SARTORIUS

User Manual

Octet® BLI Discovery

User Guide 13

Octet® BLI Discovery User Guide

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Chapter 1:

Welcome

About Octet® Systems

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

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

Table 1-1: Octet®System Software Functions

Octet® Software	Functions
Octet® BLI Discovery 	• Define quantitation or kinetic experiments and save them for future use. • Define custom assays. • Run experiments and acquire binding data. • View and save binding data.
Octet® Analysis Studio 	• Analyze binding data and view analysis results. • Export or copy analysis results. • Generate reports of quantitation or kinetic results.

For information on preparing samples for quantitation or kinetics experiments, please see the appropriate Octet® 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® Systems Safety Information

Getting Started

All users must read the following safety information.

WARNING: *Do not operate the Octet® 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 ®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® 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
	The system complies with applicable European directives.
	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	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
	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.
	Keep hands clear of moving parts.

Consignes de securite des systemes Octet®

Avant de commencer

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

WARNING: *N'utilisez pas le système Octet® 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®. 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® 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 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.
	Les interférences électromagnétiques émises par ce système se situent dans les limites approuvées par la Federal Communications Commission (Commission fédérale des communications) 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.
	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®-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
	Das System erfüllt die geltenden europäischen Richtlinien.
	Das System erfüllt die Anforderungen für elektromagnetische Verträglichkeit (EMV) in Australien und Neuseeland.
	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.
	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

Chapter 2:

Getting Started

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® 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®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®instrument in a way that makes it difficult to disconnect the power.

NOTICE: *Octet® 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® 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 provoquer 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® 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® 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®* .

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® BLI Discovery Software

NOTICE: *Octet® BLI Discovery and Octet® Analysis Studio 21 CFR Part 11 software require a compatible version of the Octet® GxP Server module. The software automatically checks the version of the Octet® 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® 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.

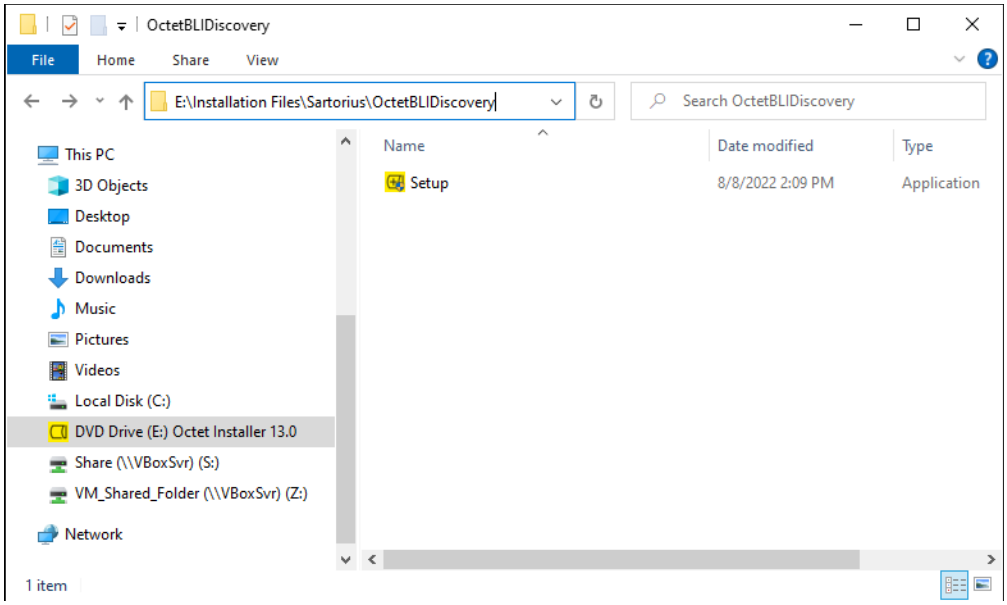


Figure 2-1: Location of Installation Program

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

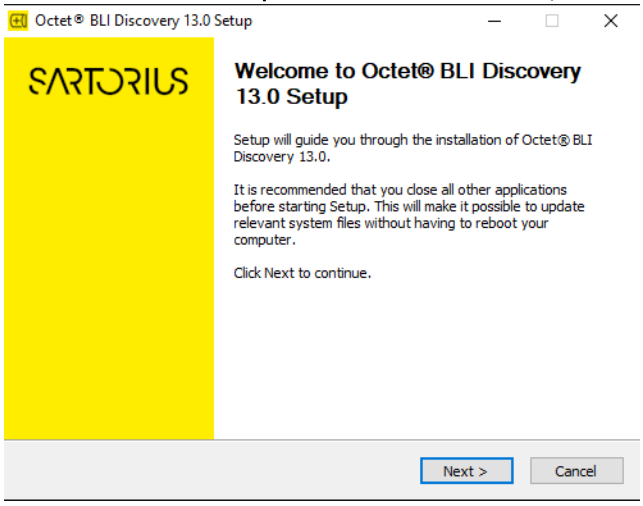


Figure 2-2: Software Setup Wizard

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

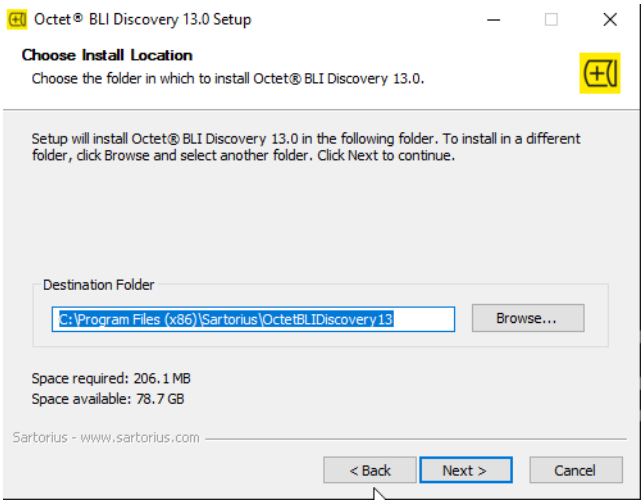


Figure 2-3: Choose Install Location Dialog Box

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

   The Choose Start Menu Folder dialog box appears.

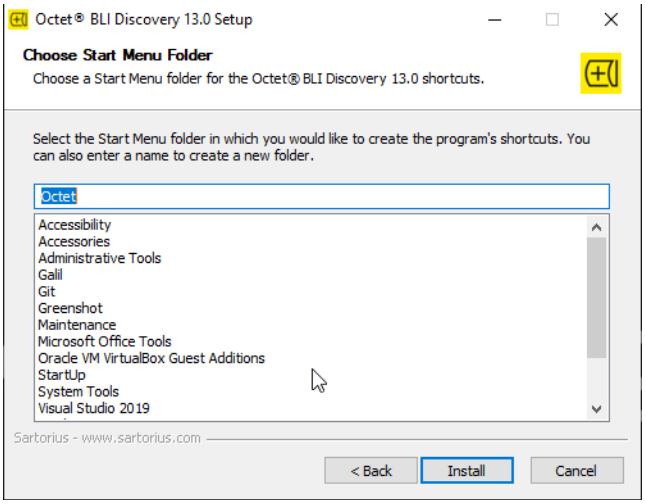


Figure 2-4: Choose Start Menu Folder Dialog Box

6. Click **Install**.

   The installation wizard takes a few seconds to install.

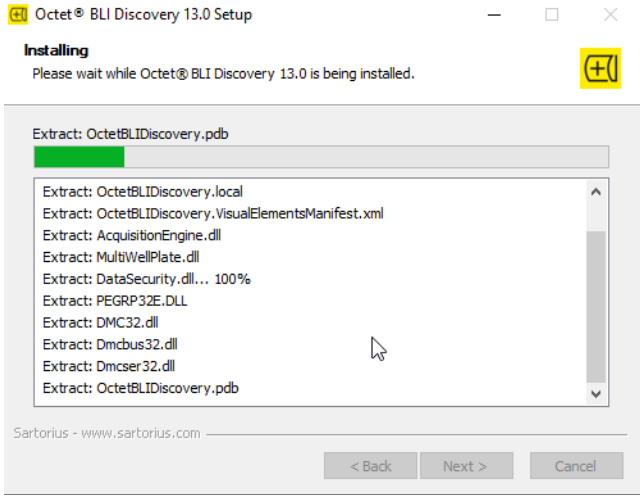


Figure 2-5: Installation Progress

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

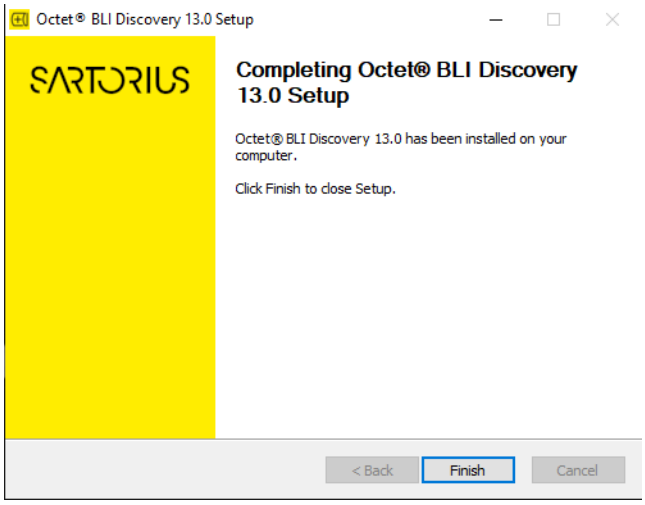


Figure 2-6: Completing the Setup

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® GxP Server Module" on page 569 for those instructions.

Starting the Octet® System and Octet® 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® BLI Discovery software by double-clicking on the Octet® BLI Discovery desktop icon

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

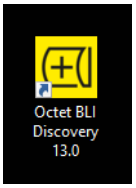


Figure 2-7: Desktop Icon

NOTICE: *When using the 21 CFR Part 11 version of the Octet® 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® BLI Discovery software **Main Screen**. Screen components along with the default windows appear are shown in Figure 2-8.

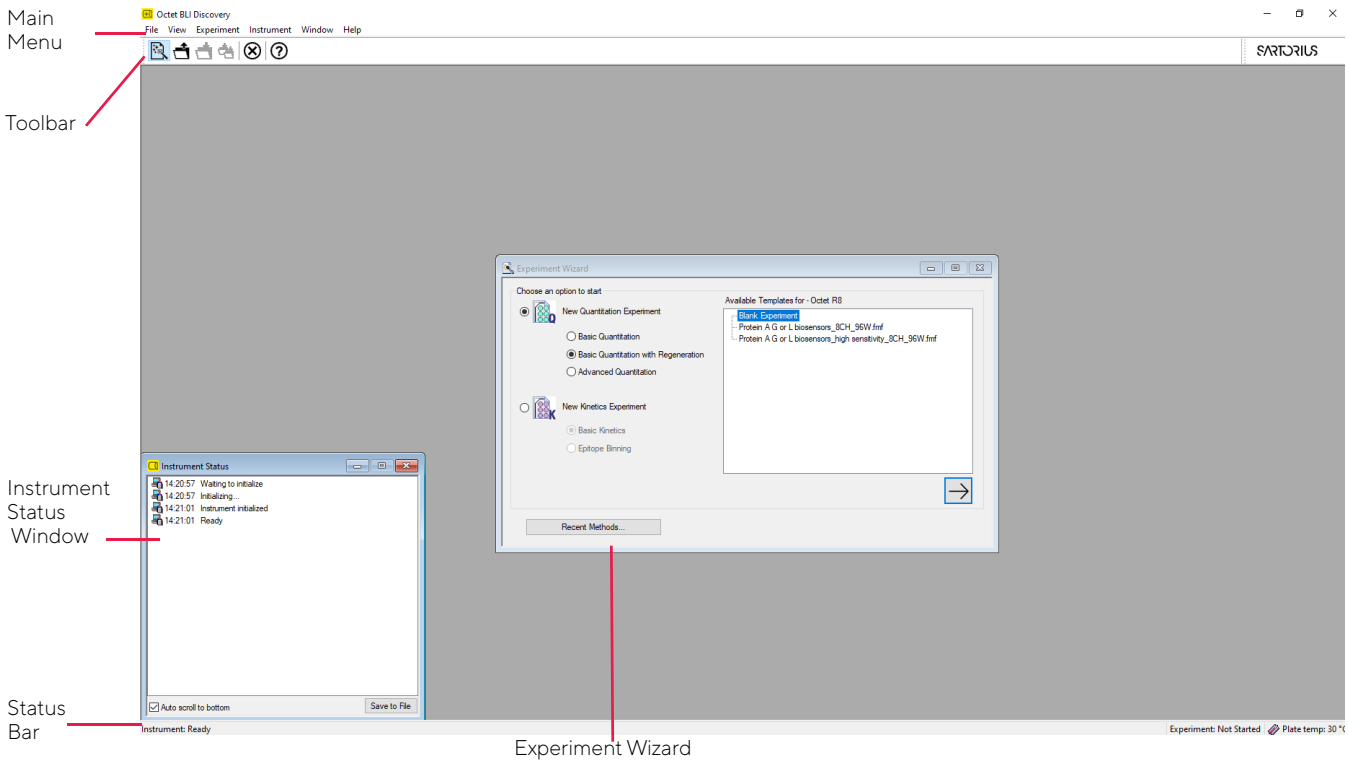


Figure 2-8: Main Screen

Main Menu and Toolbar

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

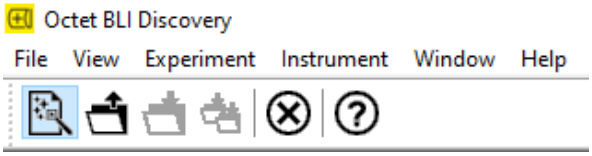


Figure 2-9: Main Menu and Toolbar

NOTICE: *The Security menu is only available in the 21 CFR Part 11 version of the Octet® 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® 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® 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.*

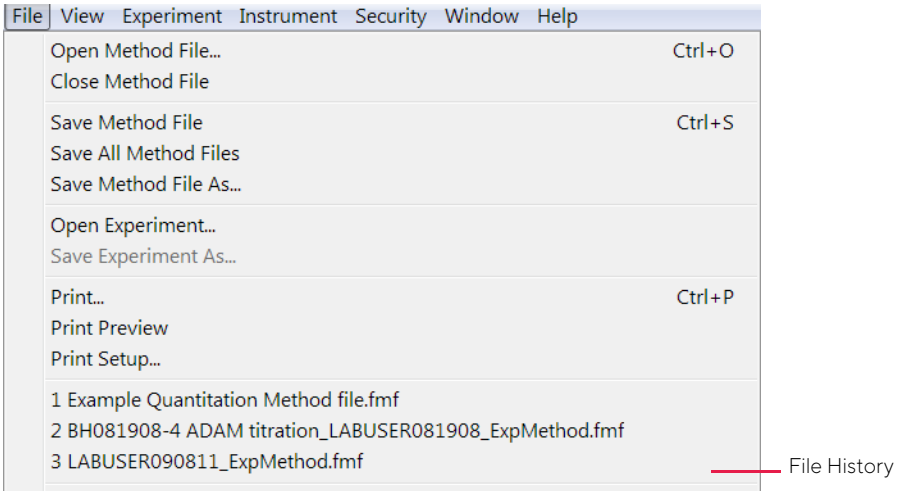


Figure 2-10: File Menu

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

Menu Command	Toolbar Button	Function
Open Method File		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		Saves the active experiment method file (.fmf).
Save All Method Files		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 **Print** 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 **Print Setup** 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 **Options** dialog box. Please refer to "Octet® BLI Discovery Options" on page 23 for more information on changing system and software 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.

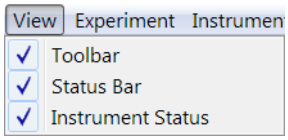


Figure 2-11: View Menu

Table 2-2: View Menu Commands

Menu Command	Function
Toolbar	Shows or hides the **Toolbar**.
Status Bar	Shows or hides the **Status bar**.
Instrument Status	Displays the **Instrument Status** window.

Experiment Menu

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

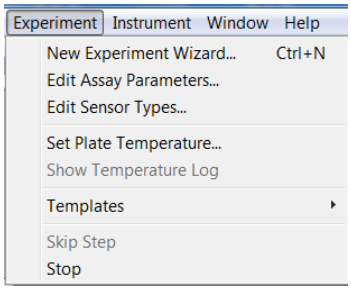


Figure 2-12: Experiment Menu

Table 2-3: Experiment Menu Commands

Menu Command	Toolbar Button	Function
New Experiment Wizard		Opens the **Experiment Wizard**.
Edit Assay Parameters	N/A	Opens the **Edit Assay Parameters** 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 **Sensor Types** dialog box to view current biosensor types, add new biosensor types and remove biosensor types. See "Managing Biosensor Types" on page 30 for more information.
Set Plate Temperature	N/A	Opens the **Temperature Setting** dialog box that displays the current sample 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 experiments only).
Stop		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® instrument.

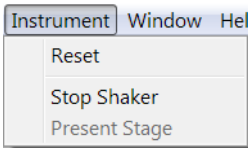


Figure 2-13: Instrument Menu

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 **Instrument Status window**.
Stop Shaker	N/A	Stops the sample plate shaker.

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

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

Security Menu

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

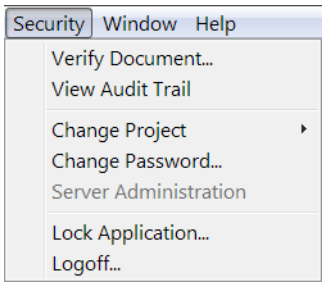


Figure 2-14: Security Menu

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.

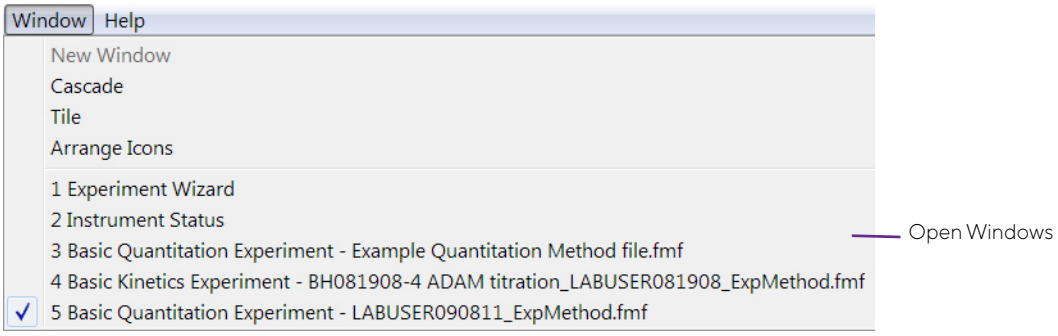


Figure 2-15: Window Menu

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.

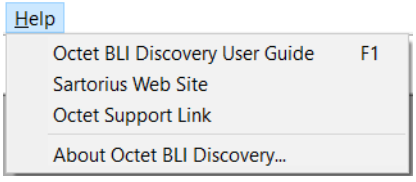


Figure 2-16: Help Menu

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® 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.sartorius.com).
Octet® Support	N/A	Opens a web browser and displays the Octet® support landing page.
About Octet® BLI Discovery	?	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® 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.

Figure 2-17: Status Bar

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

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

Instrument Status Window

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

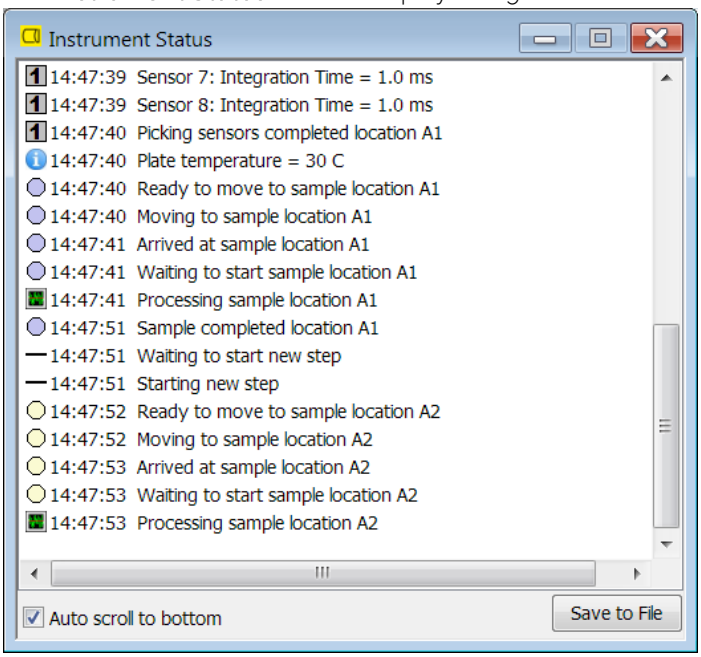


Figure 2-19: Instrument Status Window

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® 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.

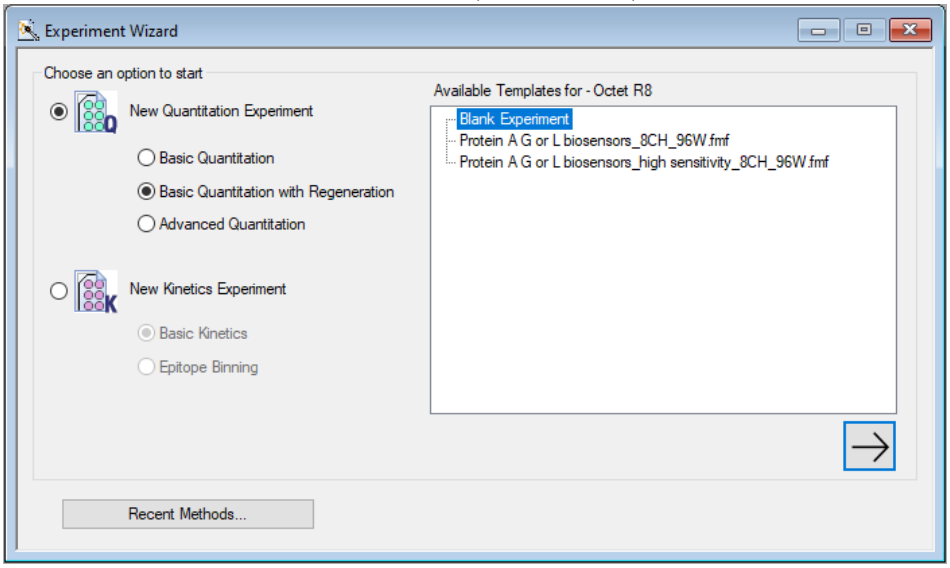


Figure 2-20: Experiment Wizard

Octet® 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**.

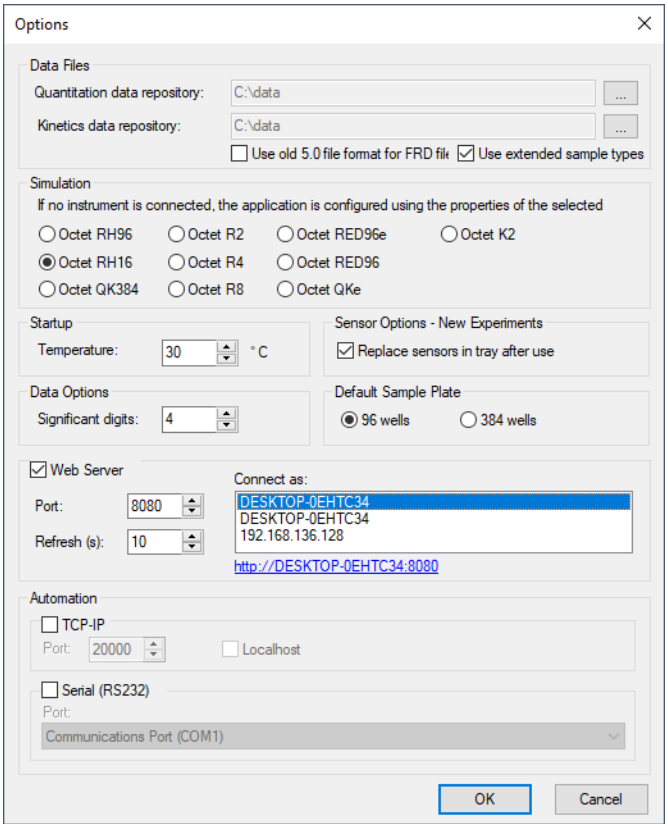


Figure 2-21: Options Dialog Box

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 **...** (**Browse**) to select a different folder. ***NOTICE:*** *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 **...** (**Browse**) to select a different folder. ***NOTICE:*** *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)

Item	Description
Use old 5.0 file format for FRD files	Select this option to save data in the earlier Octet® RED software 5.0 format. ***NOTICE:*** *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 **Sample Plate Map** and **Sample Plate Table** 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. ***NOTICE:*** *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® RH16 and Octet®QK384 systems only. For more information, please refer to Appendix A, Using Octet® RH16, Octet® RH96 and Octet® 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 f**ield (Figure 2-22) and, then click **OK**.

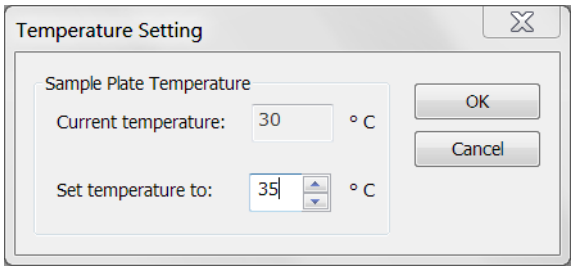


Figure 2-22: Temperature Setting

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® 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.

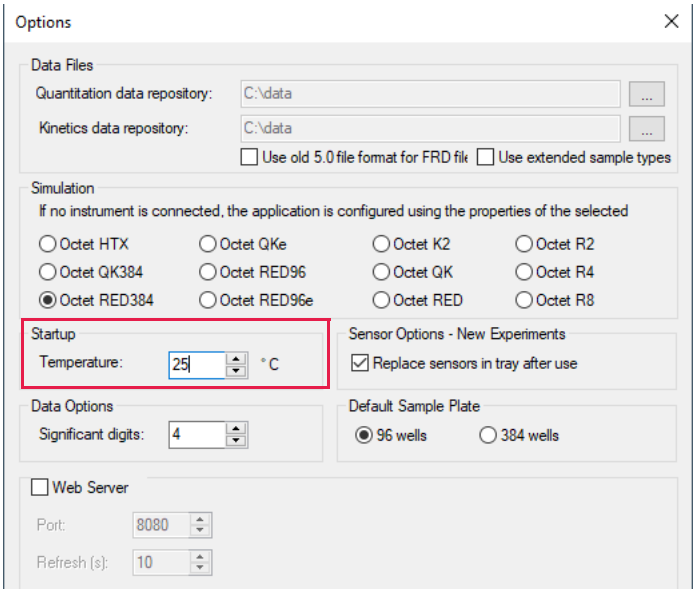


Figure 2-23: Setting the Default Startup Temperature in the Options Dialog Box

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® 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® 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**.
2. 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® system.*

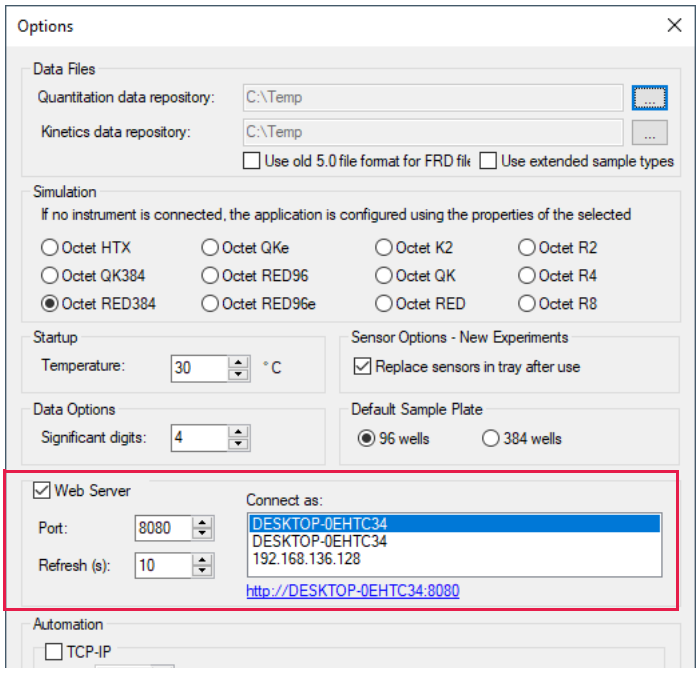


Figure 2-24: Selecting the Web Server in the Options Dialog Box

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.

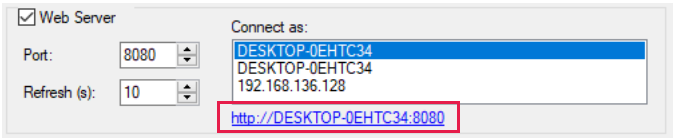


Figure 2-25: Web Server URL

4. Start the experiment in the Octet® 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® system.

NOTICE: *The remote computer or device must be on the same network as the Octet® 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).

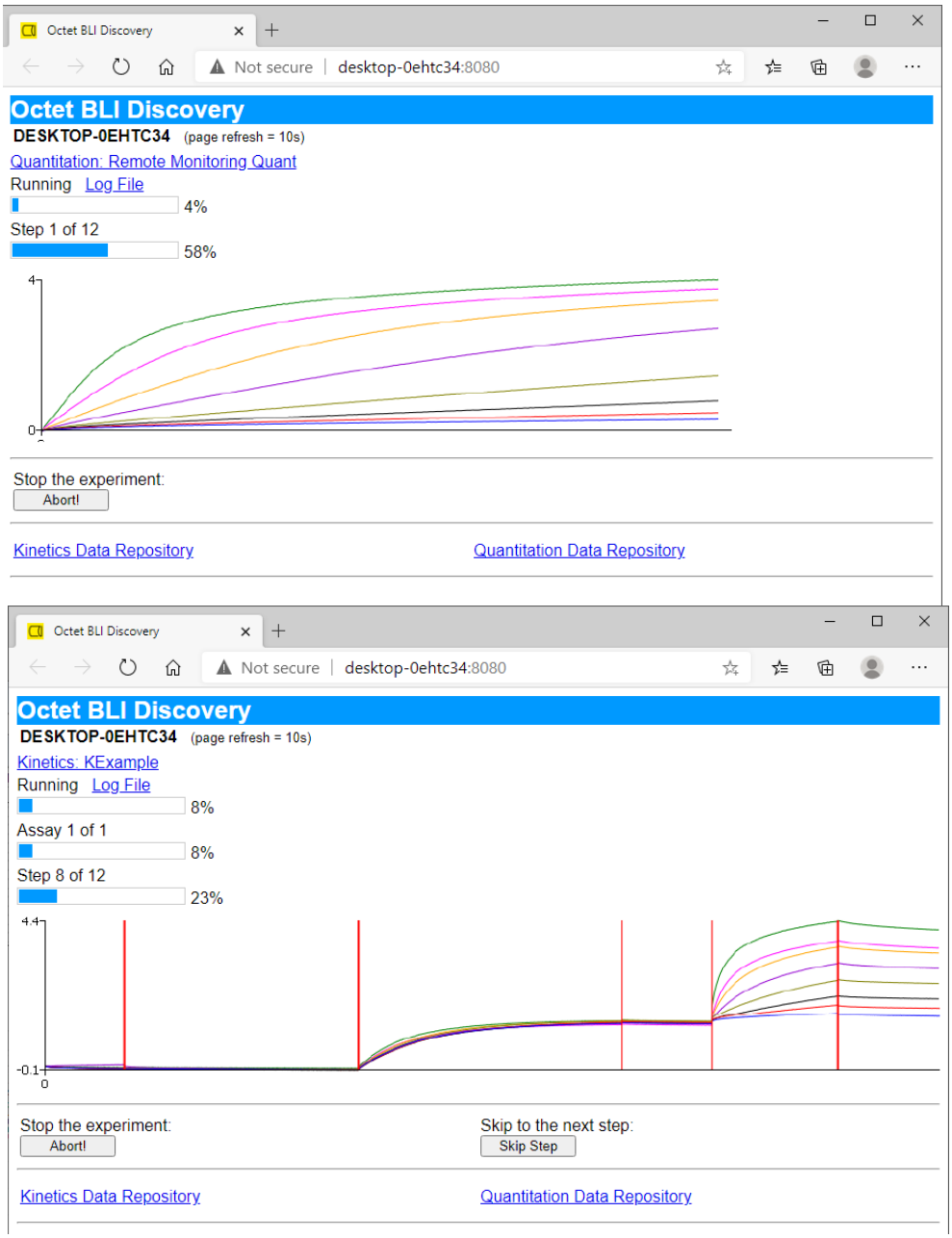


Figure 2-26: View of Quantitation Experiment (top) and Kinetics Experiment (bottom) via Web Browser

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® 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**.

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

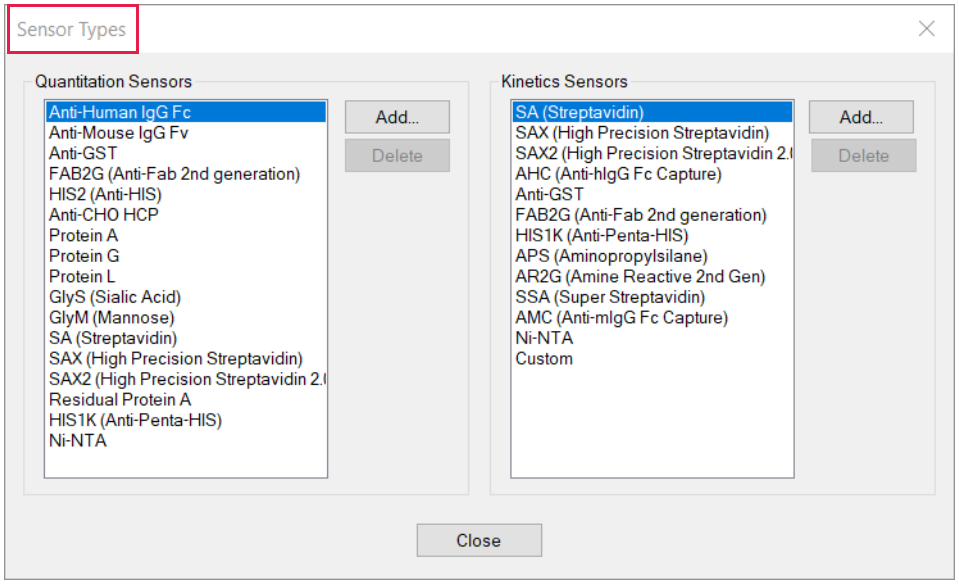


Figure 2-27: Sensor Types Dialog Box

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**.

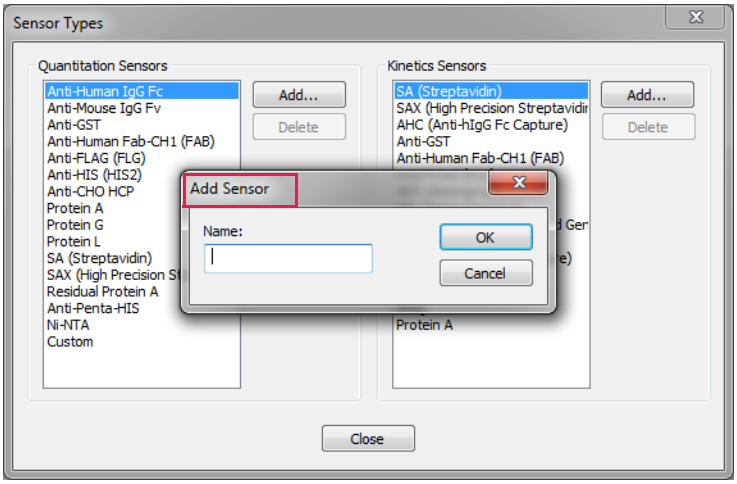


Figure 2-28: Adding a Biosensor Type

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® System Specifications and Site Requirements

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

- Octet® HTX is now the Octet® RH96
- Octet® RED384 is now the Octet® RH16

Getting Started

All users must read the following safety information.

WARNING: *Do not operate the Octet® 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® 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® 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
	The system complies with applicable European directives.
	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	The electromagnetic interference from this system is under limits approved by the Federal Communications Commission (United States).
	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.
	Keep hands clear of moving parts.

