run Experiment

In the Data File Location and Names box (Figure 11-9), you can name the experiment and the location where the files will be saved.

In the Run Settings and Advance Settings boxes you can change some of the experiment conditions if necessary, but the default settings are usually the best choice.

Click the **GO** button to start the experiment.

Oose Response Experiment - 2step_16C	H_96W_1TestAndStandard_14conc		
Plate Definition 2 Sensor Assignment	t 3 Review Experiment 4 Run Experiment		
Data File Location and Names		Prior to proceing "Go" confirm the Assau	$\leftarrow$ GC
Assay type:	Dose Response Standard Assay	Those pressing to communicate Assay.	
Quantitation data repository:	C:\Temp		
Experiment run name (sub directory):	Experiment_1		
Plate name/barcode (file prefix):	220715		
2nd Plate name/barcode:			
Auto-increment file ID start:	1		
Data files will be stored as follows:		Total experiment time: 0:25:40	
C:\Temp\Experiment_1\220715_001.frd C:\Temp\Experiment_1\220715_002.frd C:\Temp\Experiment_1\220715_003.frd	1 1 1	0.33.40	
Run Settings			
Delayed experiment start Start after (s): 600	Open runtime charts automatically     Automatically save runtime chart		
Shake sample plate while waiting	Set plate temperature (°C): 30		
Present stage at end of experiment			
Advanced Settings			
Sensor offset (mm): 3 ~	distance to sensor tip from bottom of well Default		
Warning: changing the If you are unsure of how to	ese settings could affect assay signal to noise. o use these settings, please consult the User Guide		
General Information			
User name: SarDev	Machine name: DESKTOP-MINLUQ5		
Description:			
	0		

Figure 11-9: Run Experiment Tab

## Modifying a Template: Adding More Samples

The previous experiment can be tailored to your experimental needs. This section will show you how to add additional samples and explains some additional experimental settings.

To add additional samples, select **Tab 1: Plate Definition**. Hover the cursor over a sample well (in this example we use well A7) and click the left mouse button while holding down the **Shift** key. This will select an entire set of wells to match the number of Read Head channels or biosensors that are used.



Figure 11-10: Adding Samples

While the cursor is over A7, right click to open the well pop-up menu and select **Sample**.



Figure 11-11: Setting Wells to Sample

All the unused wells in columns 7 and 8 are now samples (purple).



Figure 11-12: Sample Wells Set

Next, select wells H7 and H8 either by dragging a box around them or clicking each while holding down the **Ctrl** key. With the cursor over H7 or H8, right click and select **Reference**.



Figure 11-13: Setting Wells to Reference

Enter the concentrations for the new samples and give them a unique Sample ID. The example experiment will now look like the screen in Figure 11-14.

Dose Response Experin	ment - 2step_16CH_96V	V_1TestAndStandard_	14conc						
1 Plate Definition 2	Sensor Assignment 3	Review Experiment	4 Run E	xperiment					
In this step, all t First, check the	the information about the e assay settings. Then hig	sample plate and its we hlight one or more wells	lls will be e on the sa	entered. mple plate, and rig	ht-click to enter/modif	y well data.			
Read Head: 16	channels	$\sim$	Plate 1	Table (96 wells)					
Anna Streen Datasa an			Conce	entration units:	µg/ml ∨	Export In	nport Prin	t	
Acquisition Nate: Star	indard (5.0 Hz)	~	Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution F	^
Assay Settings Assay: Dose F	Response		H5	test sample		Reference	n/a	n/a	
Standa	ard Assay	Modify	A6	test sample		Sample	0.7815	n/a	
Single	analyte		B6	test sample		Sample	0.3907	n/a	
Buffer 180	<ol> <li>Snake speed (rpm)</li> <li>1000</li> </ol>		C6	test sample		Sample	0.1954	n/a	
Sample 300 Buffer 240	1000		D6	test sample		Sample	0.09769	n/a	
Detection 300	1000		E6	test sample		Sample	0.04884	n/a	
			F6	test sample		Sample	0.02442	n/a	
			G6	test sample		Sample	0.01221	n/a	
			H6	test sample		Reference	n/a	n/a	
			A7	test sample 2		Sample	100	n/a	
			B7	test sample 2		Sample	50	n/a	
			C7	test sample 2		Sample	25	n/a	
Plate 1 (96 wells)		✓ Modify	D7	test sample 2		Sample	12.5	n/a	
4 0 0 1	5 0 7 0	0 40 44 40	E7	test sample 2		Sample	6.25	n/a	
		9 10 11 12	F7	test sample 2		Sample	3.125	n/a	
			G7	test sample 2		Sample	1.563	n/a	
ВВВОС			H7	test sample 2		Reference	n/a	n/a	
			A8	test sample 2		Sample	0.7815	n/a	
		ăăăă.	B8	test sample 2		Sample	0.3907	n/a	
			C8	test sample 2		Sample	0.1954	n/a	
		$\bigcirc \bigcirc \bigcirc \bigcirc \bigcirc$	D8	test sample 2		Sample	0.09769	n/a	
F B B C			E8	test sample 2		Sample	0.04884	n/a	
GOOO		$\overline{)}$	F8	test sample 2		Sample	0.02442	n/a	
			G8	test sample 2		Sample	0.01221	n/a	
			H8	test sample 2		Reference	n/a	n/a	
Standard	Control 🛛 U	Inassigned	A11		n/a	Detection		n/a	~
	Reference	Reserved	<					>	

Figure 11-14: Updated Plate Definition Tab

- • • Dose Response Experiment - 2step\_16CH\_96W\_1TestAndStandard\_14conc 1 Plate Definition 2 Sensor Assignment 3 Review Experiment 4 Run Experiment In this step, sensors are assigned to samples. If you have a partial sensor tray it can be accompated by selecting the missing sensors and clicking 'Remove'. Only the first sensor tray can be a partial plate. Lį. Sensor Trav Replace sensors in tray after use 4 5 6 Well Sensor Type Lot Number Information 2 ~ А A1 SAX2 (High Precision Streptavidin 2.0) SAX2 (High Precision Streptavidin 2.0) B1 В C1 SAX2 (High Precision Streptavidin 2.0) С D1 SAX2 (High Precision Streptavidin 2.0) D E1 SAX2 (High Precision Streptavidin 2.0) Е F1 SAX2 (High Precision Streptavidin 2.0) G1 SAX2 (High Precision Streptavidin 2.0) F H1 SAX2 (High Precision Streptavidin 2.0) G A2 SAX2 (High Precision Streptavidin 2.0) Н B2 SAX2 (High Precision Streptavidin 2.0) C2 SAX2 (High Precision Streptavidin 2.0) Missing sensors Legend: Unassigned sensors D2 SAX2 (High Precision Streptavidin 2.0) E2 SAX2 (High Precision Streptavidin 2.0) Remove Fill Fill Plate Print... F2 SAX2 (High Precision Streptavidin 2.0) G2 SAX2 (High Precision Streptavidin 2.0) Plate 1 (96 wells) H2 SAX2 (High Precision Streptavidin 2.0) 10 А A3 SAX2 (High Precision Streptavidin 2.0) B3 SAX2 (High Precision Streptavidin 2.0) В  $\bigcirc$ C3 SAX2 (High Precision Streptavidin 2.0) С  $\bigcirc$  $\bigcirc$  $\bigcirc \bigcirc$ D3 SAX2 (High Precision Streptavidin 2.0) D  $\bigcirc$  $\bigcirc \bigcirc$ E3 SAX2 (High Precision Streptavidin 2.0) F3 SAX2 (High Precision Streptavidin 2.0) Е  $\bigcirc$ G3 SAX2 (High Precision Streptavidin 2.0) F  $\bigcirc \bigcirc \bigcirc$ H3 SAX2 (High Precision Streptavidin 2.0) G A4 SAX2 (High Precision Streptavidin 2.0) H  $\bigcirc$ B4 SAX2 (High Precision Streptavidin 2.0) ()C4 SAX2 (High Precision Streptavidin 2.0) Legend: O Unassigned samples D4 SAX2 (High Precision Streptavidin 2.0) v

Go to Tab 2: Sensor Assignment to see the additional biosensors (shown in green) used for the added assay.

Figure 11-15: Updated Sensor Assignment Tab



Tab 3: Review Experiment also shows the additional biosensors (shown in green) used for the added assay.

Figure 11-16: Updated Review Experiment Tab

## Modifying a Template: Changing Step Settings

To modify the assay settings, return to **Tab 1: Plate Definition** and click the **Modify** button in the Assay Settings box.

For this assay example, the detection complex is a slow binder, so we want to double the detection step time to ensure we have reached equilibrium. In the Assay Parameters window, double click the **detection time** table cell, type in the desired value, and click **OK**.



Figure 11-17: Changing the Detection Time



Figure 11-18: Assay Settings Showing Updated Detection Time

In Tab 2: Sensor Assignment, Plate 1 shows all the assays are using the same set of detection wells, displaying columns 11 and 12 in purple, yellow, and green.



Figure 11-19: All Assays Use Detection Columns 11 and 12

If you have concerns about cross contamination or consumption of the detection complex, you can change the experiment so each assay has its own set of detection wells. Click the **Modify** button in the Assay Settings box and change the Step Options for the Detection step to **Use once**.

Assay Parameters					×			
Available Assays:	Assay Parameters							
Dose Response	Single analyte     O Multiple analyte     Replicates per sensor type:     1							
Inree Step Assay	Step Type	Time (s)	Shake (rpm)	Step Options	Insert			
	Buffer	180	1000	Reuse position	Remove			
	Sample	300	1000	Online	Hemove			
	Buffer	240	1000	Reuse position				
	Detection	600	1000	Reuse position -				
				Use once				
				Use twice				
	<			Use x3				
	Regeneration	(s):	Shake sneed	Use x4				
	Regeneration: 5	*	1000 🌲	Use x5				
	Neutralization: 5	*	1000 🌲	Use x6				
			Regeneration	Use x7				
	Between assay ste	DS:	3	Use x8				
	Pre-condition senso	rs	3	Use x9				
	Post-condition sens	ors	3	Use x10				
				Reuse position				
	1			Distribute usage (	auto)			
Gray indicates a built-in assay.	ОК С	ancel						

Figure 11-20: Selecting Use Once

Changing this Step Options setting will remove the existing detection wells from the sample plate, so you will need to assign 48 new detection wells to Plate 2. Go back to **Tab 1: Plate Definition** and add 48 new detection wells. The final layout for Plates 1 and 2 should look like Figure 11-21.



Figure 11-21: Final Layout of Plate 1 and 2 Showing 48 New Detection Wells

You can confirm single use of the detection wells in Tab 2: Sensor Assignment. Plate 1 shows detection wells are set to single use, and Plate 2 is entirely composed of detection wells.



Figure 11-22: Detection Wells Showing Single Use in Plate 1, and All Wells in Plate 2 are Detection

### Modifying a Template: Adding Replicates

To get a better, more statistically meaningful, determination of dose response parameters like EC<sub>50</sub>, you should obtain multiple measurements of the same system. You can do this by adding more biosensors and repeating each assay, or you can reuse a single set of biosensors by adding regeneration steps between each assay.

In the original template, 2step\_16CH\_96W\_1TestAndStandard\_14conc.fmf, you can add replicates without reusing biosensors. In Tab 1: Plate Definition, click the **Modify** button to open the Assay Parameters window:



Figure 11-23: Assay Parameters Window

Select the Multiple analyte radio button, set the Replicates per sensor type to 3 and click OK.

Assay Parameters				
◯ Single analyte ● M	Multiple analy Replicates p	/te er sensor type:	8	
Step Type	Time (s)	Shake (rpm)	Step Options	
Step Type B Buffer -	Time (s) 180	Shake (rpm) 1000	Step Options Reuse position	

Figure 11-24: Setting Replicates Per Sensor Type

Go to **Tab 3: Review Experiment**. There you can see that where the single replicate experiment only used two sets of biosensors in Sensor Tray columns 1-4, with three replicates we are now using a full tray of biosensors. Additionally where the experiment used to have two assays, there are now six. The purple, yellow, and green set of biosensors in Figure 11-25 are for the three standards replicates. The orange, plum, and blue sets are for the three samples replicates. Note that the sample plate layout is unchanged.



Figure 11-25: Experiment Using Replicates

To demonstrate replicates with regeneration, close this method without saving and reload it in the wizard. Open the Assay Parameters window. Select the **Multiple analyte** radio button and set the **Replicates per sensor type** to **3**, then select the **Regeneration** check box. Accept all the other default settings by clicking **OK**.

) Single analyte	Mu Re	Itiple analy plicates p	te er sensor type:	3 ≑
Step Type	1	Time (s)	Shake (rpm)	Step Options
Buffer	-	180	1000	Reuse position
Sample		300	1000	Online
Buffer	:	240	1000	Reuse position
Detection	:	300	1000	Reuse position
¢				
<ul> <li>✓ Regeneration</li> <li>Regeneration:</li> <li>Not drafting to a training</li> </ul>	Time (	(s): ▼	Shake speed	(mm):
Regeneration Regeneration: Neutralization:	Time ( 5 5	(s):	Shake speed 1000 💌 1000 🐨 Regeneration	(rpm): cycles:
Regeneration Regeneration: Neutralization: Between assa	Time ( 5 5 sy steps	(s):	Shake speed 1000 1000 Regeneration 3	(rpm): cycles:
✓ Regeneration Regeneration: Neutralization: Between assa □ Pre-condition s	Time ( 5 5 sensors	(s): • • s: s	Shake speed 1000 + 1000 + Regeneration 3 + 3 +	(rpm): cycles:

Figure 11-26: Assay Parameters with Multiple Analytes and Regeneration Selected

The sample plate layout changes automatically. In Plate 1, the software has added regeneration and neutralization wells and has moved the detection and buffer wells. Plate 2 has two rows of buffer wells.



Figure 11-27: Updated Plate Layouts

To reduce plate consumption, you can move the buffer wells back to Plate 1. On Plate 1, select column 1 and 2 and assign them to buffer. This will automatically remove them from Plate 2.



Figure 11-28: Buffer Wells Moved to Plate 1

If you go back to **Tab 2: Sensor Assignment**, you will notice that the experiment uses a whole tray of biosensors instead of reusing biosensors. In the Assay Parameters window, we enabled regeneration but have not yet specified how many times to regenerate biosensors before discarding them and picking up a new set. On Tab 2, set **Times sensors will be reused** to **2**.

ors an	rs and clicking 'Remove'.			nerations sensors will be reus Apply	ed:
	Tray Format	Heterogeneo	us trays		
		Lot N	Lot Number Information		^
pe					

Figure 11-29: Setting Number of Times Sensors Will be Reused

Notice that now there are only two sets of biosensors in use (Figure 11-30). The number 2 in each denotes each biosensor is regenerated twice (used for three assays). On Tab 3: Experiment Review, you can confirm that the standards and samples are each measured three times, as desired.



Figure 11-30: Sensor Tray Showing Biosensors are Regenerated Twice

However, if you look closely at the sequence of each assay, watching which biosensors are used and in which well, you'll see the purple biosensors dipped in the standards two times and the samples once. The yellow biosensors are dipped into the standards once and the samples twice. This is more evident in Tab 2 by the colors in the standards and samples wells. The standards are two-thirds purple, one-third yellow, while the samples have the opposite pattern.



Figure 11-31: Plate Showing Biosensor Use

A better experimental design would use the same biosensor for the same wells to avoid cross-contamination. However, the standard dose response wizard only presents a limited set of options to modify an existing template. In this specific example, Octet<sup>®</sup> software is treating standards and samples equivalently, and so only meet the basic requirement of producing three replicates each. There are no options to control which specific biosensors are used in which wells. To have such control over the experimental design, you need to use the Custom Dose Response mode in the wizard.

## Setting Up a Custom Dose Response Experiment

When selecting a template in the Custom Dose Response wizard the options are the same as Kinetics, offering greater control of the overall experimental design. If you aren't already familiar with Kinetic experimental setup, we recommend reviewing the Kinetics chapters before setting up a Custom Dose Response experiment.

In this example we will duplicate the regeneration experiment described earlier but the biosensors will now be exclusive to standards or samples.

Start by selecting 2step\_16CH\_96W\_1Test\_14conc.fmf as shown in the Figure 11-32.

🖄 Experiment Wizard	
Choose an option to start	Available Templates for - Octet RH16 Blank Experiment 2 Step Assay 2 Step Assay 2 Step 3CH_96W_1Test_14conc fmf 2 step 2CH_96W_1Test_7conc fmf 3 Step Assay
Recent Methods	

Figure 11-32: Selecting the Custom Dose Response Wizard

### Tab 1: Plate Definition

Figure 11-33 shows the basic template, with one set of samples and no standards. Neither have the necessary regeneration and neutralization wells needed for biosensor reuse.

Custom Dose Response Experiment - 2step_16CH_96W_1Test_14cd	onc						- • ×
1 Plate Definition 2 Assay Definition 3 Sensor Assignment 4	Review	Experiment	5 Run Experiment				
In this step, all the information about the sample plate and its we Highlight one or more wells on the sample plate, and right-click	ells will b to enter/	e entered. /modify well dat	a.				$\leftarrow \rightarrow$
Read Head: 16 channels ~ Modify Plates				Conce	entration units:	ua/ml V	
Plate 1 (96 wells)		te 1 Table		Molar	concentration uni	its: nM V	
	Well		Peoliasta Cours	Tune	Case (up (ml)	Dilution Easter +	
			Replicate Group	Buffer	Conc (µg/mi)		
	B1			Buffer		n/a	
	C1			Buffer		n/a	
	D1			Buffer		n/a	
	E1			B Buffer		n/a	
	F1			(B) Buffer		n/a	
	G1			(B) Buffer		n/a	
	H1			(B) Buffer		n/a	
	A2			Buffer		n/a	
	B2			B Buffer		n/a	
Ultraviewed Invest Freed Pressure Birst	C2			B Buffer		n/a	
O onassigned import Export Remove Print	D2			B Buffer		n/a	
Plate 2 (96 wells)	E2			B Buffer		n/a	
	F2			B Buffer		n/a	
A0000000000000	G2			B Buffer		n/a	
BOOODOOOOOO	H2			B Buffer		n/a	
	A3	test sample		Sample			
	B3	test sample		Sample			
	C3	test sample		<ul> <li>Sample</li> </ul>			
	D3	test sample		Sample			
F0000000000000	E3	test sample		Sample			
600000000000000000000000000000000000000	F3	test sample		Sample			
	G3	test sample		Sample			
	H3	test sample		Reference     Securit			
Unassigned Import Export Remove Print	A4	test sample		Sample			
	84	test sample		Sample		×	
						,	

Figure 11-33: Basic Custom Dose Response Template

Add standards with reference wells, regeneration and neutralization wells. Use the table to add concentrations and Sample IDs for the standards. The plate definition should look like Figure 11-34.

Custom Dose Response Experiment - 2step_16CH_96W_1Test_14co	nc						- • ×
1 Plate Definition 2 Assay Definition 3 Sensor Assignment 4	Review	Experiment 5	Run Experiment				
In this step, all the information about the sample plate and its we Highlight one or more wells on the sample plate, and right-click t	lls will be o enter/i	e entered. modify well data.					$\leftarrow \rightarrow$
Read Head: 16 channels ~ Modify Plates	0.0			Concentr	tion units:	ua/ml 🗸	
Plate 1 (96 wells)	Plat	e 1 Table		Molar.com	contration units:	pM V	
			Destinate Course	Turne		Dilation Fo	
	Well A2	Sample ID	Replicate Group	Type	100		
	A3 02	test sample		Sample	50		
	03	test sample		Comple	25		
	03	test sample		Sample	12.5		
	E3	test sample		Sample	6.25		
	E3	teet eample		Sample	3 125		
	G3	test sample		Sample	1 563		
	нз	test sample		Reference	1.000		
	A4	test sample		Sample	0.7815		
HĂĂĂĂĂĂĂĂĂĂĂĂĂĂ	R4	test sample		Sample	0.3907		
	C4	test sample		Sample	0 1954		
Unassigned Import Export Remove Print	D4	test sample		Sample	0.09769		
Plate 2 (96 wells)	F4	test sample		Sample	0.04884		
	F4	test sample		Sample	0.02442		
1 2 3 4 5 6 7 8 9 10 11 12	G4	test sample		Sample	0.01221		
	H4	test sample		Reference			
BOOOOOOOOOOO	A5	test standard		Standard	100		
	B5	test standard		Standard	50		
	C5	test standard		Standard	25		
	D5	test standard		Standard	12.5		
	E5	test standard		Standard	6.25		
	F5	test standard		Standard	3.125		
G0000000000000000000000000000000000000	G5	test standard		Standard	1.563		
H = 0 = 0 = 0 = 0 = 0 = 0 = 0	H5	test standard		Reference			
	A6	test standard		Standard	0.7815		
Unassigned Import Export Remove Print	BG	test standard		Standard	0.3907		
	<			-		>	

Figure 11-34: Plate Definition After Adding Standards with References, Regeneration and Neutralization Wells
#### Tab 2: Assay Definition

Figure 11-35 shows the initial view. The upper right table shows the defined steps, and the lower right table shows the assigned steps that form the assay. The sample plate on the top left shows some of the wells striped while others, such as the standards, are solid. Striped wells are those used by one or more assays in the experiment. Clear wells have not yet been assigned. Note that this assay does not have a regeneration step.

Custom Dose Response Experiment - 2step_16CH_96W_1Test_14conc								
1 Plate Definition 2 Assay Definition 3 Sensor Assignment 4 Review Experiment 5 Run Experiment								
In this step, the assay steps will be assembled from the Step Dat Select a group of sensors and append the currently selected ste	ata List. ep into the current	assay wi	th a double click	c, or	right click for more	options.		$\leftarrow \rightarrow$
Plate 1 (96 wells)	Step Data List -					Time in (s), S	õhake speed in (rpm)	
	Add	Сору	Remove	•	Regeneration P	arams	Threshold Params	
	Name	Time	Shake speed	I Ty	уре	Threshold		
	→ Baseline	180	1000	h	Baseline			
	Sample	300	1000	r	Association			
	Baseline2	240	1000		Association			
	Delection	500	1000	<b>r</b>	7.630010011			
	Array Change Link							
	New Assay	Move U	p Move Dov	vn	Remove Rep	licate Edit	t Step Info Table	
	Assay No.	Sample	Plate Step I	Vam	ne Step Type	Sensor 1	Гуре	
Assayed samples O Unassigned samples	1 1	-	1 Baselin	е	+ 🔜 Baseline	SAX2 (Hig	h Precision Streptavic	
Plate 2 (96 wells)	1 2 3	3	1 Sample		Z Association	SAX2 (Hig	h Precision Streptavia	
	1 3	•	1 Baselin	e2	Baseline	SAX2 (Hig	h Precision Streptavic	
	1 4	1	I Detecti	on		SAX2 (Hig	n Precision Streptavic	
BOOOOOOOOOOO								
ECOCOCOCOCOCO								
Assayed reagents O Unassigned reagents								
	<						>	
							Exp.time: 0:17:50	

Figure 11-35: Assay Definition Tab Showing Striped Wells Used by One or More Assays

To add a regeneration step, click the **Add** button in the Step Data List group box. This will add a regeneration step after the detection step.

dd C	ору	Remove	Regeneration Param	s Thresh	old Param
Name	Time	Shake speed	Туре	Threshold	
Baseline	180	1000	🛌 Baseline		
Sample	300	1000	Association		
Baseline2	240	1000	🛌 Baseline		
Detection	300	1000	Association		
Regeneration	30	1000	Regeneration		

#### Figure 11-36: Adding a Regeneration Step

Check the regeneration settings by clicking the **Regeneration Params** button.

Regeneration Parame	ters	×
Step Name:	Regeneration	
	Time (s)	Shake speed (rpm):
Regeneration:	5	1000
Neutralization:	5	1000
Regeneration cycles:	3	
Total step time:	30 s	OK Cancel

Figure 11-37: Regeneration Parameters

The default regeneration setting is three regeneration cycles per regeneration step. Accept the default setting by clicking **OK**.

Next, select one of the regeneration wells in the plate map and then double click the arrow next to the regeneration step in the Step Data List table.

Plate 1 (96 wells)		Step D	ata List				Time ir	n (s), Shake s	peed in (rpm)
	C 7 0 0 40 44 40	Ade	d	Сору	Remove	Rege	neration Params	Thresho	old Params
			Name	Time	Shake speed	Туре		Threshold	
			Baseline	180	1000	🛌 Bas	eline		
B ®®®©©(			Sample	300	1000	🖊 Ass	ociation		
			Baseline2	240	1000	Bas	eline		
			Detection	300	1000	🖊 Ass	ociation		
EØØØØO		≥	Regeneration	n 30	1000	💈 Reg	generation		
F®®®®©	Regeneration (Regeneration)								
		New	Assay M	ove Up	Move Down	Remov	e Replicate	Edit Step	Info Table
		Assa	y No. Sa	nple Pl	ate Step Nam	e S	Step Туре	Sensor Ty	ре
Assayed samples	O Unassigned samples	1	1 1	1	Baseline	Ŀ	Baseline	SAX2 (High	Precision Stre

Figure 11-38: Associating a Step to a Well

You will now see a regeneration step in the assay and all the regeneration and neutralization wells will be striped.



Figure 11-39: Sensor Regeneration Added

This represents one measurement of the set of samples. To add replicates, select all the steps of this assay and click the **Replicate** button.

Assay Steps List									
New Assay Move Up M			<b>Ip</b> M	ove Down Rem	ove Replicate	Edit Step Info Table			
Assay	No.	Sample	Plate	Step Name	Step Type	Sensor Type			
1	1	1	1	Baseline	Baseline	SAX2 (High Precision Stre			
1	2	3	1	Sample	🞽 Association	SAX2 (High Precision Stre			
1	3	1	1	Baseline2	🛌 Baseline	SAX2 (High Precision Stre			
1	4	11	1	Detection	🞽 Association	SAX2 (High Precision Stre			
1	5	7	1	Regeneration -	👌 Regeneration	SAX2 (High Precision Stre			

Figure 11-40: Adding Replicates

In the Replicate Step dialog, select the Append to current assay radio button and click OK.



Figure 11-41: Replicate Steps Dialog

Now you see that the samples are measured twice.

New Assay Move Up			<b>lp</b> M	ove Down Re	Replicate	Edit Step	Info Ta	ble			
Assay	No.	Sample	Plate	Step Name	St	ер Туре	Sensor Type				
1	1	1	1	Baseline	<u>_</u>	Baseline	SAX2 (High	Precision	n Str		
1	2	3	1	Sample	K	Association	SAX2 (High	Precision	n Str		
1	3	1	1	Baseline2	h	Baseline	SAX2 (High	Precision	n Str		
1	4	11	1	Detection	K	Association	SAX2 (High	Precision	n Str		
1	5	7	1	Regeneration	\$	Regeneration	SAX2 (High	Precision	n Str		
1	6	1	1	Baseline		Baseline	SAX2 (High	Precision	n Str		
1	7	3	1	Sample	K	Association	SAX2 (High	Precision	n Str		
1	8	1	1	Baseline2	h	Baseline	SAX2 (High	Precision	n Str		
1	9	11	1	Detection	K	Association	SAX2 (High	Precision	n Str		
1	10	7	1	Regeneration ·	- 🕏	Regeneration	SAX2 (High	Precision	n Str		
<									>		
							Exp. tir	ne: 0:38			

Figure 11-42: Assay Steps List Showing Samples Measured Twice

Append another assay to get three sample measurements. Confirm in **Tab 4: Review Experiment** that the biosensors are indeed measuring the samples three times with regeneration in between.

To add the standards with the same assay step order, select the entirety of assay 1 and click the **Replicate** button. This time select the **Add as a new assay** radio button and the **Offset steps** check box. Accept the default settings Sample steps only and Horizontally by 2 columns.



Figure 11-43: Adding Replicated Steps as a New Assay

The result is shown in Figure 11-44. There are now two assays in the Assay Step List table. The colors in the table (purple and yellow) indicate the experiment will use two sets of biosensors (purple and yellow in the Sensor Assignment view, as shown before) and that one set is used exclusively for samples and the other is used exclusively for standards.



Figure 11-44: Biosensor Regeneration Added to the First Samples Assay

By inspecting the experiment in Tab 4, or by clicking each step in Assay Step List table here, you can confirm that there are three replicates for the samples and standards.

This can also be confirmed in Tab 3: Sensor Assignment where the samples and standards are now a single color (Figure 11-45).

Custom Dose Response Experiment - 2step_16CH_96W_1Test_14conc								
1 Plate Definition 2 Assay Definition 3 Sensor Assignment 4 Review Experiment 5 Run Experiment								
In this step, sensors are assigned to samples. If you have a partial sensor tray it can be accomodated by selecting the missing sensors and clicking 'Remove'. Only the first sensor tray can be a partial tray. Right click to assign a sensor type to selected sensors. Sensor Tray Reduce sensors in tray after use								
	Well	Sensor Type	Lot Number	Information	A			
	A1	SAX2 (High Precision Streptavidin 2.0)						
	B1	SAX2 (High Precision Streptavidin 2.0)						
	C1	SAX2 (High Precision Streptavidin 2.0)						
	D1	SAX2 (High Precision Streptavidin 2.0)						
	E1	SAX2 (High Precision Streptavidin 2.0)						
	F1	SAX2 (High Precision Streptavidin 2.0)						
	G1	SAX2 (High Precision Streptavidin 2.0)						
	H1	SAX2 (High Precision Streptavidin 2.0)						
	A2	SAX2 (High Precision Streptavidin 2.0)						
	B2	SAX2 (High Precision Streptavidin 2.0)						
Legend: Unassigned sensors 🕅 Missing sensors	C2	SAX2 (High Precision Streptavidin 2.0)						
	D2	SAX2 (High Precision Streptavidin 2.0)						
Remove Fill Fill Plate Print	E2	SAX2 (High Precision Streptavidin 2.0)						
Dista 1 (00 uuslis)	F2	SAX2 (High Precision Streptavidin 2.0)						
Plate I (56 Wells)	G2	SAX2 (High Precision Streptavidin 2.0)						
	H2	SAX2 (High Precision Streptavidin 2.0)						
	A3	SAX2 (High Precision Streptavidin 2.0)						
	B3	SAX2 (High Precision Streptavidin 2.0)						
	C3	SAX2 (High Precision Streptavidin 2.0)						
	D3	SAX2 (High Precision Streptavidin 2.0)						
	E3	SAX2 (High Precision Streptavidin 2.0)						
	F3	SAX2 (High Precision Streptavidin 2.0)						
	G3	SAX2 (High Precision Streptavidin 2.0)						
	H3	SAX2 (High Precision Streptavidin 2.0)						
	A4	SAX2 (High Precision Streptavidin 2.0)						
	B4	SAX2 (High Precision Streptavidin 2.0)						
Legend: Unassigned samples	C4	SAX2 (High Precision Streptavidin 2.0)						
	D4	SAX2 (High Precision Streptavidin 2.0)			¥			
]								

Figure 11-45: Solid Colors Indicate Samples and Standards Have Dedicated Biosensors

### Experimental Design Tips

#### EC<sub>50</sub>

- A sample step is required with wells of several concentrations.
- A detection step is optional but recommended if the sample response is weak and doesn't provide enough concentration vs. response resolution. All the detection wells should have the same concentration of detection molecule.
- If a sample step and detection are both present in the assay, the sample step concentrations provide the dose and the detection step provides the response of the dose-response curve.

- If a detection step is not present, or excluded in the analysis, the sample step response is used for the dose-response curve.
- If using a custom dose response template, sample and detection steps should be association step types and the step names should be Sample and Detection. This will ensure Octet<sup>®</sup> Analysis Studio software can find the dose-response input data.

#### $IC_{50}$

- A sample step is required with wells of several concentrations.
- A detection step is required. All the detection wells should have the same concentration of detection molecules.
- The sample step concentrations provide the dose and the detection step provides the response of the doseresponse curve.
- If a detection step is not present,  $IC_{50}$  determination is impossible.
- If using a custom dose response template, sample and detection steps should be association step types and the step names should be Sample and Detection. This will ensure Octet<sup>®</sup> Analysis Studio software can find the dose-response input data.

#### General considerations

- Dose-response curve fitting works best if there is data for both the upper and lower asymptotes. A scouting experiment may help to determine the highest concentrations needed.
- Dose-response curve fitting should have a minimum of 7 data points with at least 2 in the linear (mid-point) portion of the response curve. The absolute minimum number of data points for 5PL, 4PL, or 3PL dose response curve fitting are 5, 4, and 3 data points, respectively. If there are not enough data points, dose response analysis is impossible.
- More data will help with the confidence intervals of the dose-response parameters or resolving power when comparing analytes. Both increasing the number of sample concentrations and/or adding replicates will help.
- · Adding reference wells will help mitigate instrument drift and non-specific binding.
- If preparing biosensors with a loading step, include this step in the analysis to help identify outliers. See the loading z-score section in the Octet<sup>®</sup> Analysis Studio Software User Guide.
- If possible, make the sample and detection steps long enough to reach equilibrium.

# Chapter 12: Maintenance

### Troubleshooting and Service

For troubleshooting and service requests, please contact your local Sartorius representative or Technical Support at octetsupport@sartorius.com or +1-650-322-1360.

### Octet<sup>®</sup> K2, Octet<sup>®</sup> R2, Octet<sup>®</sup> R4, Octet<sup>®</sup> R8, Octet<sup>®</sup> RED96e, and Octet<sup>®</sup> QKe Systems

### Cleaning the Octet<sup>®</sup> Instrument



**WARNING:** Sample platform may be hot if the instrument has been in operation. Wait for the platform to cool before attempting to clean.



**WARNING: l**I se peut que la plateforme d'analyse des échantillons chauffe si l'appareil est en train de fonctionner. Attendez que la plateforme refroidisse avant de tenter de la nettoyer.



**WARNING:** War das Gerät in Betrieb, ist die Probenplattform möglicherweise heiß. Lassen Sie die Plattform vor der Reinigung abkühlen.

**NOTICE:** If you use the Octet<sup>®</sup> instrument regularly, clean the interior horizontal surfaces daily with a Kimwipe<sup>®</sup> tissue moistened with a 30-60% isopropyl alcohol solution. Otherwise, clean once a week or as needed.

Routine cleaning of the Octet<sup>®</sup> instrument:

- 1. Turn off the power to the instrument
- 2. Open the system door.
- 3. Wipe the biosensor and sample platform (Figure 12-1).
- 4. Carefully wipe the eight biosensor pickup tips.
- 5. Allow the surfaces to dry for at least one minute with the door open.



Figure 12-1: Octet<sup>®</sup> Instrument

#### **Cleaning Guidelines**



**WARNING:** System users are responsible for appropriate decontamination in case of spillage of hazardous materials on or inside of the equipment. If there are any doubts about the compatibility of a cleaning agent or decontamination procedure with the materials making up the Octet<sup>®</sup> system, please consult with Sartorius before proceeding.



**WARNING:** Il incombe aux utilisateurs du système de procéder à une décontamination adéquate en cas de débordement de produits dangereux sur ou à l'intérieur de l'équipement. En cas de doute sur la compatibilité d'un détergent ou d'une procédure de décontamination avec les matériaux composant le système Octet<sup>®</sup>, veuillez vous adresser à Sartorius avant toute intervention.



**WARNING:** Die angemessene Dekontamination des Systems im Falle der Freisetzung gefährlicher Substanzen auf dem oder innerhalb des Geräts liegt in der Verantwortung des Systembenutzers. Sollten Zweifel über die Kompatibilität eines Reinigungsprodukts oder Dekontaminationsverfahrens im Hinblick auf die Werkstoffe bestehen, aus denen das Octet-System gefertigt ist, wenden Sie sich vor der Reinigung bitte an Sartorius.



**WARNING:** If a large volume of liquid has been spilled in or near the instrument, turn off the power prior to cleaning, and wait at least 24 hours before attempting to restart the instrument. Never place anything on top of the instrument.



**WARNING:** En cas de débordement important de liquide à l'intérieur ou aux abords de l'instrument, éteignez l'appareil avant de procéder au nettoyage et attendez au moins 24 heures avant de tenter de le redémarrer. Ne posez rien sur l'instrument.



**WARNING:** Wenn große Mengen Flüssigkeit im Gerät oder in der Nähe des Geräts verschüttet wurden, schalten Sie das Gerät vor der Reinigung zunächst aus, und warten Sie mindestens 24 Stunden, bevor Sie es wieder in Betrieb nehmen. Platzieren Sie niemals Objekte auf der Oberseite des Gerät.

- Use a dry paper towel or cloth to wipe up accidental spills inside the instrument.
- Remove dirt or stains by wiping gently with a damp paper towel or cloth.
- To remove difficult stains or debris from the exterior surface add mild liquid soap to a damp paper towel or cloth.
- Do not use organic solvents to clean the enclosure surface.
- Remove biological contaminants by wiping the exterior surface of the instrument with a general disinfectant such as a 10% bleach solution or a Environ (1%) solution on a damp paper towel or cloth. Minimum contact time of 10 minutes.
- Consider the type of contaminant when you select the disinfectant.

#### Emptying the Waste Container

To empty the waste container:

- 1. Press on the container to open it (Figure 12-2).
- 2. Pull the container out and completely remove it from the instrument.
- 3. Remove the container insert with the biosensor tips and dispose of both in a biohazard container suitable for sharp objects.

Waste container

**NOTICE:** Sartorius recommends that the waste container be emptied after every run of a 96-biosensor tray.

Figure 12-2: Waste Container for the Octet  $^{\textcircled{R}}$  Instrument

#### **Replacing Fuses**



**WARNING:** All fuse replacements need to be performed by Sartorius service personnel. Sartorius is not responsible for personal injury incurred by unqualified personnel during fuse replacement or any other repair.



**WARNING:** Chaque remplacement de fusible doit être effectué par le personnel de maintenance de Sartorius. Sartorius décline toute responsabilité en cas de blessures dues au recours à du personnel non qualifié pour assurer le remplacement des fusibles ou toute autre réparation.



**WARNING:** Das Auswechseln von Sicherungen muss stets von Servicepersonal von Sartorius vorgenommen werden. Sartorius übernimmt keine Verantwortung für Personenschäden, die infolge der Auswechslung von Sicherungen oder der Durchführung sonstiger Reparaturen durch ungeschultes Personal entstehen.

## Octet<sup>®</sup> RH16, and Octet<sup>®</sup> QK384 Systems

### Cleaning the Octet<sup>®</sup> Instrument

**NOTICE:** If you use the Octet<sup>®</sup> instrument regularly, clean the interior horizontal surfaces daily with a Kimwipe moistened with a 30–60% isopropyl alcohol solution. Otherwise, clean once a week or as needed.

To clean the Octet<sup>®</sup> RH16 or Octet<sup>®</sup> QK384 instrument:

- 1. Present the sample plate stage (Figure 12-3).
- 2. Turn off the power to the instrument.
- 3. Open the system door.
- 4. Wipe the biosensor and sample platform.
- 5. Allow the surfaces to dry for at least one minute with the door open.



Figure 12-3: Octet<sup>®</sup> RH16 and Octet<sup>®</sup> QK384 Stage Platform

#### Cleaning the Biosensor Pickup Tips

The biosensor pickup tips hold the biosensors during an assay. Sartorius now offers Biosensor Mount Cleaning Trays (Part No: 1-5133, pack of 12) to perform periodic, regular, automated cleaning of biosensor mounts on Octet<sup>®</sup> RH96 and Octet<sup>®</sup> RH16 instruments.

With normal instrument use, plastic residue from BLI biosensor hubs can accumulate on the tips of the metal biosensor mounts inside the instrument. This accumulation can potentially lead to incorrect loading of biosensors and inconsistencies in data sensing. It is important to periodically remove the plastic residue in order to ensure continued optimal performance of the Octet<sup>®</sup> system.

Important notes:

- The Biosensor Mount cleaning procedure is preventative measure and will not remove excessive accumulations of plastic residue.
- If Biosensor Mounts are particularly dirty (indicated by visible thick white film around the tip of the mounts), OR if the instrument has been used for an extended period of time without cleaning, schedule a Preventative Maintenance visit with a e engineers before you start regular cleanings with the Cleaning Trays.
- Use Cleaning Trays for automated cleaning only with the designated method in Octet<sup>®</sup> BLI Discovery Software version 9 and up.
- Cleaning Trays single use only.

#### **Cleaning Procedure**

- 1. Remove the Cleaning Tray from plastic bag.
- 2. Spray the top of the Cleaning Tray sponge three times with 70% ethanol in a spray bottle.
- 3. Open Octet<sup>®</sup> BLI Discovery software and wait for the instrument to initialize.
- 4. From the Instrument menu, select Clean Biosensor Mounts. The instrument stage will present automatically.
- 5. Place the moistened Cleaning Tray in the Tray position on the instrument stage, and then click **OK**.
- 6. Follow the simple prompts in the software dialogue to begin the cleaning protocol. The full automated cleaning cycle will take approximately 8 minutes to complete.
- 7. Once the cleaning cycle is complete, the instrument stage will present again. Remove the used Cleaning Tray from the stage and discard.

#### Appendix A:

# Using Octet<sup>®</sup> RH16, Octet<sup>®</sup> RH96 and Octet<sup>®</sup> QK384 Systems with an Automation Interface

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### Overview

The Octet<sup>®</sup> BLI Discovery software provides support for an automation interface using a COM port (RS-232) or a Transmission Control Protocol/Internet Protocol (TCP/IP) socket/port.

An example application for testing the automation interface, called **AutomationClient.exe**, is included in the applications and Dynamic Link Libraries (DLLs) installed with the Octet<sup>®</sup> BLI Discovery software. The file is located in the C:\Program Files\Sartorius\OctetBLIDiscovery directory.

#### NOTICE:

cally.

The automation interface can only be used with the Octet<sup>®</sup> QK384, Octet <sup>®</sup> RH16, and Octet<sup>®</sup> RH96 systems. The examples that follow are illustrated using a TCP/IP connection, but the serial port connection behaves identi-

### Design of the Automation Interface

The automation interface is designed to be as universal as possible, making no assumptions about the communication medium or the language of the client application connecting to the Octet<sup>®</sup> BLI Discovery software.

The following guidelines apply:

- All commands and responses are ASCII strings, one per line.
- All lines are terminated with both carriage-return and line-feed characters ("\r\n").
- Each command starts with the name of the command and may then be followed by required and optional parameters.
- Each parameter starts with a switch definition (a la dos/unix command line) followed by the parameter itself, which allows parameters to be sent in any order.
- The command or response is terminated with a new line (CR/LF) sequence.
- Parameters containing embedded spaces need to be enclosed in double quotes.

#### Automation Interface Control Setup

Before the Octet<sup>®</sup> BLI Discovery software can be controlled using an automation interface, the correct automation options must be set. To do this, go to **File** > **Options (**Figure A-1) and select the appropriate port in the **Automation** box.

**NOTICE:** The Octet<sup>®</sup> BLI Discovery software can be controlled via automation interface through a serial port (RS-232) or a TCP/IP socket.