Octet® RED96 System Specifications and Site Requirements

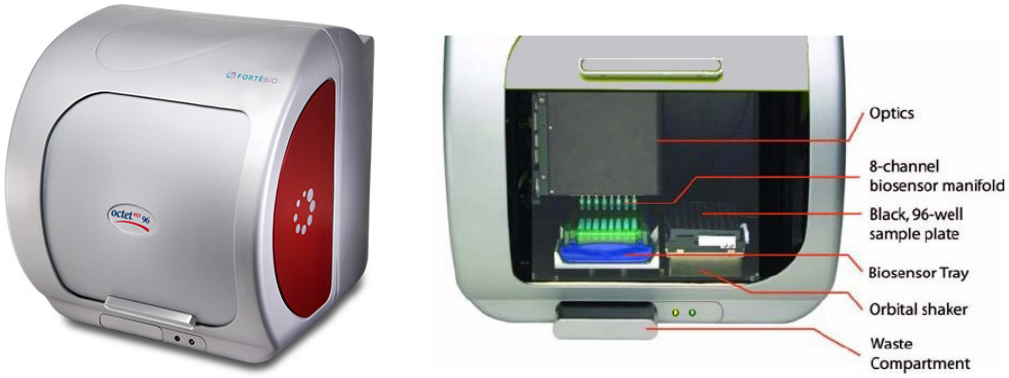


Figure 3-1: OCTET-RED96 Model Instrument—Door Closed (Left) or Open (Right)

IMPORTANT: *Using 96-well half-area plates on the Octet® 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 Model System Specifications (Sheet 1 of 2)

Item	Description
Model	OCTET-RED96
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage

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

Item	Description
Compliance	• Nemko NRTL/C, CB Scheme • CE compliance as indicated on the Instrument Identification and Safety Label. • 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.
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	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate • 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	• 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 power cords provided by Sartorius or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.*	

Octet® RH16 System Specifications and Site Requirements

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

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

NOTICE: *In Octet® 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® 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	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage
Compliance	• Nemko NRTL/C, CB Scheme • CE compliance as indicated on the Instrument Identification and Safety Label. • 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.
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening • kinetic analysis of small molecules

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

Item	Description

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 (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
Automation	• Up to 16 biosensors in parallel • Ability to integrate the Octet® instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
Optics and Mechanics	• 16-channel biosensor manifold • Optical interferometer • Sample plate platform temperature range: from 4 °C above ambient to 40 °C • 16 spectrometers (one dedicated spectrometer per biosensor)
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 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 • Power consumption: 195 W (240 W peak)

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)	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® QKe System Specifications and Site Requirements

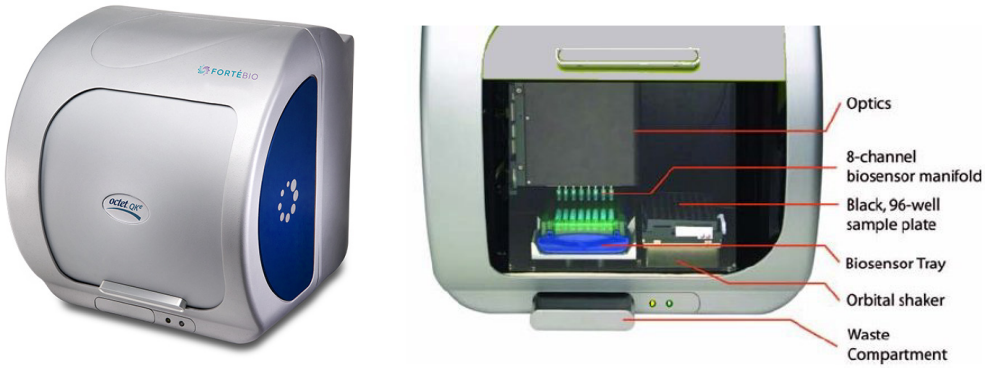


Figure 3-4: OCTET-QKE Model Instrument—Door Closed (Left) or Open (Right)

Instrument Identification and Safety Labeling

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

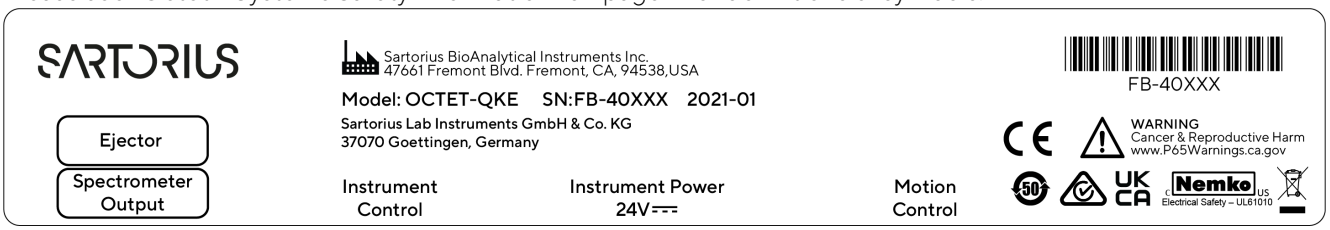


Figure 3-5: OCTET-QKE Model — Rear Panel Label

System Specifications

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

Item	Description
Model	OCTET-QKE
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage
Compliance	• Nemko NRTL/C, CB Scheme • CE compliance as indicated on the Instrument Identification and Safety Label. • 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.
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 • Biosensor re-racking • Recommended analyte molecular weight is 5,000 Da or higher
Sampling Format	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209), SBS standard microplate • Single sample plate capacity
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 regeneration 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 • Power consumption: 120 W (240 W peak)
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® QK384 System Specifications and Site Requirements

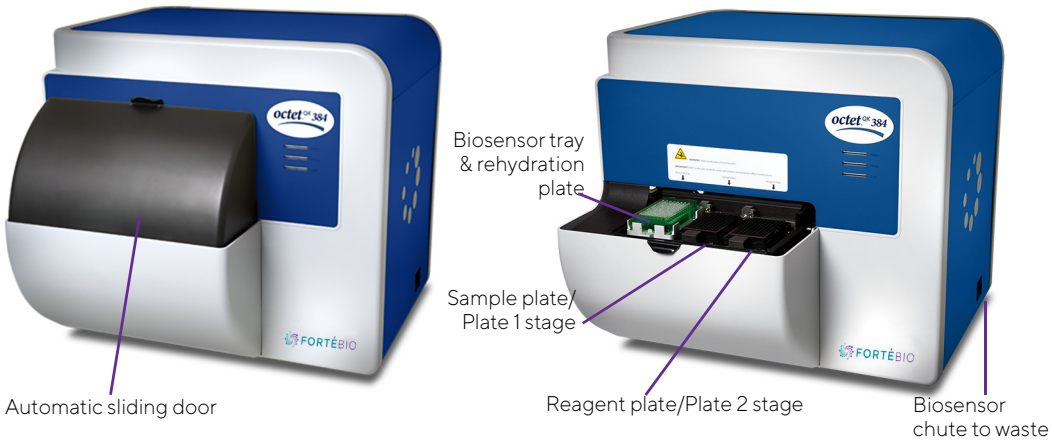


Figure 3-6: OCTET-QK384 Model —Door Closed (Left) or Open (Right)

NOTICE: *In Octet®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:

Table 3-6: OCTET-QK384 Model System Specifications (Sheet 1 of 3)

Item	Description
Model	OCTET-QK384
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage
Compliance	• Nemko NRTL/C • CE compliance as indicated on the Instrument Identification and Safety Label. • 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.
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening

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

Item	Description

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
Automation	• Up to 16 biosensors in parallel • Ability to integrate the Octet® instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
Optics and Mechanics	• 16-channel biosensor manifold • 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 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 • Power consumption: 195 W (240 W peak)

Table 3-6: OCTET-QK384 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 3-7: 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® 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® Systems Safety Information" on page 2 for definitions of symbols.

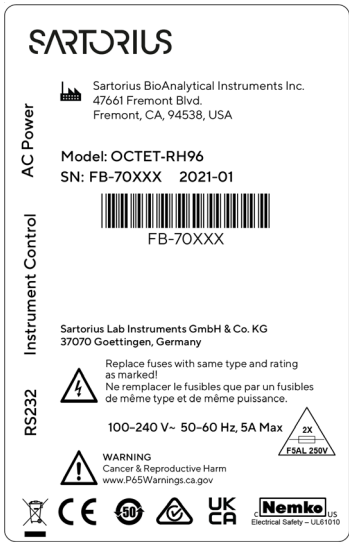


Figure 3-8: Octet-RH96 Model — Rear Panel Label

System Specifications

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

Item	Description
Model	OCTET-RH96
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage
Compliance	• Nemko NRTL/C, CB Scheme • CE compliance as indicated on the Instrument Identification and Safety Label. • 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.
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening • Small molecule kinetic analysis

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

Item	Description

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 (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
Automation	• Up to 96 biosensors in parallel • Ability to integrate the Octet® instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
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 • 16 spectrometers (selectable: one dedicated spectrometer per biosensor, up to one dedicated spectrometer per six biosensors).
Throughput	• Up to 96 biosensors in parallel, maximum of 384 tests unattended. • Two microplates, either 96- or 384-well at once. Either or both plates may be used for samples or reagents.
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 • Power consumption: 195 W (240 W peak)

Table 3-8: OCTET-RH96 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 3-9: Sensor Offset and Well Volumes for the Octet® 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® K2 Specifications and Site Requirements

The Octet® 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® 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® K2 instrument
- Package of 10 disposable tray liners for spent biosensors
- Software Installation CD
- Instrument Settings Backup CD
- Octet® mouse pad
- Octet® 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

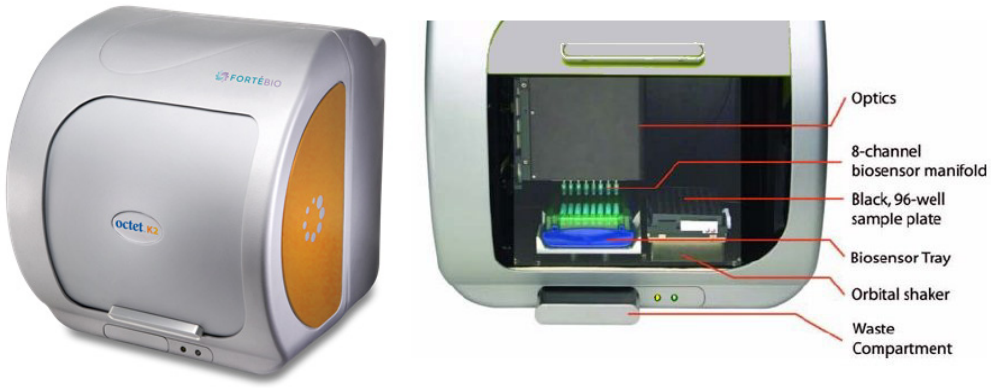


Figure 3-9: OCTET-K2 Model — Door Closed (Left) or Open (Right)

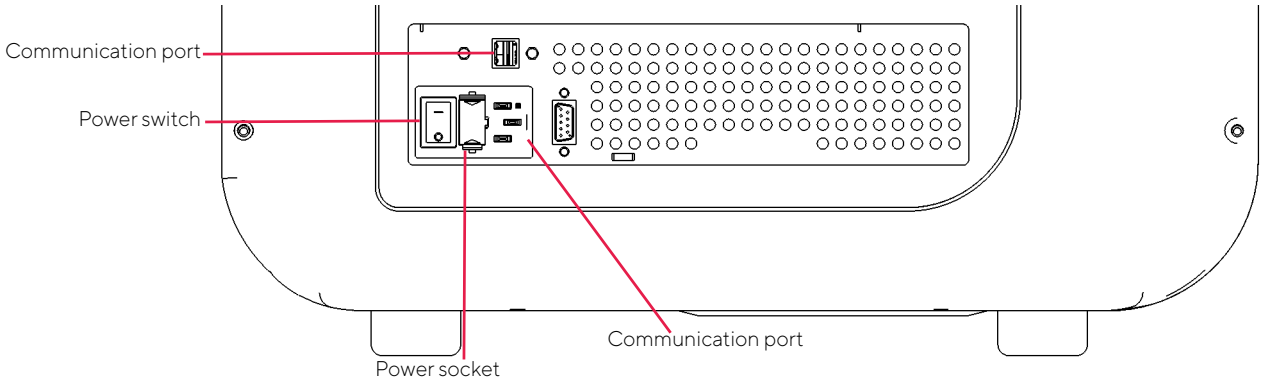


Figure 3-10: OCTET-K2 Model — Rear View

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.*

WARNING: *Using 96-well half-area plates on the Octet® 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® Systems Safety Information" on page 2 for definitions of symbols.

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	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage
Compliance	• Nemko NRTL/C, CB Scheme • CE compliance as indicated on the Instrument Identification and Safety Label. • Korea RRA/KC EMC Registration (KN11 and KN/61000-6-1:2016). • This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if it used in a domestic environment
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic analysis of proteins, peptides, and other biomolecules • Kinetic analysis of small molecule and fragment • Recommended analyte molecular weight of 150 Da or higher

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

Item	Description
Sampling Format	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate • 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 interferometer • 2 spectrometers (one dedicated spectrometer per biosensor)
Throughput	• Up to 2 biosensors in parallel, maximum of 96 tests unattended, subject to total assay time • 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)

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® R2, Octet® R4, and Octet® R8, System Specifications and Site Requirements

The Octet® R2, Octet® R4, and Octet® 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® R2, Octet® R4, and Octet® 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 x81.3 cm x 116.8 cm).

Contents of the system as shipped include:

- The Octet® R2, Octet® R4, or Octet® R8 instrument
- Package of 10 disposable tray liners for spent biosensors
- Octet® R8 only: Package of 3 evaporation covers
- Software Installation CD
- Instrument Settings Backup CD
- Octet® mouse pad
- Octet® 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

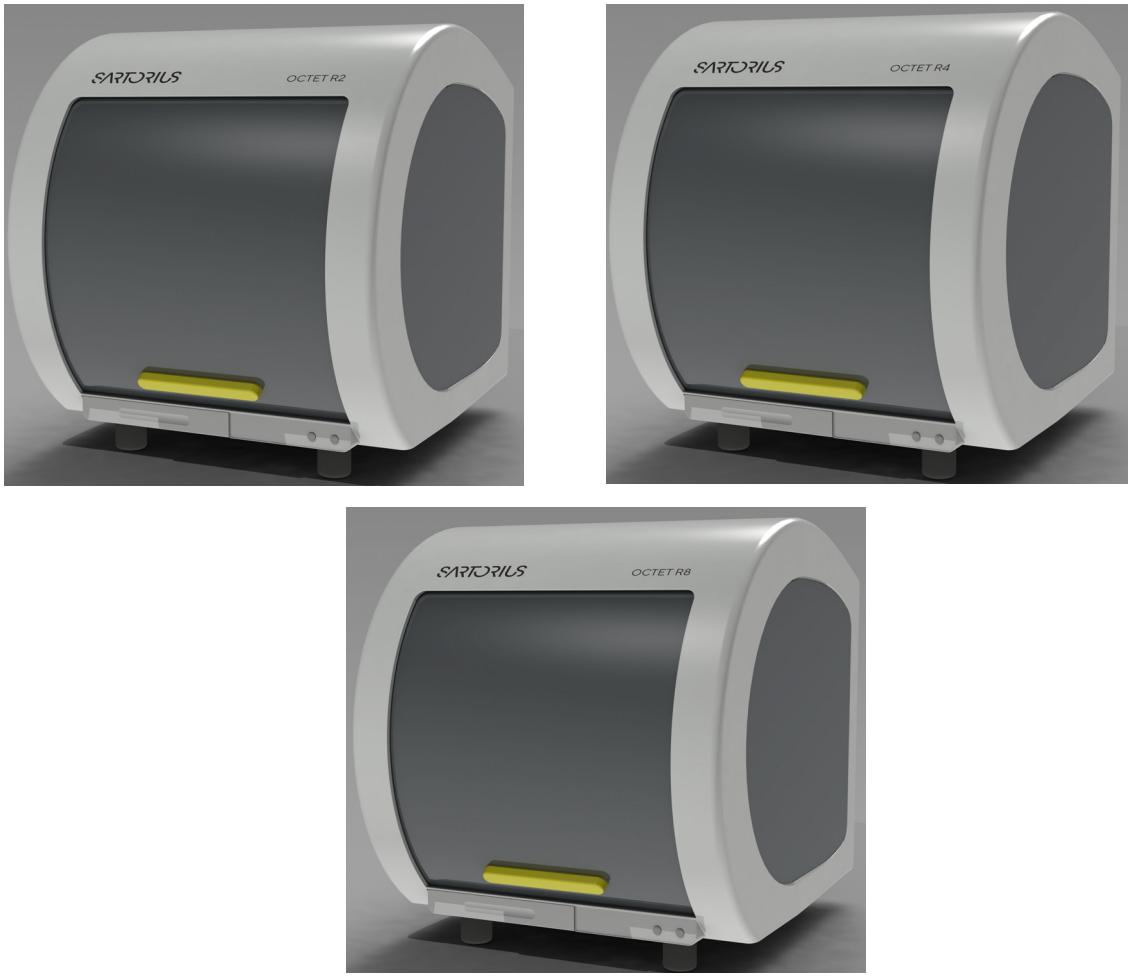


Figure 3-12: OCTET-R2, OCTET-R4, and OCTET-R8 Models — Door Closed

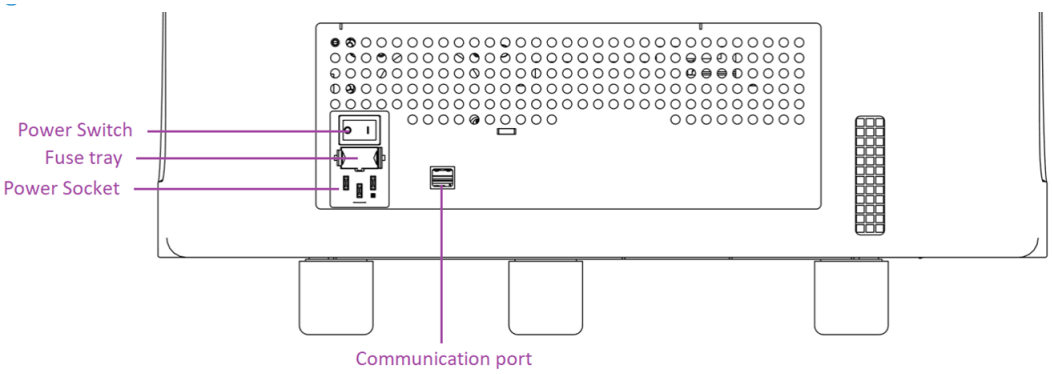


Figure 3-13: Models OCTET-R2, OCTET-R4, and OCTET-R8 Models — Rear View

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® R2, Octet ® R4, and Octet® 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® Systems Safety Information" on page 2 for definitions of symbols.

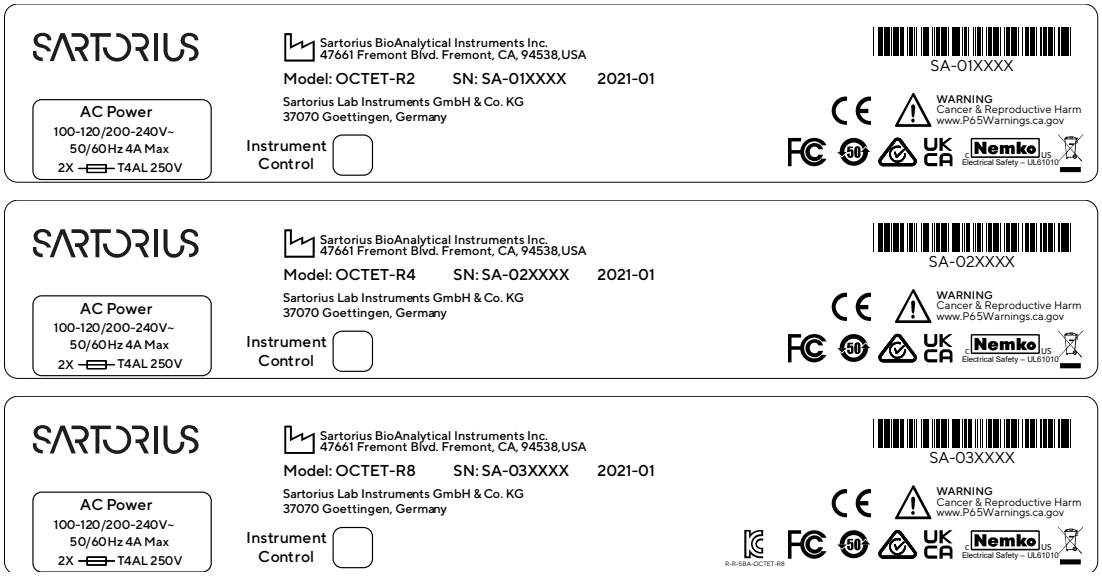


Figure 3-14: OCTET-R2, OCTET-R4, and OCTET-R8 Models — Rear Panel Labels

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	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}

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

Item	Description
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage
Compliance	• Nemko NRTL/C, CB Scheme • CE compliance as indicated on the Instrument Identification and Safety Label. • Korea RRA/KC EMC Registration (KN11 and KN/61000-6-1:2016). • This device has been tested for conformity for use in a laboratory environment. Radio interference may occur if it used in a domestic environment
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic analysis of proteins, peptides, and other biomolecules • Kinetic analysis of small molecule and fragment • Recommended analyte molecular weight of 150 Da or higher
Sampling Format	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate • 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	• OCTET-R2: 2-channel biosensor manifold • OCTET-R4: 4-channel biosensor manifold • OCTET-R8: 8-channel biosensor manifold • Optical interferometer • 2/4/8 spectrometers for Octet-R2, Octet-R4 and Octet-R8 (one dedicated spectrometer per biosensor)

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

Item	Description
Throughput	• Up to 2/4/8 biosensors in parallel, maximum of 96 tests unattended, subject to total assay time • 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	• OCTET-R2: 71 lb (32.2 kg) • OCTET-R4: 72 lb (32.7 kg) • OCTET-R8: 76 lb (34.5 kg)
Electrical Requirements	• Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max • Power consumption: 100 W (240 W peak)

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® RED96e System Specifications and Site Requirements

The Octet® 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® 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® RED96e instrument
- Package of 10 disposable tray liners for spent biosensors
- Package of 3 evaporation covers
- Software Installation CD
- Instrument Settings Backup CD
- Octet®mouse pad
- Octet®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

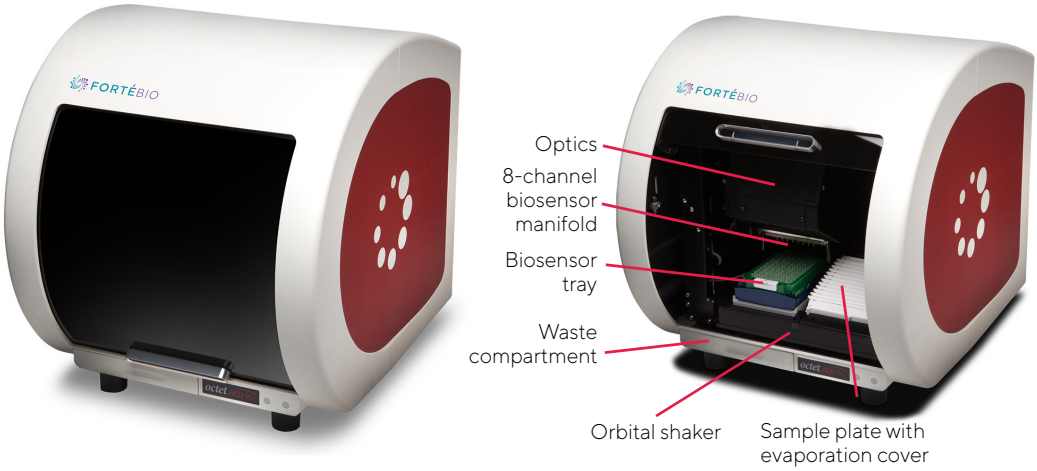


Figure 3-15: OCTET-RED96E Model — Door Closed (Left) or Open (Right)

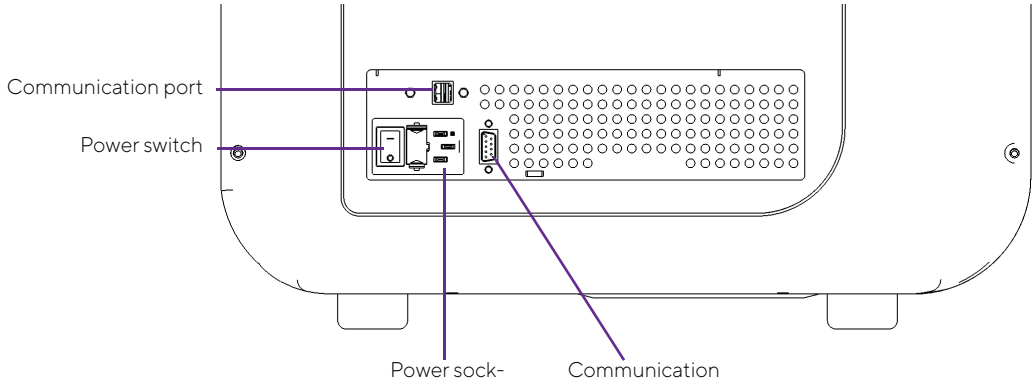


Figure 3-16: OCTET-RED96E Model — Rear View

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® 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® Systems Safety Information" on page 2 for definitions of symbols.

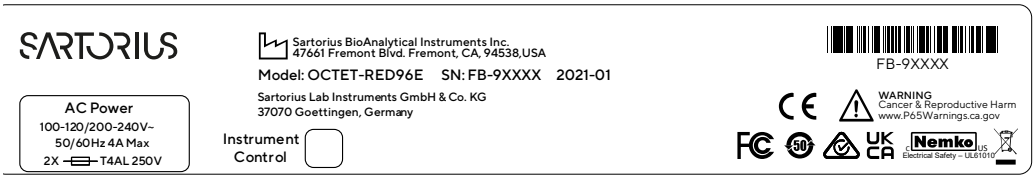


Figure 3-17: OCTET-RED96E Model — Rear Panel Label

System Specifications

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

Item	Description
Model	OCTET-RED96E
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: 0 to 40 °C • Optimum Operating Temperature: 22 ± 2 °C. ***NOTICE:*** *For optimal performance, the environmental temperature change should be less than 2 °C per hour.* • Safe Operating Temperature: 15 to 30 °C • 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 • Mains supply voltage fluctuations of +/-10% of the nominal voltage
Compliance	• Nemko NRTL/C, CB Scheme • CE compliance as indicated on the Instrument Identification and Safety Label. • 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.
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	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate • 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

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 • Power consumption: 200 W (300 W peak)

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® RED96e and the Octet® 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® RED96e and the Octet® 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.

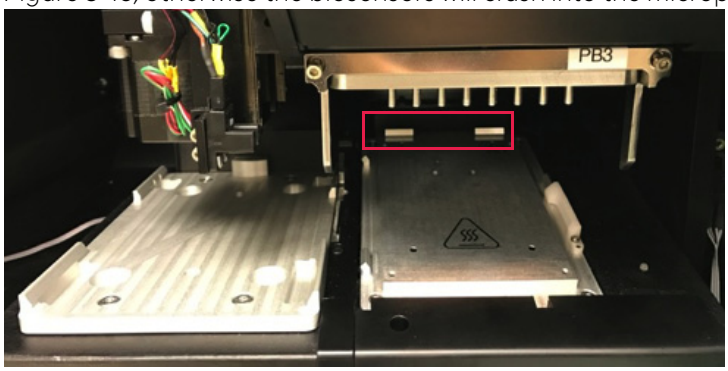


Figure 3-18: Install Push Bar

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.

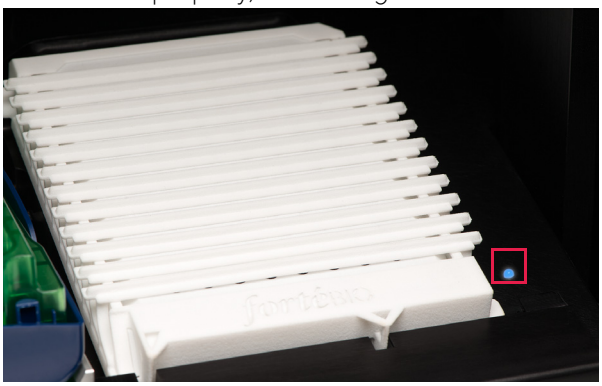


Figure 3-19: LED is Blue When Evaporation Cover is Installed Properly

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

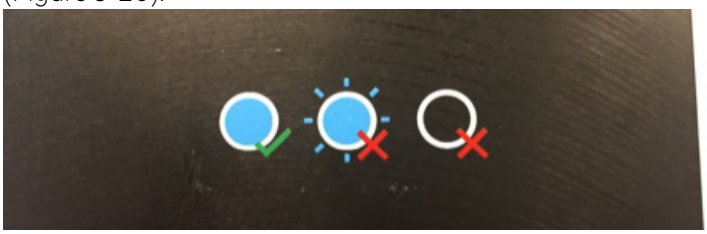


Figure 3-20: LED Status Information

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

FDA 21 CFR Part 11 Final Rule Compliance

The Octet® 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® 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® 21 CFR Part 11 software. For information, see http://www.fda.gov/regulatoryinformation/guidances/ucm125067.htm*

Octet® 21 CFR Part 11 software is comprised of three distinct software products: Octet® BLI Discovery 21 CFR Part 11 software and Octet® Analysis Studio 21 CFR Part 11 software and the Octet® GxP Server module. Octet® 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® Analysis Studio 21 CFR Part 11 software is used to analyze binding data and view analysis results. The Octet® 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® 21 CFR Part 11 software. All data acquired using Octet® 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® 21 CFR Part 11 software environment invalidates the digital signature. The Octet® 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® 21 CFR Part 11 software-environment. An Audit Trail of all activities performed in any Octet® 21 CFR Part 11 application is stored in the Octet GxP Server database.

Data files created using Octet® 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® software to ensure the integrity of the acquired data.

Administratively Controlled Application Access

The Octet® 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® 21 CFR Part 11 software uses the Octet® 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® BLI Discovery and Octet®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® 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® 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® 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® 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® 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® 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® 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® BLI Discovery 21 CFR Part 11 software is acquiring data from an Octet® instrument.

After data acquisition has begun, Octet® 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® 21 CFR Part 11 Software Overview

Octet® BLI Discovery and Octet® 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® GxP Server module to manage the information recorded during user sessions.

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

Octet® GxP Module

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

When Octet® BLI Discovery or Octet® Analysis Studio 21 CFR Part 11 software is launched, users are prompted to log on to the Octet® GxP Server module. This initiates a user session where all system, software and user events are recorded. During user sessions, the Octet® 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 ® BLI Discovery and Octet ® Analysis Studio 21 CFR Part 11 software require a compatible version of the Octet® GxP Server module. The software will automatically check the version of the Octet® 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® GxP Server module if this happens.*

Selecting a Server Location

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

When the Octet® 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® GxP Server module during the login process. The Octet® GxP Server can be run on the local host computer where Octet® BLI Discovery or Octet® Analysis Studio 21 CFR Part 11 software is installed or from a network location.

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

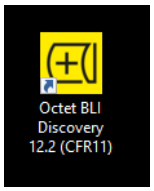


Figure 4-1: Octet® BLI Discovery 21 CFR Part 11 Software Desktop Shortcut

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

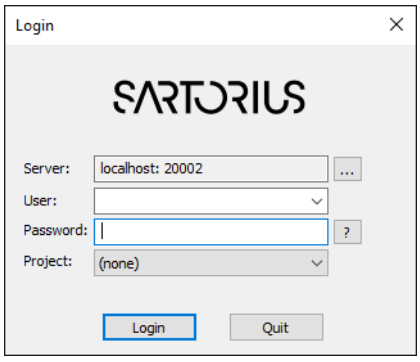


Figure 4-2: Login Dialog Box

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

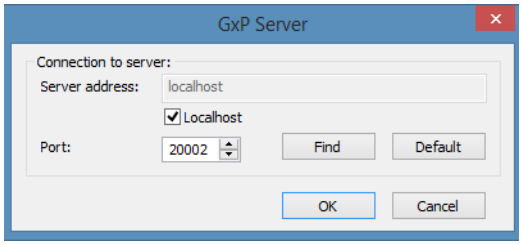


Figure 4-3: Octet® GxP Server Dialog Box

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

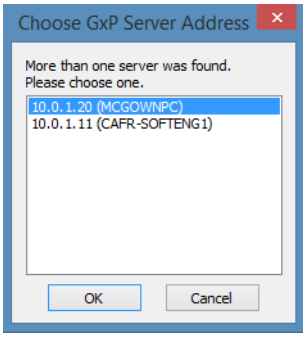


Figure 4-4: *Octet® GxP Server Address Search Results*

- ***Choosing a remote host on another subnet***—If the Octet® 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® GxP Server module (Figure 4-5).

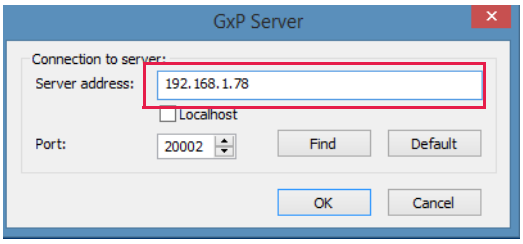


Figure 4-5: Manual Entry of Remote Host Address

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

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

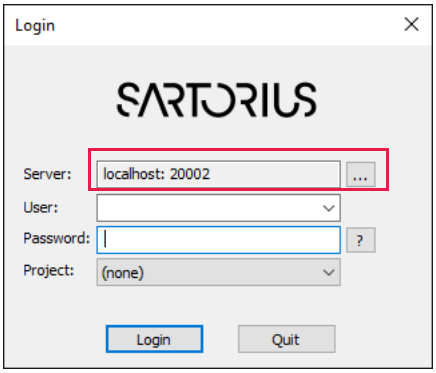


Figure 4-6: Login Dialog Box– Octet® GxP Server Location

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® GxP Server module host location that should be used.*

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

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

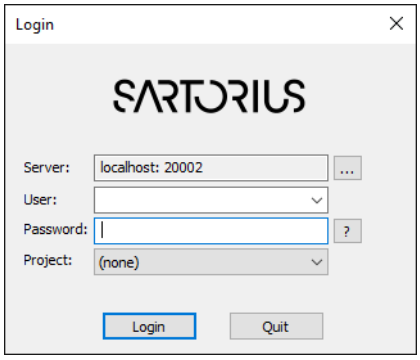


Figure 4-7: Login Dialog Box

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.*

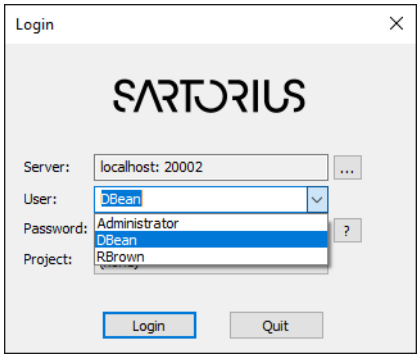


Figure 4-8: User Name Selection

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

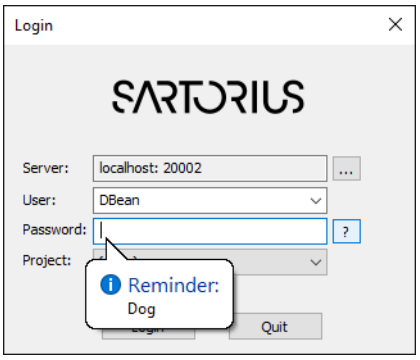


Figure 4-9: Password Reminder

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

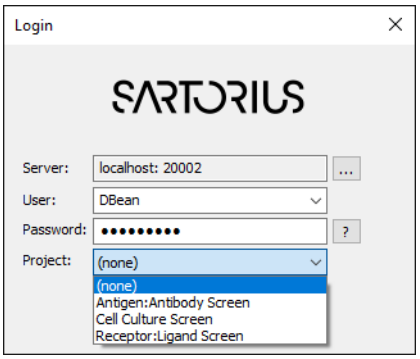


Figure 4-10: Project Selection

6. Click **Login.**

   Octet® BLI Discovery or Octet ®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).

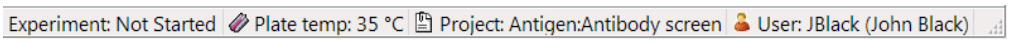


Figure 4-11: Status Bar

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® BLI Discovery and Octet® Analysis Studio software can be accessed by clicking the **Security** menu (Figure 4-12) from the software's **Main Menu**:

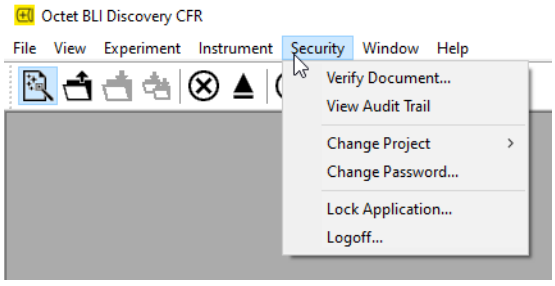


Figure 4-12: Security Menu

Experiment and Method File Compliance

When using the 21 CFR Part 11 version of Octet® 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 11-compliant software, and have not been modified outside of Octet® software.

NOTICE: *When verifying file integrity both method (.fmf) and data (.frd) files can be selected in Octet® BLI Discovery 21 CFR Part 11 software. The Octet® 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:

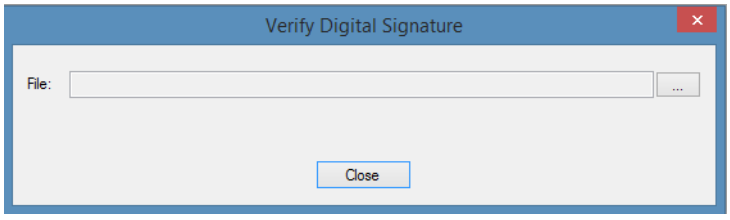


Figure 4-13: Verify Digital Signature

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

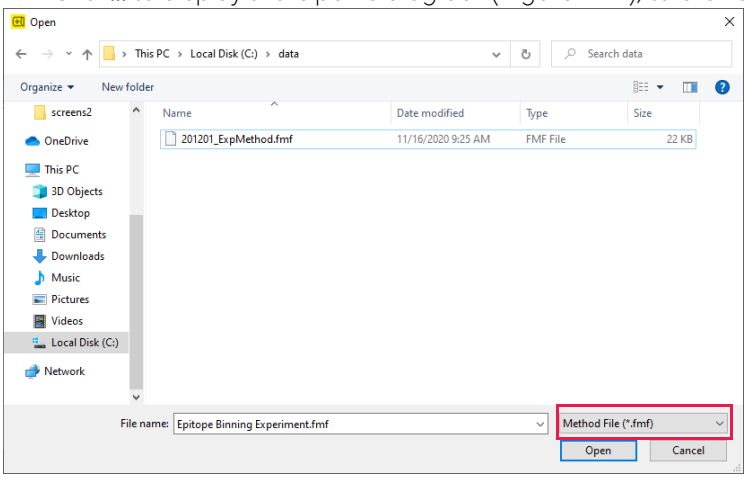


Figure 4-14: Open Dialog Box

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.

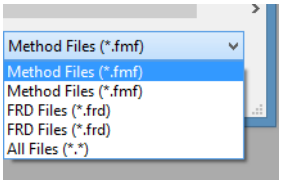


Figure 4-15: File Type Menu

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):

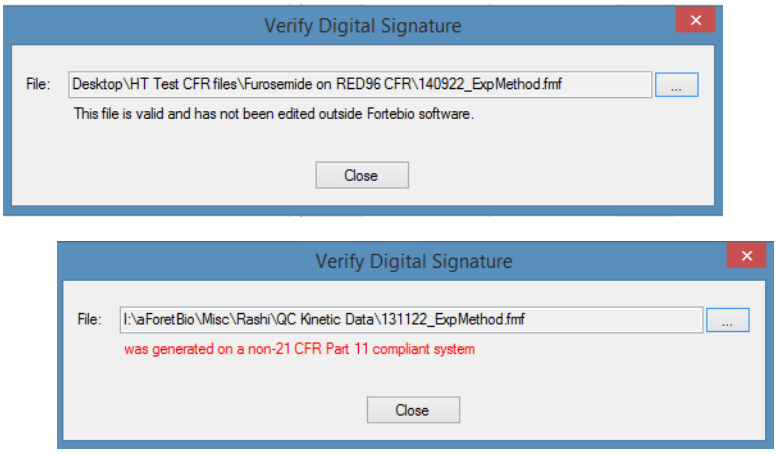


Figure 4-16: *File Compliant (top), File Not a CFR Document (bottom)*

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.

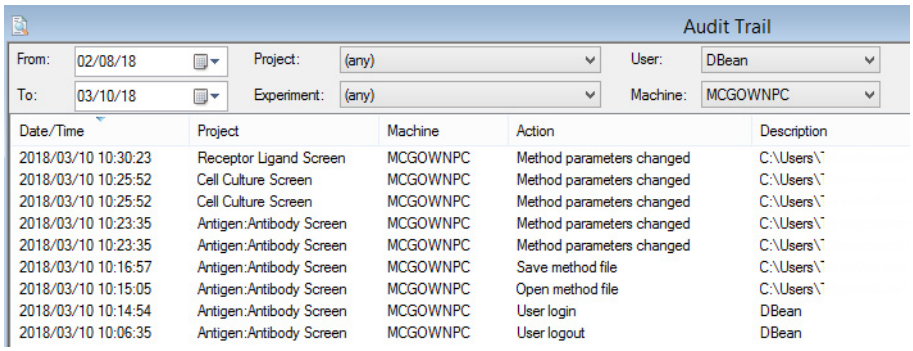


Figure 4-17: Audit Trail

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.

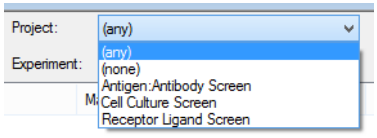


Figure 4-18: Selecting Events by Project

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).

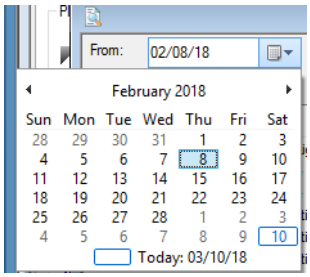


Figure 4-19: Selecting Events in a Time Period

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).

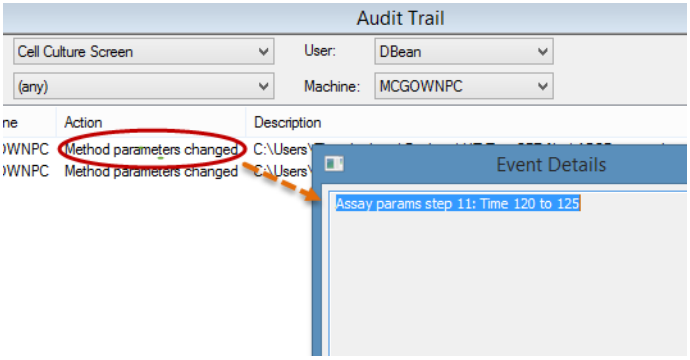


Figure 4-20: Viewing Event Details

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):

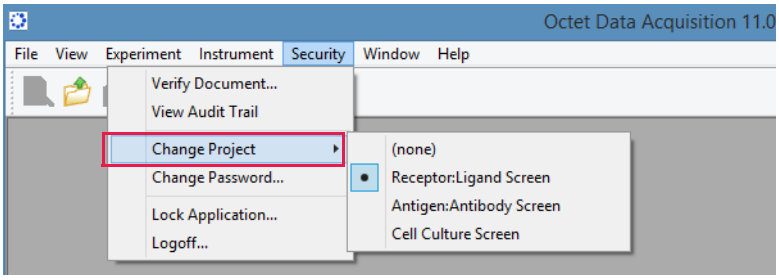


Figure 4-21: Changing Projects

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).

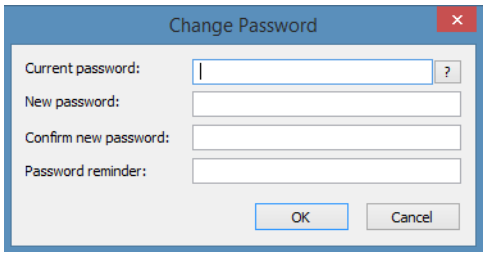


Figure 4-22: Change Password Dialog Box

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.

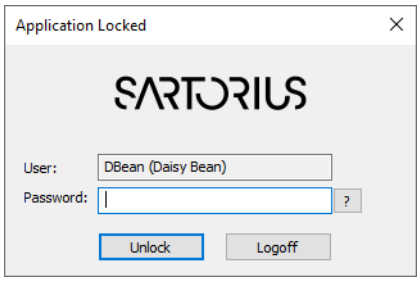


Figure 4-23: Application Locked Dialog Box

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.

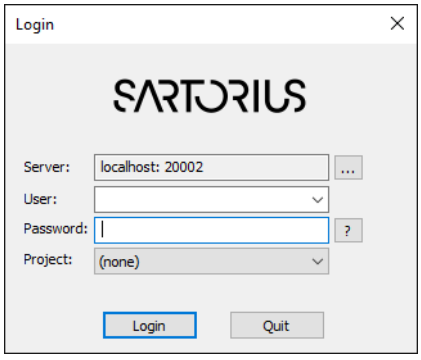


Figure 4-24: Login Dialog Box

3. **Quit** the application as needed.

Chapter 5:

Quantitation Experiments: Octet® R2, Octet® R4, and Octet® K2 System

Introduction

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

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

Software	Step	See
Octet® BLI Discovery 	1. Select a quantitation experiment in the Experiment wizard or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 84
	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® Analysis Studio 	6. Analyze the binding data. 7. Generate a report.	*Octet® Analysis Studio Software User Guide*

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® R2, Octet® R4, or Octet® 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® 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® 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 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 or 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 Regeneration	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two- or three-step quantitation assay that enables signal amplification 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.

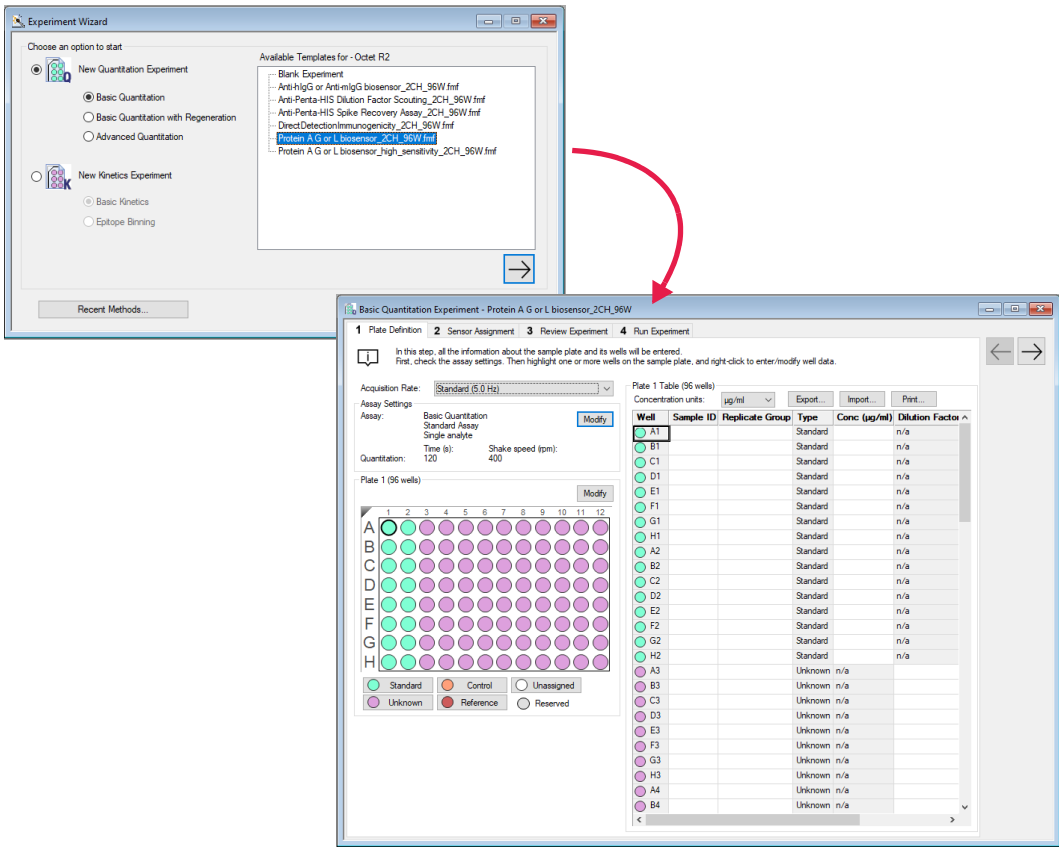


Figure 5-1: Selecting an Experiment Type in the Experiment Wizard

5. Click the → 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

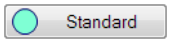
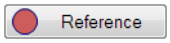
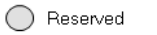
Step	See Page
1. Designate the samples.	86
2. Annotate the samples (optional).	98
3. Save the sample plate definition (optional).	104

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

Icon	Description
	Contains an analyte of known concentration. Data from the well is used to generate a standard curve during analysis.
	Contains an analyte of unknown concentration. The concentration of the analyte is calculated from the well data and the standard curve.
	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
	Provides a baseline signal which serves as a reference signal for Standard, Unknown, and Control. The reference signal can be subtracted during data acquisition in the Runtime Binding Chart and during data analysis.
	Not used during the experiment.
	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, Controls, 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 (R, N, D, or C), 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
R Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).
N Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.
D 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
C Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of interest onto the biosensor.

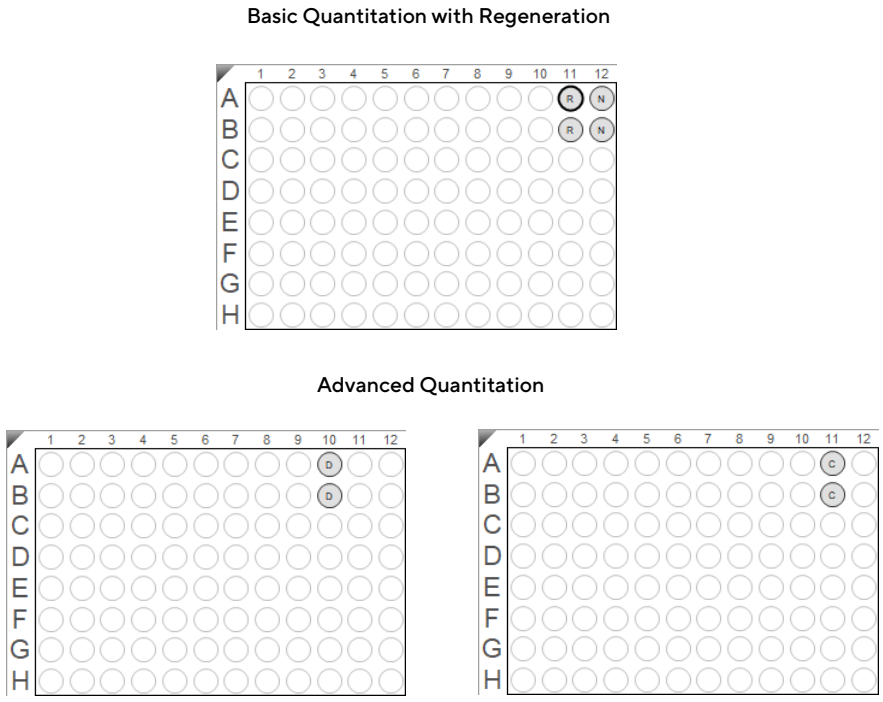


Figure 5-2: Default Locations for Reserved Wells in a 96-Well Sample Plate Map

Selecting Wells in the Sample Plate Map

NOTICE: *For the Octet® R2, Octet® R4, or Octet® 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 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 (Figure 5-3, center).
- Click a well or draw a box around a group of wells (Figure 5-3, right).

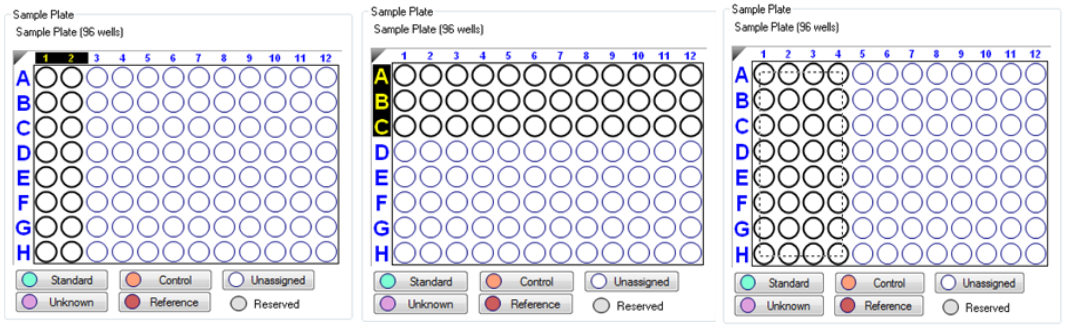


Figure 5-3: Selecting Wells in the Sample Plate Map

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.
2. 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**.

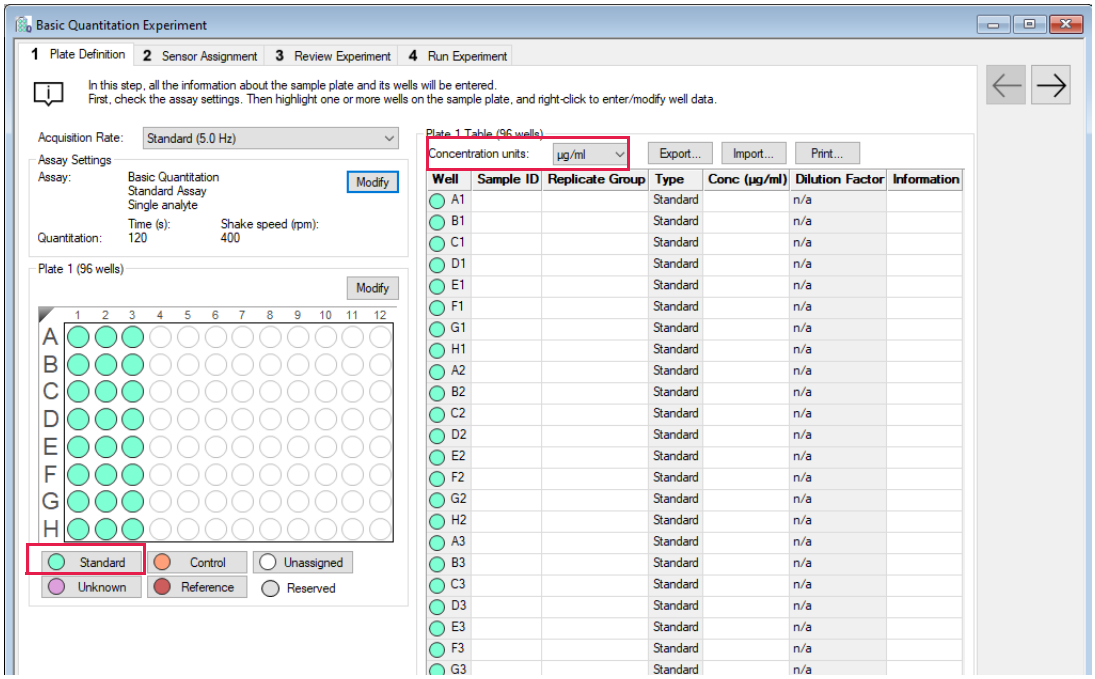


Figure 5-4: Plate Definition Window—Designating Standards

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).

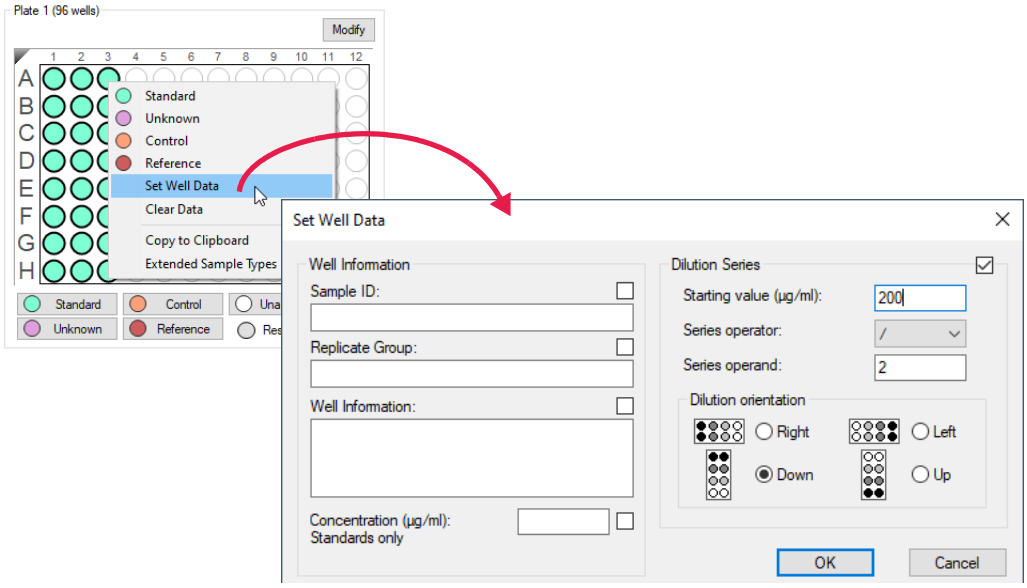


Figure 5-5: Sample Plate Map—Setting a Dilution Series

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).

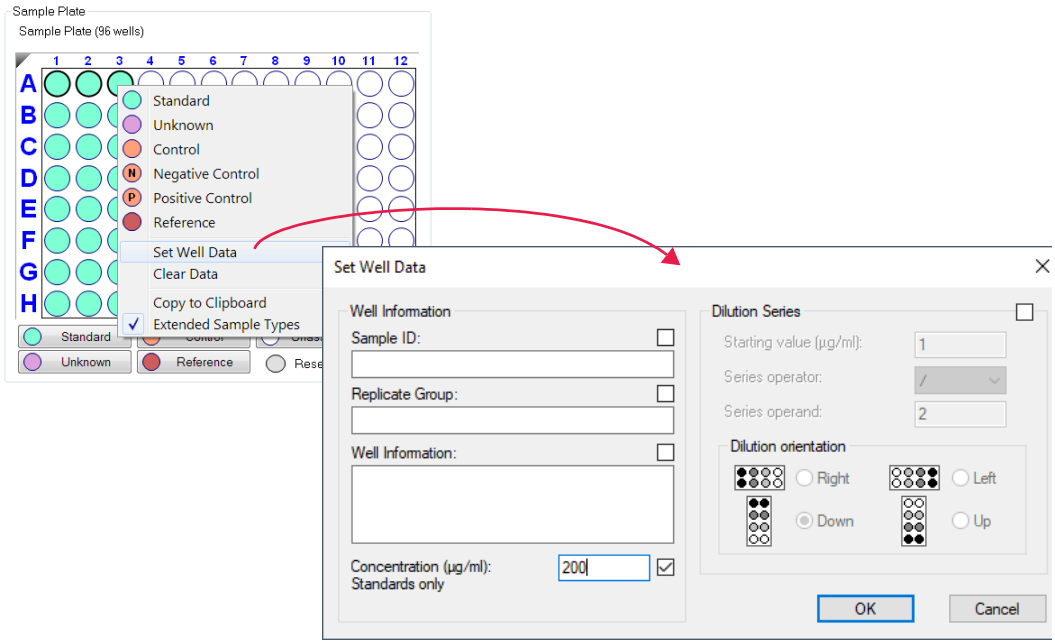


Figure 5-7: Sample Plate Map—Assigning a Standard Concentration

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).

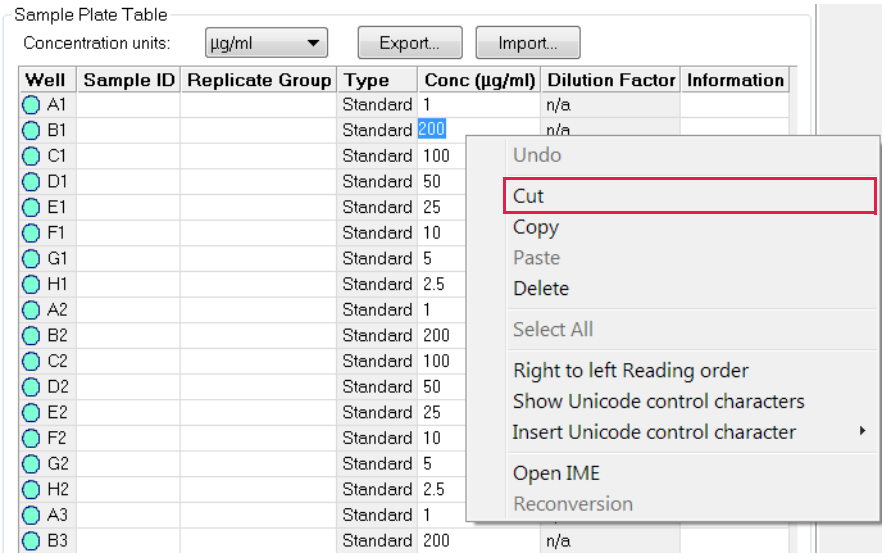


Figure 5-8: Sample Plate Table—Shortcut Menu of Edit Commands

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).

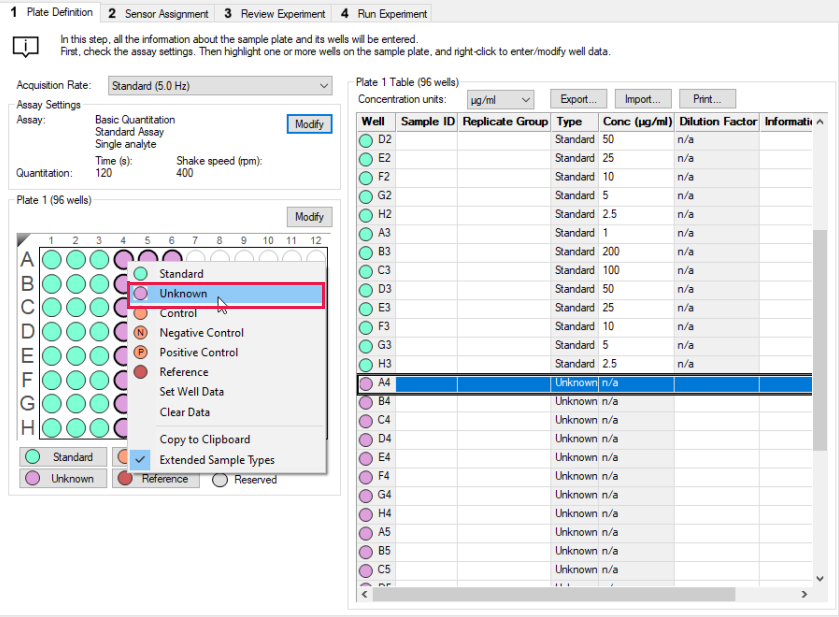


Figure 5-9: Plate Definition Window—Designate Unknown Wells

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).

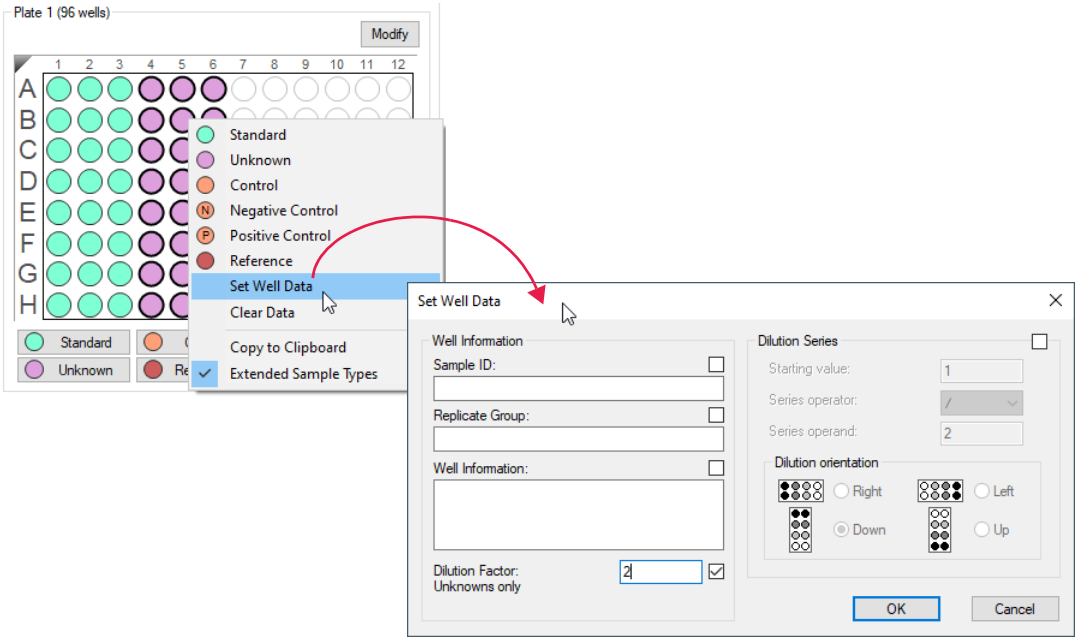


Figure 5-10: Sample Plate Map—Setting a Dilution Factor or a Serial Dilution

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).

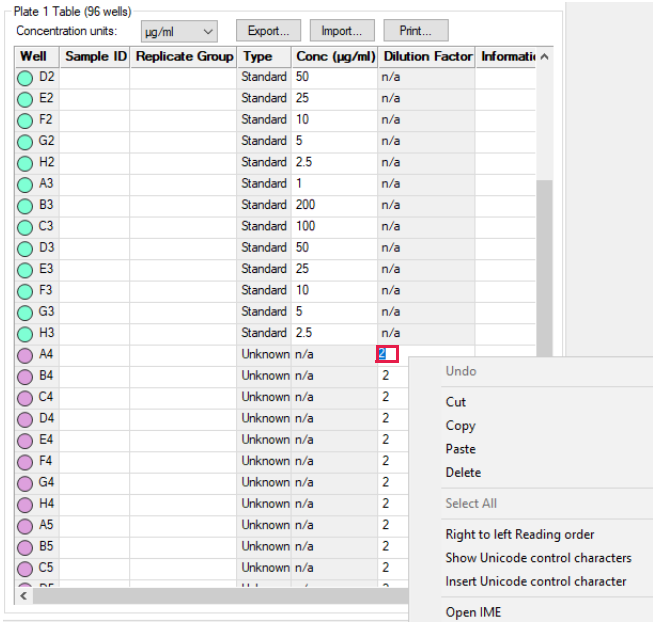


Figure 5-12: Sample Plate Table—Shortcut Menu of Edit Commands

***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).

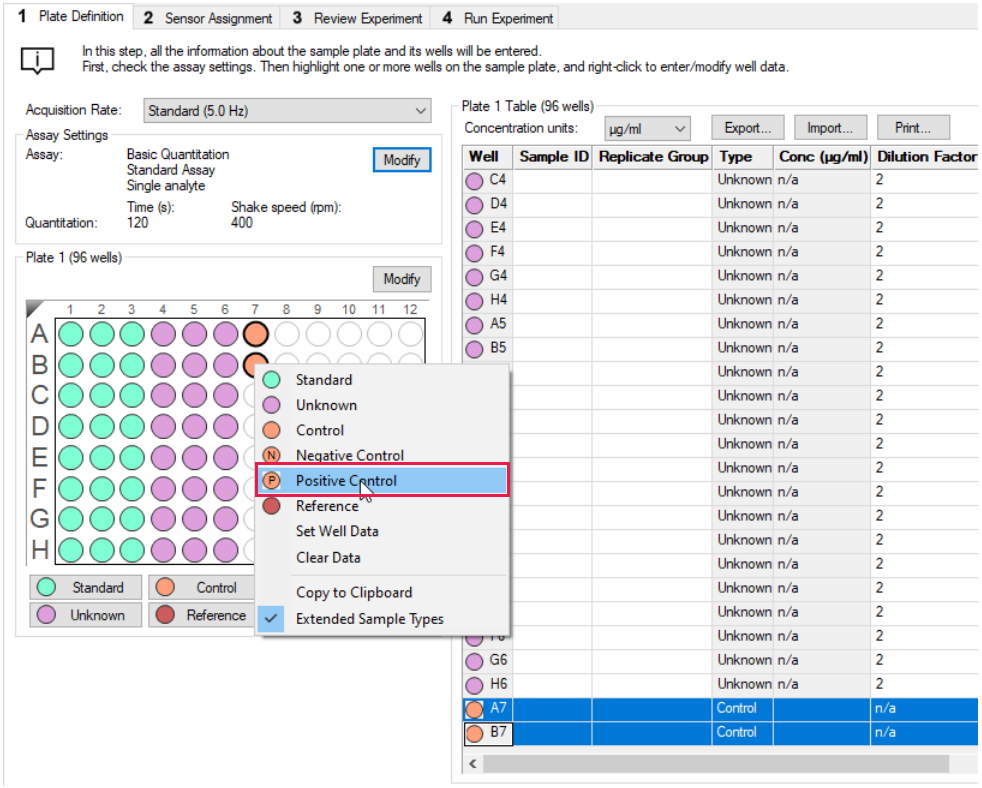


Figure 5-13: Designate Controls or Reference Wells

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**.

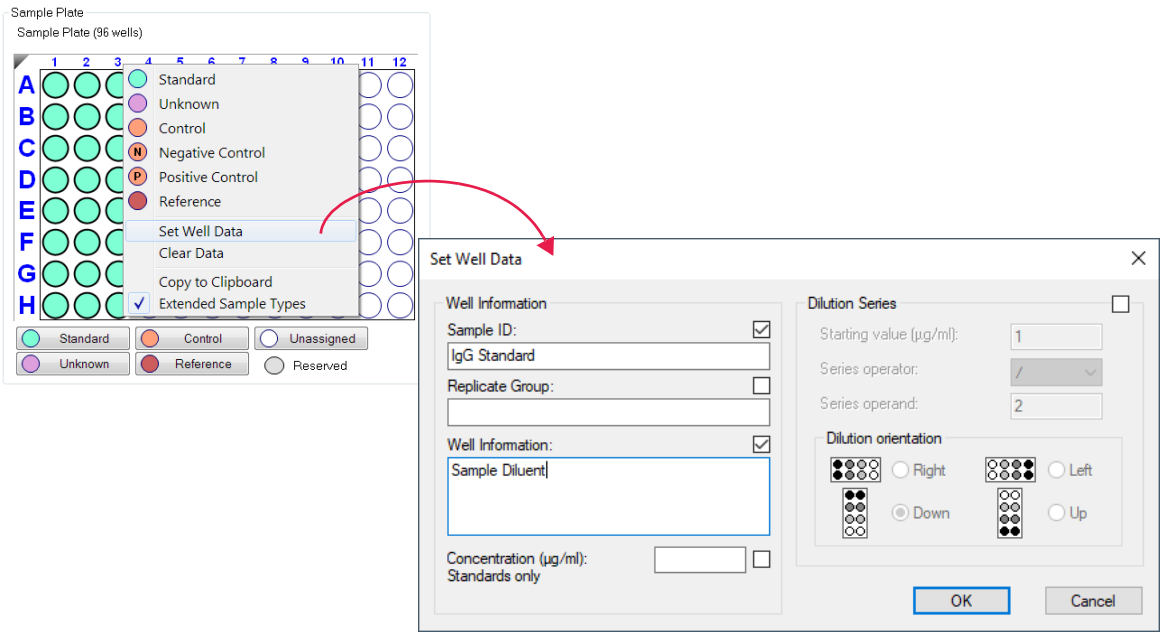


Figure 5-14: Adding Sample Annotations from the Sample Plate Map

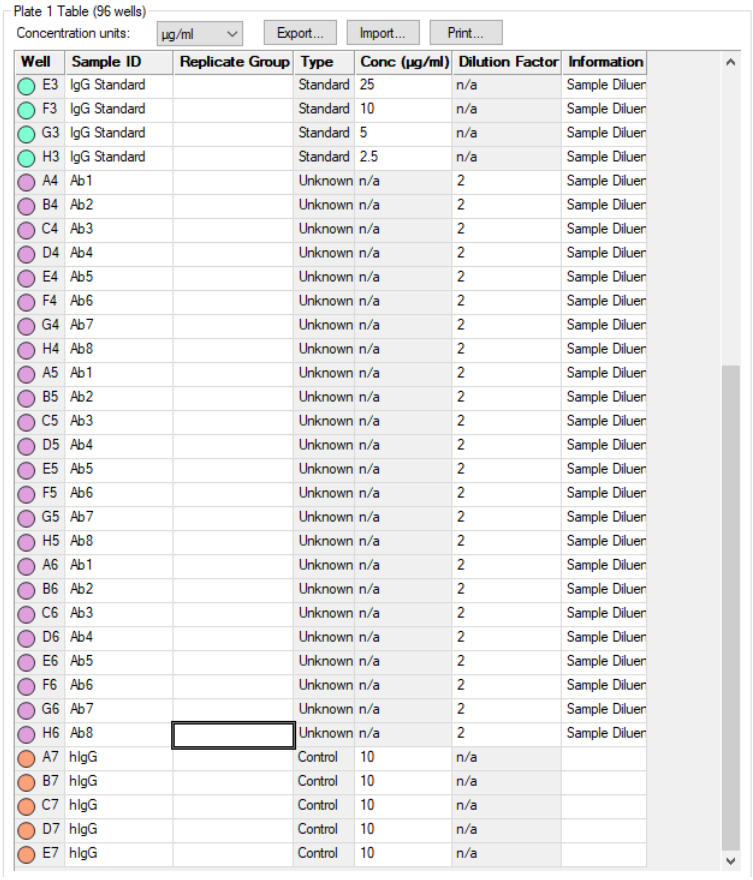
Annotating Wells in the Sample Plate Table

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**.



Well	Sample ID	Replicate Group	Type	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
H3	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	hIgG		Control	10	n/a	
B7	hIgG		Control	10	n/a	
C7	hIgG		Control	10	n/a	
D7	hIgG		Control	10	n/a	
E7	hIgG		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
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**.