Options			×			
Data Files Quantitation data repository: Kinetics data repository:	C:\data C:\data Use old 5.0 file fom	nat for FRD file 🗹	Use extended sample types			
Simulation If no instrument is connected, th Octet HTX Oct Octet QK384 Oct Coctet RED384 Oct	e application is configu et QKe ( et RED96 ( et RED96e (	red using the prop Octet K2 Octet QK	erties of the selected Octet R2 Octet R4 Octet R8			
Startup Temperature: 30	Sens C Defa	Sensor Options - New Experiments  Replace sensors in tray after use  Default Sample Plate				
Significant digits: 4		16 wells	384 wells			
Automation  TCP-P Pot: 20000  Serial (RS232) Port: Communications Port (COM1)	Localhost		DK Cancel			

Figure A-1: Options Dialog Box-Automation Interface Selection

**NOTICE:** The Localhost option can be useful in developing the automation client on the same computer that runs the Octet<sup>®</sup> BLI Discovery software.

**NOTICE:** Sartorius recommends that the Data File repositories be set using shared folders addressed by "UNC" folder names so that the internal path used by the Octet<sup>®</sup> BLI Discovery application corresponds to the external path used to access/retrieve the data files recorded during the experiment. Alternatively, the path returned by the GetRunInfo command to access the data files from another computer on the LAN.

#### Automation Client Example Application

The **Automation Client** example application can connect to the Octet<sup>®</sup> BLI Discovery software via serial port (RS-232) port or TCP/IP socket.

To connect the Automation Client example application:

- 1. In the Octet<sup>®</sup> BLI Discovery software, go to **File > Options** (see Figure A-1).
- 2. In the Automation box, select the communication port to be used (either TCP/IP or RS232, see Figure A-1).
- 3. Launch **AutomationClient.exe** located in the C:\Program Files\Sartorius\OctetBLIDiscovery directory to display the **Automation Client** dialog box (Figure A-2).

Application				
Octet BLI Disco     Octet BLI Anal     Sidekick     Octet Analysis	ysis Testing Log Studic	TCP/IP Machine: RS232 Port:	Port: 20000 🔹	Connect
Run Experiment				
Experiment name:			Show runtime window	
Method File:				!
Experiment folder:				
Skip sensor cols:	0		Continue sensor tray from last run	
Repetitions:	1 ≑			Run!
Run Sequence				
Repetitions:	1		□ Show runtime window □ Prompt for new plates □ Continue sensor tray fi (within sequence)	om last ru
Sequence:			Add Remove	

Figure A-2: Automation Client Window

- 4. Select the TCP/IP or RS-232 port selected previously in the Octet<sup>®</sup> BLI Discovery software **Options** dialog box (Figure A-1). To connect locally using **Localhost**, leave the **Machine** field blank.
- 5. Click Connect.

If the port is successfully opened, the automation client dialog will be minimized and remain minimized, indicating that the connection succeeded and the port is open. Otherwise, the automation client dialog will minimize and come back again, indicating that the connection attempt failed.

6. After a successful connection is established, send the default **Version** command (in the **Send Commands**– **Command** field) and then click **Send!** (Figure A-3).

A response similar to the following appears in the **Response** box:

	Send Commands									
	Command:	Version	✓ … Send!							
ľ	Time	Command	Response							
	2020/11	Version	12.2.0.4 OctetRED384 1.1							

Figure A-3: Send Commands–Command Field

The response indicates that the **Automation Client** has connected to the Octet<sup>®</sup> BLI Discovery software. This example indicates that version 6.1.0.75 of the Octet<sup>®</sup> BLI Discovery software is controlling an Octet<sup>®</sup> instrument using version 1.0 of the automation interface.

### Automation Commands

Table A-1 summarizes the commands supported by the Octet<sup>®</sup> BLI Discovery software automation interface.

**NOTICE:** The symbolic names are provided for C++ clients who connect using the interface as defined in the Automation API. h header file.

Table A-1: Commands Supported by the Automation Interface

Command	Symbolic Name	Purpose
Version	AUT_CMD_VERSION	Returns the version of the application being automated, the type of instrument it is controlling, and the automa- tion API version.
Reset	AUT_CMD_RESET	Stops any running experiment and resets the instrument.
GetMethodInfo	AUT_CMD_GETMETHODINFO	Returns information about the resources required by given method file.
Run	AUT_CMD_RUN	Runs an experiment using a given method file.
GetRunInfo	AUT_CMD_GETRUNINFO	Returns information about the experiment currently run- ning.
Stop	AUT_CMD_STOP	Stops a running experiment, ejecting the sensors if nec- essary.
Status	AUT_CMD_STATUS	Returns status during a running experiment: OK = ready Busy =running Waiting = waiting for a condition to be resolved Error = experiment was terminated by an error Busy is followed by descriptive information on the prog- ress of the experiment (% complete)
Present	AUT_CMD_PRESENT	Open the door and move the stage to the presentation position.
Resume	AUT_CMD_RESUME	Indicates that the "Waiting" condition has been resolved (new sensor tray installed). Continues the experiment.
Close	AUT_CMD_CLOSE	Closes the door if it is open. Homes the read head.
Cleanup	AUT_CMD_CLEANUP	Closes open MDI windows. Only valid when not busy. Useful when using the <b>Run</b> command without the <b>-s</b> option.

#### Typical Automation Session

The following is an automation session that illustrates the use of the automation commands to run an experiment.

**NOTICE:** Commands sent from the client application are designated as SEND:. Responses received from the Octet<sup>®</sup> BLI Discovery software are designated as RECV:.

Connecting to the Octet BLI Discovery Software SEND: Version\r\n RECV: 6.1.0.30 Pegasys 1.0 SEND: Status\r\n RECV: OK

Preparing for an Experiment

SEND: Cleanup RECV: OK SEND: GetMethodInfo -mC:\MethodFiles\Q001.fmf\r\n RECV: OK -p96,0 -t1 -s″Anti-Human IgG Fc″

Starting the Experiment

SEND: Version\r\n RECV: 6.1.0.30 Pegasys 1.0 SEND: Run\r\n RECV: OK

#### Getting Information about the Experiment

SEND: Version\r\n RECV: 6.1.0.30 Pegasys 1.0 SEND: GetRunInfo\n RECV: OK -n″Experiment 1″ -p″\\fbdata\Quantitation\Experiment 1″

#### Monitoring the Experiment

bool bBusy = true; while (bBusy) { Send("Status\r\n"); response = Recv();

if (response==OK)

bBusy = false; else Sleep(1000); // sleep for a second }

SEND: Status\r\n RECV: Running (5%)

SEND: Status\r\n RECV: Running (25%)

SEND: Status\r\n RECV: Running (45%)

SEND: Status\r\n RECV: Running (75%)

SEND: Status\r\n RECV: Running (95%)

SEND: Status\r\n RECV: OK

Stopping the Experiment and Presenting the Plate for Unloading

Both the Stop and the Present commands are asynchronous; they initially return OK to indicate that the command was accepted and started OK, but status must be polled until OK is returned to indicate completion.

SEND: Stop\r\n RECV: OK

SEND: Status\r\n RECV: Busy

SEND: Status\r\n RECV: Busy

SEND: Status\r\n RECV: OK

SEND: Present\r\n

RECV: OK

SEND: Status\r\n RECV: Busy

SEND: Status\r\n RECV: Busy

SEND: Status\r\n RECV: OK

#### ADVANCED AUTOMATION SESSION

If an experiment is sufficiently complex it may require more than one tray of sensors to complete the experiment. This can be detected at the start of the experiment by checking the -tN response from the GetMethodInfo command. If N is greater than 1, then the experiment requires more than one tray of sensors to complete. If this is the case, initially the experiment will start as before, but halfway through the experiment the Status command will return LoadSensors indicating that the first tray of sensors has been exhausted and another tray of sensors needs to be loaded. At this point, you must issue the Present command to allow access to the sensor plate (polled for completion) and then once the new sensor tray is in place, the Resume command must be sent to resume the experiment.

Connecting to Octet BLI Discovery

SEND: Version\r\n RECV: 6.1.0.30 Pegasys 1.0 SEND: Status\r\n RECV: OK

Preparing for an Experiment

SEND: Cleanup RECV: OK SEND: GetMethodInfo -mC:\MethodFiles\Q002.fmf\r\n RECV: OK -p96,0 -t2 -s″Anti-Human IgG Fc″

Starting the Experiment

SEND: Run -mC:\MethodFiles\Q002.fmf -bP0001 -s\r\n RECV: OK Getting Information about the Experiment

#### SEND: GetRunInfo\r\n

RECV: OK -n"Experiment 2" -p"\\fbdata\Quantitation\Experiment 2"\r\n

#### Monitoring the Experiment

bool MonitorExperiment(CCmdTransport \*pPort)
{
 // Poll the experiment until it is done.
 for (;;)
{

Sleep(200);

```
if (!SendRecv(pPort, AUT_CMD_STATUS + AUT_EOL, csResp))
return false;
```

```
int nStart = 0;
CString csStatus = csResp.Tokenize(" ", nStart);
```

```
if (csStatus == AUT_OK)
break; // SUCCESS
else if (csStatus == AUT_STOPPED)
break; // SUCCESS
else if (csStatus == AUT_RUNNING)
;
else if (csStatus == AUT_WAITING)
;
else if (csStatus == AUT_LOADSENSORS)
{
if (!LoadSensors(pPort))
return false;
}
else if (csStatus == AUT_BUSY)
;
else if (csStatus == AUT_ERROR)
return false;
}
}
```

```
bool LoadSensors(CCmdTransport *pPort)
{
    if (!SendRecv(pPort, AUT_CMD_PRESENT + AUT_EOL, csResp))
    return false;
```

```
if (csResp != AUT_OK)
return false;
```

```
if (!WaitNotBusy(pPort))
return false;
```

// At this point the robot replaces the sensor tray.. AfxMessageBox("Robot changes sensor tray...");

if (!SendRecv(pPort, AUT\_CMD\_RESUME + AUT\_EOL, csResp)) return false;

```
if (csResp != AUT_OK)
return false;
```

```
return WaitNotBusy(pPort);
}
```

```
bool WaitNotBusy(CClientResponder *pPort)
```

```
{
```

CCountdownTimerTimer(c\_uBusyTimeoutMS); CString csResp; while (!Timer.IsDone()) { Sleep(200);

```
if (!SendRecv(pPort, AUT_CMD_STATUS + AUT_EOL, csResp))
return false;
```

int nStart = 0;

CString csStatus = csResp.Tokenize(" ", nStart);

```
if (csStatus == AUT_OK)
return true;
```

```
else if (csStatus == AUT_STOPPED)
return false;
else if (csStatus == AUT_RUNNING)
return true;
else if (csStatus == AUT_WAITING)
return true;
else if (csStatus == AUT_LOADSENSORS)
return true;
else if (csStatus == AUT_BUSY)
;
else if (csStatus == AUT_ERROR)
return false;
}
TRACE1("Timeout waiting for not busy after %d ms\n",
Timer.GetElapsed());
return false;
}
AUTOMATION API.H
//
//
// Copyright (c) 2011 Sartorius.
// All rights reserved.
//
//
********
        *****
// HEADER: AutomationAPI.h
// PURPOSE: Defines the commands supported by the automation API.
// AUTHOR: BHI Nov 2008
//
#ifndef INC_ACQUISITION_AUTOMATIONAPI_H
#define INC_ACQUISITION_AUTOMATIONAPI_H
```

#### // NOTES:

// Do not position the Octet instrument such that it is difficult to disconnect the power.

// The automation interface is string based. Commands and responses are strings, one per line.

// Each command starts with the name of the command and may then be followed by required and optional parameters.

// Each parameter starts with a switch definition (a la dos/unix command

line) followed by the parameter itself. This allows parameters to be sent in any order.

// The command or response is terminated with a new line (CR/LF) sequence.

// Parameters containing embedded spaces must be enclosed in double quotes.

// Response items containing embedded spaces will be enclosed in double quotes.

#### // REVISIONS:

// 1.0 First release

// 1.1 Added (-p) plate file parameter to "Run" and "GetMethodInfo"

// commands

// Added (-u) use-last-sensor-tray option to the "Run" command.

// Added "SetValue" command to set the temperature target.

// Version of the API described in this header file. const char AUT\_API\_VERSION[] = "1.1";

```
// Status return values
const char AUT_OK[] = "OK";
const char AUT_STOPPED[] = "Stopped";
const char AUT_RUNNING[] = "Running";
const char AUT_WAITING[] = "Waiting";
const char AUT_LOADSENSORS[] = "LoadSensors";
const char AUT_BUSY[] = "Busy"; // Resetting, Presenting
const char AUT_ERROR[] = "ERROR";
const char AUT_EOL[] = "\r\n";
```

// Parameter switches for the Run command const char AUT\_SWITCH\_METHOD = 'm'; // Method file to load (required) const char AUT\_SWITCH\_FOLDER = 'f'; // Root folder for experiment data (optional) const char AUT\_SWITCH\_EXPERIMENT = 'e'; // Overide for the experiment name in the FMF file (optional) const char AUT\_SWITCH\_PLATEFILE = 'p'; // Plate file to import after method file is loaded (optional) const char AUT\_SWITCH\_BARCODE = 'b'; // Bar code of Sample plate (optional) const char AUT\_SWITCH\_BARCODE1 = '1'; // Alias for AUT\_SWITCH\_BARCODE (optional) const char AUT\_SWITCH\_BARCODE2 = '2'; // Bar code of Reagent plate (optional) const char AUT\_SWITCH\_LOTNUMBER = '1'; // Lot number of sensors (optional) const char AUT\_SWITCH\_SILENT = 's'; // Don't open the runtime window (optional) const char AUT\_SWITCH\_USELAST = 'u'; // Reuse the sensor tray as it was left after last run (optional) const char AUT\_SWITCH\_VERBOSE = 'v'; // Send back verbose status information

// Parameter switches for the SetValue command const char AUT\_SWITCH\_TEMPERATURE = 't';

// Response parameter switches for the GetMethodInfo command const char AUT\_RESPONSE\_PLATEWELLS = 'p'; const char AUT\_RESPONSE\_SENSORTRAYS = 't'; const char AUT\_RESPONSE\_SENSORTYPE = 's'; const char AUT\_RESPONSE\_EXPTYPE = 'e'; const char AUT\_RESPONSE\_RERACKING = 'r';

// Response parameter switches for the GetRunInfo command const char AUT\_RESPONSE\_EXPNAME = 'n'; const char AUT\_RESPONSE\_EXPPATH = 'p';

const char AUT\_CMD\_VERSION[] = "Version";

// Returns the version of the app being automated, the hardware platform
it controls, and the API version.
// Args: (none)
// Response: App product version (e.g. "6.0.0.120 Pegasys 1.0\r\n")

const char AUT\_CMD\_RESET[] = "Reset";
// Stops any running experiment and resets the instrument.
// Args: (none)
// Response:
// "OK\r\n"
// "Error: <reason>\r\n"

```
const char AUT CMD GETMETHODINFO[] = "GetMethodInfo";
// Returns info about a method file
// Args:
// -m <path> Method file name (required)
// Response:
// "OK -r<bool> -t<int> -s<name>\r\n"
// e.g. OK -p96,0 -t2 -s"SA (Streptavidin)\r\n"
// Response params:
         // -p<int>,<int> Sizes of the plates in use e.g. p384,96
// -t < int > Number of sensor trays required (0 .. 5) e.g. -t2
// -s<name> Name of first sensor in the tray e.g. -s"SA
(Streptavidin)"
// "Error: load method\r\n"
// "Error: bad method\r\n"
const char AUT_CMD_RUN[] = "Run";
// Runs an experiment
// Args:
// -m <path> Method file name (required)
// -p <path> Plate file to update sample plate in method settings (optional)
// -b <barcode> Sample plate bar code (optional)
```

// -1 <barcode> Sample plate bar code (optional)

// -2 <barcode> Reagent plate bar code (optional)

// -l <lotnumber> Sensor tray lot number (optional)

// -s Silent - does not open the runtime view (optional)

// -u Use the state of the sensor tray as it was left after last run

// Response:

// ″OK\r\n″

// "Error: not ready\r\n"

// "Error: bad method\r\n"

// "Error: bad barcode\r\n"

const char AUT\_CMD\_GETRUNINFO[] = "GetRunInfo";

// Returns information about an experiment that is currently running

// Args: (none)

// Response:

// "OK -n"Experiment 1" -p"\\fbdata\Quantitation\Experiment 1"\r\n"

// "Error: <reason>\r\n"

// Response params:

// -n<experiment name> Name of the experiment (folder name in repository) e.g. -n"Experiment 1"

```
// -p<experiment path> Full path to experiment folder in repository e.g. -p"\\fbdata\Quantitation\Experiment 1"
```

```
const char AUT_CMD_STOP[] = "Stop";
```

// Stops a running experiment

// Args: (none)

// Response:

// "OK\r\n"

// "Error: <reason>\r\n"

```
const char AUT_CMD_SETVALUE[] = "SetValue";
// Sets a value
// Args:
// -t <temp> Sets heater target temperature (DegC)
// Response:
// "OK\r\n"
// "Error: <reason>\r\n"
```

```
const char AUT_CMD_STATUS[] = "Status";
```

// Returns status: OK=ready, Busy=running, Error=Experiment was terminated by an error.

// Busy is followed by descriptive information on the progress of the experiment (% complete)

// Args: (none)

// Response:

// "OK\r\n"

// "Waiting\r\n"

// "Busy\r\n"

// "Running (nn%)\r\n"

// "LoadSensors\r\n"

// "Error: <reason>\r\n"

const char AUT\_CMD\_PRESENT[] = "Present"; // Pegasys only

// Open the door and move the stage to the presentation position.

// Args: (none)

// Response:

// "OK\r\n"

// "Error: <reason>\r\n"

// N.B.: Poll status waiting for "Waiting" condition to reappear

const char AUT\_CMD\_RESUME[] = "Resume";

// Indicates that the "Waiting" condition has been resolved (new sensor tray installed). Continues the experiment.

// Args: (none)

// Response:

// "OK\r\n"

// "Error: <reason>\r\n"

// Status will indicate busy until door is closed, then will return to Running state.

const char AUT\_CMD\_CLOSE[] = "Close";

// Closes the stage if it is open.

// Args: (none)

// Response:

// "OK\r\n"

// "Error: <reason>\r\n"

// Status will indicate busy until door is closed.

const char AUT\_CMD\_CLEANUP[] = "Cleanup";

// Closes open MDI windows. Only valid when not busy.

// Args: (none)

// Response:

- // "OK\r\n"
- // "Error: busy\r\n";

#endif // INC\_ACQUISITION\_AUTOMATIONAPI\_H

#### ANALYSIS AUTOMATION API

//
***************************************

//

// Copyright (c) 2011 Sartorius. // All rights reserved. // //

// HEADER: AutomationAPI.h

// PURPOSE: Defines the commands supported by the automation API. // AUTHOR: BHI Nov 2008

// #ifndef INC\_ANALYSIS\_AUTOMATIONAPI\_H #define INC\_ANALYSIS\_AUTOMATIONAPI\_H

// NOTES:

// \* The automation interface is string based. Commands and responses are
// strings, one per line.
// \* Each command starts with the name of the command and may then be
// followed by required and
// optional parameters.
// \* Each parameter starts with a switch definition (a la dos/unix command
// line) followed by the
// parameter itself. This allows parameters to be sent in any order.
// \* The command or response is terminated with a new line (CR/LF)sequence.
// \* Parameters containing embedded spaces must be enclosed in double
// quotes.

// \* Response items containing embedded spaces will be enclosed in double // quotes.

// Version of the API described in this header file. const char AUT\_API\_VERSION[] = "1.0";

```
// Status return values
const char AUT_OK[] = "OK";
const char AUT_RUNNING[] = "Running";
```

```
const char AUT_ERROR[] = "ERROR";
const char AUT_BUSY[] = "Busy";
const char AUT_STOPPED[] = "Stopped"; // Stopped by user.
const char AUT_EOL[] = "\r\n";
```

```
// Parameter switches for the LOAD command
const char AUT_SWITCH_DATASET = 'd';
```

// Parameter switches for the ANALYZE command const char AUT\_SWITCH\_PARAMS = 'p'; const char AUT\_SWITCH\_XMLINFO = 'x';

// COMMAND API // ========

const char AUT\_CMD\_VERSION[] = "Version";
// Returns the version of the app being automated, and the API version.
// Args: (none)
// Response: App product version (e.g. "6.3.1.12 1.0\r\n")

```
const char AUT_CMD_LOAD[] = "Load";

// Loads an experiment

// Args:

// -d <path> Path to experiment data files

// Response:

// "OK\r\n"

// "Error: <reason>\r\n"
```

const char AUT\_CMD\_ANALYZE[] = "Analyze"; // Runs an analysis // Args: // -p <path> Path to parameters (INI file) // -x <path> Path to XML information file (optional, can be multiple XML info files) // Response: // "OK\r\n" // "Error: <reason>\r\n" const char AUT\_CMD\_STATUS[] = "Status";

// Returns status: OK=ready, Busy=running, Error=Action was terminated by
an error.
// Busy is followed by descriptive information on the progress of the experiment (% complete)
// Args: (none)
// Args: (none)
// "Response:
// "OK\r\n"
// "Busy\r\n"
// "Running (nn%)\r\n"
// "Error: <reason>\r\n"

#endif // INC\_ANALYSIS\_AUTOMATIONAPI\_H

# Appendix B: Octet<sup>®</sup> GxP Server Module

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### Overview

The Octet<sup>®</sup> 21 CFR Part 11 software portfolio is a client-server architecture for managing digital records created with Octet<sup>®</sup> BLI systems and analyzed using Octet<sup>®</sup> analysis applications. A CFR administrator is responsible for configuring user accounts for users of the Octet<sup>®</sup> CFR system. The 21 CFR Part 11 editions of the Octet<sup>®</sup> applications, including Octet<sup>®</sup> BLI Discovery, and Octet<sup>®</sup> Analysis Studio, enforce user logins prior to performing any operations with the software. During the user session, the Octet<sup>®</sup> GxP Server records all system, software, and user events. User sessions are closed when the user logs out or after a set period of inactivity is reached. A new user session starts each time a user accesses the software.

#### Roles and Responsibilities

- **CFR Administrator:** responsible for configuring user accounts, managing user IDs, user passwords and all aspects of 21 CFR part 11, electronic signatures and audit trails.
- Windows Administrator: responsible for managing Octet<sup>®</sup> and Windows software.

### Octet<sup>®</sup> GxP Server Components

The Octet<sup>®</sup> GxP Server software has three modules.

- Octet<sup>®</sup> GxP Server is a Windows service. It handles TCP/IP network connections from the 21 CFR Part 11 client applications and interfaces with the database files.
- Octet<sup>®</sup> GxP Configuration Tool is an application for configuring basic details of the Octet<sup>®</sup> GxP Server such as the network port.
- Octet<sup>®</sup> GxP Server Administration is an application for managing all aspects of the 21 CFR Part 11 environment. The CFR Administrator uses this application to create and edit user accounts, manage permissions, configure projects, and system constants. To learn more about administrator options, see Appendix C, 21 CFR Administrator Guide on page 579.

Usually, the Octet<sup>®</sup> GxP Server and Configuration Tool are installed on one server. For easier management, you can install the Server Administration software on more than one computer
## Octet<sup>®</sup> GxP Server System Requirements

#### Table B-1: Octet<sup>®</sup> GxP Server System Requirements

Section	Description		
Operating System	Windows 10 32-bit or 64-bit, version 1607 "Anniversary Update" or newer Windows Server 2016 or 2019		
Processor	2GHz or faster		
RAM	2GB for 32-bit or 4GB for 64-bit operating system		
Hard disk space	50GB free space recommended		
Display	1920x1080 or better		
Network connection	The recommended configuration requires a local network connection to other computers using the Octet <sup>®</sup> CFR applications. This configuration does not require internet access.		

The Octet<sup>®</sup> GxP Server can run on a virtualized environment. Sartorius cannot guarantee compatibility with specific deployment topologies and custom network environments. Evaluate a test deployment before using the Octet<sup>®</sup> GxP Server in a production environment.

## Network Configuration

### **Recommended Configuration**

Install the Octet<sup>®</sup> GxP Server onto a dedicated administrator computer. This can be a dedicated physical server workstation, or a virtual machine. Typically, the Octet<sup>®</sup> GxP Server computer will be under local IT control. Ensure that the server is always on and available for client connections. The Octet<sup>®</sup> hardware controller PC must have network connectivity to the Octet<sup>®</sup> GxP Server. Other optional workstations with the Octet<sup>®</sup> Analysis Studio software, will also need network connectivity to the Octet<sup>®</sup> GxP Server.



Figure B-1: Network layout

Decide in advance how the client applications will find the server instance. If you use the IP address directly, assign a static IP address or use a DHCP reservation for the Octet<sup>®</sup> GxP Server computer. Most corporate networks can use the assigned fully qualified domain name (FQDN) of the server.

### Single Computer Configuration

If you cannot use the recommended network configuration, install the Octet<sup>®</sup> GxP Server software on a single computer along with the Octet<sup>®</sup> BLI Discovery software. This is a last resort.

See the following section for compliance considerations if using this option.



Figure B-2: Layout of a single computer with the Octet<sup>®</sup> system

### **Ensuring Compliance**

The Octet<sup>®</sup> GxP Server is a key part of keeping records compliance. Strictly control the access to the computer hosting the Octet<sup>®</sup> GxP Server software. Any Windows accounts that are Administrators on the server can directly access the user and audit trail databases to backup work. Place the server under the control of a department separate from the day-to-day users of the Octet<sup>®</sup> system, for example local IT, or the Quality department.

Control the Octet<sup>®</sup> GxP Server installation media to prevent the setup of "rogue" GxP Server instances.

*IMPORTANT:* If you are using the Single Computer Configuration, configure the Windows user accounts for Octet<sup>®</sup> system users as Windows standard accounts. Any Windows user with Administrative privileges on the computer will have access to the Octet<sup>®</sup> GxP Server database files.

## Installation of the Octet<sup>®</sup> GxP Server Module

**NOTICE:** If you are upgrading from an earlier version of Octet<sup>®</sup> GxP Server, make a backup copy of the database files before proceeding. See "Backup the Database" on page 574.

- 1. Navigate to the window that lists the files on the installation CD.
- 2. Double-click OctetGxPServer.exe to launch the installer.

3. If prompted with the *Do you want to allow this app to make changes to your device*? message, verify the publisher name and reply Yes.

The Installation wizard appears.



Figure B-3: Installation wizard

- 4. Click **Next** to display the Choose Components dialog box.
- 5. Click **Next** to display the Choose Components dialog box (Figure B-4). Install both options on the computer selected to host the Octet<sup>®</sup> GxP Server software. You can also install the Octet<sup>®</sup> GxP Administration component on other client computers such as the Octet<sup>®</sup> controller computer. In general, only install the Octet<sup>®</sup> GxP Server component on one computer in your network.



Figure B-4: Choose Components dialog box

- 6. Continue following the installation wizard. Accept the default installation path and start menu folder.
- 7. Click Install.

The "Completing the GxP Server Setup" screen appears after the installation is completed (Figure B-5).

8. Click Finish to complete the installation.



Figure B-5: Completing the GxP Server Setup

### Initial Configuration

The default configuration for Octet<sup>®</sup> GxP Server is suitable for most networks. Follow these instructions to perform the initial Administrator login. After that, if you need to change the network port the Octet<sup>®</sup> GxP Server software uses, see "Additional Server configuration options" on page 577. The default port for the Octet<sup>®</sup> GxP Server is port 20002

#### Initial Administrator Login

A new installation of Octet<sup>®</sup> GxP Server will have a single user account, "Administrator" with a blank password. The first time you login, you must create a password. Perform the initial login from the computer hosting the Octet<sup>®</sup> GxP Server software.

1. Double-click the desktop short cut (Figure B-6) to launch the Octet<sup>®</sup> GxP Server Administration tool.



Figure B-6: Octet<sup>®</sup> GxP Server Administration Shortcut

The Login dialog box appears (Figure B-7).

- 2. Choose the localhost option with the default port of 20002.
- 3. Choose "Administrator" from the dropdown list of users. Leave the password blank and click OK.

Login			×
Connection to serve Server address:	er:		
Port:	Localhost 20002	Find	Default
Credentials:		1	
User name:	Administrator		~
Password:			?
		ОК	Cancel

Figure B-7: Login Dialog Box

4. Create the Administrator password (Figure B-8). Enter a new password and set a password reminder, if desired. The password must have at least 6 characters, with at least one letter, one digit, and one special character, such as the "%" symbol.

Reset Password	×
Current password:	?
New password:	
Confirm new password:	
Password reminder:	
	OK Cancel

Figure B-8: Reset password dialog

5. After changing the password, the Octet<sup>®</sup> GxP Server Administration dialog appears. Refer to Appendix C, 21 CFR Administrator Guide on page 579 for information about adding user accounts and assigning privileges.

**IMPORTANT:** Create additional user accounts. At least two users must have the administrative privilege. If your organization requires that usernames must be associated with specific individuals, the default Administrator account can be inactivated after administrative privilege account is assigned to another user account.

The Administrator user account is set up. If you need to change the service port, follow the instructions for "Additional Server configuration options" on page 577.

Record the Octet<sup>®</sup> GxP Server IP address or fully qualified domain name, and the service port (if it was changed). This information enables all client computers to establish a connection. Post the Server's IP address and fully qualified domain name in a place that is easy to remember and to access.

### Test connectivity from client computers

Identify a client computer to use for testing connectivity to the Octet<sup>®</sup> GxP Server. This can be the Octet<sup>®</sup> hardware controller PC or a personal workstation. You must connect the client computer to the same network as the Octet<sup>®</sup> GxP Server.

You can use any of the Octet<sup>®</sup> client applications to test connectivity to the server. These include the 21 CFR Part 11 editions of Octet<sup>®</sup> BLI Discovery, Octet<sup>®</sup> Analysis Studio, or the Octet<sup>®</sup> GxP Server Administration client. The connectivity test is the same.

1. Launch the client software of your choice. This example uses the Octet<sup>®</sup> BLI Discovery software

The Login dialog box appears.

Login		×
	SVIFCTFA3	
Server:	localhost: 20002	
User:	~	
Password:		?
Project:	(none) v	
	Login Quit	

Figure B-9: Login dialog box

2. Click the... (Browse) to display the Octet<sup>®</sup> GxP Server dialog box.

GxP Server				Х
Connection to serv	/er:			
Server address:	192.168.136.1	28		
	Localhost			-
Port:	20002 🚔	Find	Default	
		OK	Cancel	

Figure B-10: GxP Server dialog box

3. Uncheck the localhost option. Use the default (20002) unless it was changed during the initial server installation. For the server address, type in the IP address or the fully qualified domain name of the Octet<sup>®</sup> GxP Server. Click **OK**.

**NOTICE:** If the Octet<sup>®</sup> GxP Server is on the same subnet as the client computer, you can click "Find" to scan for the server. If you are using a Single Computer Configuration, the server address can remain as Localhost.

4. At the login screen (Figure B-11), the user dropdown list has the usernames configured on the server. Select an appropriate username, enter the password, and click Login.

Login		×
	SVIFCTFA3	
Server:	192.168.136.128: 20002	
User:		
Password:	Administrator DBean	
Project:	GMoreno JBlack PSmith RBrown	
	Login Quit	

Figure B-11: Login screen with server address configured

- 5. After a successful login, you have access to the client application. This confirms connectivity to the Octet<sup>®</sup> GxP Server. If you are not able to log in, see the Troubleshooting section.
- 6. After you select the Octet<sup>®</sup> GxP Server address, this location becomes the default selection for the client application. You do not need to reselect it each time you initiate a new user session.

Repeat these steps to configure the server address on other client computers you may use with Octet<sup>®</sup> GxP Server.

## Backup the Database

The Octet<sup>®</sup> GxP Server module has a file-based database. To make a backup of the database, make a copy of the database file and save it to an archival location. You must be a member of the Windows Administrators group for the server to access the database file

- 1. Log on to the computer that is hosting the Octet<sup>®</sup> GxP Server Module.
- 2. Open Windows<sup>®</sup> Explorer and browse to the program data folder (C:\ProgramData\ForteBio\FBServer).
- 3. Make a copy of the FBEventLog.db and FBServer.db files.
- 4. Save the copies to another location.

## Upgrade the Octet<sup>®</sup> GxP Server Module

NOTICE: Uninstalling and reinstalling does not delete any existing GxP Server database files.

*IMPORTANT:* You must make a backup copy of the existing database before installing and upgrading to a newer version of the Octet<sup>®</sup> GxP Server.

After the upgrade, the existing audit trail database upgrades to the latest schema.

1. From the Windows Settings, choose Add or Remove Programs. Scroll to the Octet<sup>®</sup> GxP Server (if upgrading from version 12.0 or older, locate the GxP Server in the list of apps).

Settings

2. Click on Octet<sup>®</sup> GxP Server, and then click **Uninstall** (Figure B-12).

வ் Home	Apps & features	
Find a setting	Octet BLI Discovery 12.1	192 MB 10/9/2020
Apps	Octet BLI Discovery 12.1 (CFR11)	<b>192 MB</b> 10/9/2020
Ξ Apps & features		
i⊐ Default apps	Octet GxP Server 12.1 12.1.0	7.85 MB 10/9/2020
印 <u></u> Offline maps		
	Modi	fy Uninstall
Apps for websites	Office	1.72 MB
□ □ Video plavback	Microsoft Corporation	9/24/2020

Figure B-12: Uninstall Octet<sup>®</sup> GxP Server

3. To install the Octet<sup>®</sup> GxP Server module: See "Installation of the Octet<sup>®</sup> GxP Server Module" on page 569.

After the Octet<sup>®</sup> GxP Server module software starts, it updates the audit trail database to the latest database schema.

## Restoring a Database Backup

Follow these instructions to restore a database backup.

- 1. Stop the Octet<sup>®</sup> GxP Server Windows Service: Launch the Windows Services snap-in.
- 2. Locate Octet<sup>®</sup> GxP Server in the list of services, and then click **Stop the service** (Figure B-13).
- 3. Confirm that the status of the service status changes from Running to blank.
- 4. Do not close the Services window.

File Action View	Help					
⇐ ➡ 🗖 🗎	à 🗟 🛛 🖬 🕨 🖬 🕪 🖬					
Services (Local)	🔍 Services (Local)					
	Octet GxP Server	Name	Description	Status	Startup Type	Log ^
		🌼 Netlogon	Maintains a		Manual	Loci
	Stop the service	🆏 Network Connected Device	Network Co		Manual (Trig	Loci
	Restart the service	🆏 Network Connection Broker	Brokers con	Running	Manual (Trig	Loci
		🆏 Network Connections	Manages o		Manual	Loci
	Description:	🆏 Network Connectivity Assis	Provides Dir		Manual (Trig	Loci
	Authenticates users and groups for Octet applications	🆏 Network List Service	Identifies th	Running	Manual	Loci
		🆏 Network Location Awareness	Collects an	Running	Automatic	Net
		🎑 Network Setup Service	The Networ		Manual (Trig	Loci
		🎑 Network Store Interface Ser	This service	Running	Automatic	Loci
		🖏 Octet GxP Server	Authenticat	Running	Automatic (	Loc
		🎑 Offline Files	The Offline		Manual (Trig	Loci
	1	Charling Cure 21160	This convice	Dunning	Automatic (	Loc

Figure B-13: Stop the service

- 5. Restore the backup files: Copy the database backup files FBEventLog.db and FBServer.db to the folder C:\ProgramData\ForteBio\FBServer, overwriting any existing files.
- 6. Start the Octet<sup>®</sup> GxP Server Windows Service: Return to the Services window. Select Octet<sup>®</sup> GxP Server in the list of services and choose **Start** the service (Figure B-14). Confirm that the service status changes to Running.

Services (Local)	ļ
Octet GxP Server	
Start the service	
Description: Authenticates users and groups for Octet applications	

Figure B-14: Start the service

## Moving the Octet<sup>®</sup> GxP Server to a New Host

Follow these instructions to change host computer for the Octet<sup>®</sup> GxP Server software.

- 1. Create a backup of the existing database files.
- 2. Install the Octet<sup>®</sup> GxP Server software onto the new host.
- 3. Restore the database files to the new host.

- 4. Confirm the restore by logging in to the Octet<sup>®</sup> GxP Server Administration client on the new host using Localhost for the server connection.
- 5. To complete the move, uninstall the Octet<sup>®</sup> GxP Server software from the old host.
- 6. Update the server address for all client computers.

## Restarting the Octet<sup>®</sup> GxP Server Module

If you cannot find the host location of the Octet<sup>®</sup> GxP Server module during user login or if users with valid credentials are unable to login, the Octet<sup>®</sup> GxP Server module may be offline and may need to be restarted.

**NOTICE:** Contact your IT department to determine if the network or firewall settings were changed. This can prevent access to the Octet<sup>®</sup> GxP Server module.

Double-click on the OctetGxPServer.exe file (Figure B-15) in the Octet<sup>®</sup> GxP Server folder from the installed location:

) → Local Disk (C:) → Program Files (x86) ⇒	Sartorius > OctetGxPServ	er12	۹ 5 ۲
Name	Date modified	Туре	Size
🕥 Globe	9/18/2020 5:37 PM	lcon	25 KB
OctetGxPAdministration	10/1/2020 2:32 PM	Application	3,174 KB
🛃 OctetGxPConfig	10/1/2020 2:32 PM	Application	3,193 KB
🛃 OctetGxPServer	10/1/2020 2:32 PM	Application	1,432 KB
🔊 Sartorius	10/1/2020 3:19 PM	Internet Shortcut	1 KB
🎯 uninst	10/1/2020 3:19 PM	Application	217 KB



### Additional Server configuration options

The Octet<sup>®</sup> GxP Server Configuration tool allows administrators to configure the GxP service according to local network requirements. You must be a member of the Windows Administrators group to use this tool.

To configure additional options:

1. From the server hosting the Octet<sup>®</sup> GxP Server software, navigate to the installation folder and double-click on the OctetGxPConfig.exe program (Figure B-16).

:al Disk (C:) > Program Files (x86)	> Sartorius > OctetGxPSer	ver12 v	ල , Sear
Name	Date modified	Туре	Size
🔮 Globe	9/18/2020 5:37 PM	lcon	25 KB
OctetGxPAdministration	10/9/2020 11:54 AM	Application	3,174 KB
🛃 OctetGxPCqnfig	10/9/2020 11:54 AM	Application	3,193 KB
🕢 OctetGxPS	10/9/2020 11:54 AM	Application	1,432 KB
殻 Sartorius	10/9/2020 1:44 PM	Internet Shortcut	1 KB
🎯 uninst	10/9/2020 1:44 PM	Application	217 KB

Figure B-16: Octet<sup>®</sup> GxP Server Configuration Tool

2. Log in to the Octet<sup>®</sup> GxP software configuration software using the Administrator account or an account that has the administrator privileges.

www.sa	artor	ius.com		Connection	to dients: st Por UDP ping for	t: 20002 🔹	Apply & Test Default
Login Name	Full	Name	Group	Privileges	Active	Password Age	Info
Administrator	Adr	ministrator	Administrator	admin	Yes	15 days 22:16:38	Default administ
DBean RBrown PSmith GMoreno JBlack	Da Rik Pa Ge Jo	Login Credentia User r Passw	als: name: Admir rord:	iistrator	ОК	X ? Cancel	External collabor

Figure B-17: Administrator login

3. In the Connections to Clients box (Figure B-18), make changes to the server settings as needed.

Connection to clie	ents:		
Localhost	Port:	20002 ≑	Apply & Test
Support UDP	Default		

Figure B-18: Connection to clients box

4. Click **Apply & Test.** If the Octet<sup>®</sup> GxP Server module is functioning properly, this message appears (Figure B-19).



Figure B-19: Found server

To return to the originally configured Octet<sup>®</sup> GxP Server module settings, go to the Connections to clients box (Figure B-18) and click Default.

# Appendix C: 21 CFR Administrator Guide

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## Description

The Octet<sup>®</sup> 21 CFR Part 11 compliant ready software consists of three software products:

- Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software
  - Define quantitation, kinetic or custom assays.
  - Run and view experiments and binding data.
  - Analyze binding data and view analysis results.
- Octet<sup>®</sup> Analysis Studio 21 CFR Part 11 software
  - Analyze binding data and view analysis results.
- Octet<sup>®</sup> GxP Server module software
  - Manage the user database and store Audit Trail data.

NOTICE: Octet<sup>®</sup> BLI Analysis CFR is available if needed for compatibility.

### Data integrity

The CFR Administrator uses the Octet<sup>®</sup> 21 CFR Part 11 compliant ready software to ensure the integrity of the data and to control who can use the software and what they can do.

The integrity of raw data is a primary design consideration of Octet<sup>®</sup> 21 CFR Part 11 software. All data acquired using Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software is time stamped and traceable to the user who initiated data acquisition. All method files, acquired data files, and analysis settings files are digitally signed to ensure data integrity. Any modification or tampering outside of the Octet<sup>®</sup> 21 CFR Part 11 software environment invalidates the digital signature. The Octet<sup>®</sup> 21 CFR Part 11 software performs integrity checks any time a method, experiment data, or analysis settings are accessed and alerts the user if unauthorized modification has occurred.

Electronic signatures can be added to an analysis workspace to prevent further modification within the Octet<sup>®</sup> 21 CFR Part 11 software environment. An Audit Trail of all activities performed in any Octet<sup>®</sup> 21 CFR Part 11 application is stored in the Octet<sup>®</sup> GxP Server database.

Data files created using Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software are strictly bound to features that support FDA 21 CFR Part 11 regulations. As a result, these files cannot be opened or modified by the non-CFR version of Octet<sup>®</sup> software to ensure the integrity of the acquired data is intact

### User information and permissions

The CFR Administrator creates users and assigns permissions according to the user type. A user with no explicit privileges is a Guest and can only open, view, and print data and method files.

The Administrator assigns these user properties:

- Unique User Identifier or ID
- Password

The Administrator assigns these user permissions:

- Manage users and user settings
- Create and edit method templates
- Build multi-datasets
- Edit preprocess settings
- Edit analysis settings
- Edit annotation or display properties
- Convert Kinetic steps or step types into Quantitation
- Edit report pages
- Sign documents
- Set commenting requirements
- Edit experiment info
- Edit sensor and sample info
- Include/exclude wells and sensors from analysis
- Run experiments
- Import analysis settings template to a new dataset
- Export data and Excel reports
- Review Audit Trail for any user
- Remove Signature from documents
- Choose the repository directory when running an experiment

### Automatic user log out (idle timeout)

The Administrator has the option to specify the time period a program is idle before the software will automatically log out a user. The user is automatically logged out after the specified time period even if Octet<sup>®</sup> BLI Discovery 21 CFR Part 11 software is acquiring data from an Octet instrument. After data acquisition begins, Octet<sup>®</sup> 21 CFR Part 11 software continues to acquire data until the experiment is finished. The settings in Preferences determine how the data are saved, exported, and printed, whether the user is logged on or not. If no user is logged on, data acquisition cannot be stopped manually.

### Passwords

#### Expiration

The system administrator can set user passwords to expire after a period of time. If the system administrator activates the password expiration, the users must change their passwords at designated intervals. After a password expires, the software prompts the user to reset it at the next login.