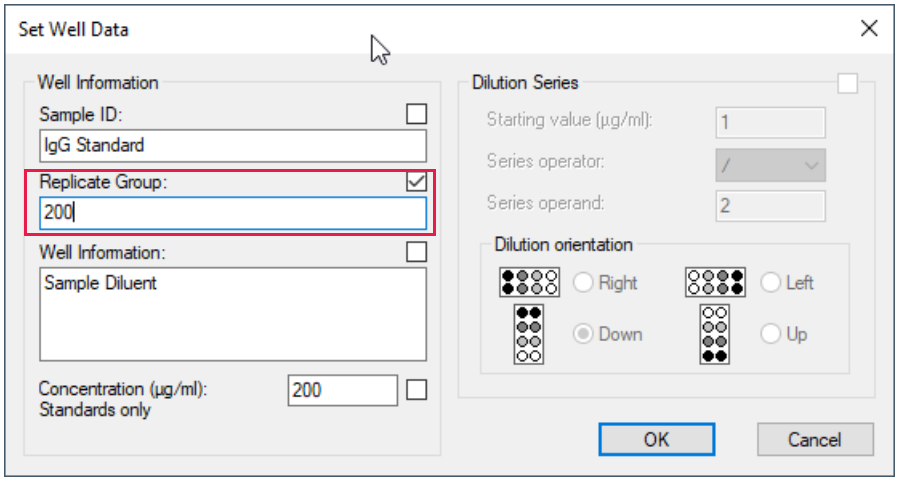


Figure 5-17: Add Replicate Group from the Sample Plate Map

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).

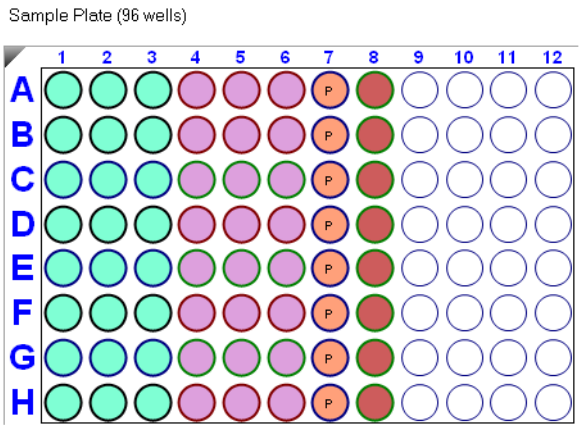
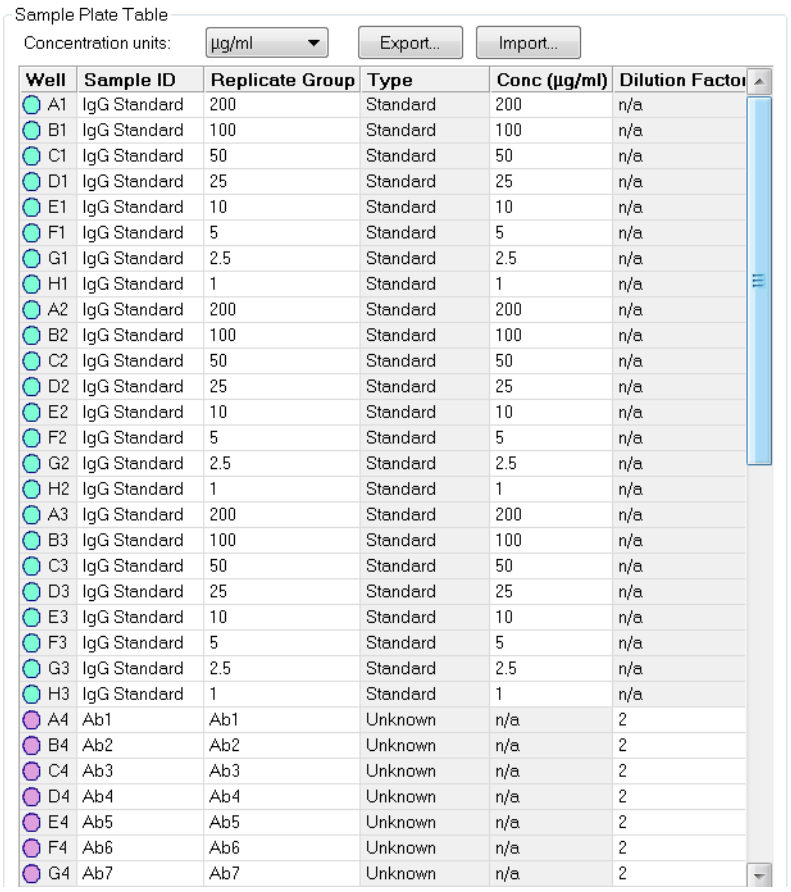


Figure 5-18: Replicate Groups in Sample Plate Map

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


Sample Plate Table

Concentration units: µg/ml Export... Import...

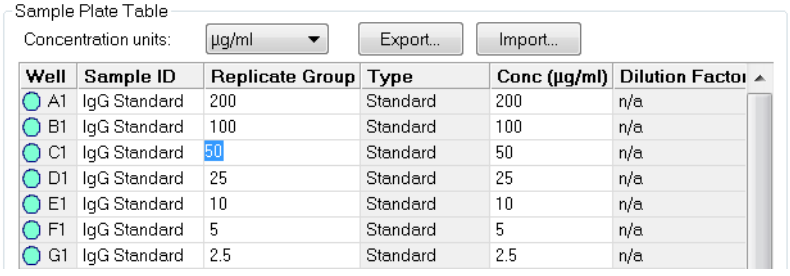
Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG Standard	2.5	Standard	2.5	n/a
H1	IgG Standard	1	Standard	1	n/a
A2	IgG Standard	200	Standard	200	n/a
B2	IgG Standard	100	Standard	100	n/a
C2	IgG Standard	50	Standard	50	n/a
D2	IgG Standard	25	Standard	25	n/a
E2	IgG Standard	10	Standard	10	n/a
F2	IgG Standard	5	Standard	5	n/a
G2	IgG Standard	2.5	Standard	2.5	n/a
H2	IgG Standard	1	Standard	1	n/a
A3	IgG Standard	200	Standard	200	n/a
B3	IgG Standard	100	Standard	100	n/a
C3	IgG Standard	50	Standard	50	n/a
D3	IgG Standard	25	Standard	25	n/a
E3	IgG Standard	10	Standard	10	n/a
F3	IgG Standard	5	Standard	5	n/a
G3	IgG Standard	2.5	Standard	2.5	n/a
H3	IgG Standard	1	Standard	1	n/a
A4	Ab1	Ab1	Unknown	n/a	2
B4	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

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG 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**.

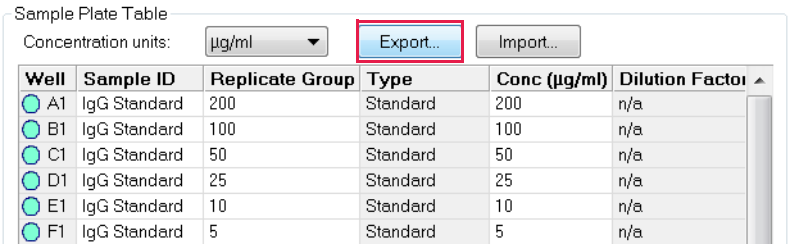


Figure 5-21: Export Button in Sample Plate Table

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

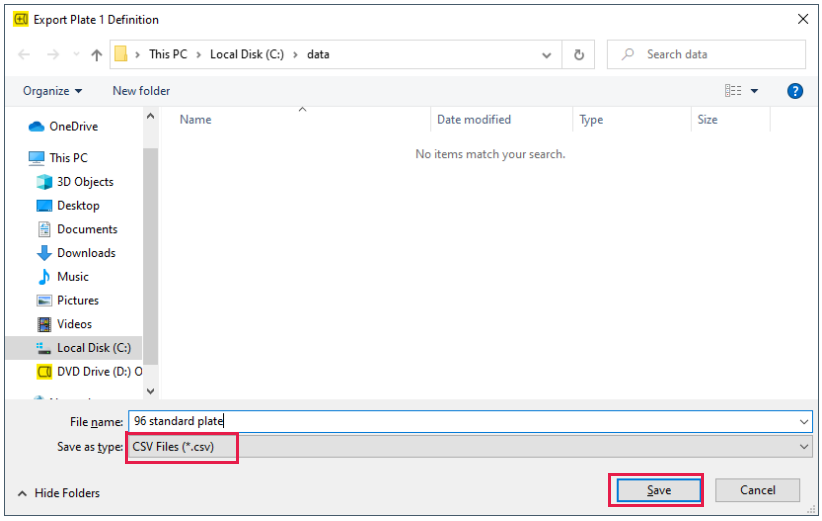


Figure 5-22: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

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

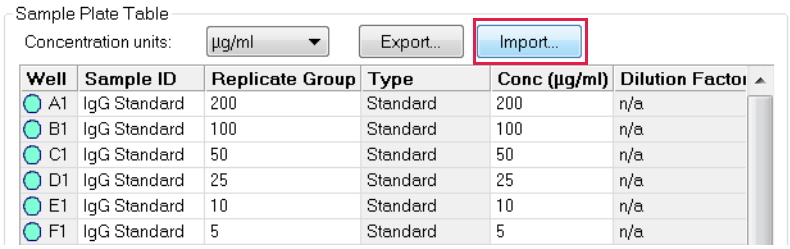


Figure 5-23: Import Button in Sample Plate Table

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

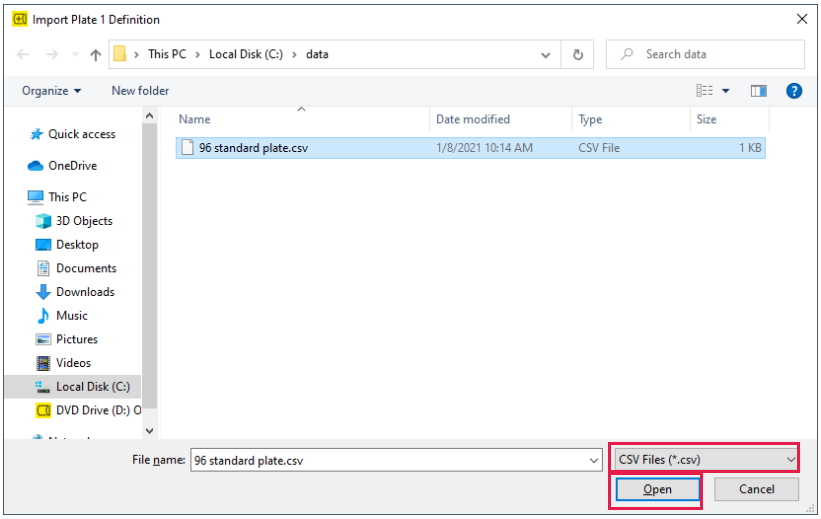
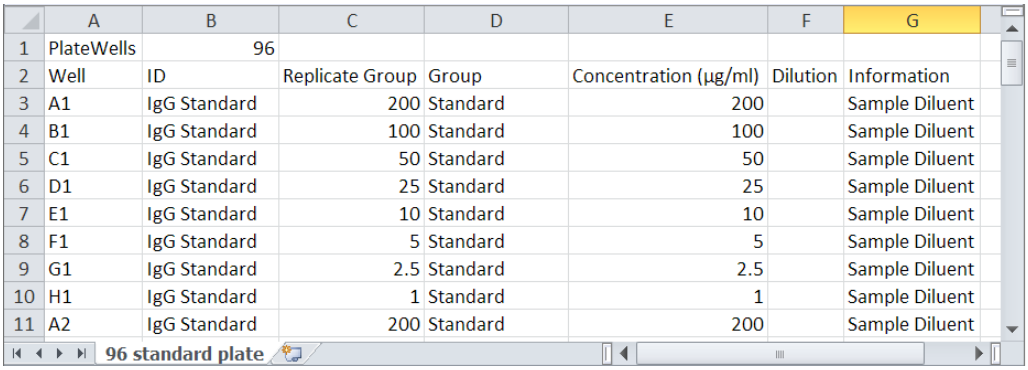


Figure 5-24: Import Plate Definition Window

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



	A	B	C	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

96 standard plate

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.

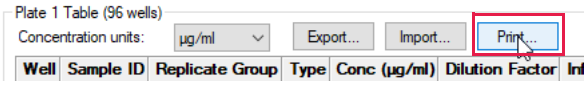


Figure 5-26: Sample Plate Print Button

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.

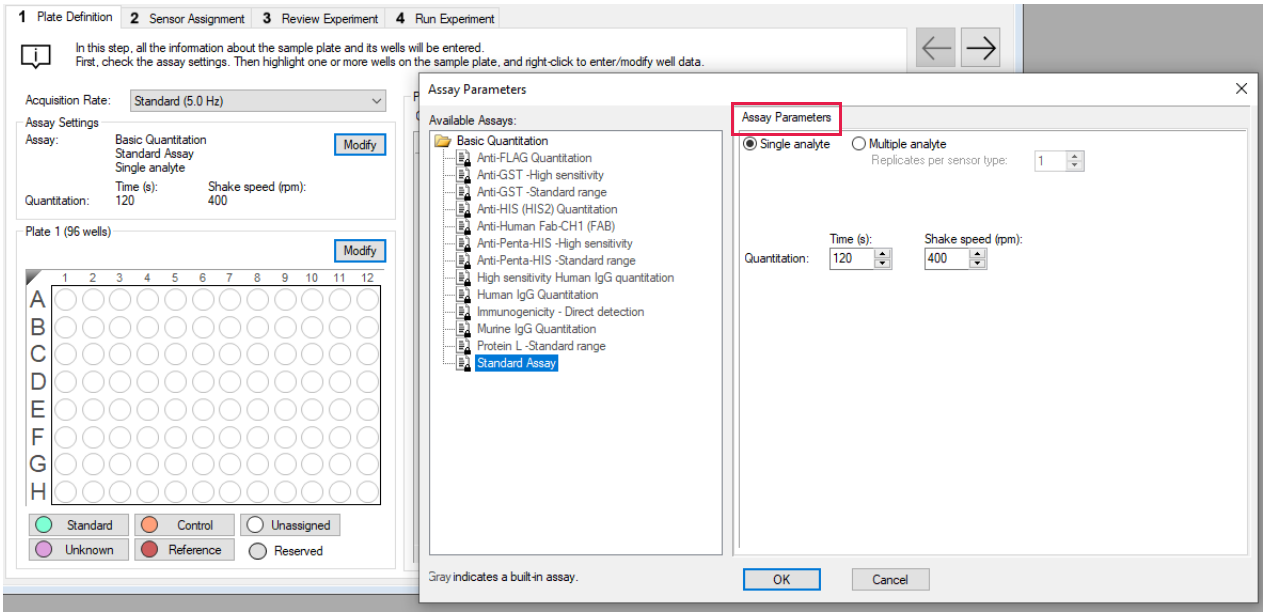


Figure 5-27: Modifying Assay Parameters

Basic Quantitation Assay Parameters

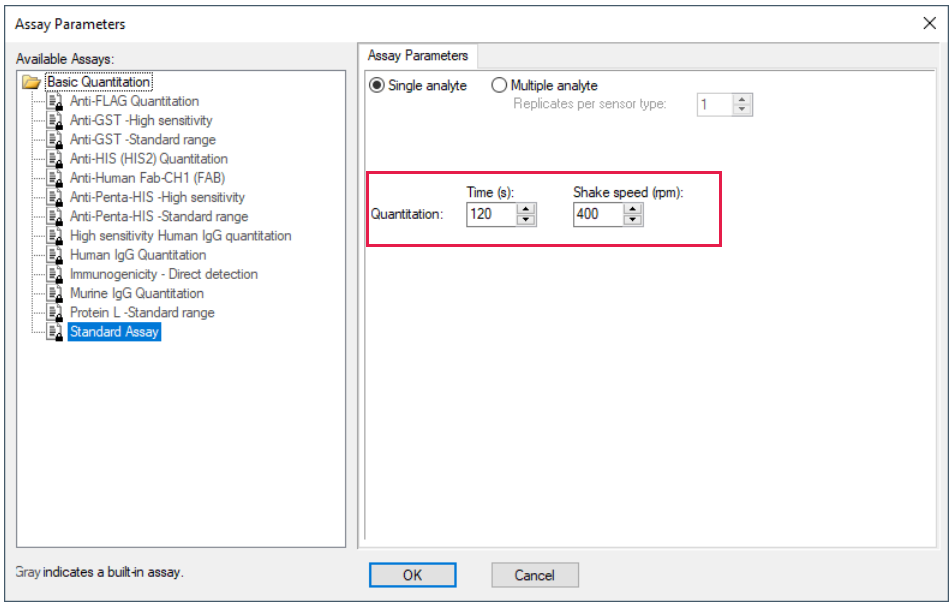


Figure 5-28: Assay Parameters—Basic Quantitation Assay

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 Replicates 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. ***NOTICE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

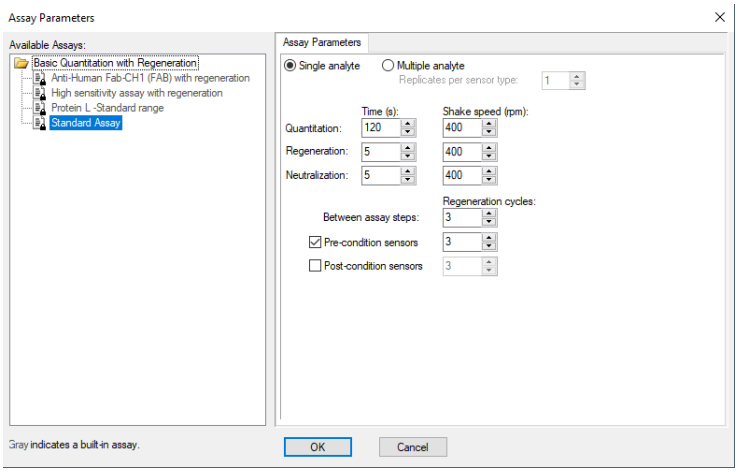


Figure 5-29: Assay Parameters—Basic Quantitation with Regeneration

Table 5-7: 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 Replicates 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). ***NOTICE:*** *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 regenerated 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.

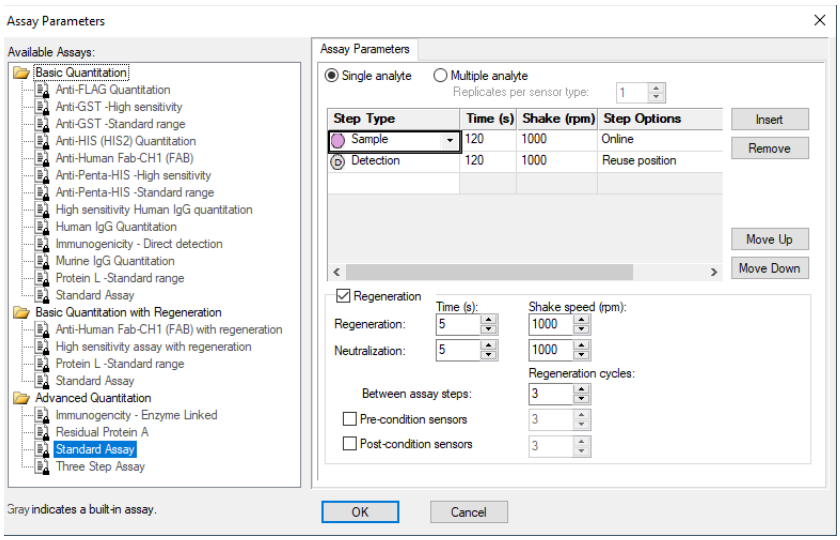


Figure 5-30: Assay Parameters—Advanced Quantitation

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 → arrow to access the Sensor Assignment window (see Figure 5-31).

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

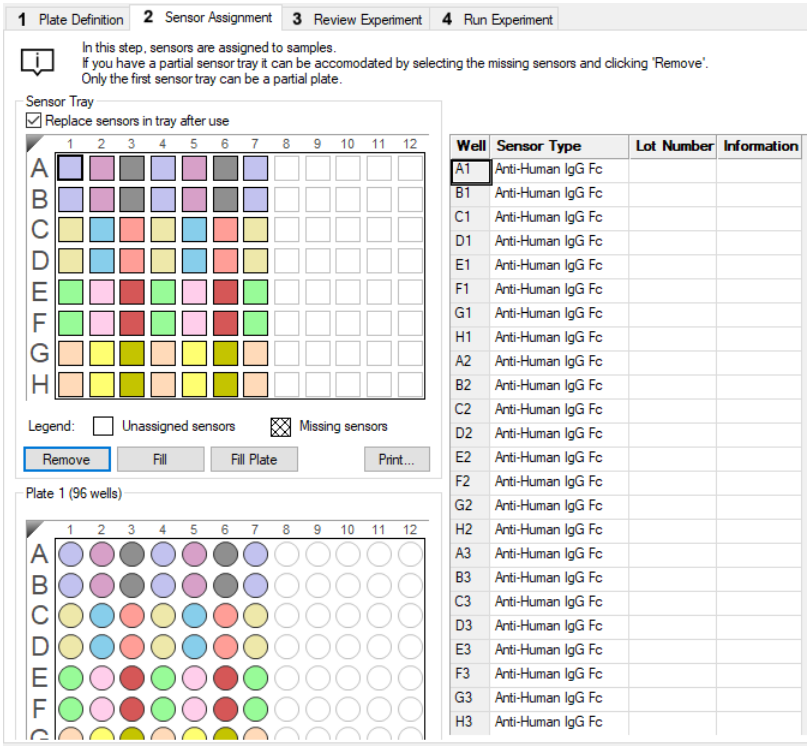


Figure 5-31: Sensor Assignment Window for Basic Quantitation without Regeneration

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.

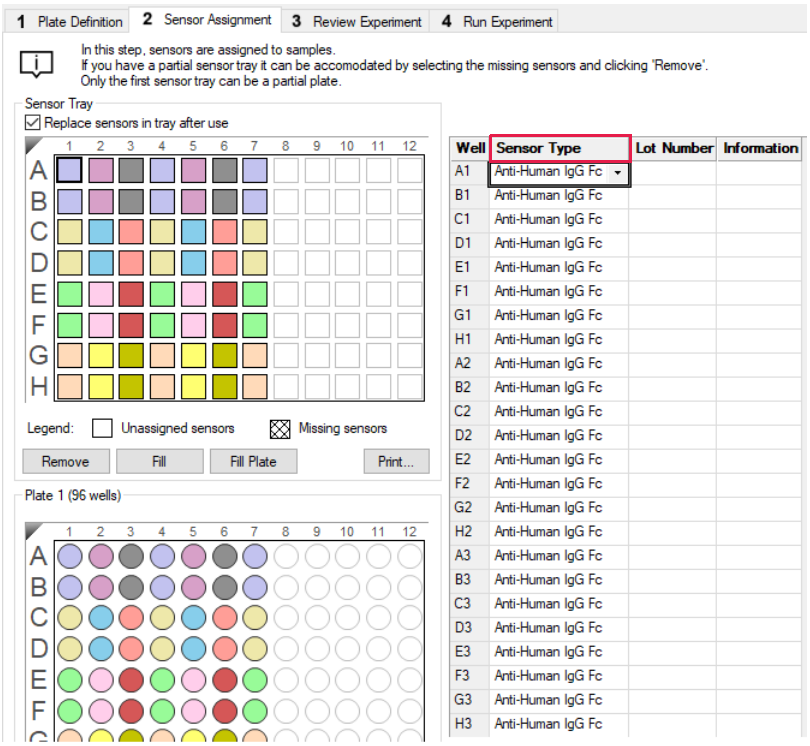


Figure 5-32: Changing Biosensor Types

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® R2, Octet® R4, or Octet® 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).

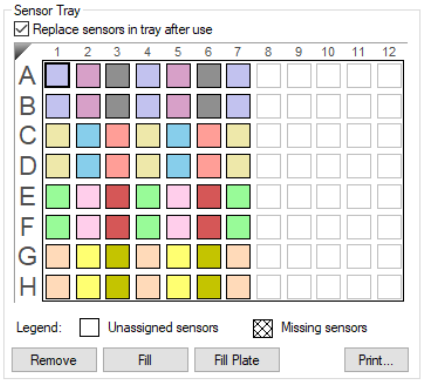


Figure 5-33: Replace Sensors in Tray After Use Check Box

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 → 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.

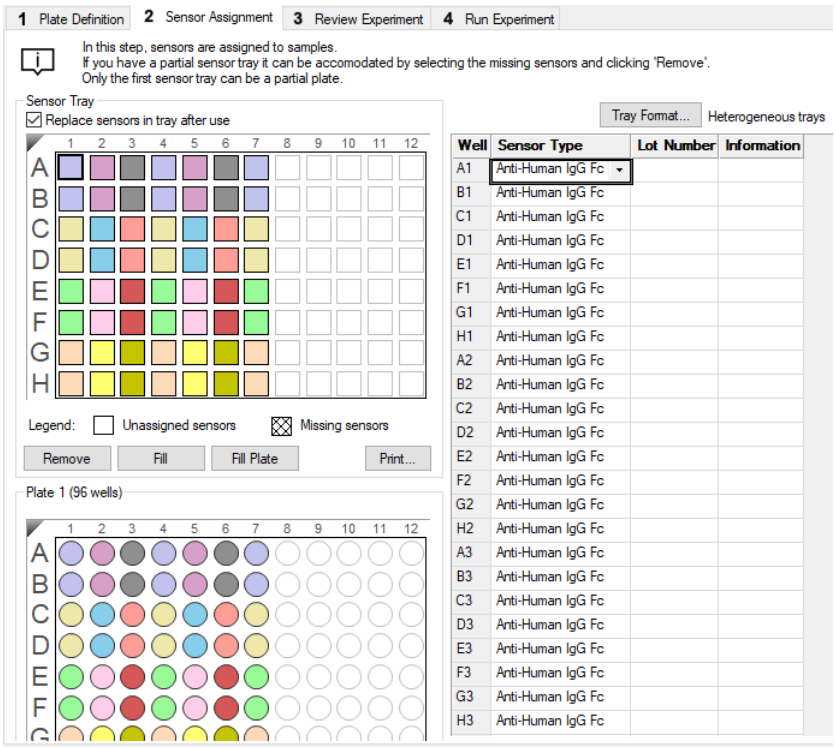


Figure 5-34: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

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 drop-down list (see Figure 5-35).

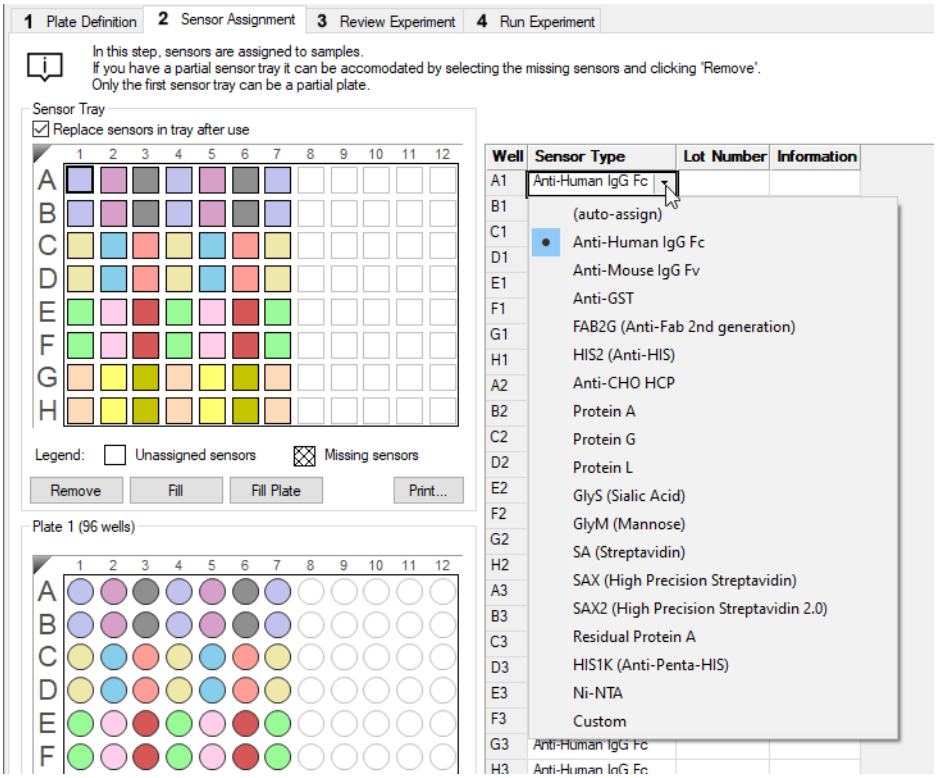


Figure 5-35: Changing Biosensor Types

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.*

1. 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**.

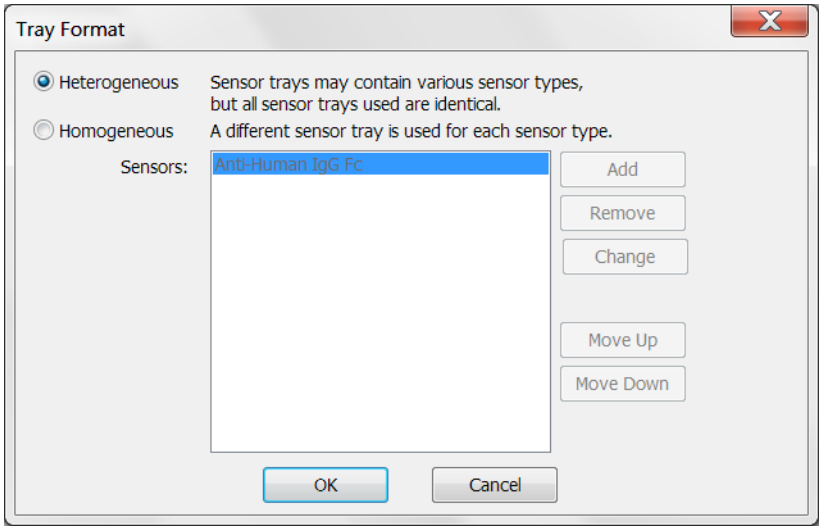


Figure 5-36: Tray Format Dialog Box

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.

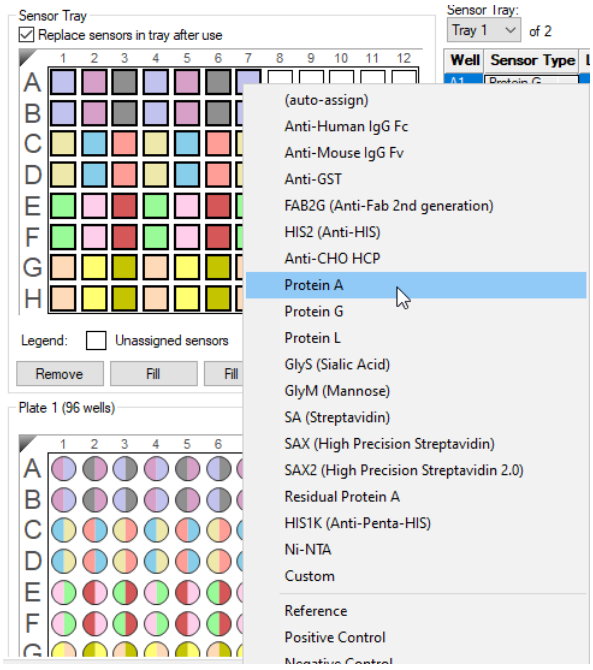


Figure 5-37: Populating the Sensor Tray Map with First Biosensor Type

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.

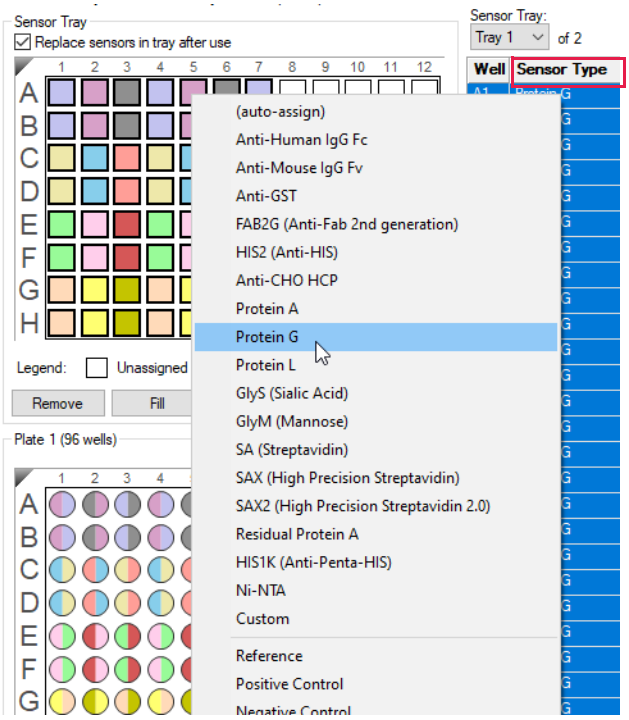


Figure 5-38: Populating the Sensor Tray Map with Second Biosensor Type

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.

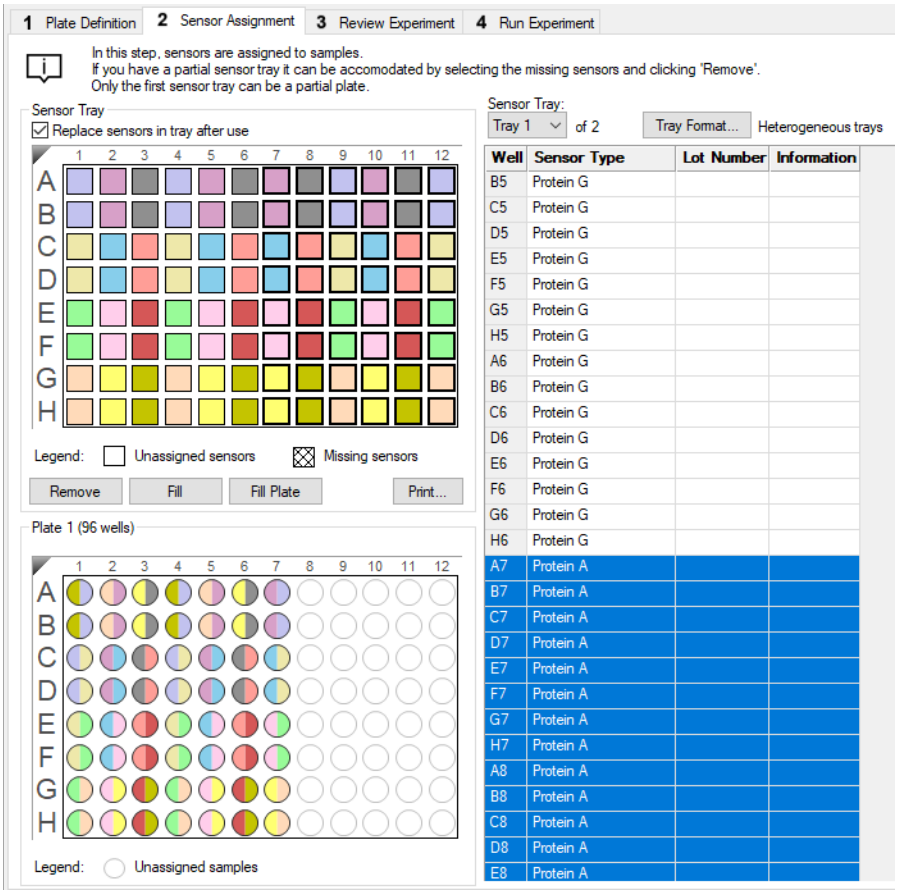


Figure 5-39: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

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).

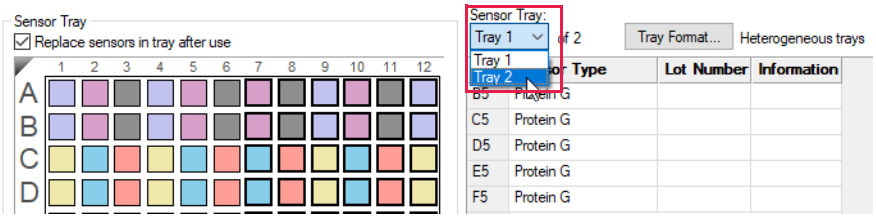


Figure 5-40: Tray Selection

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 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).

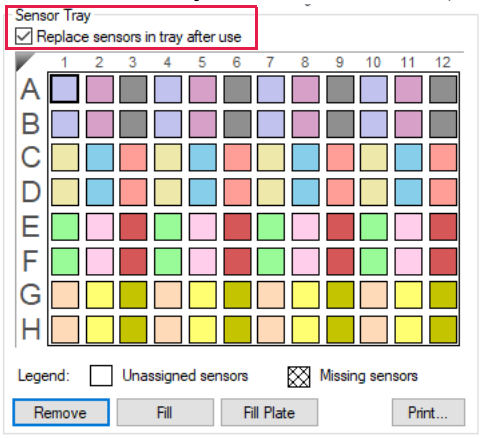


Figure 5-41: Replace Sensors in Tray After Use Check Box

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.

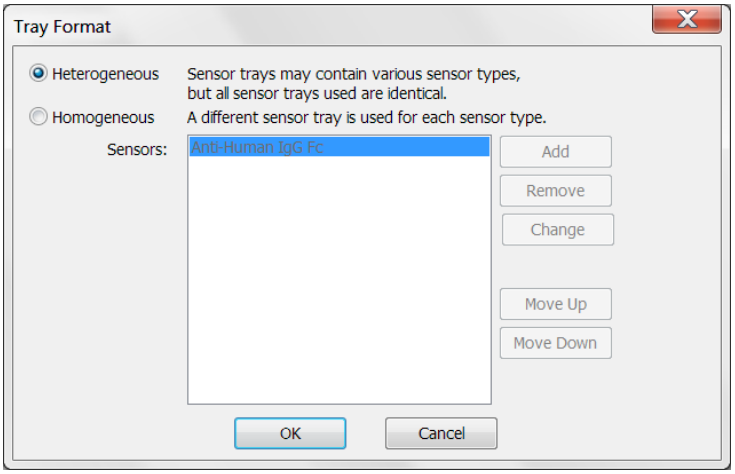


Figure 5-42: Tray Format Dialog Box

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

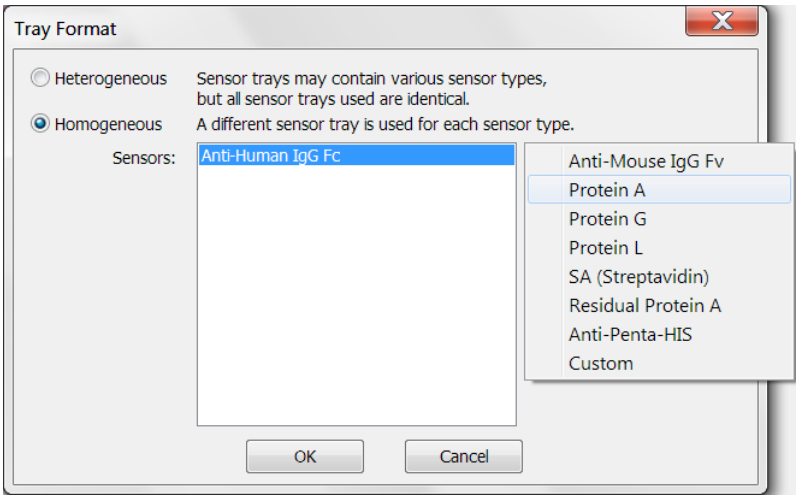


Figure 5-43: Selecting a Biosensor Type in the Tray Format Dialog Box

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.

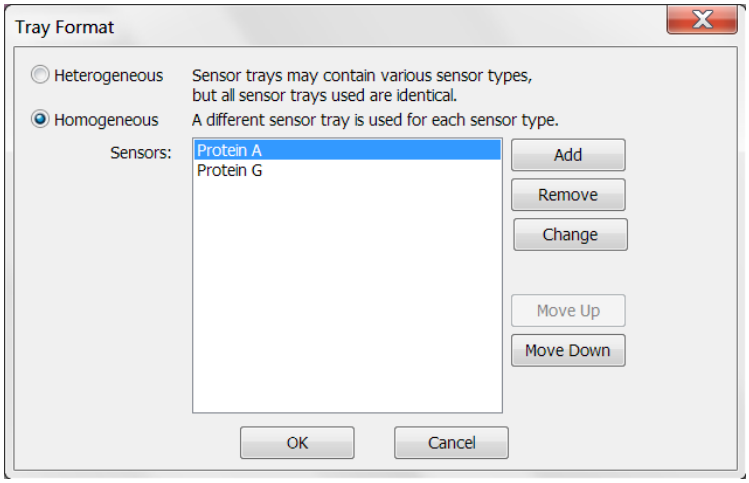


Figure 5-44: Biosensor Types List Order in Sensor Box

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.

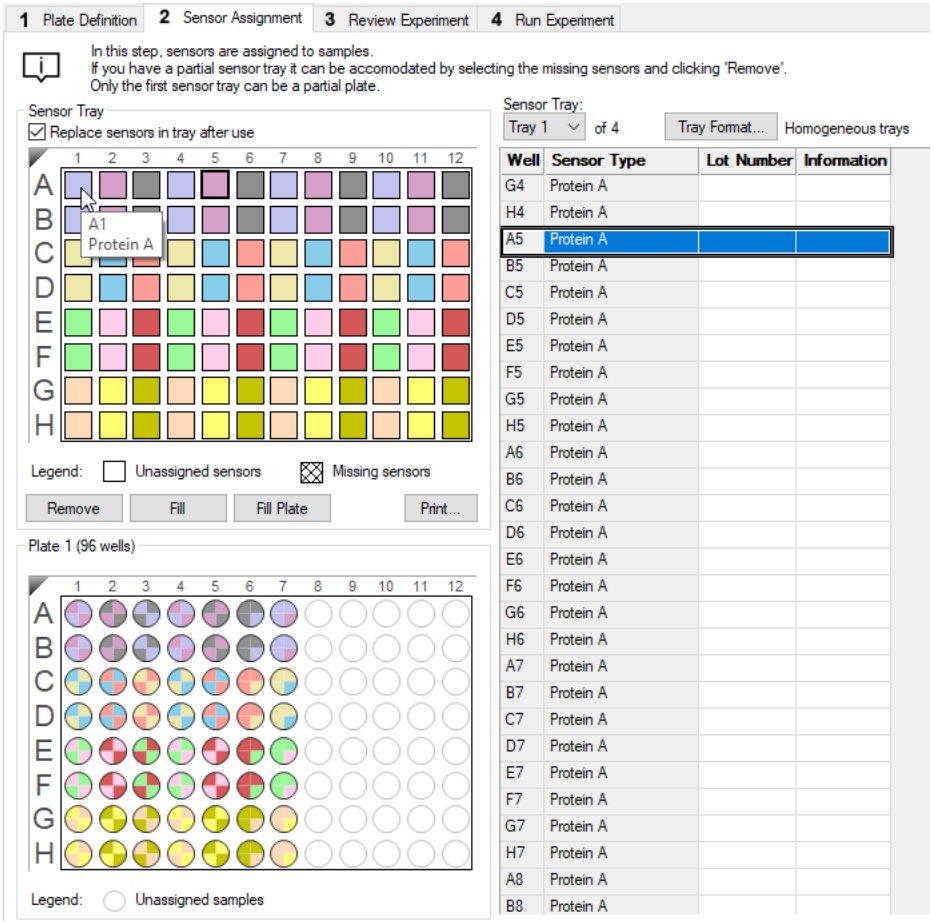


Figure 5-45: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

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

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

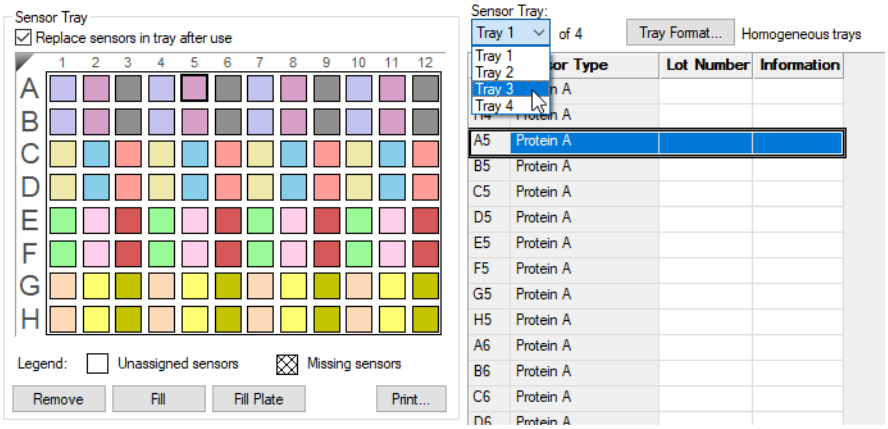


Figure 5-46: Tray Selection

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).

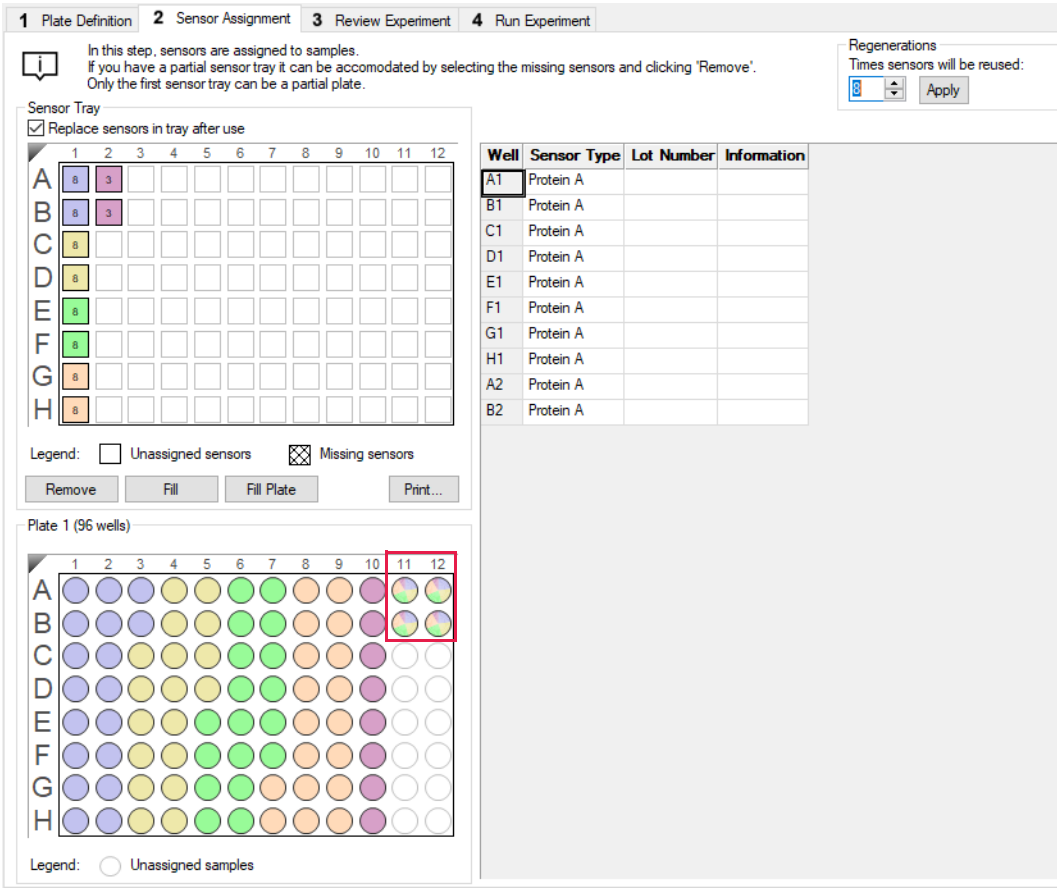


Figure 5-48: Fractional Use of Regeneration and Neutralization Wells

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).

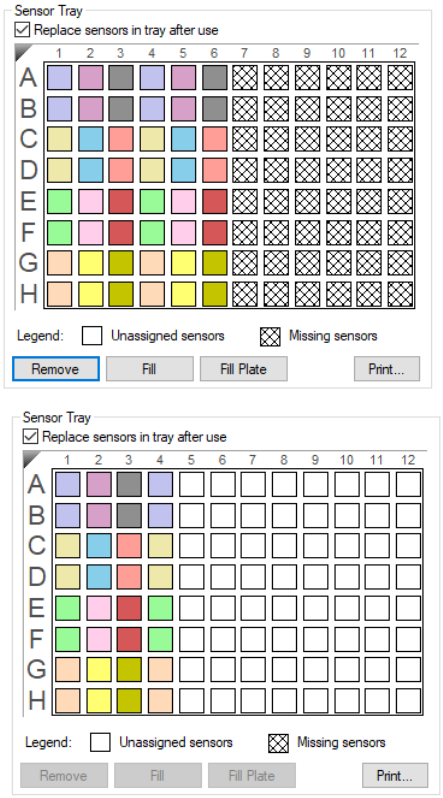


Figure 5-49: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray

   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® 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 ← → arrows to select an assay.

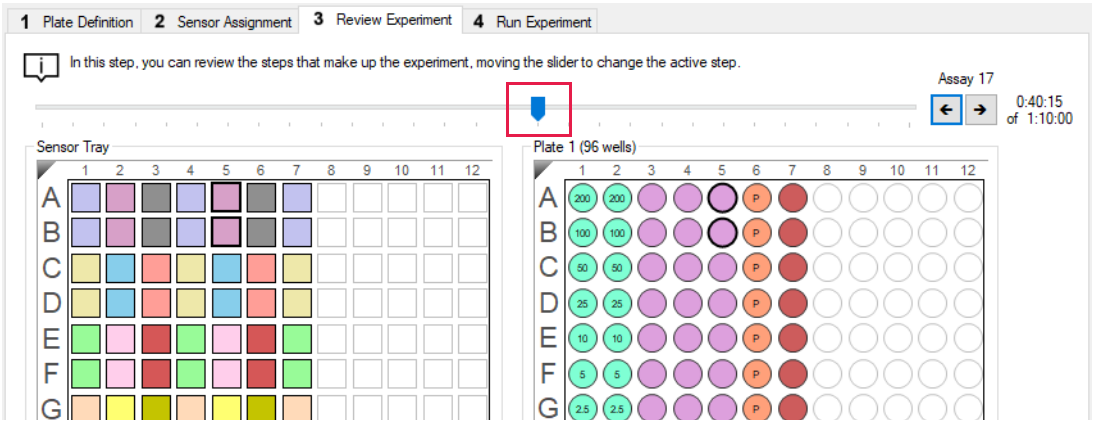


Figure 5-50: Review Experiment Window

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.*

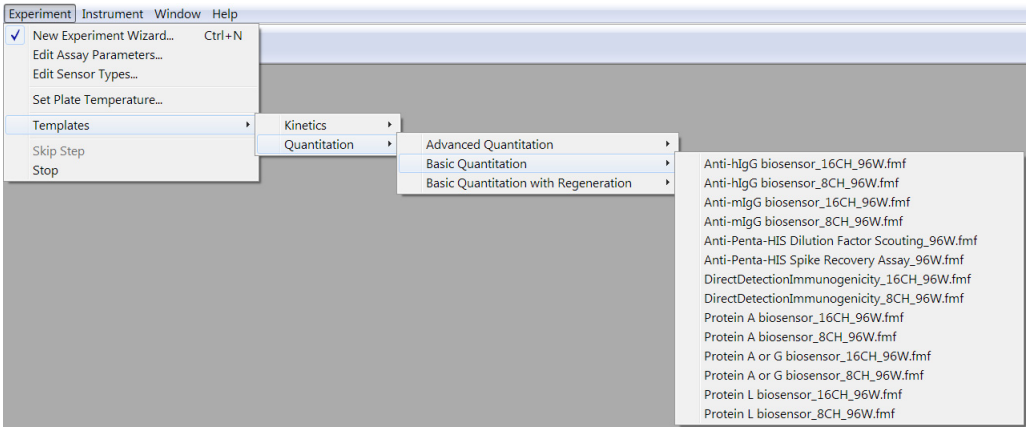


Figure 5-51: Experiments in the Template Folder

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® 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).

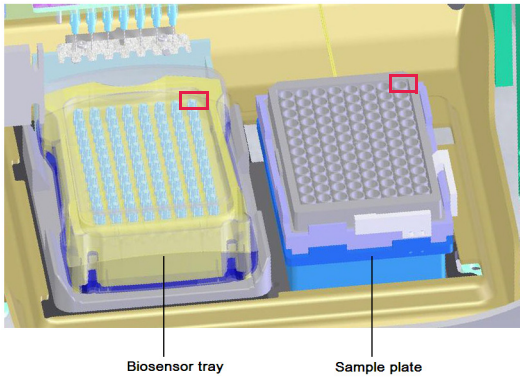


Figure 5-52: Biosensor Stage (left) and Sample Stage (right)

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

4. Close the Octet® 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).

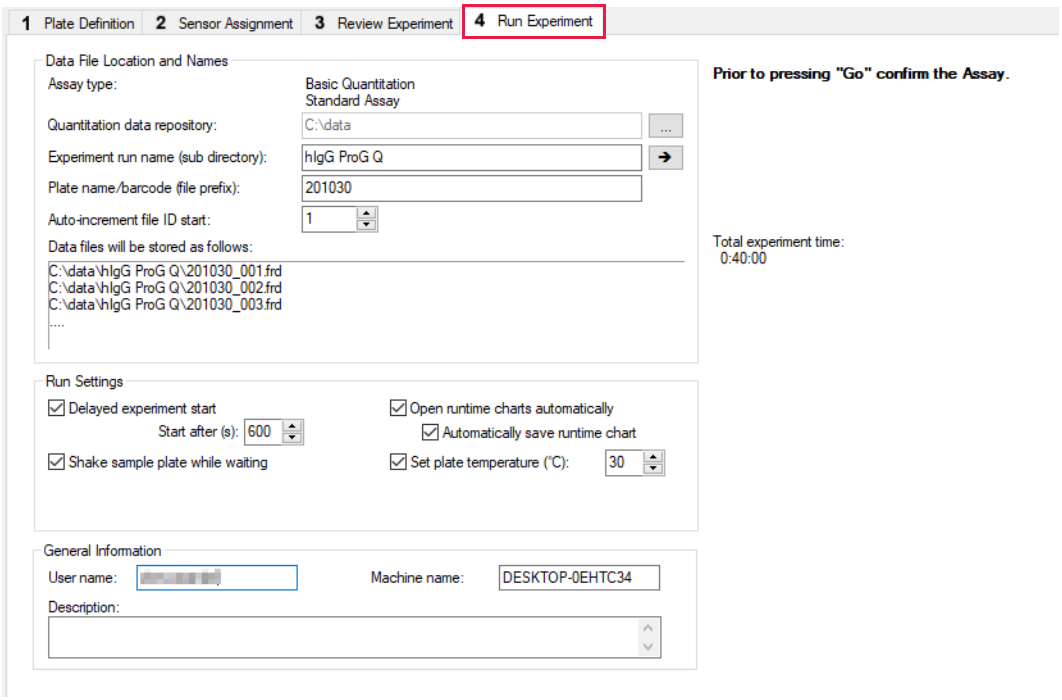


Figure 5-53: Run Experiment Window

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.*

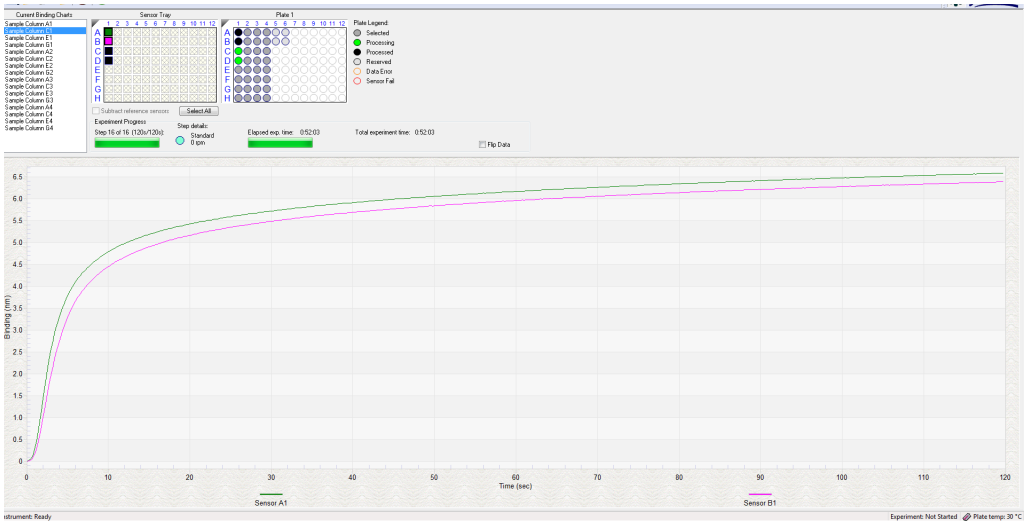


Figure 5-54: Runtime Binding Chart

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® 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® 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.*

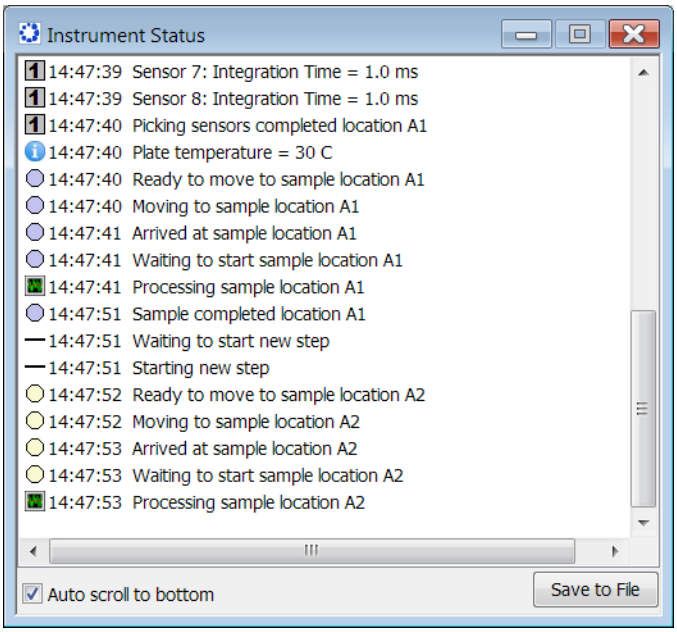


Figure 5-55: Instrument Status Log

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 **Browse** to select another data location. ***NOTICE:*** *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 **GO**.
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 **Runtime Binding Chart** for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the **Runtime Binding Chart**. 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 **File > Options**. The factory set default temperature is 30 °C. ***NOTICE:*** *If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet® 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® 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	***NOTICE:*** *For the Octet® R2, Octet® R4, or Octet® K2 system, acquisition rate settings are available on the Plate Definition Tab.* • 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. • 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. • 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.
Sensor offset (mm)	Recommended sensor offset for quantitation—3 mm. ***NOTICE:*** *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® 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 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® 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 bit-map image generated at the end of the run.*

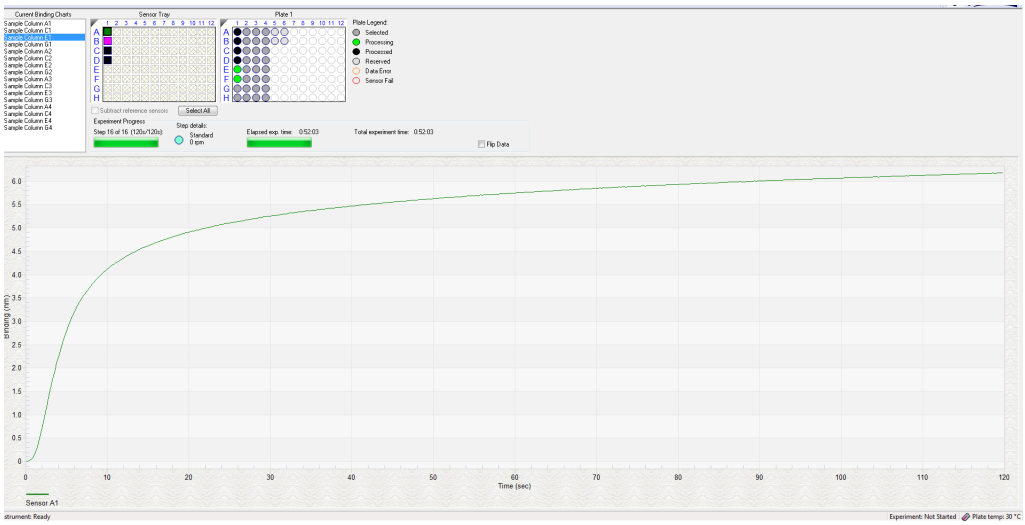


Figure 5-56: Runtime Binding Chart Window

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**.

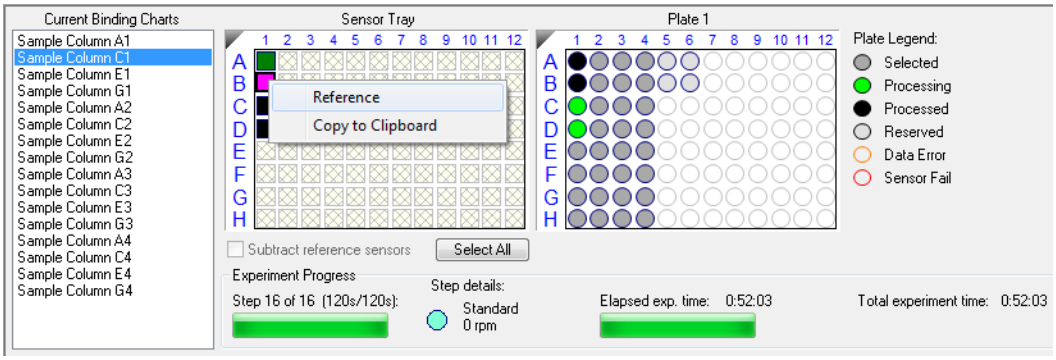


Figure 5-57: Designating a Reference Biosensor in the Runtime Binding Chart

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.

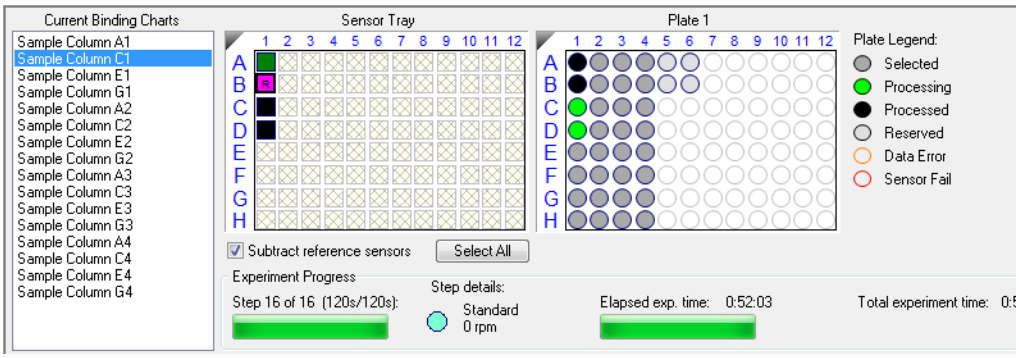


Figure 5-58: Subtract Reference Sensors check box in the Runtime Binding Chart

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.

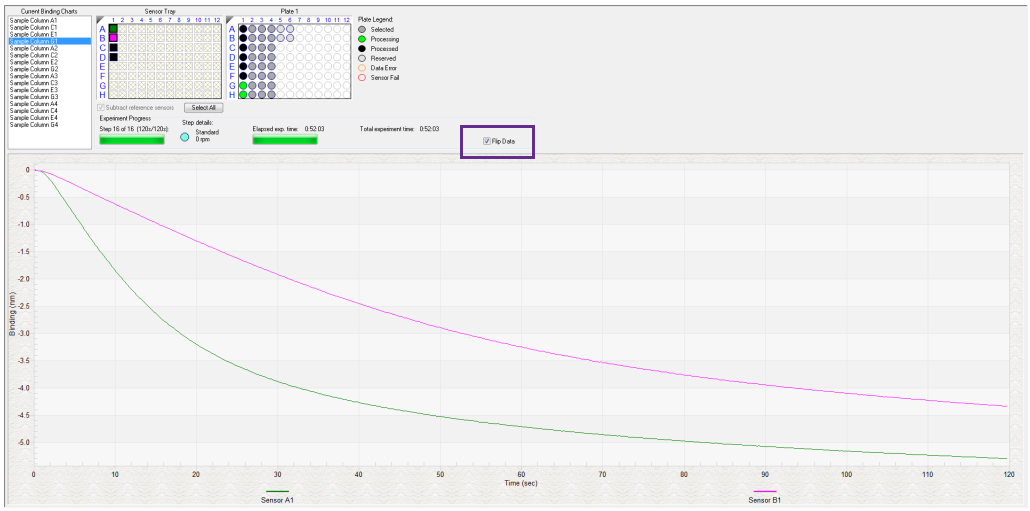


Figure 5-59: Data Inverted Using Flip Data Function

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

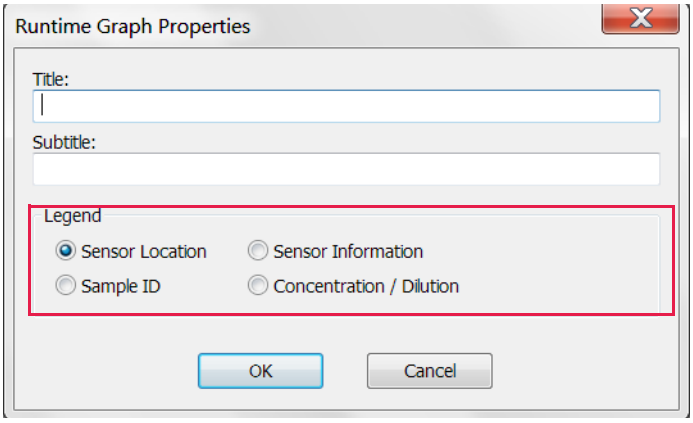


Figure 5-60: Selecting a Runtime Binding Chart Legend

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**.

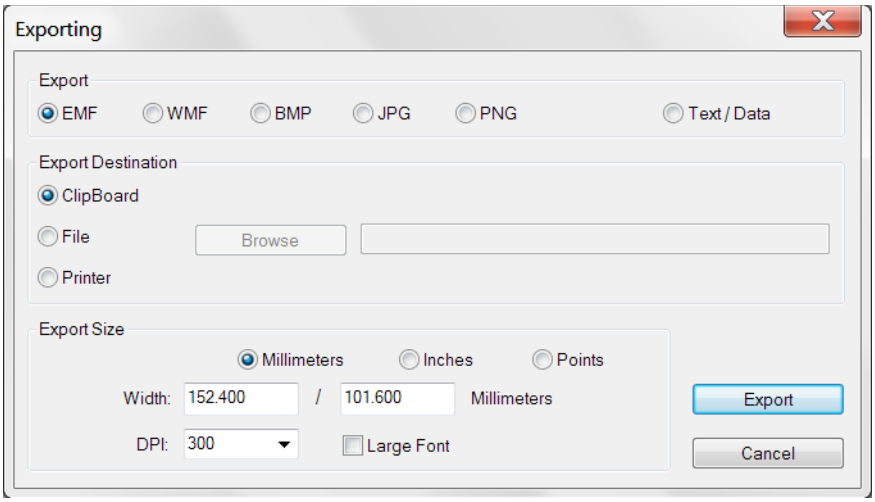


Figure 5-61: Exporting Dialog Box

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 binding data	✓		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 biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard

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

Task	Export	Option	Export Destination	Result
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

Managing Experiment Method Files

After you run an experiment, the Octet® 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® 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 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 5-62.

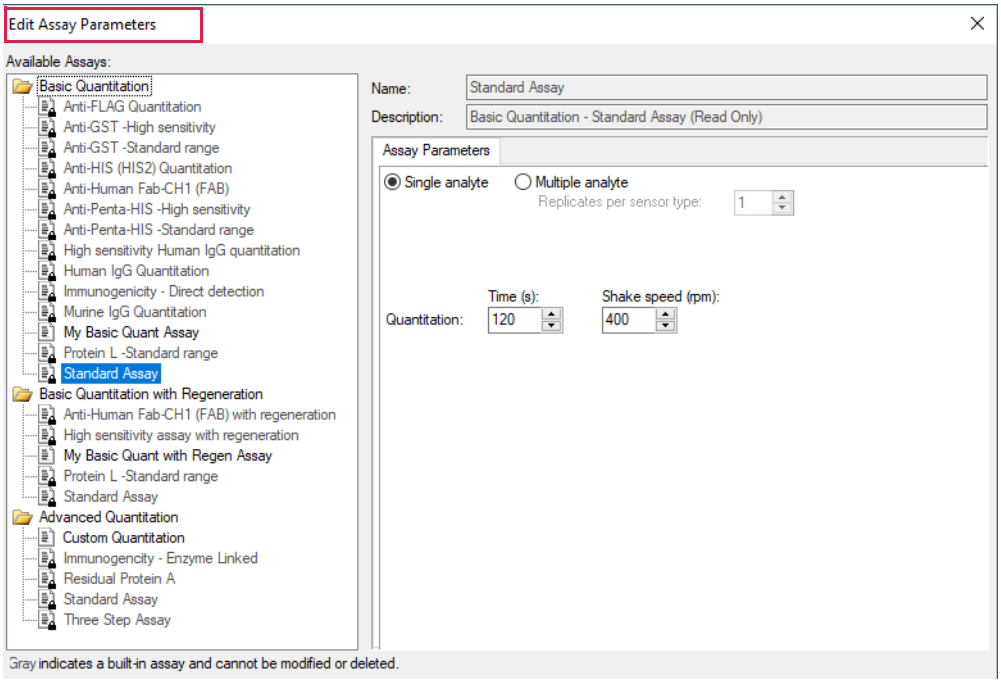


Figure 5-62: Edit Assay Parameters Dialog Box

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).

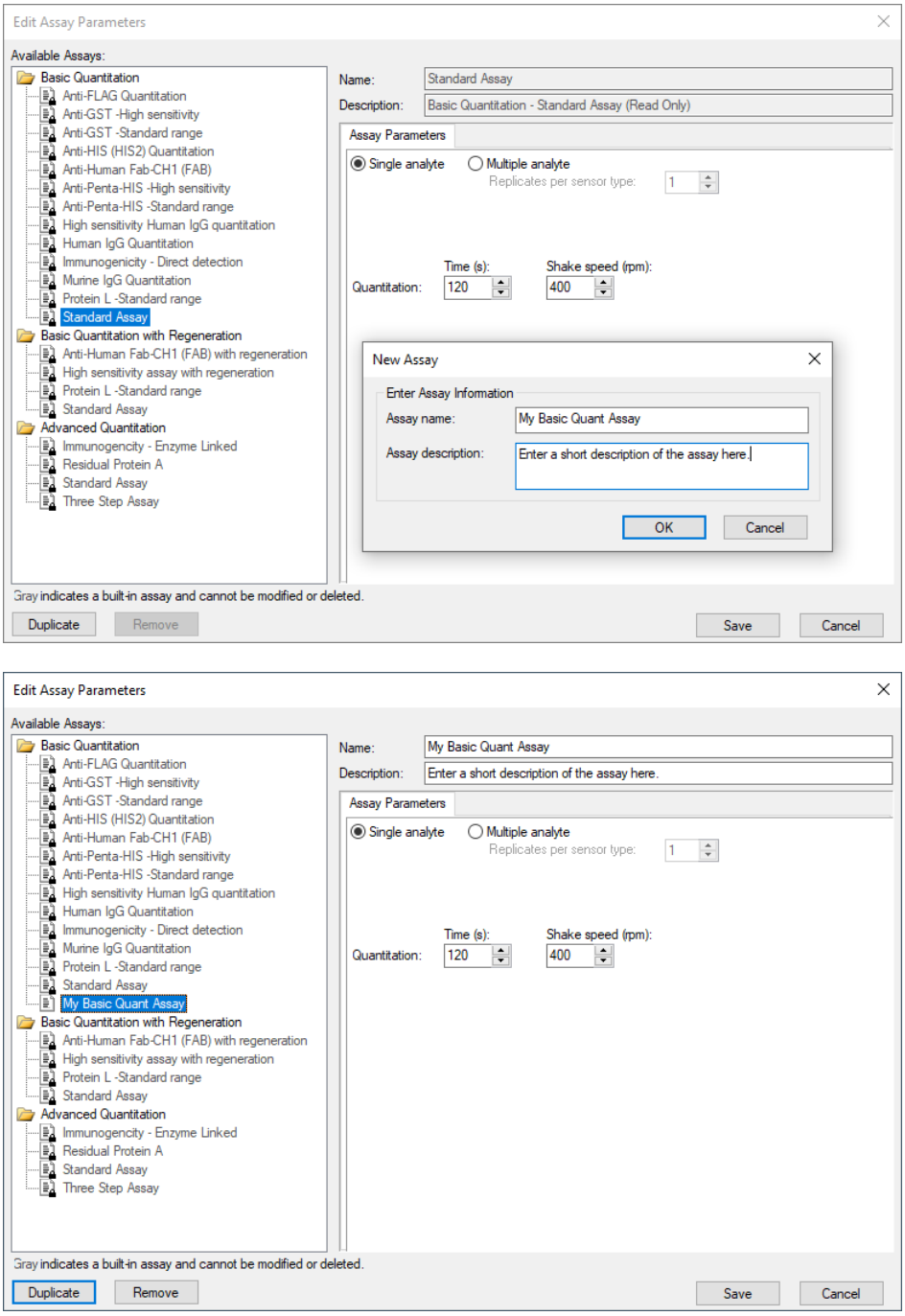


Figure 5-63: Defining a New Assay

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

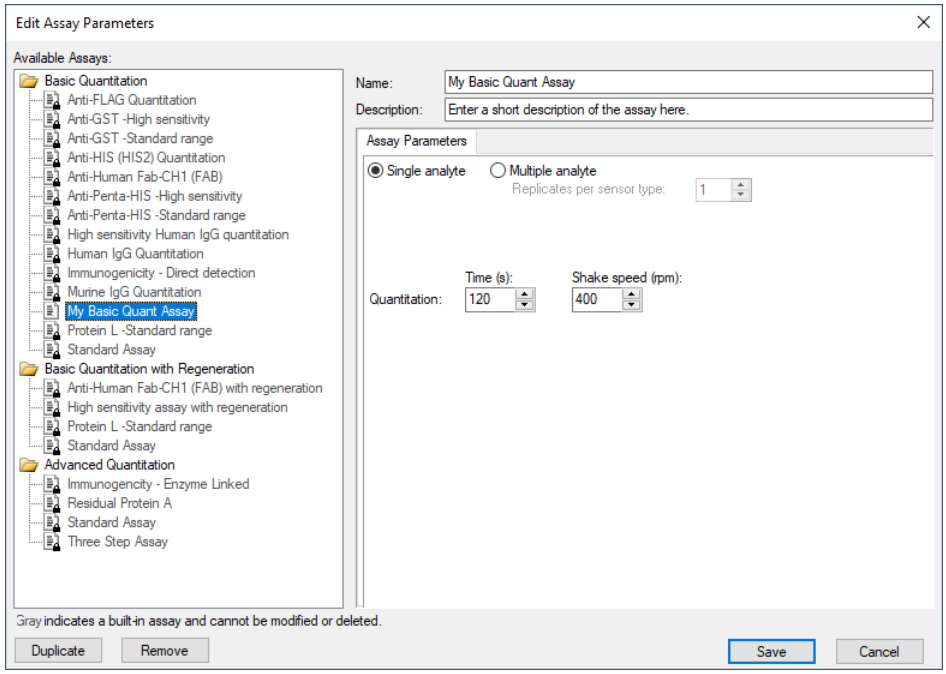


Figure 5-64: Assay Parameters—Basic Quantitation Assay

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 Replicates 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. ***NOTICE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

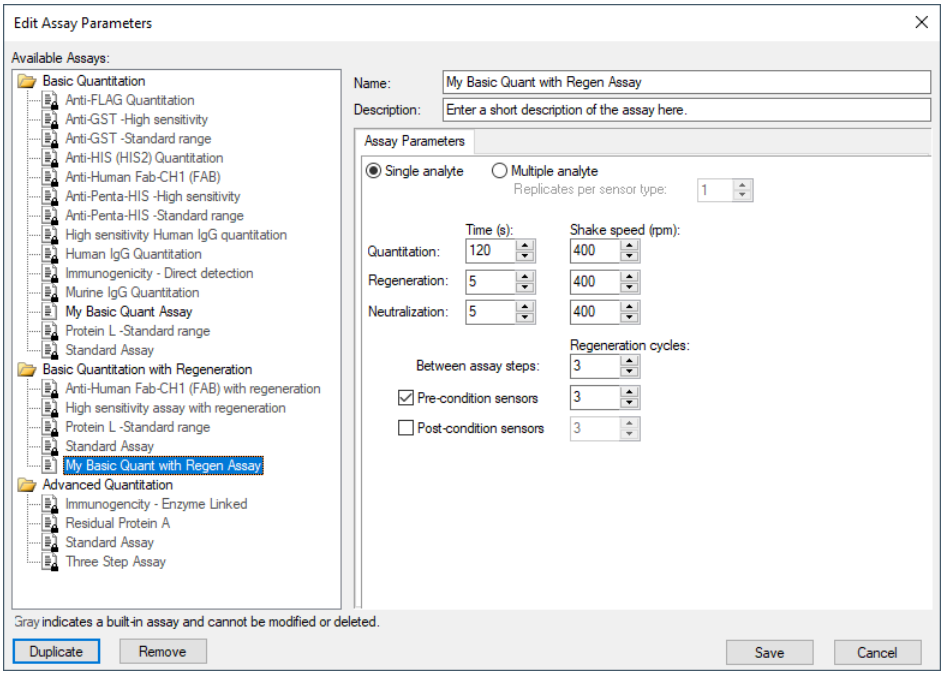


Figure 5-65: Assay Parameters—Basic Quantitation with Regeneration

Table 5-15: 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 Replicates 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). ***NOTICE:*** *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 regenerated 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.