#### Requirements

The system administrator can set the minimum number of characters a password must have and the level of password complexity. Complexity involves requiring passwords to have at least one alpha, one numeric, and one punctuation character. The administrator can assign a common password (such as "Welcome@2021") for the user's first session, and then instruct them to change it during that first session. Users can change their password after they logon

#### Security

The system administrator can set the maximum number of failed login attempts. If the user tries to log in with the incorrect information for the set number of tries, the account is locked, and this action is logged into the Audit Trail.

The administrator can unlock the user and reset the user password.

If a user leaves the group or company, the system administrator can inactivate the user, to help prevent unauthorized use of the software. User accounts can be inactivated, not deleted.

## Administrator Options

### Administrator Checklist

The following is list of the steps for using the Options.

- 1. Select the appropriate Constants for your group.
- 2. Review the groups and assign permissions.
- 3. Create user accounts and implement other options as required.

### Administrator Account Setup

Refer to Appendix B Octet GxP Server Module page 629, "Initial Administrator Login".

### Accessing Administrator Options

Access administrator options using the Octet<sup>®</sup> GxP Server Administration tool.

• Double-click the Octet<sup>®</sup> GxP Server Administration Desktop Shortcut (Figure C-1):



Figure C-1: Octet® GXP Server Administration Desktop Shortcut

The Octet<sup>®</sup> GxP Server Administration tool can be installed on the primary Octet<sup>®</sup> GxP Server computer as well as other client computers to enable remote administration. To install the administration client on additional computers, follow the instructions for Installing the Octet<sup>®</sup> GxP Server module, and choose ONLY the Octet<sup>®</sup> GxP Administration component during setup (Figure C-1).

s Groups	Projects	Const		ents						
gin Name F	ull Name	Active	Locked	Group	Privileges	Password Age	Info			
		Lo	gin						×	
			Connectio	n to ser	ver:					
			Server a	ldress:					_	
						nost				
			Port:		20002	÷ Fine	ł	Default		
			Credentia	s:						
			User nam	e:	Administ	trator			7	
			Password	:					?	
						OK		Cancel		

The Octet<sup>®</sup> GxP Server Administration window displays (Figure C-2):

Figure C-2: Octet<sup>®</sup> GxP Server Administration with Login Dialog Box

From the **User name** drop down list, select **Administrator**. Enter your **Password**. Click '**?**' for a password reminder if needed. Click **OK** to dismiss the Login dialog which then displays the Octet<sup>®</sup> GxP Server Administration Users Tab (Figure C-3).

www.sar	torius.com					[	Print Login.
sers Groups	s Projects Con	stants	Events				
Login Name	Full Name	Active	Locked	Group	Privileges	Password Age	Info
Administrator	Administrator	Yes		Administrator	admin	1 day 21:28:53	Default administrator user
DBean	Daisy Bean	Yes		Developer	analysis, plate, run	1 day 21:27:06	
RBrown	Richard Brown	Yes		Supervisor	review	1 day 21:26:37	
PSmith	Paul Smith	Yes		Lab User	run	00:01:22	
GMoreno	George Moreno	Yes		Guest	(none)	00:00:47	External collaborator
JBlack	John Black	Yes		Developer	analysis, plate, run	00:00:00	

Figure C-3: Octet<sup>®</sup> GxP Server Administration Users Tab

Five tabs are available in the Octet<sup>®</sup> GXP Server Administration window:

- Users Tab-Allows user and password management and individual privileges selection
- Groups Tab-Allows user group management and group privileges selection
- Projects Tab-Allows project management and setup
- **Constants Tab**—Allows setup of password requirements, cached server credentials and screen lock due to inactivity.
- Events Tab-Displays event logs for individual user accounts, projects or machines

Click on a tab to view its information.

Each tab has a context-sensitive Tab menu that can be accessed by right-clicking in the tab window. The menu displayed depends on the tab currently selected and the position of the cursor when you right-click.

Contents of tabs can also be sorted. Clicking the header of a column sorts content alphabetically or chronologically, and data in other columns are also sorted to maintain data association.

## Users Tab

Linese a

The Users Tab **(**Figure C-4) allows administrators to add and inactivate user accounts and set and change individual user account privileges and passwords. Click on any column header to sort the table. For example, the users in (Figure C-4) are sorted alphabetically according to Group.

Administrator         Yes         Administrator         admin         1 day 21:28:53         Default administrator user           DBean         Daisy Bean         Yes         Developer         analysis, plate, run         1 day 21:27:06         Developer           DBlack         John Black         Yes         Developer         analysis, plate, run         00:00:00           SMoreno         George Moreno         Yes         Guest         (none)         00:00:122           Smown         Richard Brown         Yes         Supervisor         review         1 day 21:26:37	ogin Name	Full Name	Active	Locked	Group	Privileges	Password Age	Info
DBean         Daisy Bean         Yes         Developer         analysis, plate, run         1 day 21:27:06           JBlack         John Black         Yes         Developer         analysis, plate, run         00:00:00           GMoreno         George Moreno         Yes         Guest         (none)         00:00:47         External collaborator           PSmith         Paul Smith         Yes         Lab User         run         00:01:22           RBrown         Richard Brown         Yes         Supervisor         review         1 day 21:26:37	Administrator	Administrator	Yes		Administrator	admin	1 day 21:28:53	Default administrator user
JBlack         Yes         Developer         analysis, plate, run         00:00:00           GMoreno         George Moreno         Yes         Guest         (none)         00:00:47         External collaborator           PSmith         Paul Smith         Yes         Lab User         run         00:01:22           RBrown         Richard Brown         Yes         Supervisor         review         1 day 21:26:37	DBean	Daisy Bean	Yes		Developer	analysis, plate, run	1 day 21:27:06	
GMoreno         Guest         (none)         00:00:47         External collaborator           PSmith         Paul Smith         Yes         Lab User         run         00:01:22           RBrown         Richard Brown         Yes         Supervisor         review         1 day 21:26:37	JBlack	John Black	Yes		Developer	analysis, plate, run	00:00:00	
PSmith Paul Smith Yes Lab User run 00:01:22 RBrown Richard Brown Yes Supervisor review 1 day 21:26:37	GMoreno	George Moreno	Yes		Guest	(none)	00:00:47	External collaborator
RBrown Richard Brown Yes Supervisor review 1 day 21:26:37	PSmith	Paul Smith	Yes		Lab User	run	00:01:22	
	RBrown	Richard Brown	Yes		Supervisor	review	1 day 21:26:37	

Figure C-4: User Tab Information Sorted by Group

### Creating a New User Account

1. Right-click in a blank area in the Users Tab. The Tab menu appears,.



Figure C-5: Users Tab Menu New User Option

New User		×
Login name:	1	
Full name:		
Information:		
Password:		
Confirm password:		
Password reminder:		_
Group:	Lab User	~
	Lab Users can only run experiments	
Privileges:	Administration Manage users and user settings Analysis and Change Create and edit method template Build multi-dataset Edit preprocess settings Edit anotation/display properties Convert Kinetic step/step type into Quantitation Edit report pages (LEGACY) Change Review Sign document Review Edit range requirement Review Edit and trail for any user Edit experiment info Edit experim	<
Options:	Password does not expire	
Options:	User must change password at next login OK Cance	9

2. Select **New User** to display the New User dialog box.

Figure C-6: New User Dialog Box

- 3. Assign Account Details. Enter the user's Login name, Full name, Information (optional), Password, and Password reminder (optional).
- 4. *Assign to a User Group.* Select a user group from the **Group** drop down list. The following default group selections are available:
  - Administrators-can manage Users and Group settings including add, delete, edit and view all events
  - Supervisors-can review data and events
  - **Developers**—can create, run, save and export data
  - Lab Users-can only run experiments
  - Guests-have no explicit privileges, these must be assigned by the administrator

If other user groups have been created by an administrator, they are also available for selection in the **Group** drop down box. For more information, see "Creating a New User Group" on page 590.

#### 5. *Modify Privileges.* The default privilege sets for each group type are shown in Table C-1.

 Table C-1: Default User Group Privileges

Privilege Set	Administration	Analysis and Change	Review	Plate Settings	Run Experiment
Administrator	$\checkmark$				
Supervisor			$\checkmark$		
Developer		$\checkmark$		$\checkmark$	$\checkmark$
Lab User			$\checkmark$		
Guest					

**NOTICE:** Analysis and Change, Review and Plate applies only to the Octet<sup>®</sup> BLI Analysis software, not the Octet<sup>®</sup> Analysis Studio user guide for more details.

Individual privilege sets for each user are shown in Figure C-6. To add/remove a specific privilege for a User, select/deselect the corresponding check box.

6. **Options**–Select the **Password does not expire** check box if desired. This check box is deselected by default so that user account passwords expire according to the set PasswordTTL constant. For more information on setting constants please see "Constants Tab" on page 594. Selecting **User must change password at next login** forces the new user to personalize their password before using the system.

 Table C-2: Default User Privileges (Sheet 1 of 2)

		Default Group Privileges						
	Administrator	Supervisor	Developer	Lab User	Guest			
Administration								
Manage users and user settings	$\checkmark$							
Analysis and Change								
Create and edit method template			$\checkmark$					
Build multi-dataset			$\checkmark$					
Edit preprocess settings			$\checkmark$					
Edit analysis settings			$\checkmark$					
Edit annotation/display properties			$\checkmark$					
Convert Kinetic step/step type into Quantitation			$\checkmark$					

#### Table C-2: Default User Privileges (Continued) (Sheet 2 of 2)

		Default C	Group Privileges		
	Administrator	Supervisor	Developer	Lab User	Guest
Edit report pages			$\checkmark$		
(LEGACY) Change			$\checkmark$		
Review					
Sign Document		$\checkmark$			
Set commenting require- ment		$\checkmark$			
Review Audit Trail for any user		$\checkmark$			
Remove signature from document					
Plate Settings					
Edit experiment info			$\checkmark$		
Edit sensor and sample plate info			$\checkmark$		
Include/exclude wells and sensors from analysis			$\checkmark$		
(LEGACY) Plate			$\checkmark$		
Run Experiment					
Run Experiment			1	$\checkmark$	
Import analysis settings template to new dataset				$\checkmark$	
Export data and Excel report				$\checkmark$	
Choose repository direc- tory when running an experiment					

#### Viewing and Changing User Account Settings

1. Position the cursor on the account and right-click to display the Tab menu.

New User
Edit User
Set Password
Inactivate User
Lock User

Figure C-7: User Tab Menu

2. Select Edit User: Make changes to privileges by selecting/deselecting check boxes.





**NOTICE:** To prevent system lockout, users with the Administrator privilege cannot remove their own Administrator privilege. A different administrator account must be used to revoke the privilege.

3. Click **OK** to save changes and exit.

#### Inactivating a User Account

- 1. Position the cursor on the account and right-click to display the Tab menu (Figure C-7).
- 2. Select Inactivate User.
- 3. Click OK to save changes and exit.

**NOTICE:** The default Administrator account can be inactivated when at least one other user account has the administrator privilege.

#### Changing User Account Passwords

1. Right-click on the user account and select **Set Password** from the Tab menu.

The Reset Password dialog box displays (Figure C-9):

Reset Password	×
Current password:	?
New password:	
Confirm new password:	
Password reminder:	
User must change pass	sword at next login
	OK Cancel

Figure C-9: Change Password Dialog Box

- 2. Enter the **New password** for the user account.
- 3. Re-enter the new password. Password reminder is optional.
- 4. Check **User must change password at the next login** if you want the user to personalize their password before using the system.
- 5. Click **OK** to save changes and exit.

**NOTICE:** Users Each user can change their own password by logging into the Octet <sup>®</sup> GxP Server Administration software with their Username and password. They can then change their password by right-clicking on their account, and following the same steps as described above.

#### Changing the Administrator Password

1. Right-click on the Administrator account and select **Set Password** from the Tab menu.

The Change Password dialog box displays (Figure C-10):

Change Password	×
Current password:	?
New password:	
Confirm new password:	
Password reminder:	
	OK Cancel

Figure C-10: Change Password Dialog Box

- 2. Enter the Current password then enter the New Password.
- 3. Re-enter the New Password. Password reminder is optional.
- 4. Click **OK** to save changes and exit.

**NOTICE:** The Administrator password can also be changed within the Octet<sup>®</sup> BLI Discovery application when logged in as administrator. Select **Change Password** from the Security menu then follow the prior steps.

## Group Tab

The Groups Tab (Figure C-11) allows administrators to add and delete user groups as well as set and change group privileges. The columns contain information about each group. The table can be sorted by clicking on any column header. For example, the Groups in Figure C-11 have been sorted alphabetically according to Name.

	torius.com	Print Login						
Users Groups	Projects Constan	ts Events						
Name	Privileges	Info						
Administrator	admin	Administrators can add/delete/edit users and groups						
Supervisor	review	Supervisors can review data and events						
Developer	analysis, plate, run	Developers can create, run, save and export data						
Lab User	run	Lab Users can only run experiments						
Guest	(none)	Guests have no explicit privileges						

Figure C-11: Groups Tab

When a user account is assigned to a user group, the privileges defined in the group are also applied to the individual user account. The following default user groups are available and the detailed privileges are given above under User Account Administration.

- Administrators Can manage Users and Group settings including add/delete/edit and view all events
- Supervisors Can review data and events
- Developers Can create, run, save and export data
- Lab Users Can only run experiments
- Guests Have no explicit privileges, these must be assigned by the administrator

### Creating a New User Group

1. Right-click in a blank area in the **Groups** Tab to display the Tab menu (Figure C-12).

New Group
Edit Group
Delete Group

Figure C-12: Group Tab Menu

Click **New Group** to display the New Group dialog box (Figure C-13). 2. New Group × Group name: Information: Privileges: Administration Manage users and user settings Analysis and Change Create and edit method template Build multi-dataset Edit preprocess settings
 Edit analysis settings Edit annotation/display properties Convert Kinetic step/step type into Quantitation Edit report pages (LEGACY) Change Review Sign document Set commenting requirement Review Audit Trail for any user harn fra Cancel OK

Figure C-13: New Group Dialog Box

- 3. Enter the Group name and (if desired) Information.
- 4. **Assign Privileges** Each group can be assigned specific privileges. Add group privileges by selecting or deselecting the check boxes next to each privilege.

The categories include the following:

- Administration Can administer the user database
- Analysis and Change Can change methods and configuration values
- Review Can review changes and events
- Plate Settings Can change sample plate properties
- Run Experiment Can run experiments and analyses
- 5. Click **OK** to save changes and exit.

### Viewing and Changing Group Settings

1. Right-click on a group to display the Tab menu (Figure C-14).

New Group
Edit Group
Delete Group

Figure C-14: Group Tab Menu with All Options Active

2. Select Edit Group to display the Edit Group dialog box (Figure C-15).



Figure C-15: Edit Group Dialog Box

- 3. If needed, modify the group settings. For more details on individual settings, please refer to "Creating a New User Group" on page 590.
- 4. Click **OK** to save changes and exit.

#### Deleting a User Group

- 1. Right-click on the group to display the Tab menu and select **Delete Group**.
- 2. Click **OK** to save and exit.

## Project Tab

The Projects Tab (Figure C-16) allows administrators to add and delete user projects. Projects are selected when a new user session is initiated in Octet<sup>®</sup> BLI Discovery, or Octet<sup>®</sup> Analysis Studio software, allowing all user, system and software events for a particular project to be monitored. The columns contain information about each user. The table can be sorted by clicking on any column header. For example, the Projects in Figure C-16 have been sorted alphabetically according to Name.



Figure C-16: Projects Tab

#### Creating a New Project

1. Right-click in a blank area in the **Projects** Tab to display the Tab menu (Figure C-17).

New Project
Edit Project
Delete Project

Figure C-17: Projects Tab Menu.

2. Select New Project to display the New Project dialog box (Figure C-18).

		New Project		×
Project name: Information:				
			ОК	Cancel

Figure C-18: New Project Dialog Box

- 3. Enter the **Project name** and (if desired) **Information**.
- 4. Click **OK** to save and exit.

Viewing and Changing Project Settings

1. Right-click on a project to display the Tab menu (Figure C-19).

New Project
Edit Project
Delete Project

Figure C-19: Projects Tab Menu with All Options Active

2. Select Edit Project to display the Edit Project dialog box (Figure C-20).



Figure C-20: Edit Project Dialog Box

- 3. If needed, modify the **Project name** or **Information**.
- 4. Click **OK** to save changes and exit.

#### Deleting a Project

- 1. Right-click on the project to display the Tab menu and select **Delete Project**.
- 2. Click **OK** to save and exit.

## Constants Tab

Use the Constants Tab to set Octet<sup>®</sup> GxP Server constant settings.

<mark>∠( </mark> ∞	ww.sarto	rius.	<u>com</u>		Print Login					
Users	Groups	Pro	jects	Constants	Events					
Name			Value	Descriptio	'n					
Passw	ordTTL		180	Password	expiration duration (days)	1				
Passw	ordMinLer	ngth	6	Minimum r	number of characters in password					
Passw	ordSecure	e	1	Level of p	evel of password complexity; 0 for none and 1 for secure					
Creder	ntialsTTL		5	Offline us	age duration (days)					
UserIdleMin 15 Idle			15	Idle time	ne duration before locking login session (mins)					
Passw	ordLock		5	Number o	f failed login attempts before locking account					

#### Figure C-21: Constants Tab

#### Table C-3: Administrator Constants

Constant	Description	Default Value	Value Range
CredentialsTTL	The number of days that the server settings are stored in the cache. This allows the software to oper- ate in case the server is temporarily down. Data is saved on the local computer and, upon the next con- nection to the database, the cached events automat- ically upload to the database.	5	Minimum=0, no max value
PasswordMinLength	Minimum number of characters that a password must contain.	0	Minimum=0, no max value
PasswordSecure	Level of password complexity. Setting the constant to 0 has no password restrictions. Setting the con- stant to 1 requires passwords to contain at least one alpha, one numeric, and one punctuation character.	0	0-1
PasswordTTL	Amount of time in days that a password is allowed to remain unchanged.	180	Minimum=0, no max value
UserldleMin	Idle time in minutes allowed during a user session after which the session is automatically closed and requires the user to log back in.	15	Minimum=0, no max value
PasswordLock	Number of failed login attempts before the account is locked.	3	Minimum=3, no max value

**NOTICE:** To prevent system lockout, the password lock does not apply to user accounts with the administrator privilege. The software closes instead to limit the speed passwords can be entered.

#### Viewing and Changing Constants

- 1. Right-click on the constant to display the Tab menu which displays a single option: Edit Constant.
- 2. Click **Edit Constant** to display the Edit Constant dialog box (Figure C-22).

		Edit Constant		×
Constant name:	PasswordMinLer	ngth		
Value:	6			
			ОК	Cancel

Figure C-22: Edit Constant Dialog Box

- 3. If needed, modify the Value. For more information on value range, please see Table C-3.
- 4. Click **OK** to save changes and exit.

## **Events** Tab

Use the Events Tab to view all the users, system and software event information that is recorded by the Octet<sup>®</sup> GxP Server module. Audit trails are stored on the Octet<sup>®</sup> GxP Server, not in individual files.

<u>~(</u>	www.sartorius.com					Print	Login	
Users	Groups Projects	Constants Eve	ents					
User:	(any)	✓ Pressed of the second se	oject: (ar	іу)	✓ Machine:	(any)	$\sim$	
Date:	9/ 9/2020 🔲 🔻	to 10/ 9/2020	-	Experiment: (any)			$\sim$	
Date	/Time	Login Name	Project	Machine	Software	Туре	Info ^	]
10/7	/2020 4:18:55 PM CDT	Administrator		DESKTOP-0EHTC34	Octet GxP Server	User login	Administrator	
10/7	/2020 4: 19:03 PM CDT	Administrator		DESKTOP-0EHTC34	Octet GxP Server	Password changed	Administrator	
10/7	/2020 4:20:50 PM CDT	Administrator		DESKTOP-0EHTC34	Octet GxP Server	User added	DBean	
10/7	/2020 4:21:19 PM CDT	Administrator		DESKTOP-0EHTC34	Octet GxP Server	User added	RBrown	
10/7	/2020 4:21:27 PM CDT	Administrator		DESKTOP-0EHTC34	Octet GxP Server	User logout	Administrator	
10/7	/2020 4:22:38 PM CDT	Administrator		DESKTOP-0EHTC34	Octet GxP Server	User login	Administrator	
10/7/2020 4:22:48 PM CDT Administrate		Administrator		DESKTOP-0EHTC34	Octet GxP Server	Project added	Antigen:Antibod	
10/7/2020 4:22:55 PM CDT Adminis				DESKTOP-0EHTC34	Octet GxP Server	Project added	Cell Culture Scre	

Figure C-23: Events Tab

**NOTICE:** Octet<sup>®</sup> GxP Server module versions 8.2 and higher also display event information that was recorded in BLItz Pro software for BLitz systems.

Events are tracked for individual user accounts, projects and machines. By default, a historical log of all events recorded on the active Octet<sup>®</sup> GxP Server module displays:

- Date and Time When the event occurred
- Login Name User name associated with the event
- Project Name of project associated with the event
- Machine Name of instrument used (includes both Octet<sup>®</sup> and BLItz instruments for Octet<sup>®</sup> GxP Server module versions 8.2 and higher)
- **Software** Which software the event was logged in (available in Octet<sup>®</sup> GxP Server module versions 8.2 and higher only, includes Octet<sup>®</sup> BLI Discovery and BLItz Pro software events)

- Type Event type
- Info Any additional information recorded with the event

You can filter the Events Log according to User, Project, Machine and Experiment by selecting items in the corresponding drop down menus. For example, Figure C-24 shows a drop down menu for selecting events by User Name.