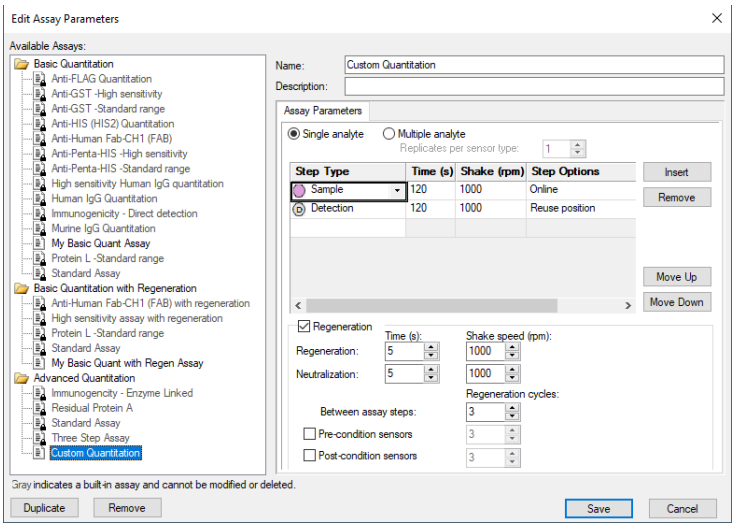


Figure 5-66: Assay Parameters—Advanced Quantitation

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 **Edit Assay Parameters** dialog box appears (see Figure 5-67).

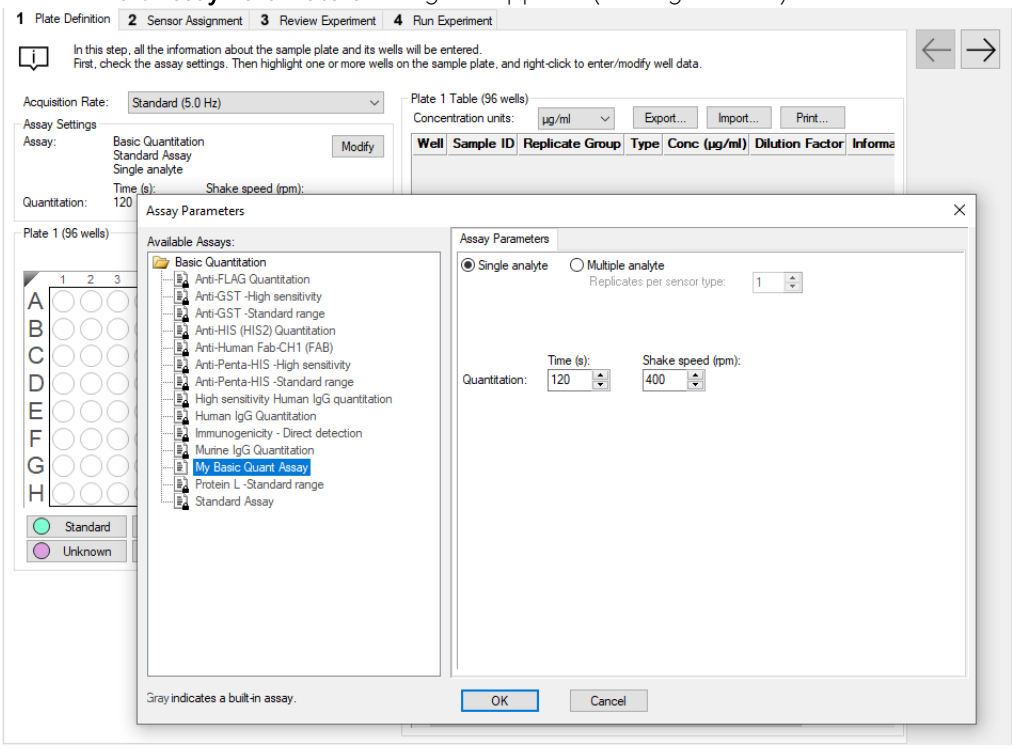


Figure 5-67: Selecting a Custom Assay

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® Analysis Studio software.

After starting the Octet® system and the Octet® 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:

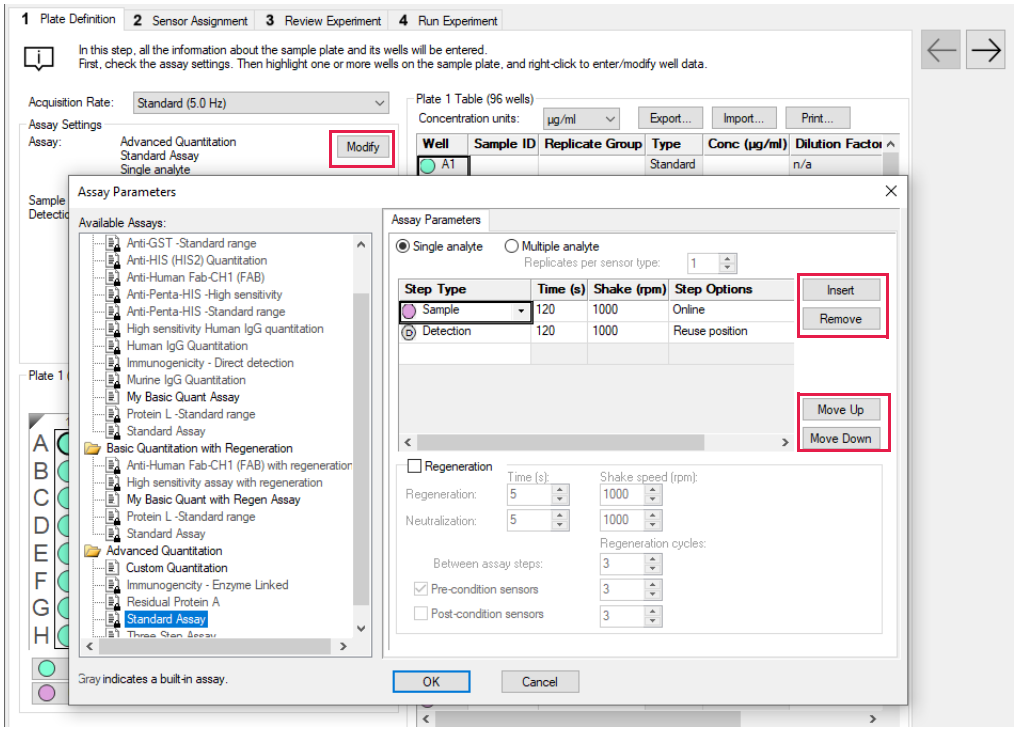


Figure 5-68: Assay Parameters Window.

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® R8, Octet® RED96e, and Octet® QKe

Introduction

Quantitation experiment determine the analyte concentration of a sample using a reference set of standards. After starting the Octet® system hardware and the Octet® 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® BLI Discovery 	1. Select a quantitation experiment in the **Experiment wizard** 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® Analysis Studio 	6. Analyze the binding data. 7. Generate a report.	*Octet® Analysis Studio Software User Guide*

IMPORTANT: *Using 96-well half-area plates on the Octet® R8, Octet® RED96, or Octet® 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® 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® 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 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  or click **Experiment** > **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 Regeneration	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two- or three-step quantitation assay that enables signal amplification 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.

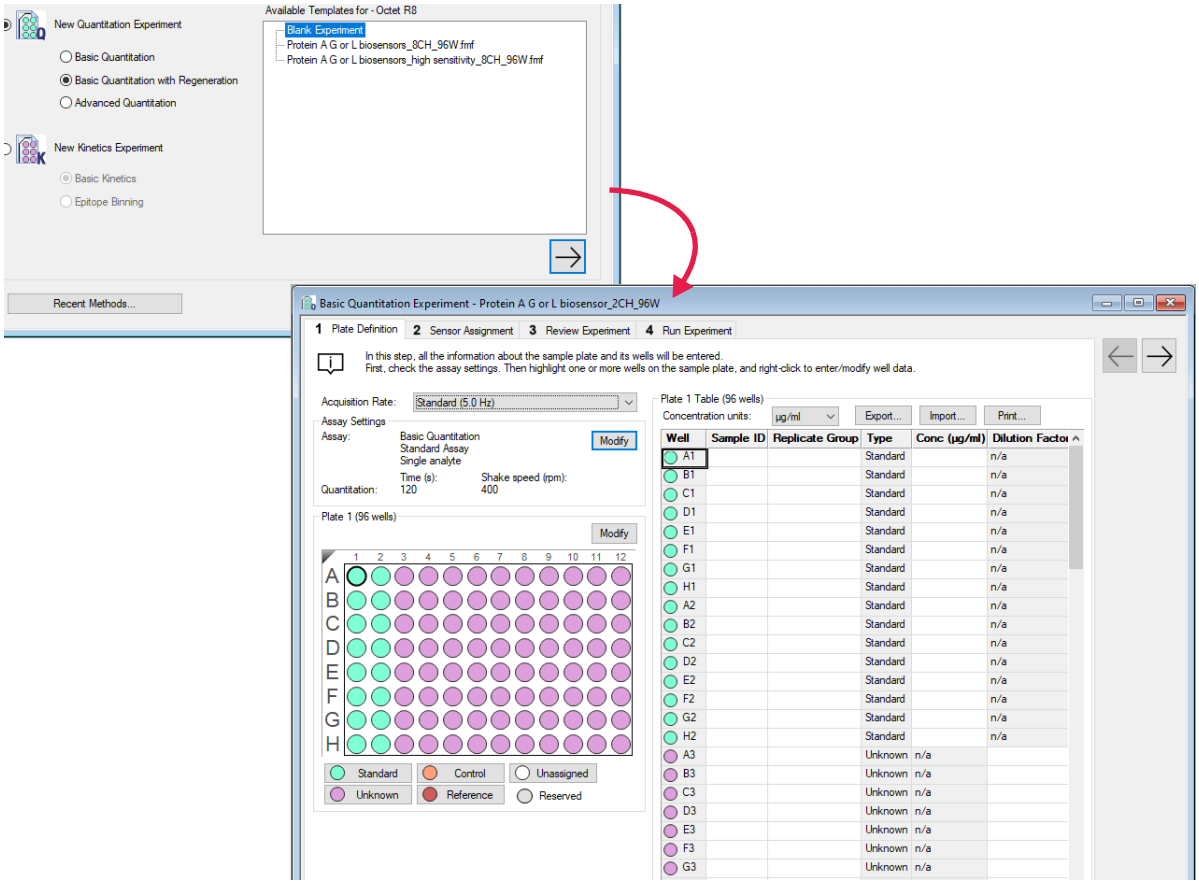


Figure 6-1: Selecting an Experiment Type in the Experiment Wizard

5. Click the → 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
3. 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

Icon	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 **Unknowns**, **Controls**, and **Standards**. 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), **Detection** (D), or **Capture Antibody** (C). Reserved wells are not available for use as **Standards**, **Unknowns**, **Controls**, 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 location. To change the location of a reserved column (R, N, D, or C) 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
R Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).
N Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

Table 6-5: Reserved Well Requirements

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

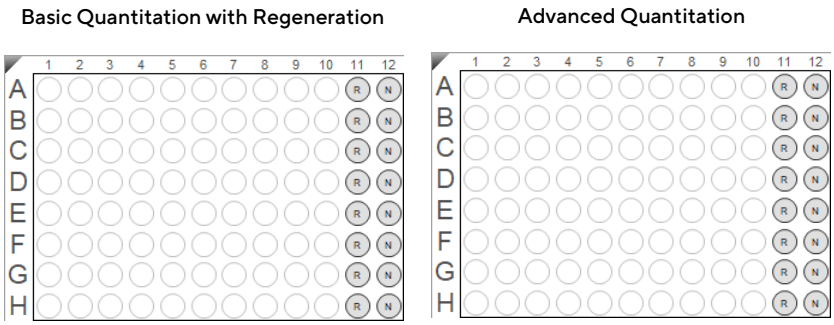


Figure 6-2: Default Locations for Reserved Wells in a 96-Well Sample Plate Map

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 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 (Figure 6-3, center).
- Click a well or draw a box around a group of wells (Figure 6-3, right).

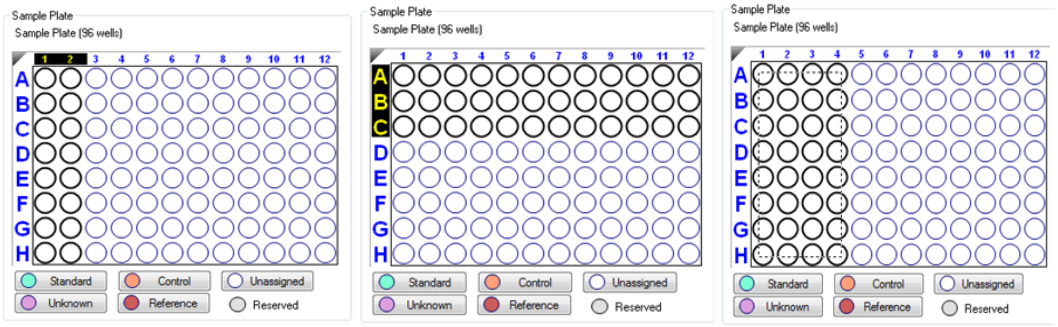


Figure 6-3: Selecting Wells in the Sample Plate Map

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.
2. 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**.

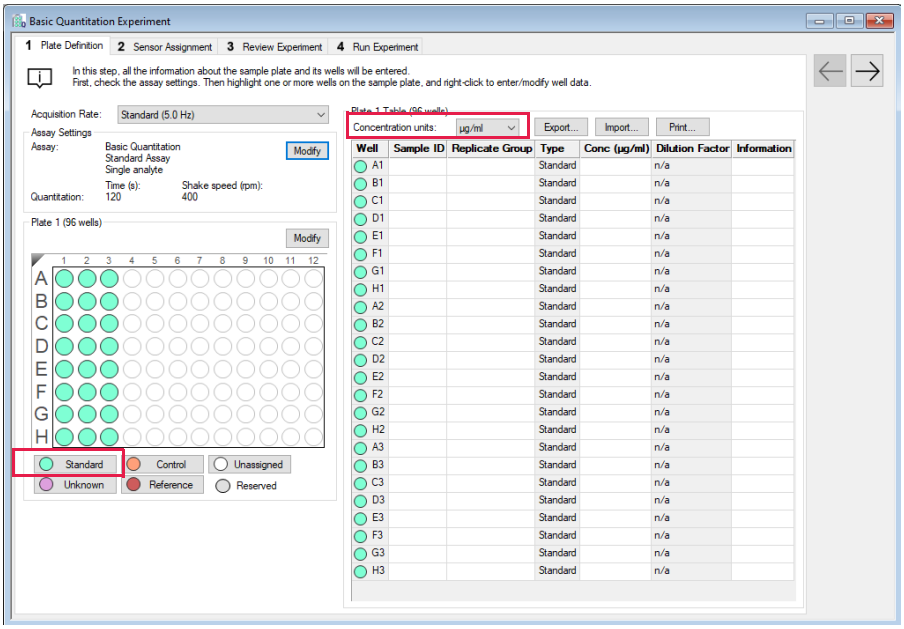


Figure 6-4: Plate Definition Window—Designating Standards

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).

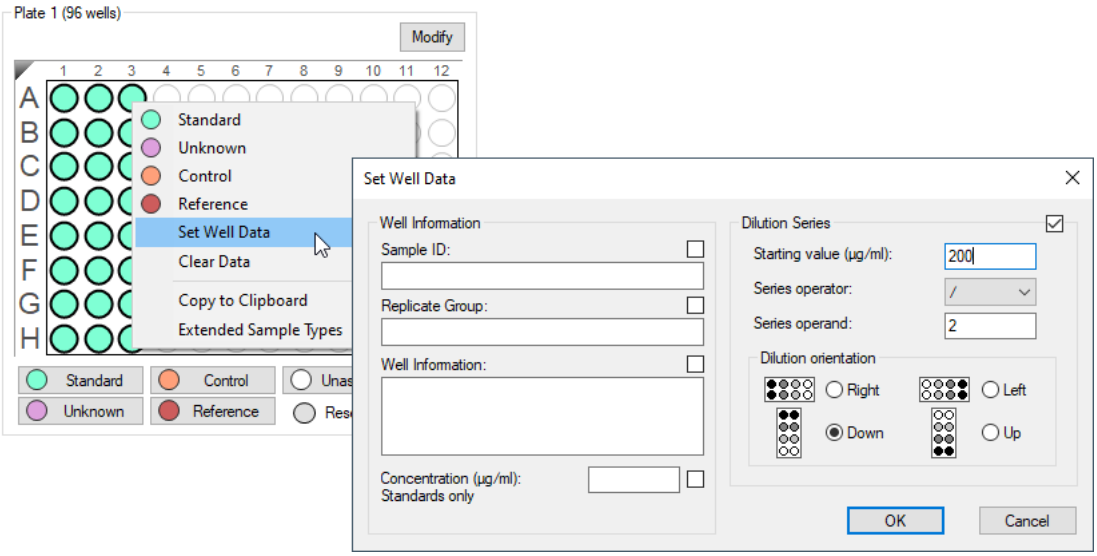


Figure 6-5: Sample Plate Map—Setting a Dilution Series

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).

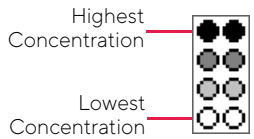


Figure 6-6: Concentration Representation in Dilution Series

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).

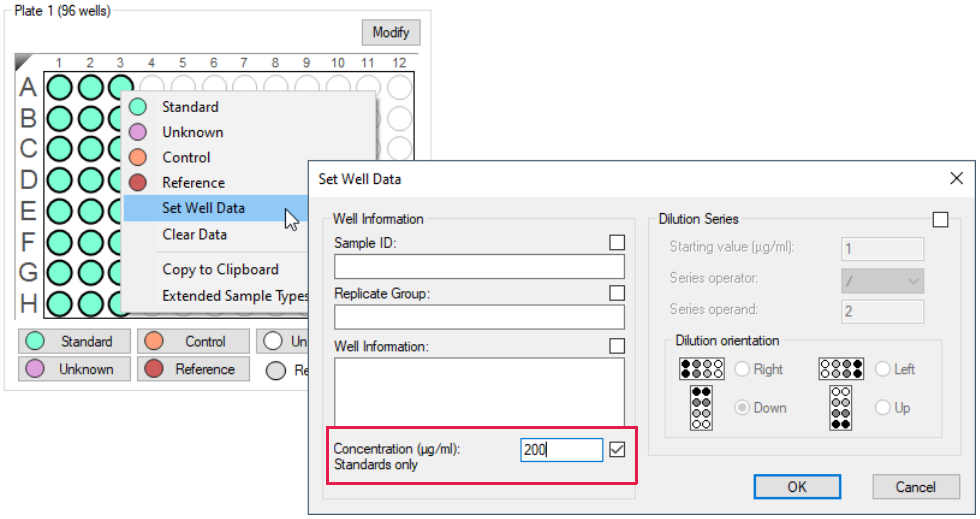


Figure 6-7: Sample Plate Map—Assigning a Standard Concentration

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).

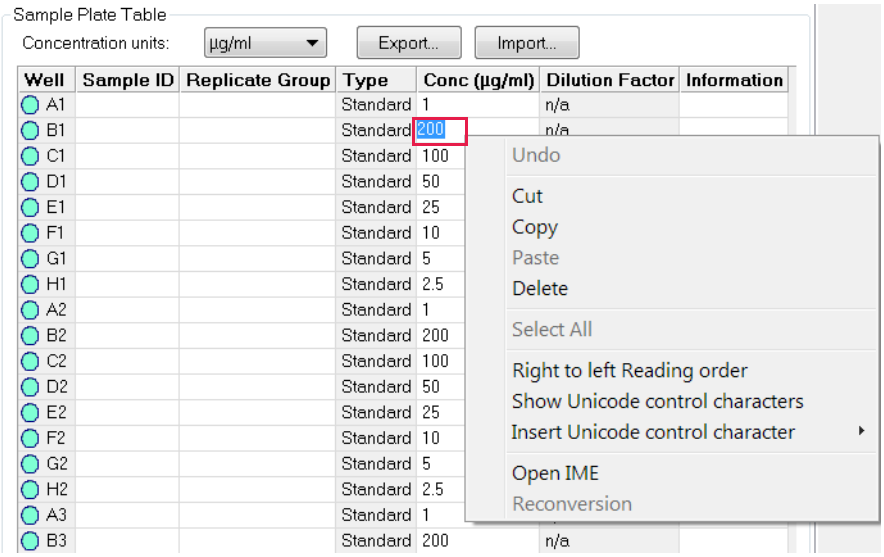


Figure 6-8: Sample Plate Table—Shortcut Menu of Edit Commands

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).

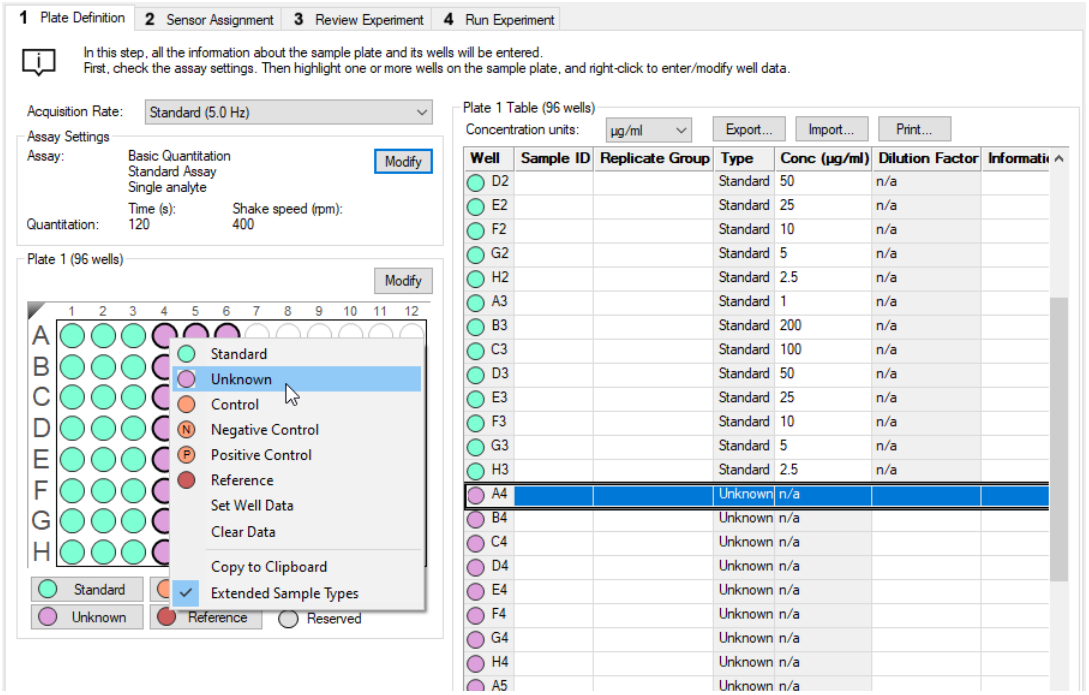


Figure 6-9: Plate Definition Window—Designate Unknown Wells

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).

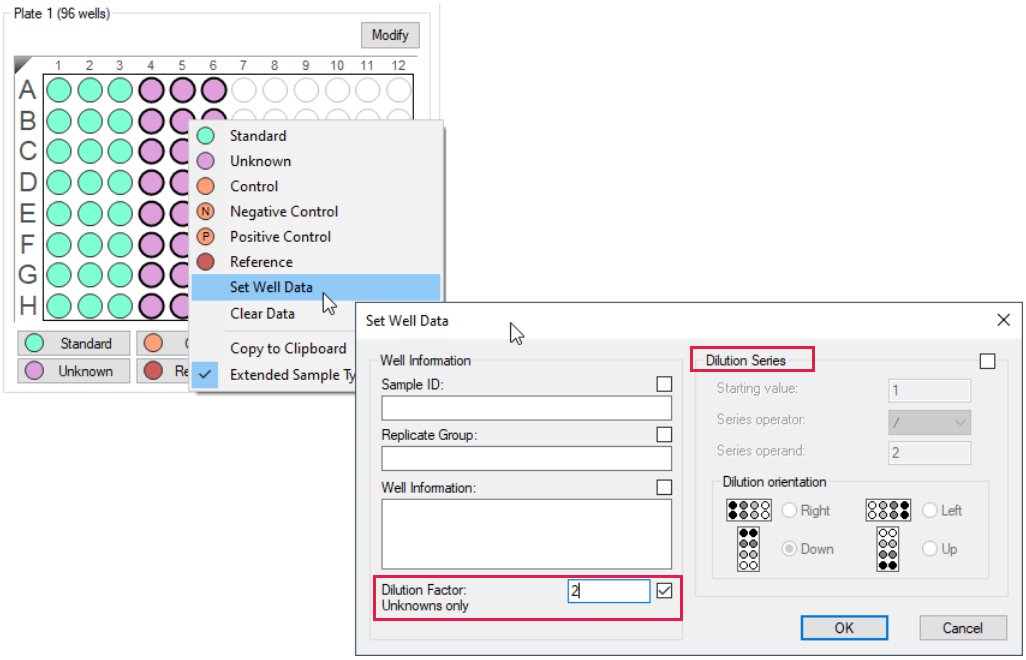


Figure 6-10: Sample Plate Map—Setting a Dilution Factor or a Serial Dilution

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)

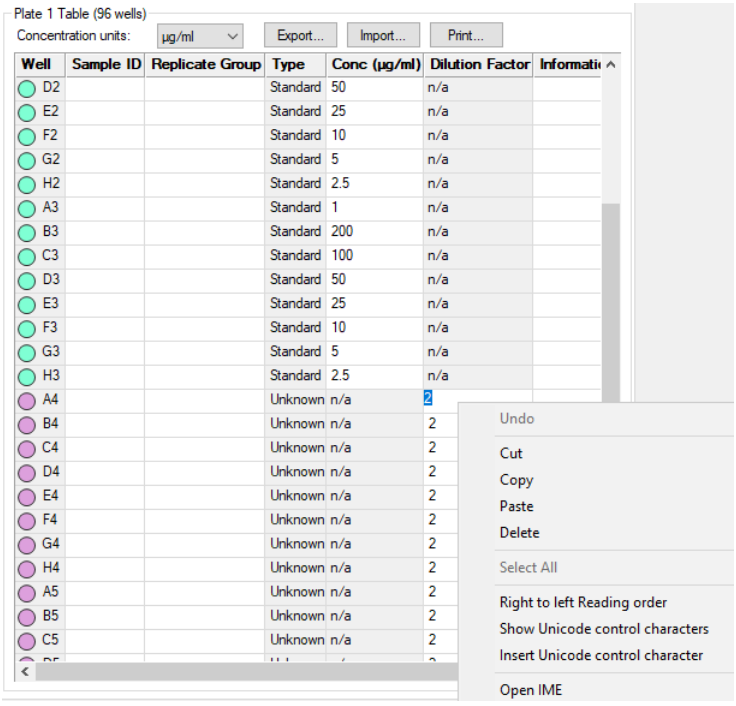


Figure 6-12: Sample Plate Table—Shortcut Menu of Edit Commands

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).

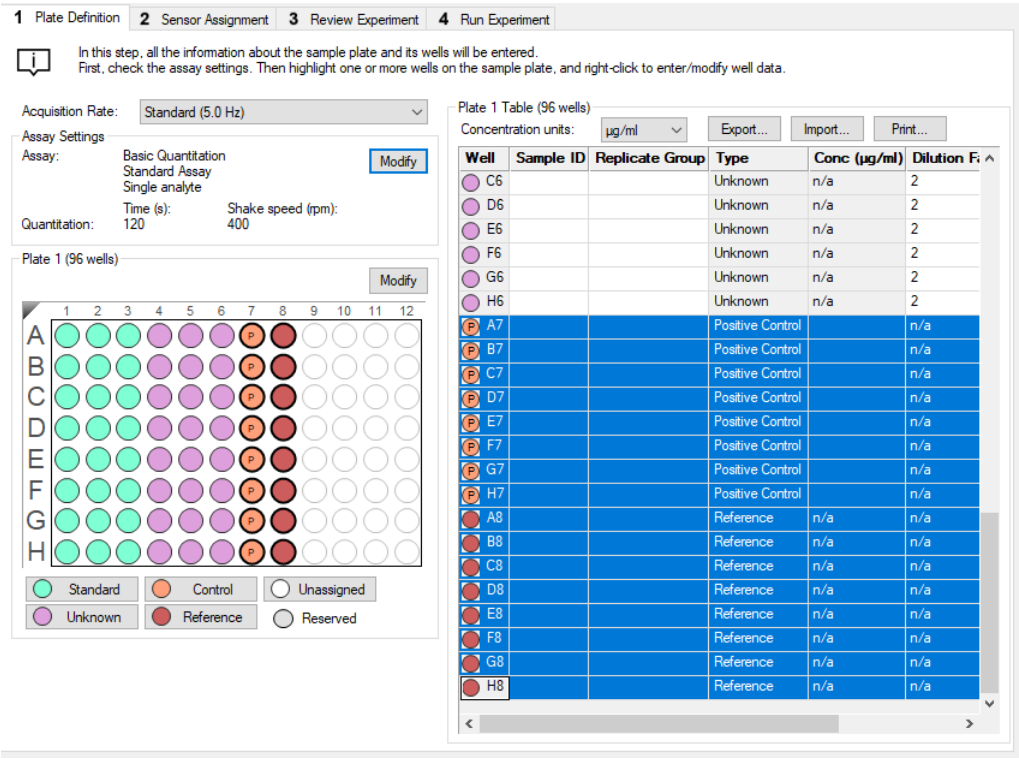


Figure 6-13: Designate Controls or Reference Wells

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 **Run-time 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**.

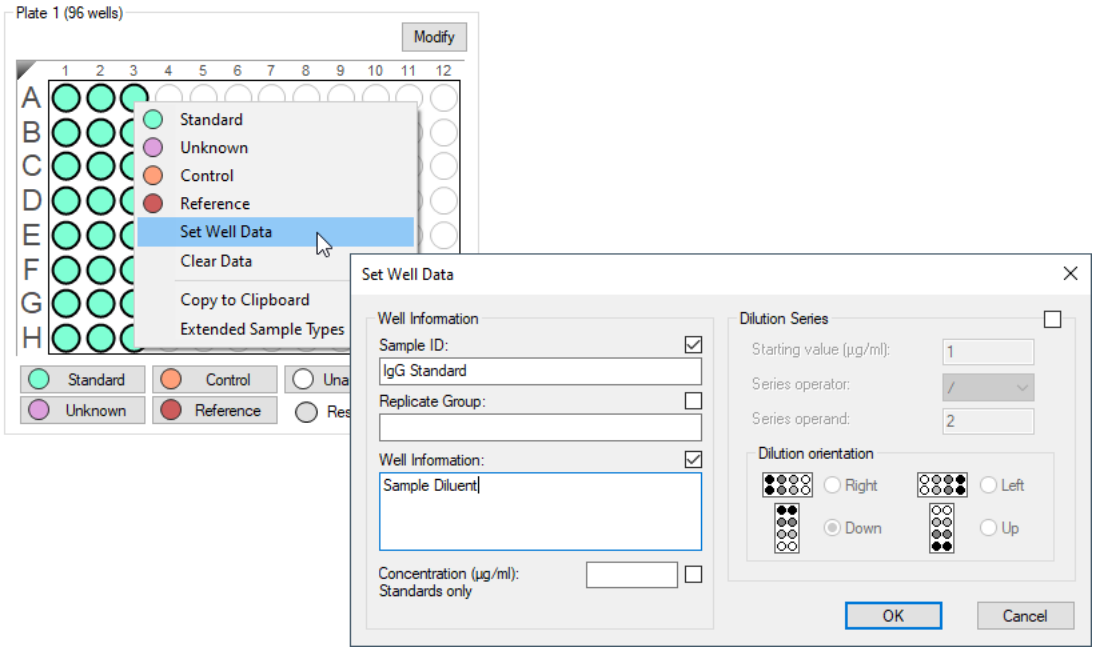


Figure 6-14: Adding Sample Annotations from the Sample Plate Map

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.