<u></u>	www.sarto	rius.com							P	rint	Login	I
Users	Groups	Projects	Constants	Eve	ents							
User:	(any)		~	Pro	oject: (a	ny)	`	Machir	ne: (any)		$\sim$	
Date:	<mark>(any)</mark> Administra	tor		2020		Experiment: (a	ny)				$\sim$	_
Date	RBrown			me	Project	Machine	Soft	ware	Туре	Info		^
10/3	7/2020 4:18	:55 PM CD	T Administ	ator		DESKTOP-0EHTC	34 Octe	t GxP Serv	er User login	Admi	nistrator	
10/3	7/2020 4:19	:03 PM CD	T Administr	ator		DESKTOP-0EHTC	34 Octe	t GxP Serv	er Password cha	inged Admi	nistrator	
10/3	7/2020 4:20	:50 PM CD	T Administr	ator		DESKTOP-0EHTC	34 Octe	t GxP Serv	er User added	DBea	in	
10/3	7/2020 4:21	: 19 PM CD	T Administ	ator		DESKTOP-0EHTC	34 Octe	t GxP Serv	er User added	RBro	wn	

Figure C-24: Selecting Events by User Name

You can also limit your search to a specific time period by choosing the start/stop day from the calendar drop down menus (Figure C-25).

	www.sartorius.com												
		U	lsers	Groups	Pro	jects	Constants	Eve	nts				
		U	lser: (	any)			~	Pro	ject:	(ar	ıy)		$\sim$
		D	ate:	9/ 9/20	20	•	to 10/ 9/	2020		]-	Experiment:	(any)	
4		Sept	tembe	r 2020		•	Login Nan	ne	Proje	ect	Machine		Softwa
Sun	Mon	Tue	Wed	Thu	Fri	Sat	Administra	ator			DESKTOP-0EH	TC34	Octet (
30	31	1	2	3	4	5	Administr	ator			DESKTOP-0EH	TC34	Octet (
6	7	8	9	10	11	12	Administra	ator			DESKTOP-0EH	TC34	Octet
13	14	15	16	17	18	19	Administr	ator			DESKTOP-0EH	TC34	Octet
20	21	22	23	24	25	26	Administr	ator			DESKTOP-0EH	TC34	Octet
27	28	29	30	1	2	3	Administr	ator			DESKTOP-0EH	TC34	Octet
4	5	6	7	8	9	J	Administr	ator			DESKTOP-DEH	TC34	Octati
			Today	: 10/9/	2020		Administra	ator			DESKTOP-DEH	TC34	Octet
			10/7/2	020 7.2	22,00	FIFT CD	Administr	ator			DESKTOP-DEH	1034	Uctet

Figure C-25: Events Displayed for User Name

If any action is a change in Method parameters, details about the changes can be viewed by double-clicking on the event which brings up the Event Details Box (Figure C-26).

2018/03/08 11:21:39 DBean	MCGOWNPC	Octet Acquisition	Method parameters changed	C:\
2018/03/08 11:24:29 DBean	MCGOWNPC	Octet Acquisition	Method parameters changed	C:1
		Event De	etails	×
	Sample plate def	initions were chang	ed	
		-		
			Close	

Figure C-26: Event Details Describing Method Parameter Changes

# Appendix D: Ambr<sup>®</sup> 15 and Octet<sup>®</sup> Data Exchange

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## Overview

Octet<sup>®</sup> Software version 13 has been designed to work with the Ambr<sup>®</sup> 15 Software in a way that allows easy transfer of sample information. Plate maps can be quickly and easily configured to run on Octet<sup>®</sup> systems. After analyzing the Octet<sup>®</sup> data, results can be exported back to Ambr<sup>®</sup> 15 Software for review in aggregate with other key metrics from the cell culture assay.



Figure D-1: Octet<sup>®</sup> and Ambr<sup>®</sup>Software Workflow

Ambr<sup>®</sup> 15 Software transfers data using .csv files. It generates plate map files in the designated export folder as samples are extracted from the bioreactors. Ambr<sup>®</sup> 15 Software also monitors a designated import folder. When results from Octet<sup>®</sup> Software are placed in the import folder, Ambr<sup>®</sup> 15 Software automatically reads and imports the Octet<sup>®</sup> data.

## Initial Setup

### Step 1: Plan Data Exchange Locations

For the Ambr<sup>®</sup>-Octet<sup>®</sup> data exchange to work correctly, Ambr<sup>®</sup> and Octet<sup>®</sup> Software should both have access to a shared folder location. If the Ambr<sup>®</sup> and Octet<sup>®</sup> Software are running on the same computer, this can be any local folder. If the Ambr<sup>®</sup> and Octet<sup>®</sup> Software are on two different computers, we recommend you set up a shared network folder and map the drive on both the Ambr<sup>®</sup> and the Octet<sup>®</sup> computer. If a shared network folder is not possible, data can still be exchanged, but the files must be moved back and forth manually using other means, such as an external USB drive.

You will need to define two folders. Keep in mind that the terms import and export have different meanings depending on each software's point of view.

- 1. The Ambr<sup>®</sup> export folder is the same as the Octet<sup>®</sup> import folder. This is the folder where the Ambr<sup>®</sup> Software will create plate maps when sampling from the bioreactors. This folder is used with the Octet<sup>®</sup> BLI Discovery Software to plan and run the method. A recommended folder name is AmbrOutOctetIn.
- 2. The Octet<sup>®</sup> export folder is the same as the Ambr<sup>®</sup> import folder. Results from the Octet<sup>®</sup> analysis will be exported to this folder and automatically loaded into Ambr<sup>®</sup> Software. A recommended folder name is Ambrl-nOctetOut.

In the following steps, we will use a network folder mapped as the S: drive on the Octet<sup>®</sup> and Ambr<sup>®</sup> computers. The drive has the following folders defined:



Figure D-2: S: Drive Folders

### Step 2: Install Ambr<sup>®</sup> 15 Software

The Ambr<sup>®</sup> 15 Software needs a license for automatic data export and import.

Confirm that the data import/export license is installed correctly by looking at the Configuration tab in the Ambr<sup>®</sup> 15 Software. If you do not see Data Import and Data Export listed, contact Ambr<sup>®</sup> support to resolve the license issue.

Culture Station 2 Results Audit Configuration Se	tup Cell Counts Status
Edit Configuration Pipette Cleaning Compress Log Files Current Ambr® Configuration Ambr® cell culture 24 vessels without cooling MODDE Data Import Data Export	Edit LIMS Configuration
Self Test Offline Template Editor	

Figure D-3: Ambr<sup>®</sup> Software Configuration Tab

### Step 3: Install Octet<sup>®</sup> Software

Install the version 13 Octet<sup>®</sup> Software by running the installers for Octet<sup>®</sup> BLI Discovery and Analysis Studio Software. Version 13 can be installed side-by-side with previous versions.

## Step 4: Configure Ambr<sup>®</sup> 15 Import and Export Locations

On the Ambr<sup>®</sup>15 computer, look under the C:\Ambr<sup>®</sup>\Configuration\Parameters\ folder for a configuration file named **BRM Liquid Handler Parameters.txt**. Edit the text file and configure the folder locations for import and export.

#### Example:

BRM Liquid Handler Parameters.txt - Notepad           File         Edit         Format         View         Help
BRM_LIQUID_HANDLER Parameters
Sample_Data_Export_Directory = S:\AmbrOctetExchange\AmbrOutOctetIn; Sample_Data_Import_Directory = S:\AmbrOctetExchange\AmbrInOctetOut; Enable_Sample_Data_Export = true; Enable_Sample_Data_Import = true;
;This Parameters file has been screened to remove parameters that are as set in the software ;It is as set on BRM#1 9 April 2010.
; Parameters that are used to define the Configuration of the BRM Liquid Handler ;
DEFAULT_Z_STANDBY_CURRENT=1.0;
;; ; THESE ARE BRM SPECIFIC OFFSETS BETWEEN THE Z AXIS HEADS ;

Figure D-4: BRM Liquid Handler Parameters File

## Step 5: Configure Octet<sup>®</sup> Import and Export Locations

Open Octet<sup>®</sup> BLI Discovery Software and choose **File** > **Options...** from the main menu.

Locate the setting for the default folder for data import and click on the **Browse (...)** button to choose the location of the AmbrOutOctetIn folder.

Click **OK** to save the setting.
Options				×
Data Files				
Quantitation data repository:	C:\Temp			
Kinetics data repository:	C:\Temp			
Simulation	Use old 5.0	) file format for FRL	D file Use extended	sample types
If no instrument is connected,	the application is	configured using	the properties of the s	elected
Octet RH96     Octet     Octet RH96     Octet	t R2 Oct	et RED96e	Octet K2	
Octet QK384 Octe	t R8 O Oct	et RED96 et QKe		
Startup		Sensor Options	- New Experiments	
Temperature: 30	<b>€</b> °C	Replace se	nsors in tray after use	
Data Options		Default Sample	Plate	
Significant digits: 4	▲ ▼	96 wells	◯ 384 wells	
Web Server				
Port: 8080 🜲				
Refresh (s): 10 🔶				
Octet®-Ambr® Data Bridge				
Default folder for data	S:\AmbrOctet	Exchange \AmbrO	utOctetIn	
Automation				
TCP-IP Port: 20000	Localhost			
Serial (RS232)				
Port:				$\sim$
			ОК	Cancel

Figure D-5: Selecting the AmbrOutOctetIn Folder Location

Open Octet<sup>®</sup> Analysis Studio Software and choose **File** > **Preferences...** from the main menu.

Locate the setting for the default export folder and click on the **Browse (...)** button to choose the location of the AmbrInOctetOut folder.

Click **OK** to save the setting.

Preferences	×
Analysis Preference Open all Kinetic data in this mode: Determined By	v Input Experiment File V
Export Use legacy Excel 97-2003 Format (.xls)	
Octet®-Ambr® Data Bridge Default export location: S:\AmbrOctetExchange\	AmbrinOctetOut
ОК Са	ancel

Figure D-6: Selecting the AmbrInOctetOut Folder Location

### Step 6: Test the Ambr® Data Export

Prior to running a full experiment on the Ambr<sup>®</sup> 15, we recommend performing a simple test experiment to confirm automatic data export is configured correctly. See Appendix E on page 611 for instructions on how to create a simple Ambr<sup>®</sup> experiment to test the data export function.

Configure a process step to Sample Liquid from Culture Vessel.

Scheduled Tim	e: 16/Dec/2021 11:40:24		Re
Status:	Not Started	$\sim$	schedule
Priority	50	<b></b>	
Resource:	LH		
Method:	Sample Liquid From Culture Vessel		
Group:		~	Add Group
Hide	Parameters Volumes		
Hide Details Culture Statio	Parameters Volumes		
Hide Details Culture Statio Bioreactor 1,	Parameters Volumes in 1 2,3,4,5,6,7,8,9,10,11,12		
Hide Details Culture Statio Bioreactor 1, Aspirate SAN	Parameters Volumes In 1 2,3,4,5,6,7,8,9,10,11,12 IPLE		
Hide Details Culture Statio Bioreactor 1, Aspirate SAN Plate PLATE	Parameters         Volumes           n 1         2,3,4,5,6,7,8,9,10,11,12           IPLE         1           1         14,19,16,11,12		
Hide Details Culture Statio Bioreactor 1,. Aspirate SAM Plate PLATE Target Wells Discense SA	Parameters         Volumes           in 1         2,3,4,5,6,7,8,9,10,11,12           IPLE         1           [A1.B1,C1.D1,E1,F1,G1,H1,A2,B2,C2,D2]           MPLE		
Hide Details Culture Statio Bioreactor 1, Aspirate SAN Plate PLATE Target Wells Dispense SA 100 ul	Parameters         Volumes           n 1         2,3,4,5,6,7,8,9,10,11,12           2,3,4,5,6,7,8,9,10,11,12         IPLE           1         [A1,B1,C1,D1,E1,F1,G1,H1,A2,B2,C2,D2]           MPLE         Image: Control of the second seco		
Hide Details Culture Statio Bioreactor 1, Aspirate SAN Plate PLATE Target Wells Dispense SA 100 ul Mapping Use	Parameters         Volumes           n 1         2,3,4,5,6,7,8,9,10,11,12           2,3,4,5,6,7,8,9,10,11,12         PIEE           1         [A1,B1,C1,D1,E1,F1,G1,H1,A2,B2,C2,D2]           MPLE         ar Defined		
Hide Details Culture Static Bioreactor 1, Aspirate SAN Plate PLATE Target Wells Dispense SA 100 ul Mapping Use 1 ml Tip	Parameters         Volumes           n 1         2,3,4,5,6,7,8,9,10,11,12           12,3,4,5,6,7,8,9,10,11,12         1           14         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1           1         1		

Figure D-7: Configuring a Process Step

After the Ambr<sup>®</sup> 15 performs this sampling step, confirm that a plate map was automatically created and saved into the AmbrOutOctetIn folder.

# Walk-through

#### Setup and Run an Ambr<sup>®</sup> Experiment

See Appendix E on page 611 for instructions on how to create a simple Ambr<sup>®</sup> experiment.

#### Import Samples to Octet<sup>®</sup> Software

Application-specific questions such as choosing a BLI biosensor type, or the specific steps required for the assay are out of the scope of this guide. We will demonstrate import of the Ambr<sup>®</sup> sample data using a Basic Quantitation experiment template, but any Quantitation experiment can be used.

In the Experiment Wizard screen, choose the blank template for **Basic Quantitation**, then click the arrow to begin defining the method.

🖄 Experiment Wizard	
Choose an option to start	Available Templates for - Octet RH96  Anti-hlgG or AntimgG biosensor_16CH_96W fmf Anti-hlgG or AntimgG biosensor_8CH_96W fmf Anti-Penta-HIS Dilution Factor Scouting_16CH_96W fmf Anti-Penta-HIS Dilution Factor Scouting_8CH_96W fmf Anti-Penta-HIS Spike Recovery Assay_16CH_96W fmf DirectDetectionImmunogenicity_16CH_384W fmf DirectDetectionImmunogenicity_8CH_96W fmf DirectDetectionImmunogenicity_8CH_96W fmf Protein A G or L biosensor_16CH_96W fmf Protein A G or L biosensor_high_sensitivity_8CH_96W fmf
Recent Methods	$\rightarrow$

Figure D-8: Experiment Wizard

In **Tab 1 Plate Definition**, click the **Import** button above the sample table. Choose **Import Ambr data file** from the menu.

be es	e entere sample	ed. plate, and rigl	ht-click to enter/mod	ify well o	data.			
	Plate 1 Conce	Table (96 wel entration units:	ls) µg/ml	×*	Export	Import 🔻	Print	
	Well	Sample ID	Replicate Group	Туре	Conc (µg/m	Import C	SV file	
						Import a	mbr data file	

Figure D-9: Importing Ambr<sup>®</sup> Data File

From the prompt, select one or more plate maps to import. To choose multiple files, hold the **Ctrl** key while clicking on the files to import. Click **Open** to load the plate map(s) into the import wizard.

🔂 Import Ambr® Plate Definitions											
← → × ↑ 📴 > This PC > Share (\\VBoxSvr) (S:) > AmbrOctetExchange > AmbrOutOctetIn v 🖸 🖉 Search AmbrOutOctetIn											
Organize 🔻 New f	older					•					
💻 This PC	Name	Date modified	Туре	Size							
3D Objects	Plate24-1[2-16-22]	8/3/2022 10:54 PM	Microsoft Excel C	19 K	3						
Desktop	🔯 Plate24-2[2-16-22]	8/3/2022 10:54 PM	Microsoft Excel C	19 K	3						
Documents	Plate24-3[2-16-22]	8/3/2022 10:54 PM	Microsoft Excel C	19 K	3						
Downloads	Plate24-4[2-16-22]	8/3/2022 10:54 PM	Microsoft Excel C	19 K	3						
👌 Music											
Pictures											
📑 Videos											
🏪 Local Disk (C:)											
🛫 Share (\\VBoxSv											
🛖 VM_Shared_Fold											
鹶 Network	v										
Fil	le name:			~ CSV	Files (*.csv)	~					
	L				Open Ca	incel					

Figure D-10: Selecting Plate Maps

The Ambr<sup>®</sup> Data Import wizard allows you to review the incoming data before loading into the current method.

🔳 aml	or® Dat	a Import											$ \Box$ $\times$
Index	Import	Original Well	Well	Plate Station	Vessel	Timestamp	System ID	Experiment	Barcode	Information	Dilution Factor		3 9 10 11 12
1		A1	A1	platen: CS1	1	04/Feb/2022	ambr1234	Test1	DF445678	Clone-1a			
2		A2	A2	platen: CS1	2	04/Feb/2022	ambr1234	Test1	DF445678	Clone-1b			
3		A3	A3	platen: CS1	3	04/Feb/2022	ambr1234	Test1	DF445678	Clone-1c		BOOOOOOOO	$\mathbf{)}0000$
4		A4	A4	platen: CS1	4	04/Feb/2022	ambr1234	Test1	DF445678	Clone-1d			
5	P	A5	A5	platen: CS1	5	04/Feb/2022	ambr1234	Test1	DF445678	Clone-1e			
6		A6	A6	platen: CS1	6	04/Feb/2022	ambr1234	Test1	DF445678	Clone-1f			
7		A7	A7	platen: CS1	7	04/Feb/2022	ambr1234	Test1	DF445678	Clone-2a			
8		A8	A8	platen: CS1	8	04/Feb/2022	ambr1234	Test1	DF445678	Clone-2b			
9	V	A9	A9	platen: CS1	9	04/Feb/2022	ambr1234	Test1	DF445678	Clone-2c			
10		A10	A10	platen: CS1	10	04/Feb/2022	ambr1234	Test1	DF445678	Clone-2d		$\mathbf{F}()()()()()()()()()()()()()()()()()()()$	
11		A11	A11	platen: CS1	11	04/Feb/2022	ambr1234	Test1	DF445678	Clone-2e			
12		A12	A12	platen: CS1	12	04/Feb/2022	ambr1234	Test1	DF445678	Clone-2f		$ \mathbf{G}  () () () () () () () () () () () () () $	
												Ready to Import	
	Choose I	Files									Clear		
Inform Sam Repl	ation ma ple ID: icate Gro	Culture S Culture S oup: (leave bl	itation- ank)	Vessel (CS1-1)	~	Well mapp Select pos	ing sitions on the 24 well to TL BL	import table to 96 well: TR BR	apply a wel	ll mapping sche	Left	Up Right Down	Cancel
													Cancer

Figure D-11: Reviewing Incoming Data

The following options are available in the import wizard:

- Click Choose Files... to select and load additional files. Multiple plate maps can be loaded at once and arranged onto the plate. The Ambr<sup>®</sup> Software creates a plate map for each culture station sampled, even if the samples were placed into the same plate.
- Click the **Clear** button to clear the staged data. This is helpful if you have loaded the wrong plate map.
- Information table:
  - The gray columns indicate data provided by the Ambr<sup>®</sup> system which should not be edited.
  - If there are any rows that should not be imported, simply uncheck the **Import** option for that row.
  - The Original Well indicates the well position where the Ambr<sup>®</sup> liquid handler placed the sample. The Well column indicates the position where the sample will be when analyzed by the Octet<sup>®</sup> system. There are many ways to manipulate the location of the sample. See the information on Well mapping that follows.
  - A Dilution Factor may be entered at this time if desired, or it can be added later after finalizing the import to the Octet<sup>®</sup> method.
  - The information table can be sorted by clicking on any of the column headings.
- Information mapping You may choose to map certain information about the bioreactor to Octet<sup>®</sup> sample data fields. Ambr<sup>®</sup> data fields can be mapped to the Octet<sup>®</sup> Sample ID and/or Replicate Group fields using the dropdown options. The required Ambr<sup>®</sup> data is automatically mapped into the Octet<sup>®</sup> Sample Information field.
- **Well mapping -** Click and drag to select rows in the information table. Then click on one of the well mapping buttons to arrange the wells on the Octet<sup>®</sup> sample plate. The plate graphic on the right side of the wizard shows the final well locations.
  - **Direct -** The Original Well provided by the Ambr<sup>®</sup> Software is used directly as the Octet<sup>®</sup> plate location.
  - **24 to 96-well -** Maps a bank of 24 wells to a quadrant on a 96-well plate (e.g. A1:D6, A7:D12, E1:H6, E7:H12). The original well locations must be in the range A1:D6.
  - 96 to 384-well Maps a 96-well plate into an equivalent grid of a 384-well plate. You must choose a 384-well plate from the method editor before importing Ambr<sup>®</sup> data (only for 384-well capable Octet<sup>®</sup> instruments).
  - **Manual mapping -** Select a row or rows in the information table, and then click **Up/Down/Left/Right** to move the selected wells onto the Octet<sup>®</sup> plate. You can also manually type in the Octet<sup>®</sup> plate location directly in the information table.
  - Import status The import status is displayed live below the plate graphic. You should resolve any errors before continuing with the import. Possible errors include missing or invalid Octet<sup>®</sup> well locations, or multiple rows that map to the same Octet<sup>®</sup> well.

When you are satisfied with the plate definition, click **Import** to load the definition into the Octet<sup>®</sup> method editor.