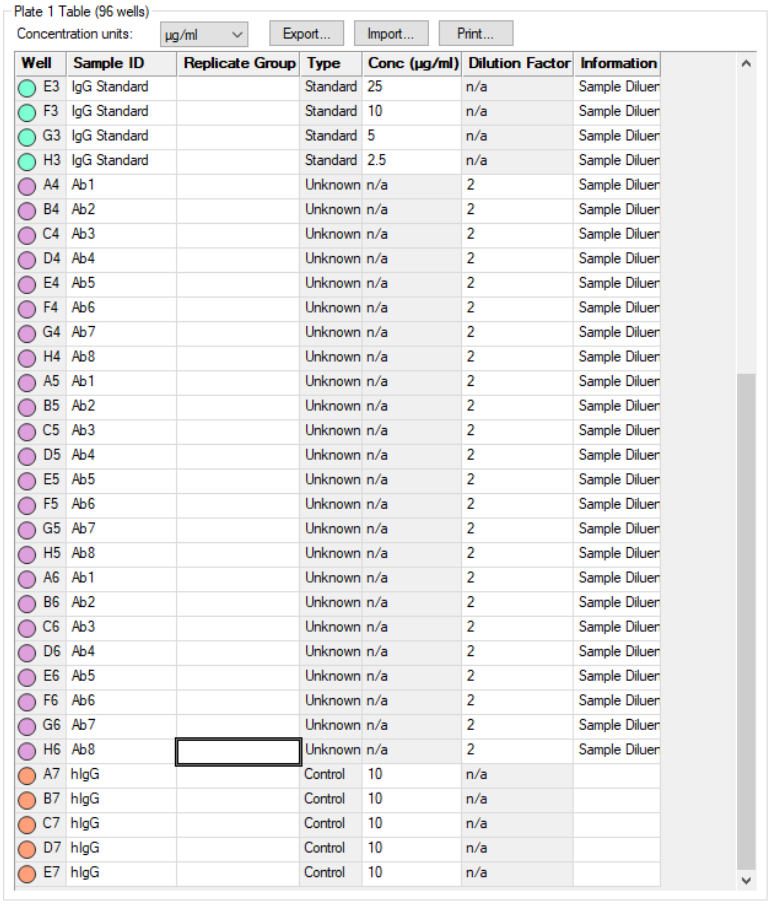


Plate 1 Table (96 wells)

Concentration units: µg/ml Export... Import... Print...

Well	Sample ID	Replicate Group	Type	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
H3	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	hIgG		Control	10	n/a	
B7	hIgG		Control	10	n/a	
C7	hIgG		Control	10	n/a	
D7	hIgG		Control	10	n/a	
E7	hIgG		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**.

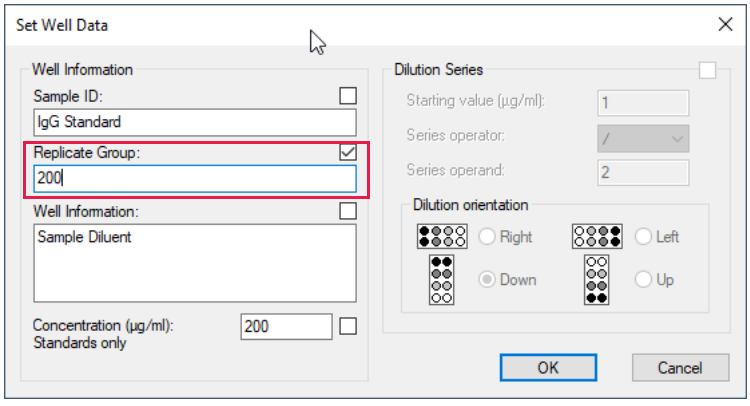


Figure 6-17: Add Replicate Group from the Sample Plate Map

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).

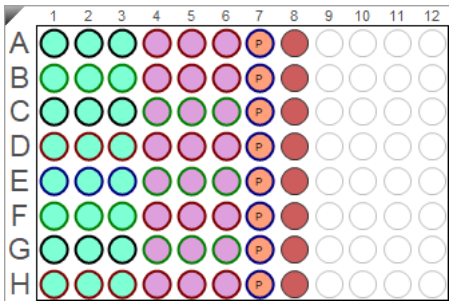


Figure 6-18: Replicate Groups in Sample Plate Map

The **Sample Plate Table** updates with the **Replicate Group** names entered (see Figure 6-19).

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG Standard	2.5	Standard	2.5	n/a
H1	IgG Standard	1	Standard	1	n/a
A2	IgG Standard	200	Standard	200	n/a
B2	IgG Standard	100	Standard	100	n/a
C2	IgG Standard	50	Standard	50	n/a
D2	IgG Standard	25	Standard	25	n/a
E2	IgG Standard	10	Standard	10	n/a
F2	IgG Standard	5	Standard	5	n/a
G2	IgG Standard	2.5	Standard	2.5	n/a
H2	IgG Standard	1	Standard	1	n/a
A3	IgG Standard	200	Standard	200	n/a
B3	IgG Standard	100	Standard	100	n/a
C3	IgG Standard	50	Standard	50	n/a
D3	IgG Standard	25	Standard	25	n/a
E3	IgG Standard	10	Standard	10	n/a
F3	IgG Standard	5	Standard	5	n/a
G3	IgG Standard	2.5	Standard	2.5	n/a
H3	IgG Standard	1	Standard	1	n/a
A4	Ab1	Ab1	Unknown	n/a	2
B4	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 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).

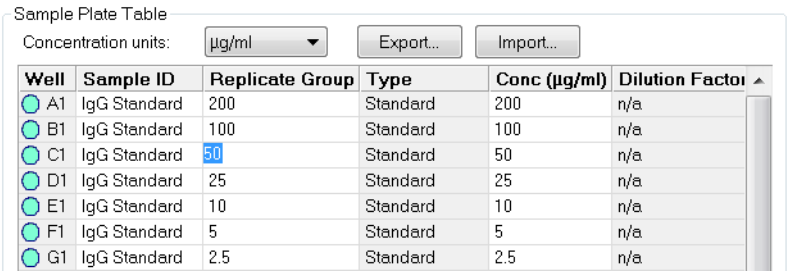


Figure 6-20: Add Replicate Group from the Sample Plate Table

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® 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**.

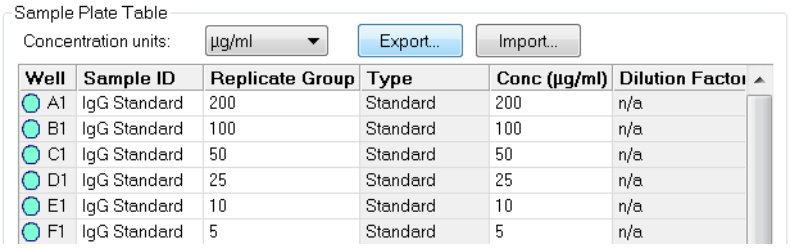


Figure 6-21: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 6-22), select a folder, enter a name for the plate (.csv), and click **Save**.

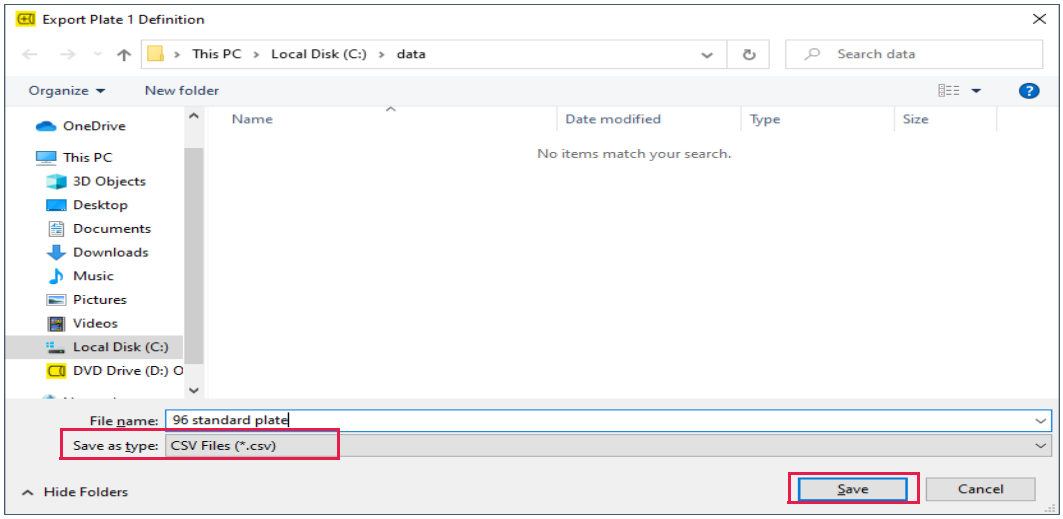


Figure 6-22: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the **Sample Plate Table** (see Figure 6-23), click **Import**.

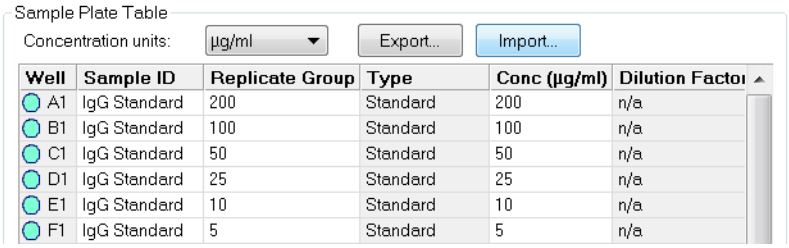


Figure 6-23: Import Button in Sample Plate Table

2. In the **Import Plate Definition** window (see Figure 6-24), select the plate definition (.csv), and click **Open**.

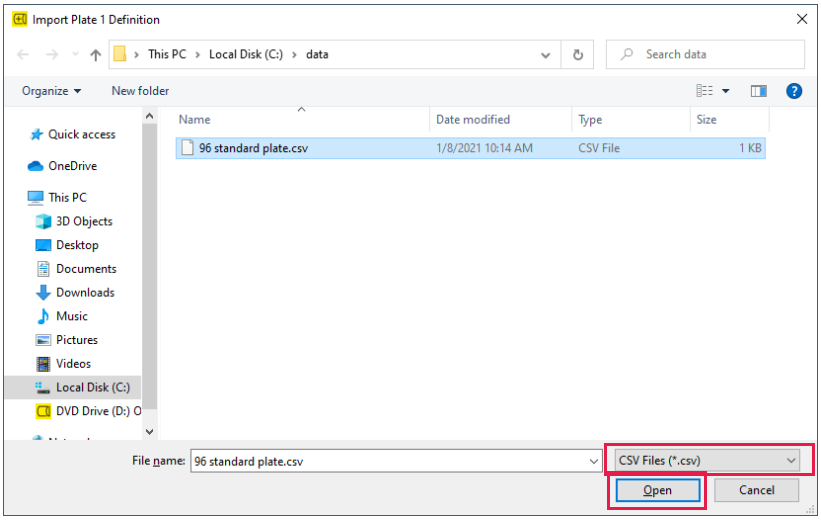


Figure 6-24: Import Plate Definition Window

NOTICE: *You can also create a .csv file for import. Figure 6-25 shows the appropriate column information layout.*

	A	B	C	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

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**.

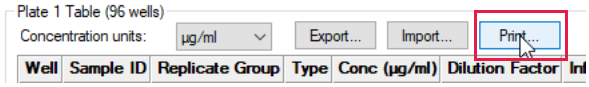


Figure 6-26: Sample Plate Print Button

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.

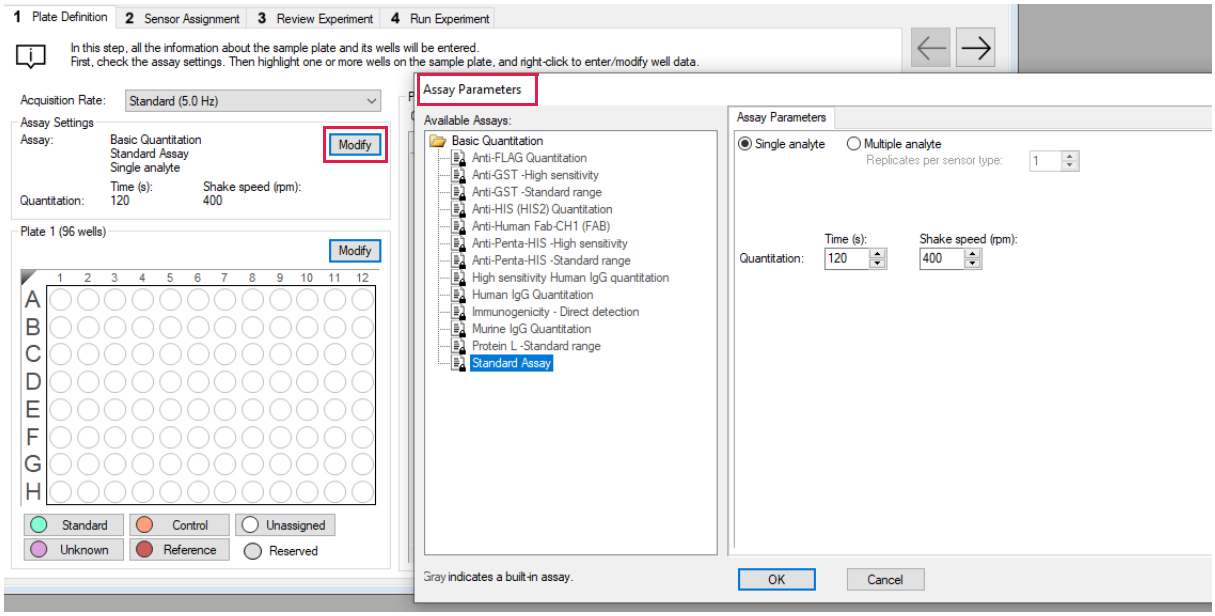


Figure 6-27: Modifying Assay Parameters

Basic Quantitation Assay Parameters

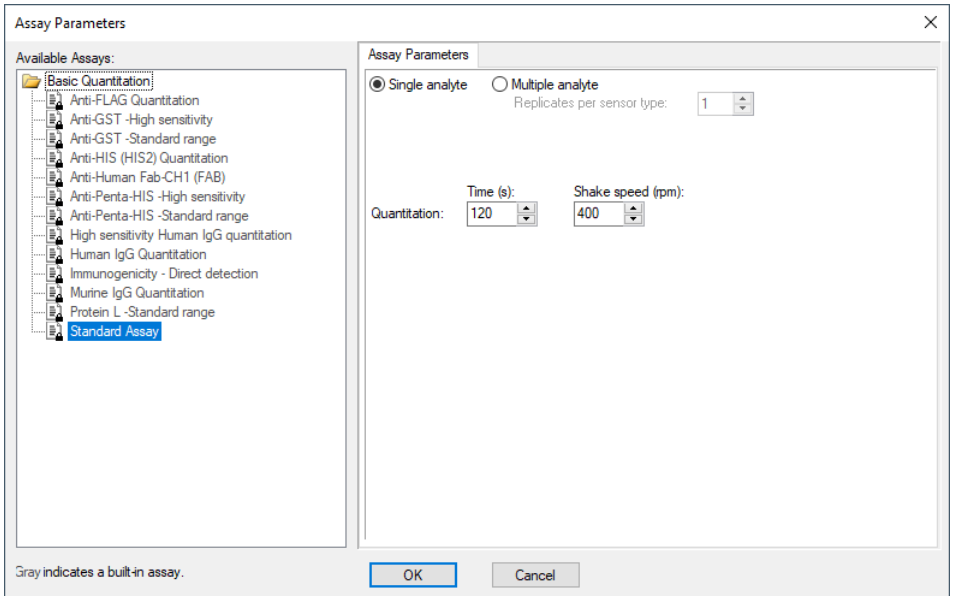


Figure 6-28: Assay Parameters—Basic Quantitation AssayBasic Quantitation with Regeneration Assay Parameters

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 Replicates 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. ***NOTICE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

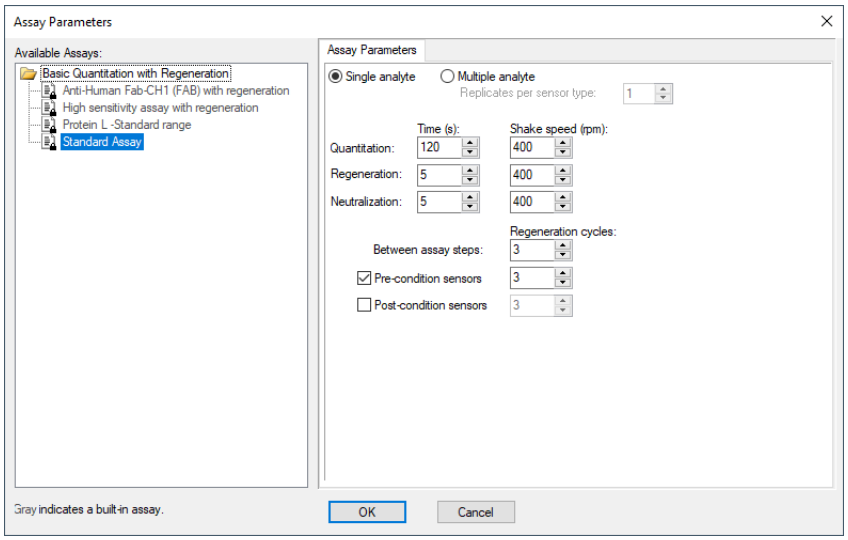


Figure 6-29: Assay Parameters—Basic Quantitation with Regeneration

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 Replicates 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). ***NOTICE:*** *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 regenerated 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.

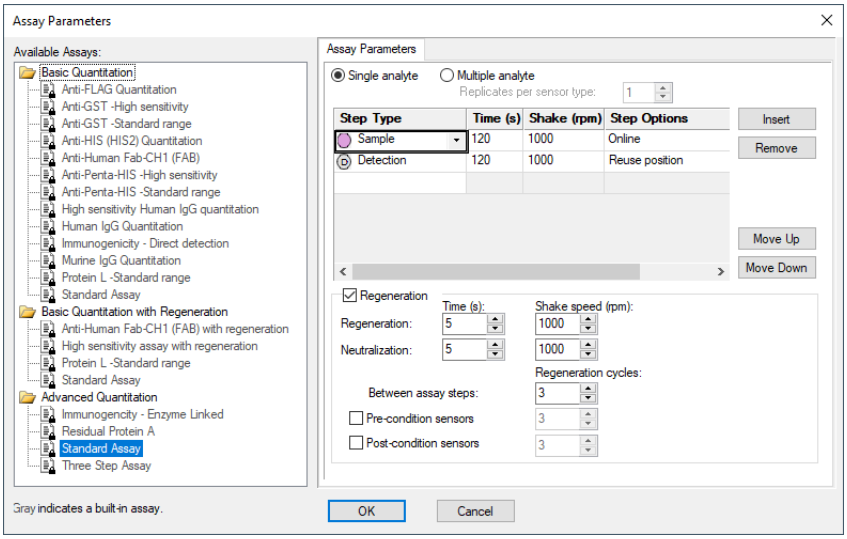


Figure 6-30: Assay Parameters—Advanced Quantitation

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 → 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.

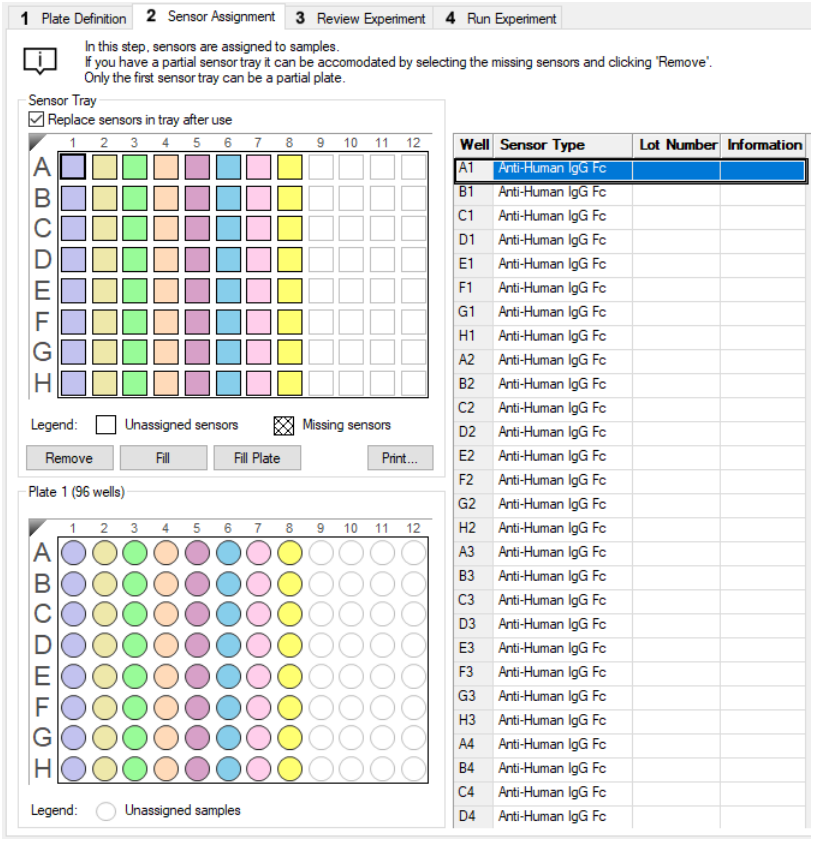


Figure 6-31: Sensor Assignment Window for Basic Quantitation without Regeneration

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).

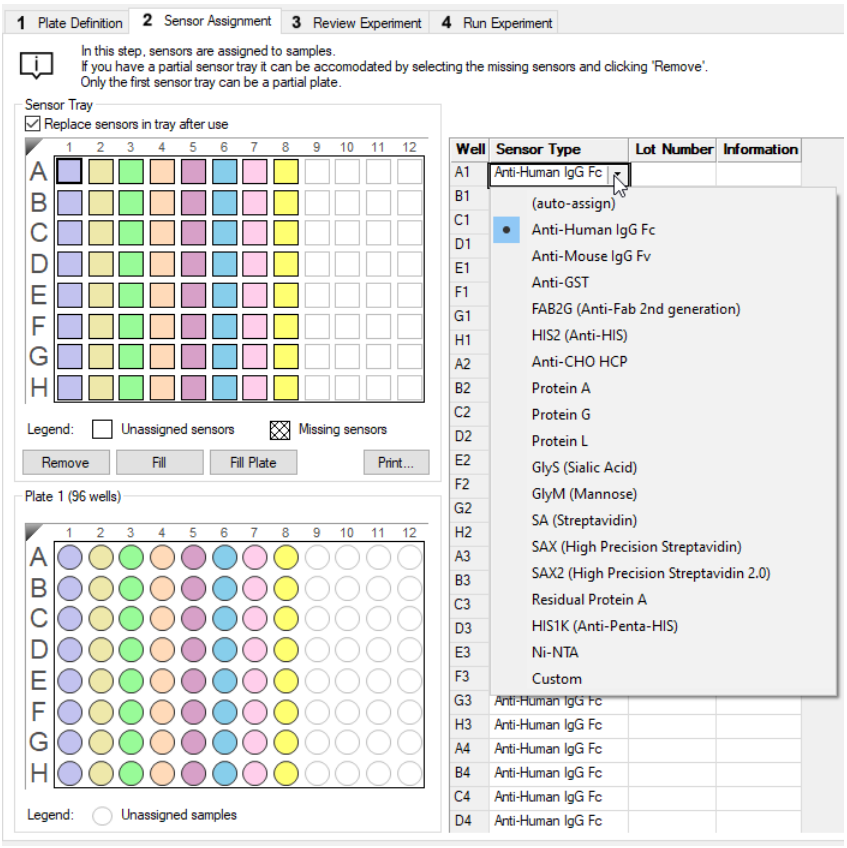


Figure 6-32: Changing Biosensor Types

2. 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® RED96 and the Octet® 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).

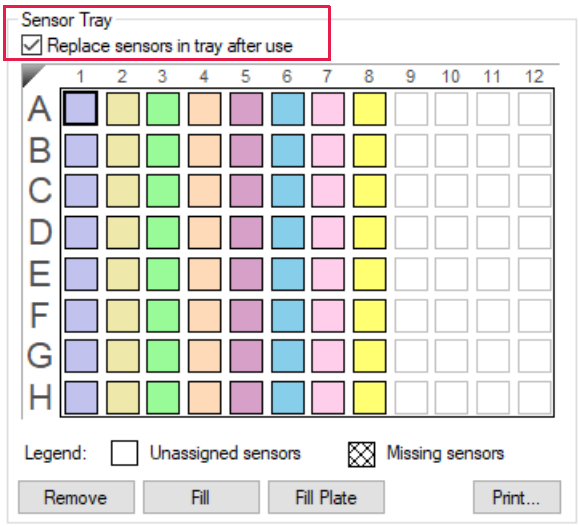


Figure 6-33: Replace Sensors in Tray After Use Check Box

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 → 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.

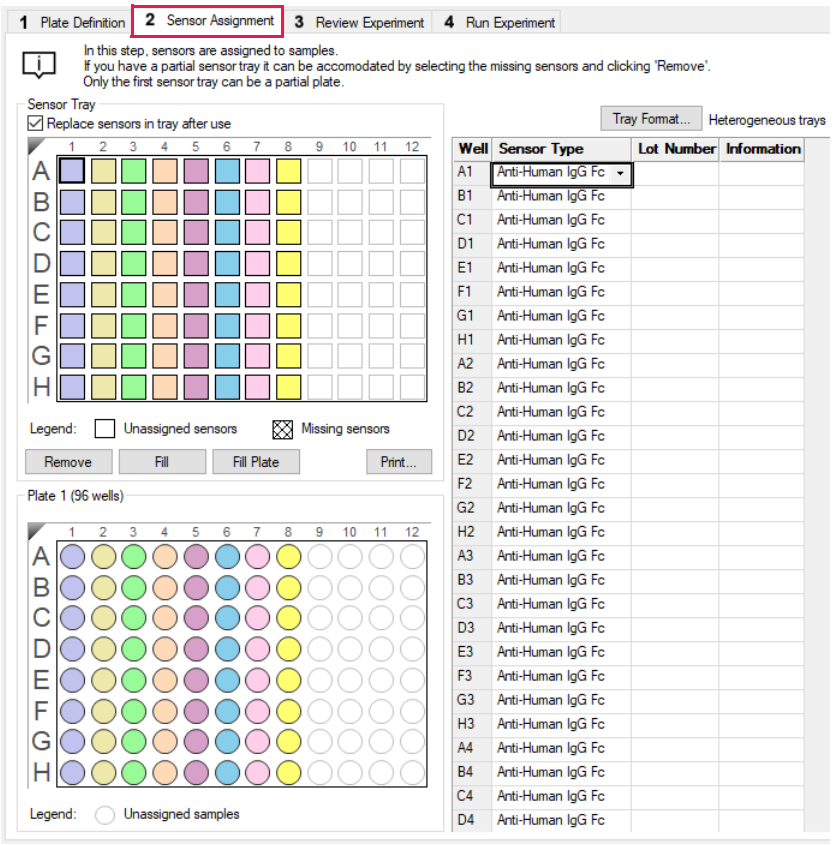


Figure 6-34: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

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 drop-down list (see Figure 6-35).

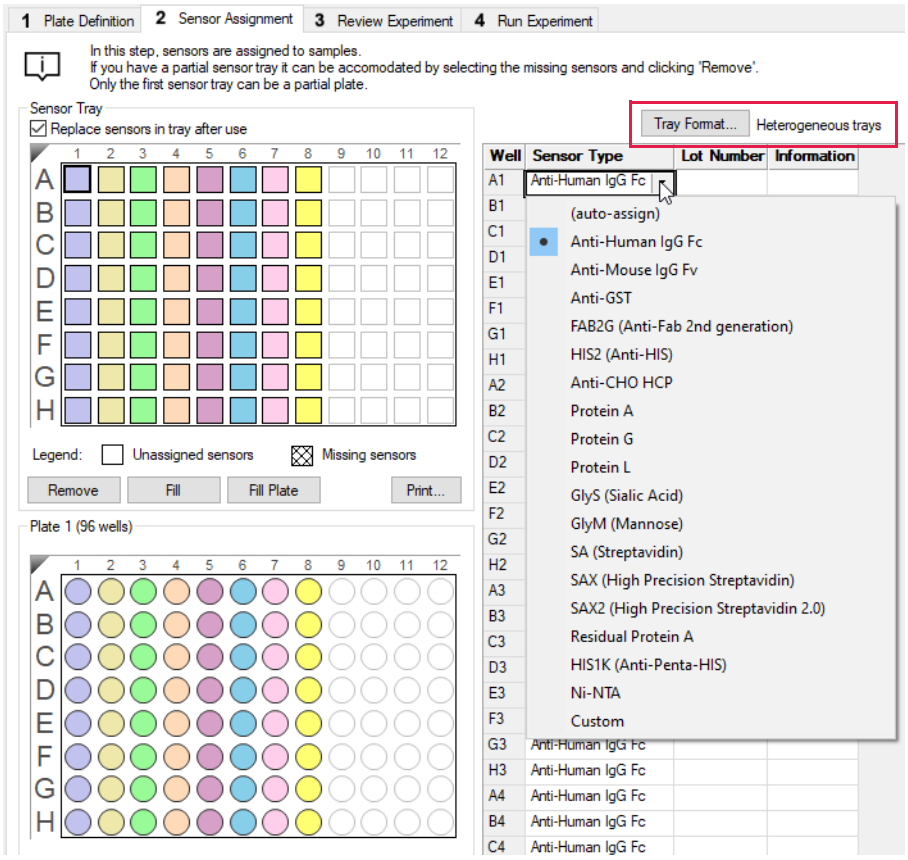


Figure 6-35: Changing Biosensor Types

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.*

1. 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**.

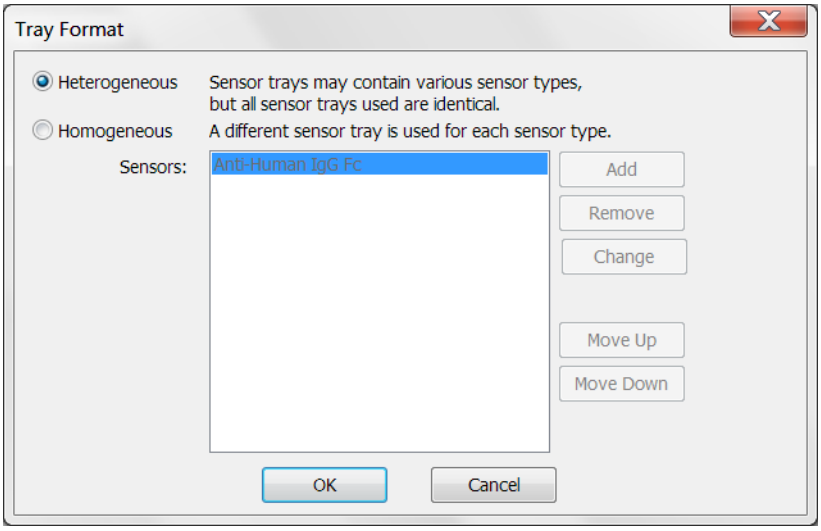


Figure 6-36: Tray Format Dialog Box

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.

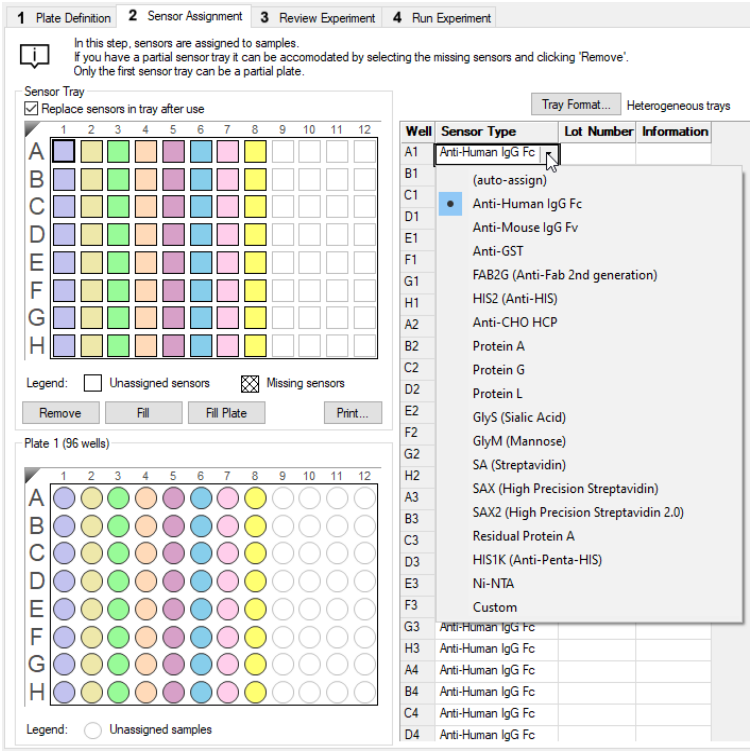


Figure 6-37: Populating the Sensor Tray Map with First Biosensor Type

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.

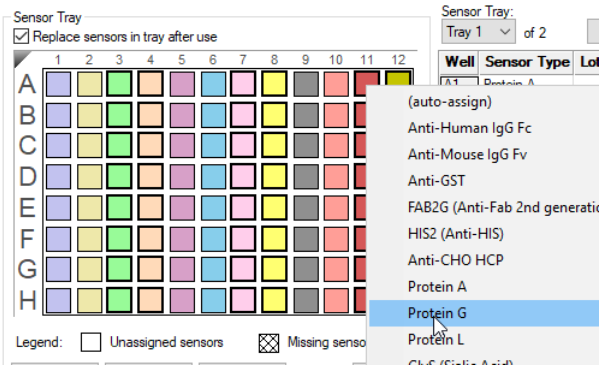


Figure 6-38: Populating the Sensor Tray Map with Second Biosensor Type

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 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.

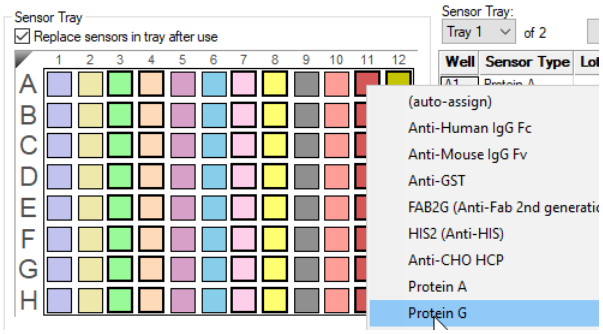


Figure 6-39: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

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).

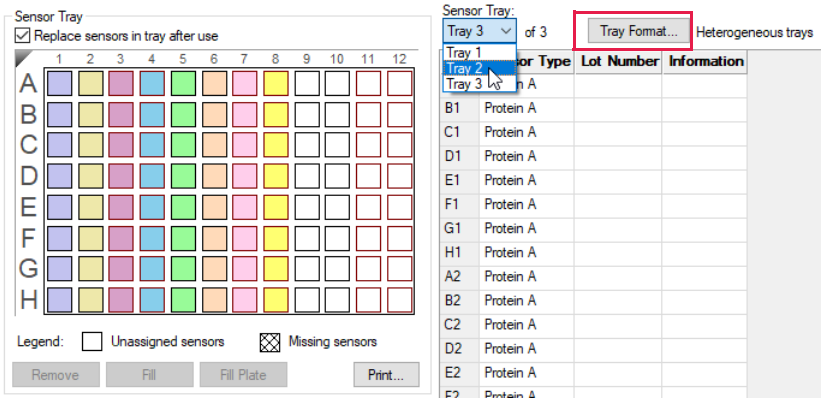


Figure 6-40: Tray Selection

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 6-41).

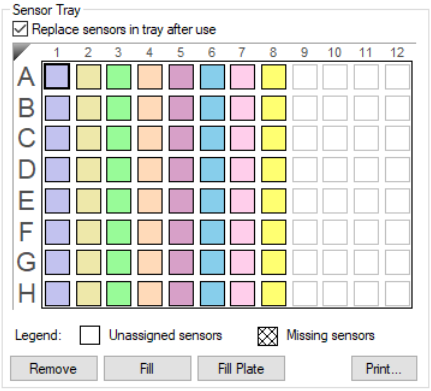


Figure 6-41: Replace Sensors in Tray After Use Check Box

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.

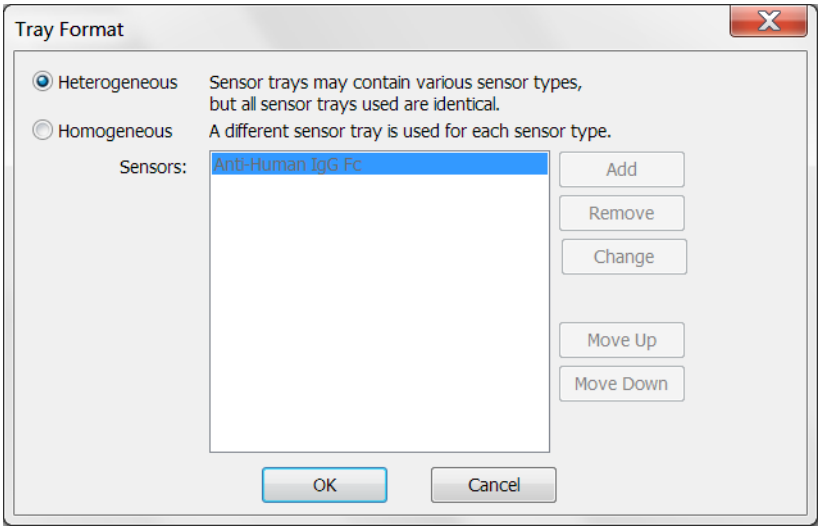


Figure 6-42: Tray Format Dialog Box

2. Select **Homogeneous**. Click **Add** to select the first biosensor type (see Figure 6-43).

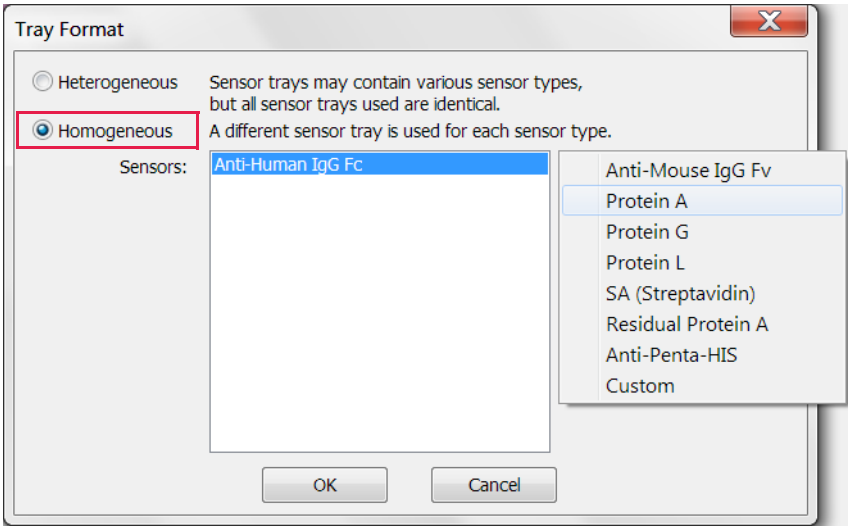


Figure 6-43: Selecting a Biosensor Type in the Tray Format Dialog Box

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).