4 Plate Definition	<b>0</b>		in a Dur f							
In this e	step, all the information of the step set th	tion about the sample pla tings. Then highlight one of	te and its wells w or more wells on t	ill be entered the sample p	l. late, and right	-click to enter/modify	well data.			
Acquisition Rate:	Standard (5.0	Hz)	$\sim$	Plate 1 T	able (96 wells	)				
Assav Settings				Concer	tration units:	$\mu$ g/ml $\sim$	Ex	port Impor	t 🔻 Print.	
Assay:	Basic Quantitatio	n	Modify	Well	Sample ID	Replicate Group	Туре	Conc (µg/ml)	Dilution Factor	Information
	Standard Assay		modify	💭 A1	CS1-1		Unknown	n/a		ambr1234,Te
	Single analyte			A2	CS1-2		Unknown	n/a		ambr1234,Te
Quantitations	Time (s):	Shake speed (rpm):		A3	CS1-3		Unknown	n/a		ambr1234,Te
Quantitation:	120	400		A4	CS1-4		Unknown	n/a		ambr1234,Te
Plate 1 (96 wells)				A5	CS1-5		Unknown	n/a		ambr1234,Te
1 1010 1 (00 11010)			Modify	A6	CS1-6		Unknown	n/a		ambr1234,Te
			wouny	A7	CS1-7		Unknown	n/a		ambr1234,Te
1 2	3 4 5	6 7 8 9 1	0 11 12	A8	CS1-8		Unknown	n/a		ambr1234,Te
				A9	CS1-9		Unknown	n/a		ambr1234,Te
				A10	CS1-10		Unknown	n/a		ambr1234,Te
B			) ( ) ( ) (	A11	CS1-11		Unknown	n/a		ambr1234,Te
				A12	CS1-12		Unknown	n/a		ambr1234,Te
C (-)(-)			)())()							

Figure D-12: Import Button in Tab 1 Plate Definition

Note in Figure D-12 that the Octet<sup>®</sup> Information column has been populated with the Ambr<sup>®</sup> sample data. Do not edit this information manually. The Sample ID, Replicate Group, and Dilution Factor can be edited without affecting Ambr<sup>®</sup> data exchange.

### Run the Octet<sup>®</sup> Experiment

Configure other aspects of the method as needed. Prepare the sample plate(s) and run the Octet<sup>®</sup> experiment.

#### Analyze Octet<sup>®</sup> Results

When the experiment is complete, use the Octet<sup>®</sup> Analysis Studio Software to analyze the data. Quantitation analysis is outside the scope of this document. Refer to the Octet<sup>®</sup> Analysis Studio User Guide for details.

### Export Octet<sup>®</sup> Results

When you are satisfied with the results of the analysis, export back to the Ambr<sup>®</sup> Software by clicking the **Export** button and choosing **Export to Ambr format**.



Figure D-13: Selecting Export to Ambr Format

The export dialog appears. You may choose to export up to four different results from the analysis. For variables 1 through 4, choose an Octet<sup>®</sup> result to export or leave as (none), and type the variable name that should be used in the Ambr<sup>®</sup> Software.

Export to ambr for	mat	×
Export Options		
	Octet Result	Ambr Variable Name
Variable 1	Well Conc. ~	TITER
Variable 2	(none) ~	
Variable 3	(none) ~	
Variable 4	(none) ~	
Save To: D:\data\	AmbrOctetExchange\AmbrIn(	OctetOut\Export to Ambr_2022_03_07 15_46_1:
		Export Cancel

Figure D-14: Adding Variable Names For Ambr® Software

By default, the results will be exported to the AmbrInOctetOut folder you selected in the initial setup.

**NOTICE:** When the Ambr<sup>®</sup> Software successfully imports the results, it will automatically delete the file.

# Appendix E: Ambr<sup>®</sup> 15 and Octet<sup>®</sup> Data Exchange Simulation

## **Experiment Simulation**

Presented here is a simple, simulated Ambr<sup>®</sup> experiment where you can confirm that the Ambr<sup>®</sup> output data exchange function is working correctly.

Start up the application and click the **Create New Experiment** button.



Figure E-1: Creating a New Experiment

When asked if you would like to base the new experiment on a previous one, click **No**.



Figure E-2: Experiment Dialog

Click **Yes** to start the New Experiment Wizard.

111		
4	Ambr® 15 cell culture software	$\times$
	Do you want to use the New Experiment Wizard	
1	Yes No	

Figure E-3: Experiment Wizard Dialog

In the New Experiment tab, type in an experiment name and a start date at least an hour into the future. The exact start date/time doesn't matter as we will reset it to the present when you are ready to start the experiment.

Keep the other options at their defaults and click **Next**.

New Experiment Wizard			
New Experiment Add Media CS1 Add Media CS2 Condition Media Add	d Cells Add Feed Add Base Sample	Add Glucose Add Antifoam Add Background Bas	e Calendar Mimic
New Experiment Add Media CS1 Add Media CS2 Condition Media Add Experiment Details Name Octet E Start Date 27/ Jul /2022 22:16:25 ✓ Save To File Load From File	d Cells Add Feed Add Base Sample Plates and Pipettes ✓ Auto Insert Plate Loadings ✓ Auto Insert Pipette Loadings Culture Station 1 ✓ 1 ✓ 2 ✓ 3 ✓ 4 ✓ ✓ 7 ✓ 8 ✓ 9 ✓ 10 ✓ Culture Station 2 ✓ 1 ✓ 2 ✓ 3 ✓ 4 ✓ ✓ 7 ✓ 8 ✓ 9 ✓ 10 ✓	Add Glucose Add Antifoam Add Background Bas Design Of Experiment Auto Add DOE Tags 5 ☑ 6 Set All 11 ☑ 12 Clear All 11 ☑ 12 Clear All	e Calendar Mimic
OK		Next	Cancel

Figure E-4: New Experiment Tab

The first media plate will be pipetted into Culture Station 1. It will be placed onto Deck 1.

Check the **Add Media Plate** and **Add Media To Vessels** checkboxes in the Add Media CS1 Tab. Set the volume to **4** ml.

New Experimen	New Experiment Wizard											
New Experiment	Add Media CS1	Add Media	CS2 Condition Me	lia Add Cells	Add Feed	Add Base	Sample	Add Glucose	Add Antifoam	Add Background Base	Calendar	Mimic
🗹 Add Media Pla	ate	Tue 26 Jul 1	3:24:55					YY	<u>Y Y Y</u>			
Name	Media1		Location Dec	c1		$\sim$						
Plate Type	TAP BIOSYST	TEMS 24 DEE	P WELL 🗸		ls Li	dded 🗌	þ		$\square$	$\square$		
Add Media To	Vessels			Initial Vo	olume 0.0	ml	U					
		Re	euse Pipette Tips 🗹									
Volume 4	.0 ml per t	ransfer										
Plate to Cult	ure Station 1 Ma	pping										
24 well A1I	B6 -> V112	~	Define									
A1 A2	A3 A4 A	45 Å6	Transfer From Well									
7 8 B1 B2	9 10 11 B3 B4 B	12 85 B6	To Bio react	r								
ок			Pr	evious				Nex	t			Cancel

Figure E-5: Add Media CS1 Tab

The second media plate will be pipetted into Culture Station 2. It will be placed onto Deck 2 in the Add Media CS2 Tab.

New Experimen	t Wizard											
New Experiment	Add Media CS1	Add Media CS2	Condition Media	Add Cells	Add Feed	Add Base	Sample	Add Glucose	Add Antifoam	Add Background Base	Calendar	Mimic
Name Plate Type	Media2 TAP BIOSYST	EMS 24 DEEP V	Deck 2 Deck 1 ELL V Deck 3 Deck 4	2		~						
						Plate to	Culture St	ation 2 Mappin	g			
						24 well /	\1B6 -> '	V112	Define	ê		
						A1 /	42 A3	A4 A5	A6			
						7 8 B1	32 <sup>9</sup> B3	10 11 B4 B5	12 B6			
ОК			Previo	us				Ne	đ			Cancel

Figure E-6: Add Media CS2 Tab

Check the **Condition Media** checkbox in the Condition Media Tab.

New Experiment	Wizard											
New Experiment	Add Media CS1	Add Media CS2	Condition Media	Add Cells	Add Feed	Add Base	Sample	Add Glucose	Add Antifoam	Add Background Base	Calendar	Mimic
Condition Med	lia	Tue 26 Jul 13:24:	55									
Temperature	36	т	arget DO	50								
Stirring RPM	500	L	lpper pH Limit	6.8								
	Up Stirring O Down Stirr	g ring										
-												
e												
ок			Previ	ous				Nex	t			Cancel

Figure E-7: Condition Media Tab

New Experiment Wizard New Experiment Add Media CS1 Add Media CS2 Condition Media Add Cells Add Feed Add Base Sample Add Glucose Add Antifoam Add Background Base Calendar Minic Phase Location Plate ID Plate Type Lidded Plate Loadings Media 1 Deck 1 Media1 TAP BIOSYSTEMS 24 DEEP WELL False 1 Media 2 Deck 2 Media2 TAP BIOSYSTEMS 24 DEEP WELL False 1 Deck 1  $\cap$ С  $\bigcirc$  $\cap$  $\cap$ CDeck 2 Deck 3 Deck 4 ОК Previous Next Cancel

Skip the remaining tabs and go to the last one, Mimic. Click OK.

Figure E-8: Mimic Tab

When asked if you want to create the experiment, click Yes.



Figure E-9: Create Experiment Dialog

In the main screen, click the **Mimic** Tab.

This shows the placement of plates and pipettes on the Ambr<sup>®</sup> system. The Culture Stations are CS1 and CS2. In the tables, it shows Media Plate 1 is reserved for Deck 1 and Media Plate 2 is reserved for Deck 2. 4 ml pipettes will be loaded into location Pipette 2.



Figure E-10: Mimic Tab in Main Screen

We need to add a 96-well plate that will contain the culture station samples to be analyzed in the Octet<sup>®</sup> system. We will put this plate on Deck 4.

Click the **Edit Labware Configuration** radio button. Click the **add...** button for Deck 4. A dialog will display with a selection of plate types. Select **GREINER 96 WELL**. When prompted for a plate name, type in any name. In this example we chose OCTET SAMPLE.



#### Figure E-11: Selecting a Plate Type

On the right next to Pipette 1, click add... and follow the prompts to add 1 ml pipettes.

Introduction Experiment Mimic Process Definition Process Steps Calendar	Vessel Data Overview Culture Station 1 Culture Station 2 Results Audit	Configuration Setup Cell Counts Status	
○ Show Whats Loaded Now			Vessel Data
○ Preview Bed Lavout Over Time	Pipette 2		Load Layout
			Check Layout
Edit Labware Configuration	ID:1 ml Back Left 1 ml & 4 ml		Save Layout
			Clear Layout
	Deck 1		Deck Map
			Layout Definition Wizard
	Plate Pipette Box Lid Holder		
Deck 2 Deck 3	bood Beek dooloo		
Plate Plate	ID:Octet Sample Plate Lid Holder		
Plates		Pipettes	
Deck 1 Empty v add		Load Plate Pipette 1 1 ml Back Left v add	Load Full Box
Deck 2 Empty v add Deck 3 Empty v add		Uhload Plate Pipette 2 Empty add	Load Partial Box
Deck 4 Octet Sample v add		Plate Info	Unload Tip Box
Plate Id Bed Location Loaded Barcode Lidded Plate Type		Show Plate in Poette Box Id Loaded Free Platte Type	Show Tip Box in
Media1         Deck 1         No         No         TAP BIOSYSTEMS           Meria2         Deck 2         No         No         TAP BIOSYSTEMS	24 DEEP WELL	Pades Ruse	Delete To Box
Octet Sample Deck 4 No Yes GREINER 96 WEL		1 mi Back Left Pipete 1 No 1 mi	Delete tip Box
		Edt Plate	Auto Create

When you are finished adding the necessary labware, the Mimic tab should look like this:

Figure E-12: Mimic Tab

In the main screen, click the **Process Steps** tab.

The steps below, created by the New Experiment Wizard, show the loading of the 4 ml pipettes and media trays onto the Ambr<sup>®</sup> system. What is missing is the 96-well Octet<sup>®</sup> sample plate and the 1 ml pipettes to transfer culture station samples to this plate.

Ambr® 15 cell cu	ture software: Experiment N	lame (Octet E) Starte	d [26/Jul/2022	) 26/Jul/20	22 22:19:29								-	- 0	:
Start Pause	All Pause LH / Abandor Edit Step	Stop	Stopped CS1 CS2 LH RDR PC					In Simulation Mode						op Liquid Ha	andler
troduction Experime	nt Mimic Process Definition	n Process Steps Ca	lendar Vessel I	Data Over	view Culture	Station 1 Culture Station 2 Results	Audit Configur	ation Setup Cell Counts Status							
Date Time	Time From Inoculation	Group	Completed	Priority	Resource	Method	DOE Tag	Parameter 1	Parameter 2	Parameter 3	Parameter 4	Paramete	ņ.	Elter	
Wed 27 Jul 2022													P.	Filler	
ad 27 Jul 22:16:26			Not Started	50	User	Reload Pipette Box		Please load the pipette box	4 ml Back Right				a Adva	anced Ontin	-
ad 27 Jul 22:16:27		Add Media	Not Started	50	User	Load Plate On To System		Please load the plate	Plate MEDIA2	0 Seconds		1	Ç		
ed 27 Jul 22:16:28		Add Media	Not Started	50	User	Load Plate On To System		Please load the plate	Plate MEDIA1	0 Seconds				Insert Step.	
ed 27 Jul 22:16:29		Add Media	Not Started	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA1	Source Wells [A1.A2.A3.A4.A5.A6.B1.B2.B3.B4.B5.B6] Media	Aspirate Media_4ml_Tips	Culture Station 1	Bioreacto			
ed 27 Jul 22:17:29		Add Media	Not Started	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA2	Source Wells [A1.A2.A3.A4.A5.A6.B1.B2.B3.B4.B5.B6] Media	Aspirate Media_4ml_Tips	Culture Station 2	Bioreacto		Goto Now	
ed 27 Jul 22:18:29		Condition Media	Not Started	50	CS1	Set Temperature	Temperature	36.00 °C							
ed 27 Jul 22:19:29		Condition Media	Not Started	50	CS1	Start Stirring	Speed	Up Stir 500 RPM					D	OF Summar	v
ed 27 Jul 22:20:29		Condition Media	Not Started	50	CS1	Start Control DO pH	Gassing	DO Set Point 50.00 %	Upper pH Limit 6.80	Starting N2 0.20	Starting O2 75.00				
ad 27 Jul 22:21:29		Condition Media	Not Started	50	CS2	Set Temperature	Temperature	36.00 °C					Ch	eck DOE T	ags
ed 27 Jul 22:22:29		Condition Media	Not Started	50	CS2	Start Stirring	Speed	Up Stir 500 RPM							
ed 27 Jul 22:23:29		Condition Media	Not Started	50	CS2	Start Control DO pH	Gassing	DO Set Point 50.00 %	Upper pH Limit 6.80	Starting N2 0.20	Starting O2 75.00			Check Time	
ed 27 Jul 22:24:29		Condition Media	Not Started	50	Monitor	Start Monitor									<u> </u>
													Pmo	ess Sen Va	~~
													DOE	Process St	eos
													Rea	agent Mappi	ing
															-

Figure E-13: Process Steps Tab

In the next few screens, we will add a step to place the 1 ml pipettes on the Ambr<sup>®</sup> system.

Right click any step and select **Process Steps | Insert New**.

Ambr® 1	5 cell cult	ure soft	tware: Experiment N	lame [(	Octet E] Si	tarted [26/Jul/2022 ]	26/Jul/20	22 22:20:41				
Start	Pause Al	Pa	use LH / Abandon Edit Step		Stop	Stopped	cs	1 CS2 LH	RDR PC			
Introduction	Experiment	Mimi	c Process Definition	Proc	ess Steps	Calendar Vessel D	)ata Oven	view Culture	Station 1 Culture Station 2 Results	Audit Configura	ation Setup Cell Counts Status	
Dat	e Time	Time	From Inoculation	Grou	ıp	Completed	Priority	Resource	Method	DOE Tag	Parameter 1	Pa
Wed 27 J	ul 2022											
ed 27 Jul 2	2:16:26	_	CL 1/2			Not Started	50	User	Reload Pipette Box		Please load the pipette box	k 4 m
ed 27 Jul 2	2:16:27		Change view	•	Andia	Not Started	50	Lleer	Load Plate On To System		Please load the plate	Pla
ed 27 Jul 2	2:16:28		Process Steps	•	In	sert New	Ctrl+N	r	Load Plate On To System		Please load the plate	Pla
ed 27 Jul 2	2:16:29		Process Groups	•	De	elete	Del		Add Liquid To Culture Vessel	Add Media	Plate MEDIA1	So
ed 27 Jul 2	2:17:29		DOE	•	Re	e-Run			Add Liquid To Culture Vessel	Add Media	Plate MEDIA2	So
ed 27 Jul 2	2:18:29		Advanced Editing	•	A	bandon			Set Temperature	Temperature	36.00 °C	
ed 27 Jul 2	2:19:29		Undo		C	ору	Ctrl+C		Start Stirring	Speed	Up Stir 500 RPM	
ed 27 Jul 2	2:20:29		Phases	•	Pa	iste	Ctrl+V		Start Control DO pH	Gassing	DO Set Point 50.00 %	Up
ed 27 Jul 2	2:21:29	_		Cond		ultiple Dacte	Ctrl+M		Set Temperature	Temperature	36.00 °C	
ed 27 Jul 2	2:22:29			Cond		dd Stans Ta Casur	Chill C		Start Stirring	Speed	Up Stir 500 RPM	
ed 27 Jul 2	2:23:29			Cond		ad steps to droup	. cm+o		Start Control DO pH	Gassing	DO Set Point 50.00 %	Up
ed 27 Jul 2	2:24:29			Cond	d Ke	emove Steps From G	roup	hitor	Start Monitor			
					Ex	port Steps To File						
					Sł	now Equation Details	5					

Figure E-14: Inserting a New Process Step

When **Insert New...** is selected, the Process Step Toolbox is shown.

When a step is inserted, its placement in the order of steps is set by its start time. We want this new step at the beginning. Click the **Reschedule** button and in the Change the date and time dialog, set the **Time** to a few seconds before the time stamp of the first step in the table. Time stamps are in the Date Time column on the far left.

oduction	Experiment	Mimic Proce	ss Definition	Process Steps Ca	lendar V	essel Data Overvie	ew Culture Stati	ion 1 Culture Station 2 Results Audit Configuration Setup Cell Cou	unts Status
Date	e Time T	ime From Inc	oculation	Group	Comp	Process Step Tool	lbox		
Ved 27 J	ul 2022					Process Step 100			
d 27 Jul 2	2:16:26				Not St	Scheduled Time:	27/Jul/2022 22	2:16:31	Re
d 27 Jul 2	2:16:27			Add Media	Not St	Status:	Not Started		schedule
d 27 Jul 2	2:16:28			Add Media	Not St	Priority:	50		<b>÷</b>
d 27 Jul 2	2:16:29			Add Media	Not St	P noncy.			~
1 27 Jul 2	2:17:29			Add Media	Not St	Resource:			
127 Jul 2	2:18:29			Condition Media	Not St	Method:			
1 27 Jul 2	2:19:29			Condition Media	Not St	Group:			<ul> <li>Add Group</li> </ul>
d 27 Jul 2	2:20:29			Condition Media	Not St	Parameters:	Edit		
d 27 Jul 2	2:21:29			Condition Media	Not St	l'alameters.	Parameters		
d 27 Jul 2	2:22:29			Condition Media	Not St	Show		Change the date and time	
127 Jul 2	2:23:29			Condition Media	Not St	Details			
127 Jul 2	2:24:29			Condition Media	Not St			Edit when the process step will run	
					_				
								Specify using date and time	
						Cours			
						Save		27/ Jul /2022 22:16:25	
								Specify using day and time	
								Day 1 Day is the offset from the	
								Time 10:16:25 PM +	
								OK Cancel	

Figure E-15: Rescheduling the Process Step

In the Process Step Toolbox, set the Resource to **User** and in the tree view, select **Reload Pipette Box**. Then click the **Edit Parameters** button.

		ototoo	
Process Step Tool	box		
Scheduled Time: Status: Priority: Resource:	27/Jul/2022 22:16:25 Not Started 50 User	Re schedule	User Pause     Plate Handling     Load Plate On To System     Remove Plate From The System     Pipette Handling
Method: Group: Parameters: Show Details	Reload Pipette Box Contemporation Contemporation C	Add Group	

Figure E-16: Selecting Reload Pipette Box in the Process Step Toolbox Dialog

Click the **Edit Parameters** button to bring up the Process Step Editor. In the Pipette Box settings, choose **1 ml Back Left** for the Station. Click **Save**.

Process Step Editor	
Process Step Editor Message Pipette Box	Please identify which pipette box should be loaded
Save	Station: 1 ml Back Left 4 ml Back Right 1 ml Back Left Previous

Figure E-17: Process Step Editor

To add the 96-well plate to the Ambr<sup>®</sup>system, go to step view, right click a step and select **Process Steps | Insert New**.

In the Process Step Toolbox, reschedule the start time before the first step in the list, set the Resource to **User**, and select **Load Plate On To System** in the tree. Click the **Edit Parameters** button.

Process Step Too	lbox		
Scheduled Time: Status: Priority: Resource: Method: Group:	27/Jul/2022 22:16:24 Not Started 50 User ✓ Load Plate On To System ✓	Re schedule	User Pause     Plate Handling     Remove Plate From The User     Final     Email     Equations     Anual Operations     Audt
Parameters: Show Details Save	Edit Parameters		Cancel

Figure E-18: Selecting Load Plate On To System

In the **Plate** selector, choose **OCTET SAMPLE** (or the name you gave the 96-well plate previously defined in the Mimic tab). Click **Save**.

Process Step Editor	
Message Plate Timeout	Please identify which plate the user should load onto the system.
Save	Plate ID: MEDIA1 MEDIA1 Previous OCTET SAMPLE

Figure E-19: Selecting the Plate

Below is an example of what the step list should look like at this point. The most important things to note are that the pipettes, media plates, and 96-well sample plates are added to the beginning of the experiment so they are available for the actions that take place later.

Date Time	Time From Inoculation	Group	Completed	Priority	Resource	Method	DOE Tag	Parameter 1	Parameter 2	Parameter 3	Parameter 4	F
Wed 27 Jul 2022												
ed 27 Jul 22:16:24			Not Started	50	User	Load Plate On To System		Please load a plate.	Plate MEDIA1	0 Seconds		
ed 27 Jul 22:16:25			Not Started	50	User	Reload Pipette Box		Please load a pipette box	1 ml Back Left			
ed 27 Jul 22:16:26			Not Started	50	User	Reload Pipette Box		Please load the pipette box	4 ml Back Right			
ed 27 Jul 22:16:27		Add Media	Not Started	50	User	Load Plate On To System		Please load the plate	Plate MEDIA2	0 Seconds		
ed 27 Jul 22:16:28		Add Media	Not Started	50	User	Load Plate On To System		Please load the plate	Plate MEDIA1	0 Seconds		
ed 27 Jul 22:16:29		Add Media	Not Started	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA1	Source Wells [A1,A2,A3,A4,A5,A6,B1,B2,B3,B4,B5,B6] Media	Aspirate Media_4ml_Tips	Culture Station 1	E
ed 27 Jul 22:17:29		Add Media	Not Started	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA2	Source Wells [A1,A2,A3,A4,A5,A6,B1,B2,B3,B4,B5,B6] Media	Aspirate Media_4ml_Tips	Culture Station 2	E
ed 27 Jul 22:18:29		Condition Media	Not Started	50	CS1	Set Temperature	Temperature	36.00 °C				
ed 27 Jul 22:19:29		Condition Media	Not Started	50	CS1	Start Stirring	Speed	Up Stir 500 RPM				
ed 27 Jul 22:20:29		Condition Media	Not Started	50	CS1	Start Control DO pH	Gassing	DO Set Point 50.00 %	Upper pH Limit 6.80	Starting N2 0.20	Starting O2 75.00	
ed 27 Jul 22:21:29		Condition Media	Not Started	50	CS2	Set Temperature	Temperature	36.00 °C				
ed 27 Jul 22:22:29		Condition Media	Not Started	50	CS2	Start Stirring	Speed	Up Stir 500 RPM				
ed 27 Jul 22:23:29		Condition Media	Not Started	50	CS2	Start Control DO pH	Gassing	DO Set Point 50.00 %	Upper pH Limit 6.80	Starting N2 0.20	Starting O2 75.00	
ed 27 Jul 22:24:29		Condition Media	Not Started	50	Monitor	Start Monitor						

#### Figure E-20: Step List

Now that all the experiment hardware has been programmed into the experiment, the final step of taking culture samples and putting them onto the 96-well sample plate will be added.

In the Process Steps table, right click and choose Insert Step.

In this case, we don't need to reschedule the step. The default setting puts it as the last step, which is where we want it.

For Resource, select LH (liquid handler) and select Sample Liquid From Culture Vessel in the tree.

Process Step Tool	lbox		
Scheduled Time: Status: Priority: Resource: Method: Group: Parameters: Show Details	27/Jul/2022 22:24:34 Not Started 50 LH Sample Liquid From Culture Vessel Edit Parameters	Re schedule	Add to Bioreactor     Sample Liquid From Culture Vessel     Double Sample Liquid From Culture Vessel     Double Sample Liquid From Culture Vessel     Paused Sample Liquid From Culture Vessel     Paused Sample Liquid From Culture Vessel     Remove Liquid F
Save			Cancel

Figure E-21: Selecting Sample Liquid From Culture Vessel

To make this step a little easier to spot, we'll create an Octet<sup>®</sup> group and assign this step to it. Click the **Add Group** button and type **Octet** into the **Add Group Name** dialog.

Joneduleu Time.	2//Jul/2022 22:24:34	Re He Add to Bioreactor
Status: Priority: Resource: Method: Group: Parameters:	Not Started 50 LH Sample Liquid From Culture Vessel Edit Parameters	Schedule Sc
Show Details	Add Group Name Octet	

Figure E-22: Adding the Group Name

Click the **Edit Parameters** button to access the Process Step Editor.

In the Plate settings, set the sample destination by choosing the 96-well plate (OCTET SAMPLE in this example).

Process Step Editor	
Process Step Editor Intro Culture Station Plate Mapping Volume Aspirate Method Name Dispense Method Name Reuse Pipettes Keep Cap Off	Please identify the plate that the sample will be placed into
Save	Plate ID: OCTET SAMPLE MEDIA1 MEDIA2 OCTET SAMPLE

Figure E-23: Selecting the Sample Destination

The mapping option sets which culture stations will be sample and the destination of each sample. The default setting is only sampling culture station 1, vessel 1. To change it, click **Edit**.

Process Step Editor	
Intro Culture Station Plate	Please specify which culture station sample will be added to which well
Mapping	
Volume	Edit
Aspirate Method Name	Vessel 1->Well A1
Dispense Method Name	
Reuse Pipettes	
Keep Cap Off	
Save	Previous Next Cancel

Figure E-24: Sample Mapping

In the mapping editor, the initial view shows vessel 1 mapped to sample plate well A1. For this experiment, we want to sample all 12 vessels.



Click the Mapping drop down and select V1... 12 -> 96 well A1... A12. Click Load to update the mapping view.

Figure E-25: Mapping Editor



This view now shows all 12 vessels mapped to the first row of the sample plate. Click **Update** to accept this mapping.

Figure E-26: Updated Mapping

In the **Volume** settings, set the amount of sample to pipette. All other options can be left at their default settings. Click **Save**.

Process Step Editor								
Intro	Please specif	mple volume	2					
Culture Station		,						
Plate								
Mapping								
Volume								
Aspirate Method Name								
Dispense Method Name								
Reuse Pipettes			1					
Keep Cap Off	Mapping	Volume	Units					
	Vessel 1 -> Well A1	4	ul					
	Vessel 2 -> Well A2	4	ul					
	Vessel 3 -> Well A3	4	ul					
	Vessel 4 -> Well A4	4	ul					
	Vessel 5 -> Well A5	4	ul					
	Vessel 6 -> Well A6	4	ul					
	Vessel 7 -> Well A7	4	ul					
	Vessel 8 -> Well A8	4	ul					
	Vessel 9 -> Well A9	4	ul					
	Vessel 10 -> Well A10	4	ul					
	Vessel 11 -> Well A11	4	ul					
	Vessel 12 -> Well A12	4	ul					
	All the Same	Import		Export				
Save	Previous	Next		Cancel				

Figure E-27: Setting the Amount of Sample to Pipette

This is the view of the final test experiment:

Date Time	Time From Inoculation	Group	Completed	Priority	Resource	Method	DOE Tag	Parameter 1
Wed 27 Jul 2022								
ed 27 Jul 22:16:24			Not Started	50	User	Load Plate On To System		Please load a plate.
ed 27 Jul 22:16:25			Not Started	50	User	Reload Pipette Box		Please load a pipette box
ed 27 Jul 22:16:26			Not Started	50	User	Reload Pipette Box		Please load the pipette bo
ed 27 Jul 22:16:27		Add Media	Not Started	50	User	Load Plate On To System		Please load the plate
ed 27 Jul 22:16:28		Add Media	Not Started	50	User	Load Plate On To System		Please load the plate
ed 27 Jul 22:16:29		Add Media	Not Started	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA1
ed 27 Jul 22:17:29		Add Media	Not Started	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA2
ed 27 Jul 22:18:29		Condition Media	Not Started	50	CS1	Set Temperature	Temperature	36.00 °C
ed 27 Jul 22:19:29		Condition Media	Not Started	50	CS1	Start Stirring	Speed	Up Stir 500 RPM
ed 27 Jul 22:20:29		Condition Media	Not Started	50	CS1	Start Control DO pH	Gassing	DO Set Point 50.00 %
ed 27 Jul 22:21:29		Condition Media	Not Started	50	CS2	Set Temperature	Temperature	36.00 °C
ed 27 Jul 22:22:29		Condition Media	Not Started	50	CS2	Start Stirring	Speed	Up Stir 500 RPM
ed 27 Jul 22:23:29		Condition Media	Not Started	50	CS2	Start Control DO pH	Gassing	DO Set Point 50.00 %
ed 27 Jul 22:24:29		Condition Media	Not Started	50	Monitor	Start Monitor		
ed 27 Jul 22:24:34		Octet	Not Started	50	LH	Sample Liquid From Culture Vessel		Culture Station 1

Figure E-28: Final Test Experiment

When we started the New Experiment Wizard, the start time was set in the future. This made it easier to program the loading steps at the beginning of the experiment. In our example, we set the experiment to start a day from now. If we were to click Start, the experiment would go through the initiation phase and then sit idle for a whole day.

To start running the experiment now, go to the **Experiment tab** in the main view. In the Day O field, make sure the date is set for **today**. Next, click the **Reschedule** button.

Introduction Experiment Mimi	receive Process Definition Process Steps Calendar Vessel Data Overview Culture Station 1 Culture Station 2 Results Audit Configuration Setup Cell Counts Status
Experiment	Process Definition Experiment Notes
Name Octet E	Create New Experiment Import Experiment Save To File Load From File
Day 0 Tue 26 Jul 2022	Set Day 0         Create DOE Experiment         Export DOE Results         Re schedule         Clear
Process Notes	
To do	
Date Time	What the user needs to do
Wed 27 Jul 2022	
22:16:24	Load 'MEDIA1' plate. 'Please load a plate.'.
22:16:25	Load '1 ml Back Left' pipette box.
22:16:26	Load '4 ml Back Right' pipette box.
22:16:27	Load 'MEDIA2' plate. 'Please load the plate'.
22:16:28	Load 'MEDIA1' plate. 'Please load the plate'.
22:16:29	Fill 'MEDIA1' plate, wells [A1,A2,A3,A4,A5,A6,B1,B2,B3,B4,B5,B6] Each well containing a minimum volume of 5 ml.
22:17:29	Fill 'MEDIA2' plate, wells [A1,A2,A3,A4,A5,A6,B1,B2,B3,B4,B5,B6] Each well containing a minimum volume of 5 ml.

Figure E-29: Rescheduling the Experiment for Today

Set the **Day** to **O** and **Time** to 3-4 minutes from the current time.

Change the date and time								
Change experiment start								
<ul> <li>Specify using date and time</li> </ul>								
26/ Jul /2022 22:40:58           ● Specify using day and time								
Day 0 Day is the offset from the experiment start date								
OK								

Figure E-30: Setting the Experiment Date and Time

Click the **Start** button to start the experiment.

roduction	Experiment	Mimic	Process	Definition	Process Steps	Calendar	Vessel Data	Overview	Culture Station 1	Culture Sta	tion 2 Result	s Audit	Configuration	Setu
Experiment	t										Process Defini	tion		
No. 0.115				Creat	te New Experim	ent	Import Experiment				Lord From Fla			
Name	Uctet E					L	oad Experiment	t	Export Experiment	ile Load From File				
Day 0	Day 0 Tue 26 Jul 2022 Set Day 0				Creat	te DOE Experim	nent	Export DOE Resul	Re schedu	e	Clear			
Process N	otes													
<b>T</b>														
To do														
To do Date Ti	ime	1	Vhat th	e user ne	eeds to do									
To do Date Ti Tue 26	ime Jul 2022		Vhat th	e user ne	eeds to do									
To do Date Ti Tue 26 22:40:50	ime Jul 2022 8	, , ,	Vhat th .oad 'N	e userne 1EDIA1' p	eeds to do plate. 'Please	e load a p	late.'.	_						
To do Date Ti Tue 26 22:40:5 22:40:5	ime Jul 2022 8 9	1	Vhat th oad 'N oad '1	e user ne 1EDIA1' p ml Back	eeds to do plate. 'Please Left' pipette	e load a p box.	late.'.		_	_	_	_	_	
To do Date Ti Tue 26 22:40:53 22:40:53 22:41:00	ime Jul 2022 8 9 0		Vhat th oad 'N oad '1 oad '4	e user ne 1EDIA1' p ml Back ml Back	eeds to do plate. 'Please Left' pipette Right' pipett	e load a p box. e box.	late.'.		_		_		_	
To do Date Ti Tue 26 22:40:5 22:40:5 22:41:0 22:41:0	ime Jul 2022 8 9 0 1		Vhat th oad 'N oad '1 oad '4 oad 'N	e user ne MEDIA1' p ml Back ml Back MEDIA2' p	eeds to do plate. 'Please Left' pipette Right' pipett	e load a p box. e box. e load the	late.'. plate'.	_	-		_			
To do Date Ti Tue 26 22:40:50 22:41:00 22:41:00 22:41:00 22:41:00	ime Jul 2022 8 9 0 1 2		Vhat th oad 'N oad '1 oad '4 oad 'N oad 'N	e user ne IEDIA1' p ml Back ml Back IEDIA2' p IEDIA1' p	eeds to do plate. 'Please Left' pipette Right' pipett plate. 'Please plate. 'Please	e load a p box. e box. e load the e load the	late.'. plate'. plate'.			_	_			
To do Date Ti Tue 26 22:40:53 22:41:03 22:41:00 22:41:00 22:41:00 22:41:00 22:41:00	ime Jul 2022 8 9 0 1 2 3		Vhatth oad 'N oad '1 oad '4 oad 'N oad 'N	e user ne IEDIA1' p ml Back ml Back IEDIA2' p IEDIA1' pla	eeds to do late. 'Please Left' pipette Right' pipett plate. 'Please plate. 'Please ate, wells [A1,	e load a p box. e box. e load the e load the A2,A3,A4.	late.'. plate'. plate'. ,A5,A6,B1,B2	2,83,84,8	5.B6] Each well	containing	a minimum	volume	: of 5 ml.	

Ambr® 15 cell culture software: Experiment Name [Octet E] Started [26/Jul/2022 ] 26/Jul/2022 22:35:56

Figure E-31: Starting the Experiment

During initiation you will see the calibration screen. You can leave the settings alone, but the Batch field needs a setting. Enter any number and click **Update**.

Figure E-32: Setting the Batch Number

In the next phase of initiation, you will see several warnings with the option to continue or cancel the experiment. Most warnings can be ignored but look for the message **Using unloaded plate**. This is an indication that one the plate loading steps is missing or the loading step is set with a time stamp after it is needed. If you see this error, cancel the experiment and fix the error in the Process Steps tab.

verview Culture Station 1	Culture Station 2 Res	ults Audit Configuration Setu	Cell Counts Status				
	Process De	finition	Experiment Notes				
Import Experiment	t Save To	File Load From File					^ Edit
Export Experiment	it		Starting I	Progress			
Export DOE Result	Its Re sche	dule Clear	🗹 Ch	ecking Pipette	Box Lids	Status	Message
			⊡ Ch	ecking Plate L	ds		User interaction scheduled out side working
			Z Ch	ecking Valid P	rocess Stens	Δ	Hours for a weekday [Load Plate On To
				ooking Dinette			System - Tue 26 Jul 22:40:58]
				lecking Pipelle	Usage		Using unloaded plate [OCTET SAMPLE] in
	Process Start Warning	9			Limits		Method[Sample_Liquid_From_Culture_Vess
	Warnings				rerflow		at [Tue 26 Jul 2022 22:49:08]
	Using unloade	ed plate [OCTET SAMPL	E] in Method[Sample_Lic	quid_From_Culture_	Vessel] s		
3,84,85,86] Each well					ration		
3,84,85,86] Each well							
					ess Steps		
					Calibration		
	Yes	Do you	want to continue	?	№ s Step Time	s	
				ecking Muted	Alarm		
			- Ch	ecking Gas Pr	accura		
				lecking das Ph	555010		
			OK				

Figure E-33: Using Unloaded Plate Warning

At the end of initiation, the Starting Progress dialog should be all green. Click **OK** to run the first step. Return to the **Process Step** tab.





The first few steps will inform you to place plates and pipettes onto the Ambr<sup>®</sup> system. Click **OK** for each prompt.

Pause	CS1 CS2 LH RDR PC	In Simulation	on Mode
Calendar Vesse	Labware Loading / Unloading		
Completed		Load Plate	
Running Not Started		Please load a plate.	
Not Started Not Started Not Started Not Started Jia Not Started Jia Not Started Jia Not Started	ID Media1 Location Deck 1 PlateType TAP BIOSYSTEMS 24 DEE Without a Lid Press OK when completed	P WELL	Barcode You may optionally enter the barcode of the plate if it has one present.
dia Not Started dia Not Started dia Not Started dia Not Started Not Started		Pipette 1 Pipette 2	
		D:Media1	
	Deck 2 Deck 3	Deck 4	
	ОК		Cancel

Figure E-35: Load Plate Prompt

The Process Steps table shows the status of each step in the experiment. When completed, all steps should be green.

P HILD -	ro cen cuite	ine solutione: experiment i	anne foeter i jotare		1 20/200/	2022 20110.00								
Start	Pause All	Pause LH / Abandon Edit Step	n Stop	Stoppe	d	S1 CS2 LH	RDR	PC						
Introduction	Experiment	Mimic Process Definitio	n Process Steps C	alendar Vessel	Data Ov	erview Culture	e Station 1	Culture Station 2	Results A	udit	Configuration	Setup	Cell Counts	Status
Dat	e Time T	Time From Inoculation	Group	Completed	Priority	Resource	Method				DOE Tag	Para	meter 1	
Tue 26 J	ul 2022													
Je 26 Jul 2	2:59:37			Completed	50	User	Load PI	ate On To Syst	tem			Plea	se load a p	late.
ue 26 Jul 2	2:59:38			Completed	50	User	Reload	Pipette Box				Plea	se load a p	ipette box
Je 26 Jul 2	2:59:39			Completed	50	User	Reload	Pipette Box				Plea	se load the	pipette bo
ue 26 Jul 2	2:59:40		Add Media	Completed	50	User	Load PI	ate On To Syst	tem			Plea	se load the	plate
Je 26 Jul 2	2:59:41		Add Media	Completed	50	User	Load PI	ate On To Syst	tem			Plea	se load the	plate
ue 26 Jul 2	2:59:42		Add Media	Completed	50	LH	Add Liq	uid To Culture '	Vessel		Add Media	Plate	MEDIA1	
Je 26 Jul 2	3:00:42		Add Media	Completed	50	LH	Add Liq	uid To Culture '	Vessel		Add Media	Plate	MEDIA2	
ue 26 Jul 2	3:01:42		Condition Media	Completed	50	CS1	Set Ten	nperature			Temperature	36.00	°C	
Je 26 Jul 2	3:02:42		Condition Media	Completed	50	CS1	Start Sti	rring			Speed	Up S	tir 500 RPN	1
ue 26 Jul 2	3:03:42		Condition Media	Completed	50	CS1	Start Co	ntrol DO pH			Gassing	D0 S	et Point 50	.00 %
Je 26 Jul 2	3:04:42		Condition Media	Completed	50	CS2	Set Ten	nperature			Temperature	36.00	°C	
.ie 26 Jul 2	3:05:42		Condition Media	Completed	50	CS2	Start Sti	rring			Speed	Up S	tir 500 RPN	1
Je 26 Jul 2	3:06:42		Condition Media	Completed	50	CS2	Start Co	ntrol DO pH			Gassing	D0 S	et Point 50	.00 %
.ie 26 Jul 2	3:07:42		Condition Media	Completed	50	Monitor	Start Mo	onitor						
Je 26 Jul 2	3:07:47		Octet	Completed	50	LH	Sample	Liquid From C	ulture Vess	sel		Cultu	re Station 1	

Ambr® 15 cell culture software: Experiment Name [Octet F] Started [27/Jul/2022 ] 26/Jul/2022 23:10:55

Figure E-36: Process Steps Table Showing All Steps as Completed

The output directory should contain the plate map CSV file needed by the Octet<sup>®</sup>system.

→ • ↑ 📘	<ul> <li>This</li> </ul>	This PC > Local Disk (C:) > AMBR > DataInterface > Out			
1 Quish serves		Name	Date modified	Туре	Size
Desktop	*	[OCTET SAMPLE][26 Jul 20	022 231018] 7/26/2022 11:10 PM	Microsoft Excel C	1 KB
Downloads	*				
Documents	*				
Pictures	*				

Figure E-37: Output Directory
	А	В	с	D	E	F	G	н	I.	J
1	Version 1									
2	System	Experiment	Station	Vessel	When	Plate	Well	Barcode	Volume	Info
3	ambr24-R2-XXX	Octet F	CS1	1	7/26/2022 23:08	Octet Sample	A1		0.004	
4	ambr24-R2-XXX	Octet F	CS1	2	7/26/2022 23:08	Octet Sample	A2		0.004	
5	ambr24-R2-XXX	Octet F	CS1	3	7/26/2022 23:08	Octet Sample	A3		0.004	
6	ambr24-R2-XXX	Octet F	CS1	4	7/26/2022 23:08	Octet Sample	A4		0.004	
7	ambr24-R2-XXX	Octet F	CS1	5	7/26/2022 23:08	Octet Sample	A5		0.004	
8	ambr24-R2-XXX	Octet F	CS1	6	7/26/2022 23:08	Octet Sample	A6		0.004	
9	ambr24-R2-XXX	Octet F	CS1	7	7/26/2022 23:09	Octet Sample	A7		0.004	
10	ambr24-R2-XXX	Octet F	CS1	8	7/26/2022 23:09	Octet Sample	A8		0.004	
11	ambr24-R2-XXX	Octet F	CS1	9	7/26/2022 23:09	Octet Sample	A9		0.004	
12	ambr24-R2-XXX	Octet F	CS1	10	7/26/2022 23:09	Octet Sample	A10		0.004	
13	ambr24-R2-XXX	Octet F	CS1	11	7/26/2022 23:09	Octet Sample	A11		0.004	
14	ambr24-R2-XXX	Octet F	CS1	12	7/26/2022 23:10	Octet Sample	A12		0.004	
15										
16										
17										
18										
10										
(OCTET SAMPLE)(26 Jul 2022 2310 (+)										

Figure E-38: Plate Map CSV File

## Sales and Service Contacts

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