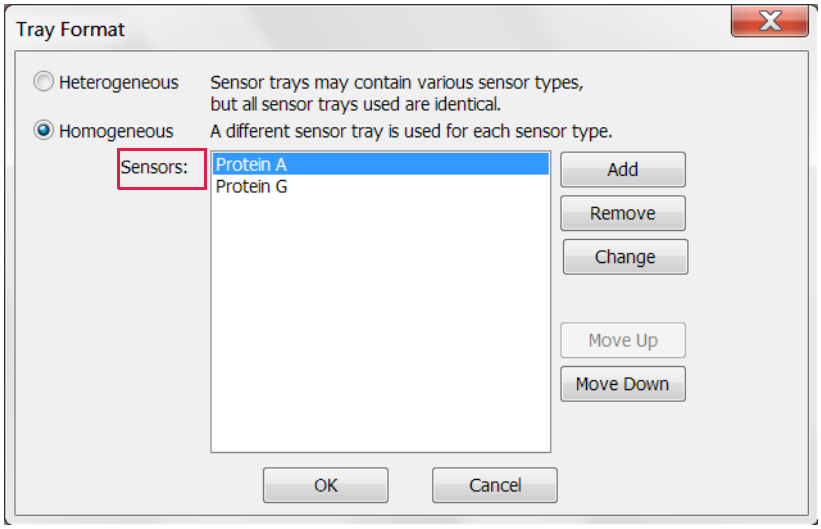


Figure 6-44: Biosensor Types List Order in Sensor Box

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.

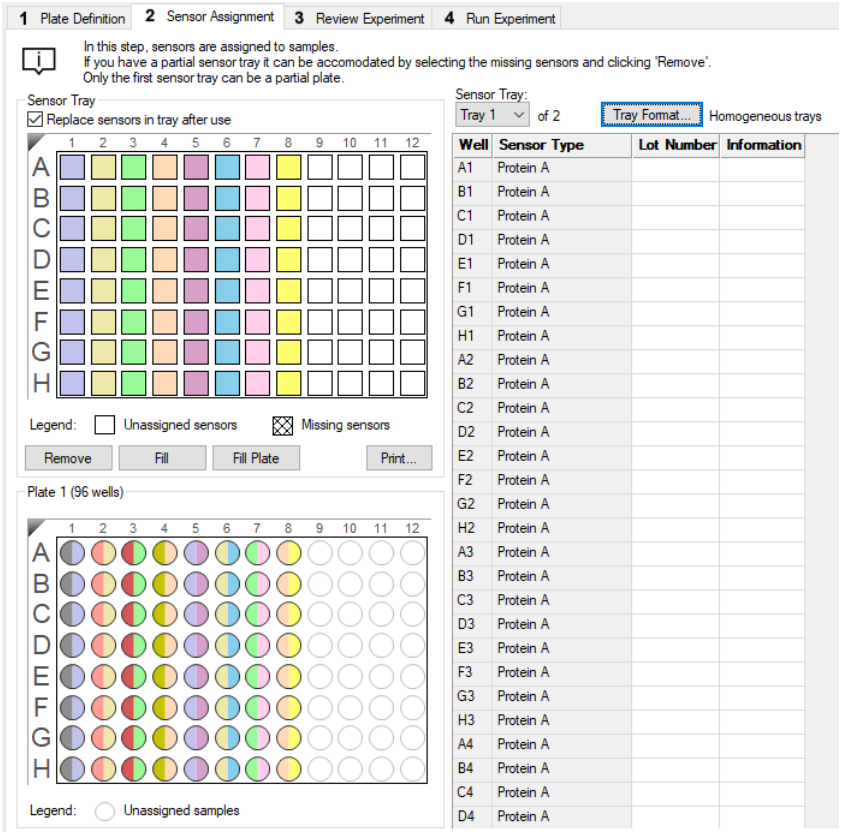


Figure 6-45: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

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).

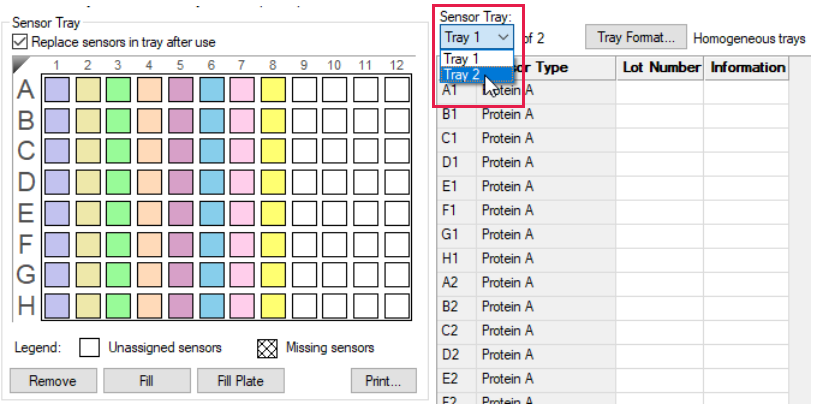


Figure 6-46: Tray Selection

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).

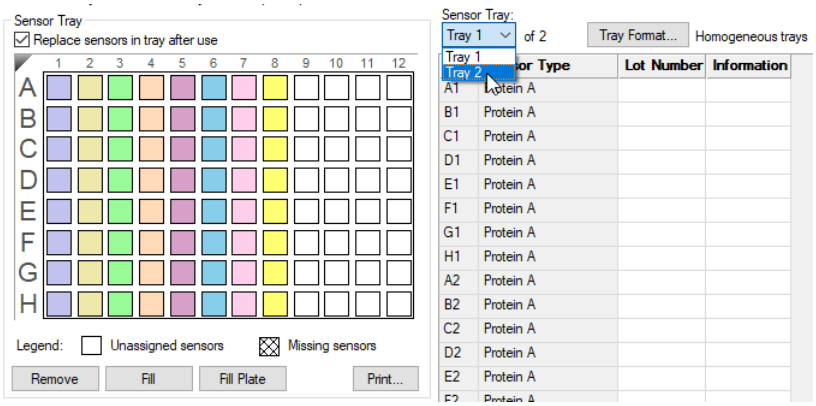


Figure 6-47: Replace Sensors in Tray After Use Check Box

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).

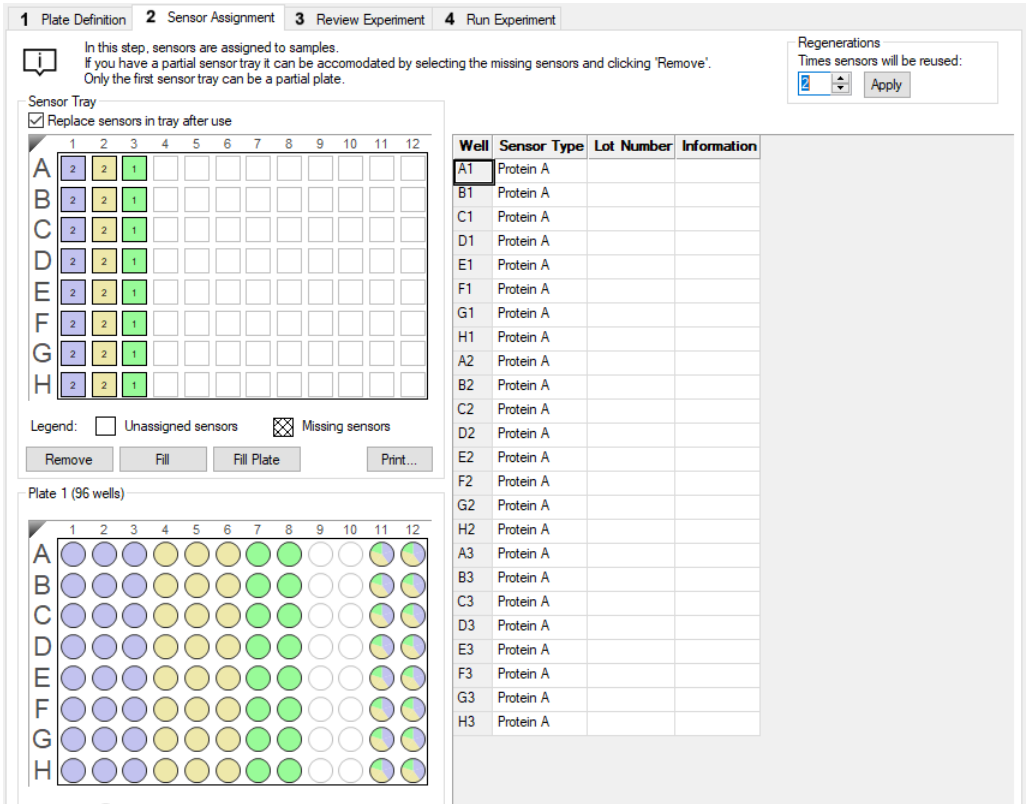


Figure 6-48: Fractional Use of Regeneration and Neutralization Wells

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).

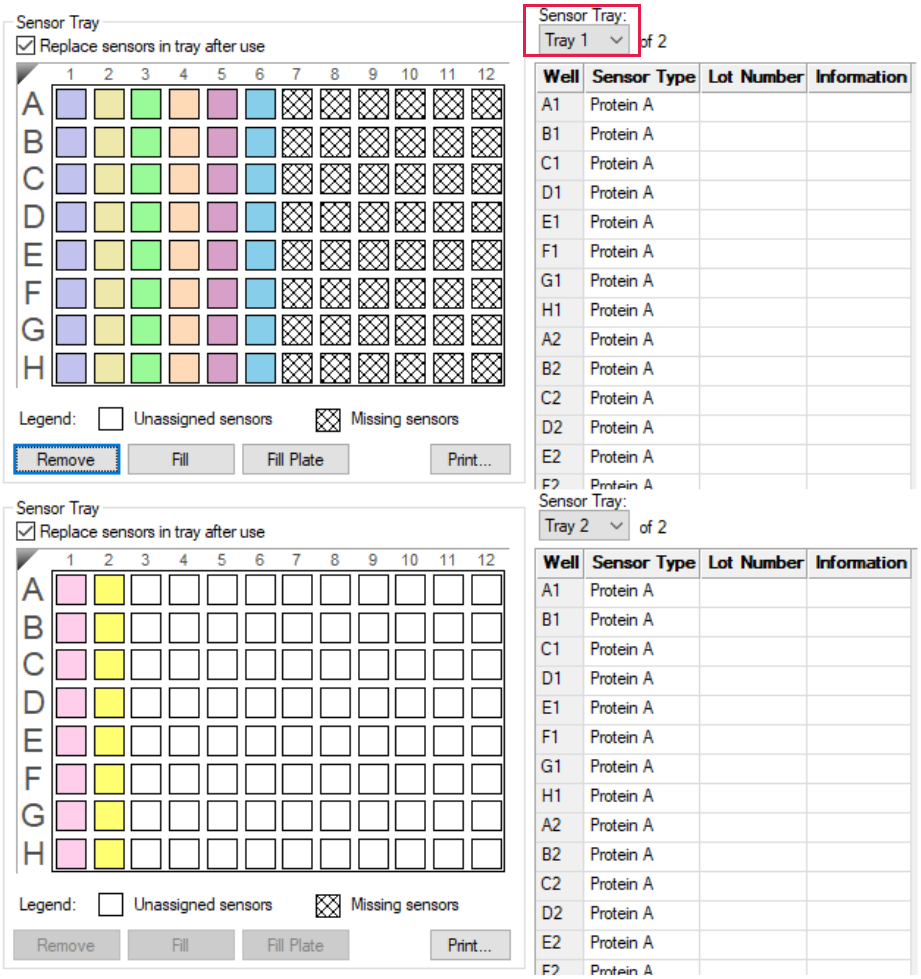


Figure 6-49: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray

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® 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 (← →) arrows to select an assay.

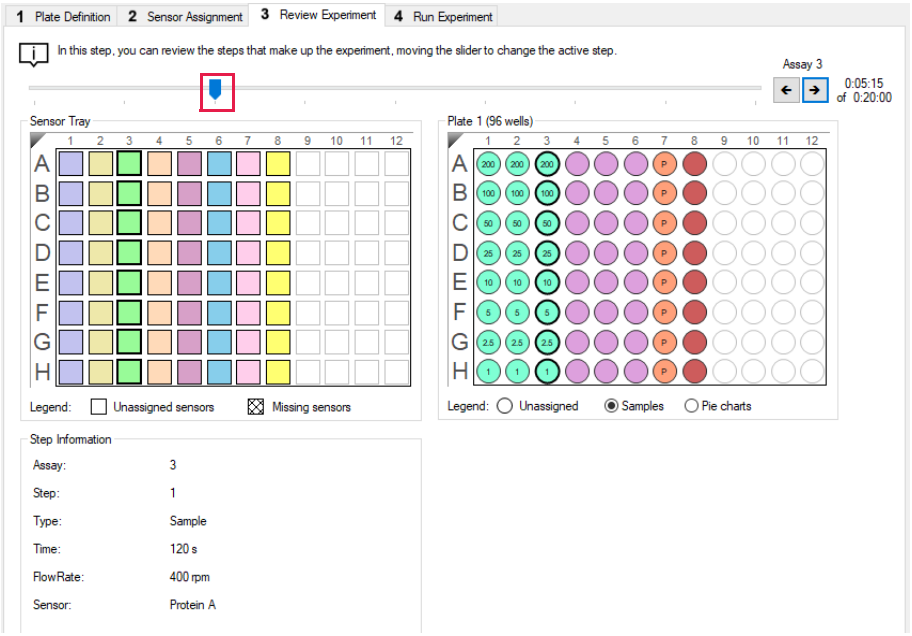


Figure 6-50: Review Experiment Window

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.*

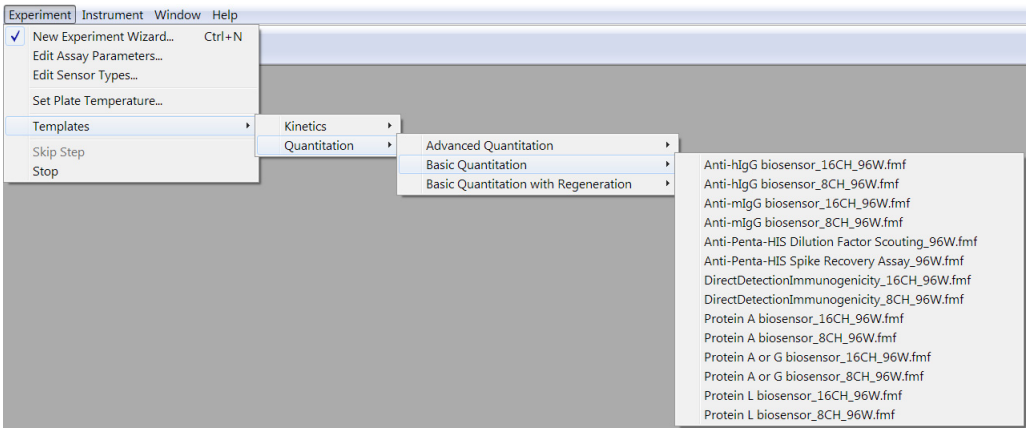


Figure 6-51: Experiments in the Template Folder

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® 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).

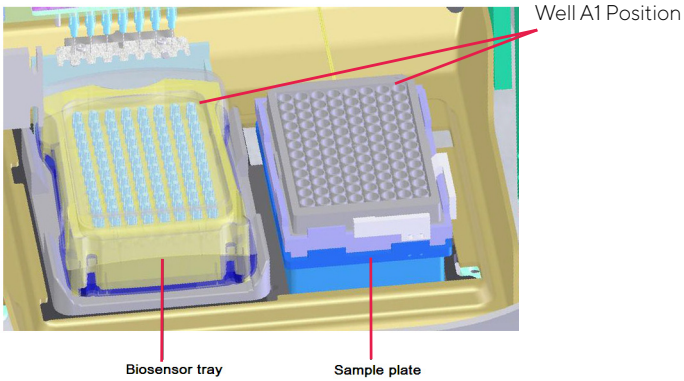


Figure 6-52: Biosensor Stage (left) and Sample Stage (right)

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

4. **Octet® RED96e and Octet® 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® RED96e and the Octet® 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® 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® 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 → to access the Run Experiment window (see Figure 6-53).

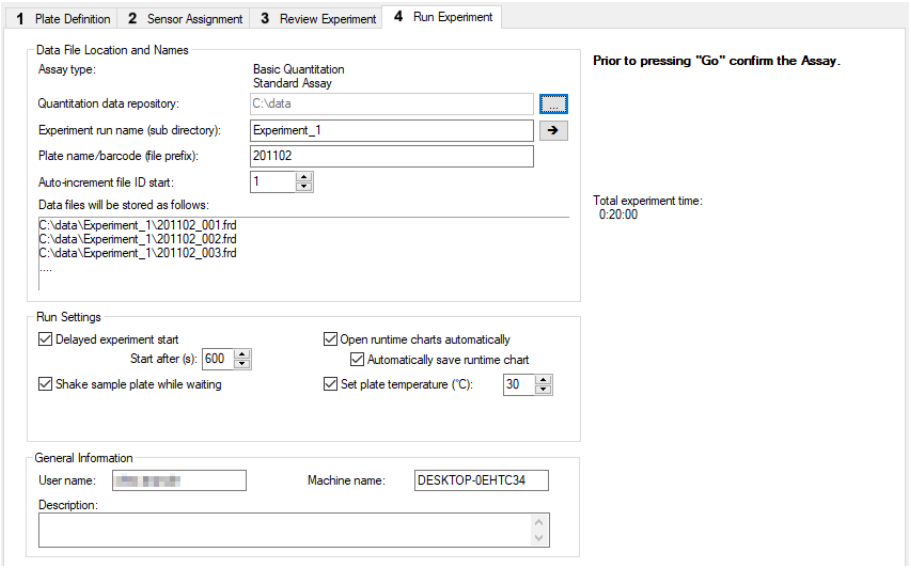


Figure 6-53: Run Experiment Window

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.*

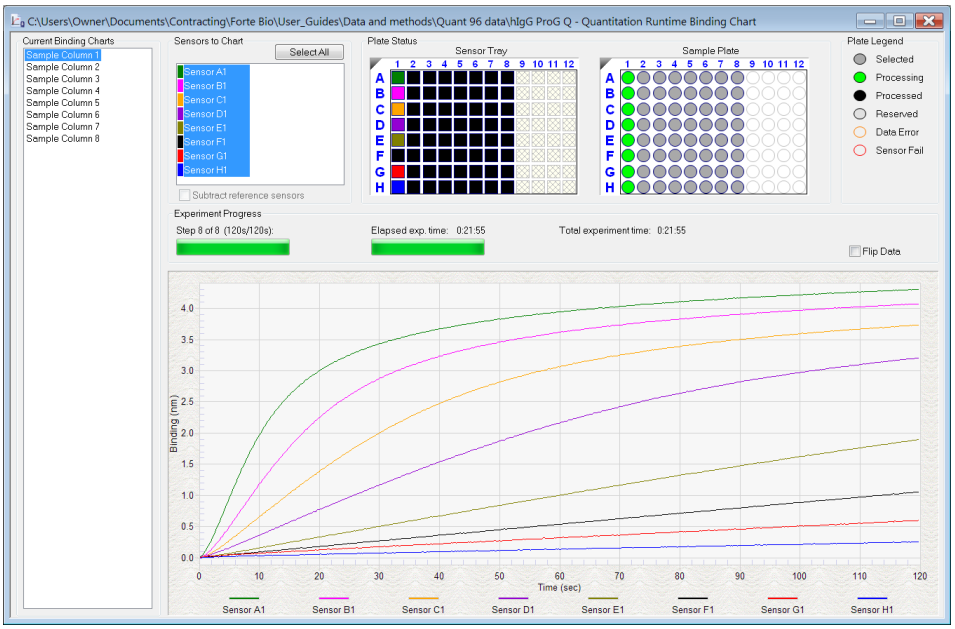


Figure 6-54: Runtime Binding Chart

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® 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® 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.*

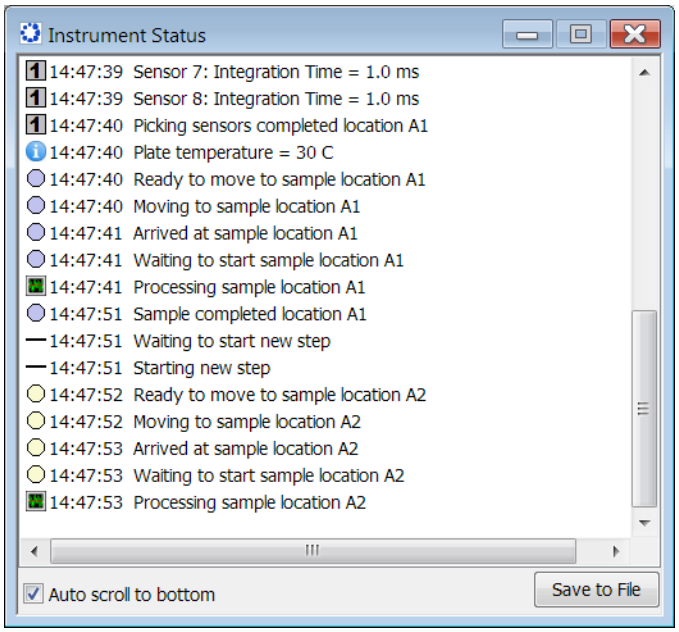


Figure 6-55: Instrument Status Log

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. ***NOTICE:*** *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 **GO** .
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 **Runtime Binding Chart** for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the **Runtime Binding Chart**. 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 **File > Options**. The factory set default temperature is 30 °C. ***NOTICE:*** *If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet® 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® 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® 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. • 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.
Acquisition rate: • Octet® RED96 • Octet® RED96e • Octet® R8	***NOTICE:*** *For the Octet® RED, Octet® RED96, Octet® RED96e, and Octet® R8 systems, acquisition rate settings are available on the Plate Definition Tab.* • 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. • 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. • 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.
Sensor offset (mm)	Recommended sensor offset for quantitation—3 mm. ***NOTICE:*** *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® 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 color-coded (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® 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.*

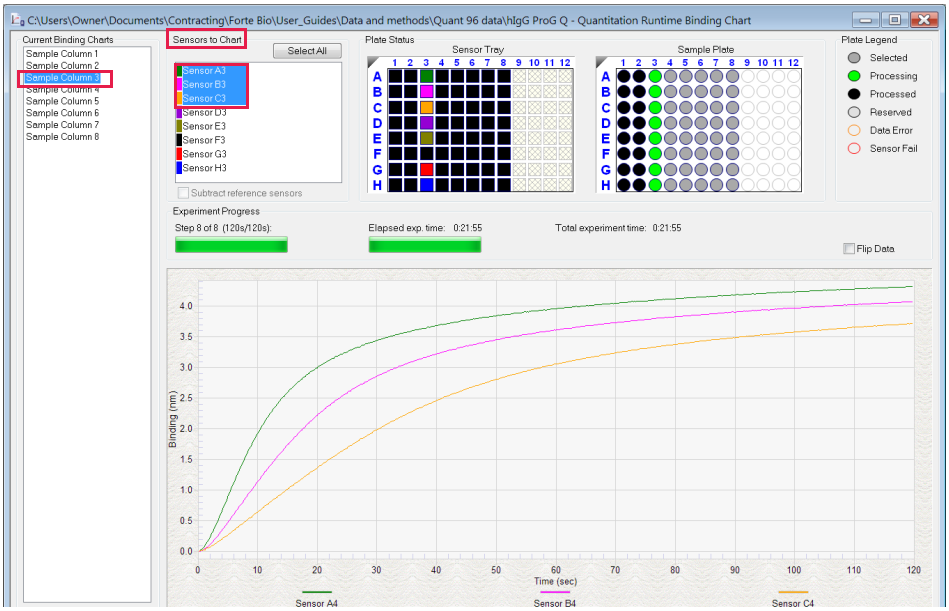


Figure 6-56: Runtime Binding Chart Window

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).

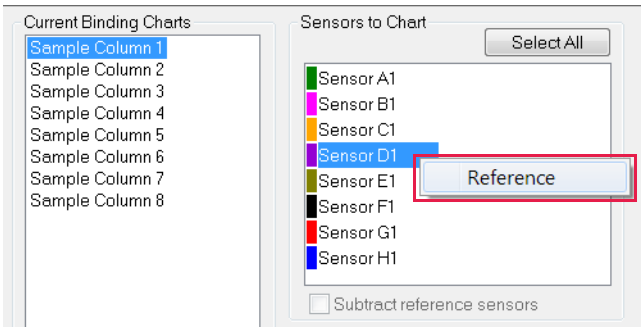


Figure 6-57: Designating a Reference Biosensor in the Runtime Binding Chart

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).

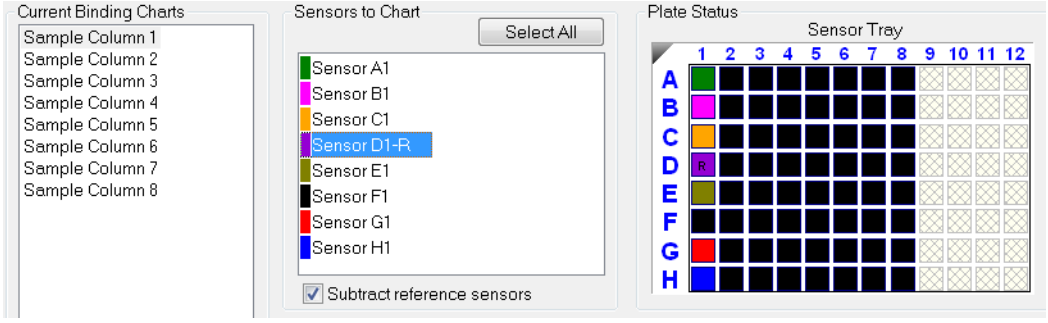


Figure 6-58: Subtract Reference Sensors check box in the Runtime Binding Chart

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.

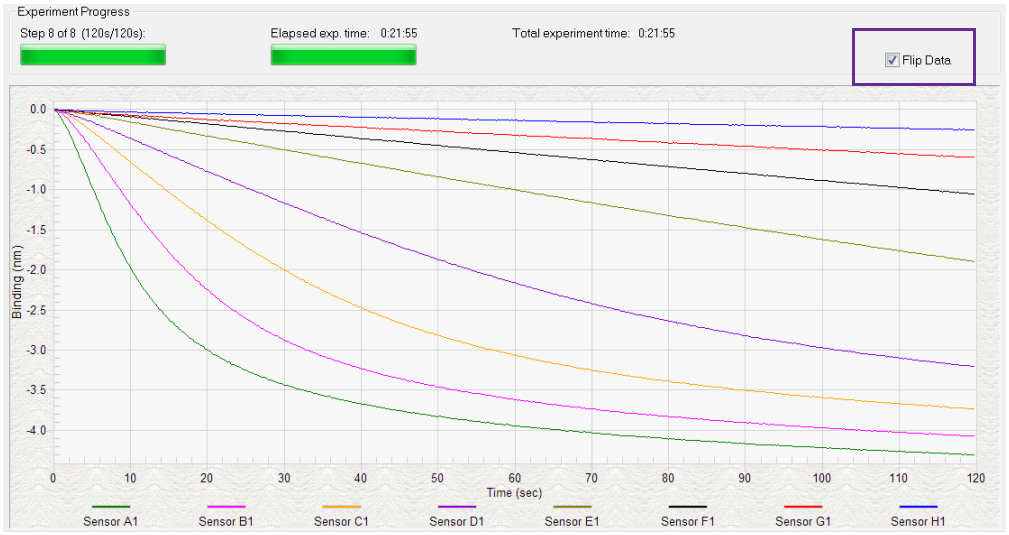


Figure 6-59: Data Inverted Using Flip Data Function

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

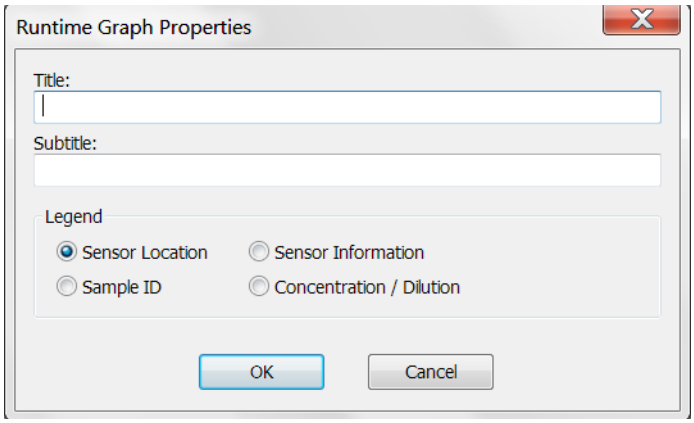


Figure 6-60: Selecting a Runtime Binding Chart Legend

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**.

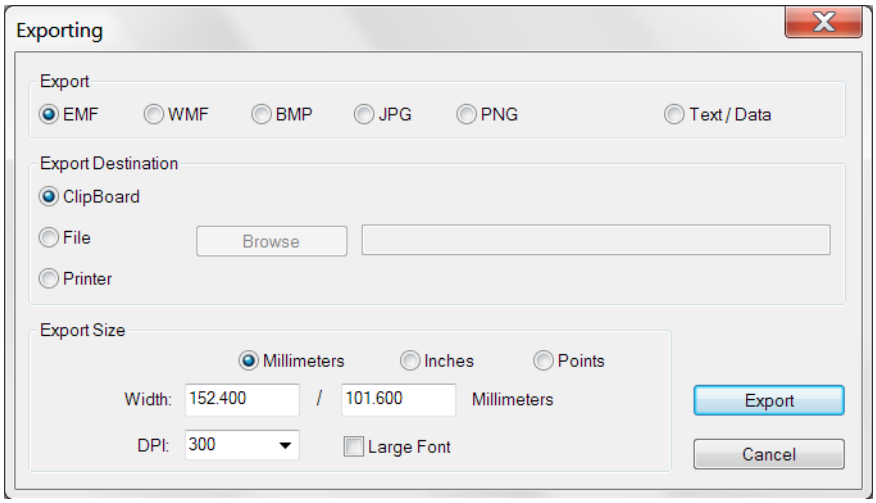


Figure 6-61: Exporting Dialog Box

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 **File** > **Browse** 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 **File** > **Browse** to select a folder and enter a file name.	Creates a graphic image.
Copy the Run-time Binding Chart		✓	Clipboard	Copies the chart to the system clip-board
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

Managing Experiment Method Files

After you run an experiment, the Octet® 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® 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 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 6-62.

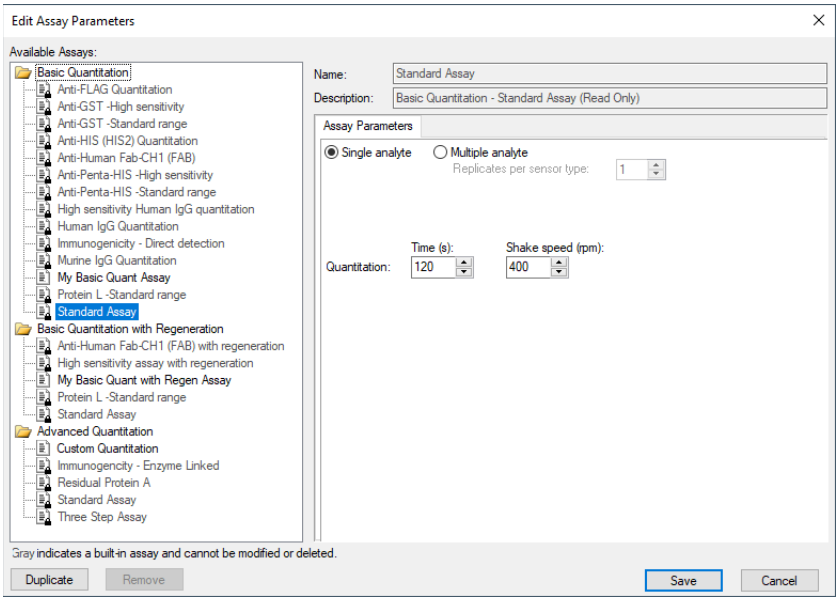


Figure 6-62: Edit Assay Parameters Dialog Box

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).

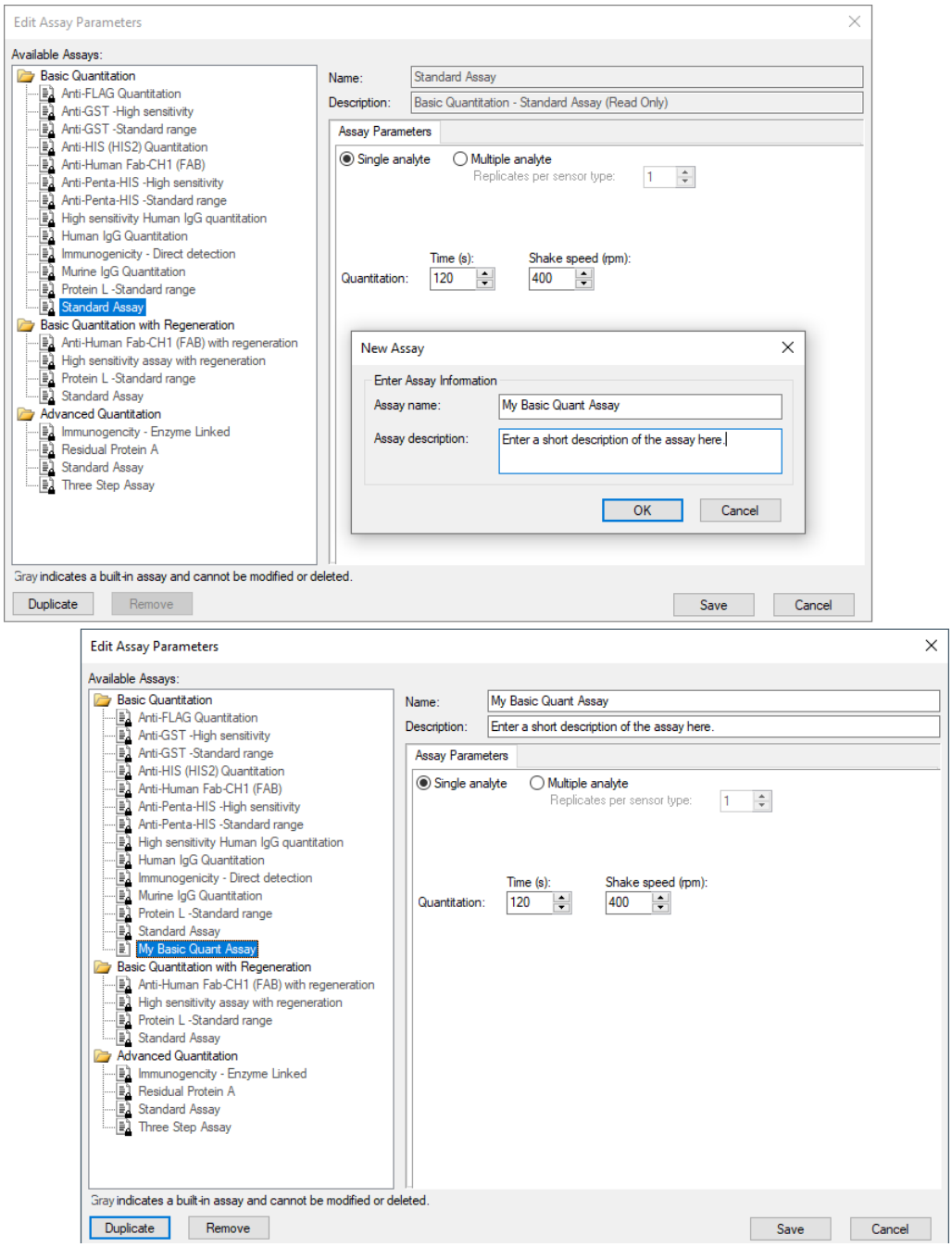


Figure 6-63: Defining a New Assay

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

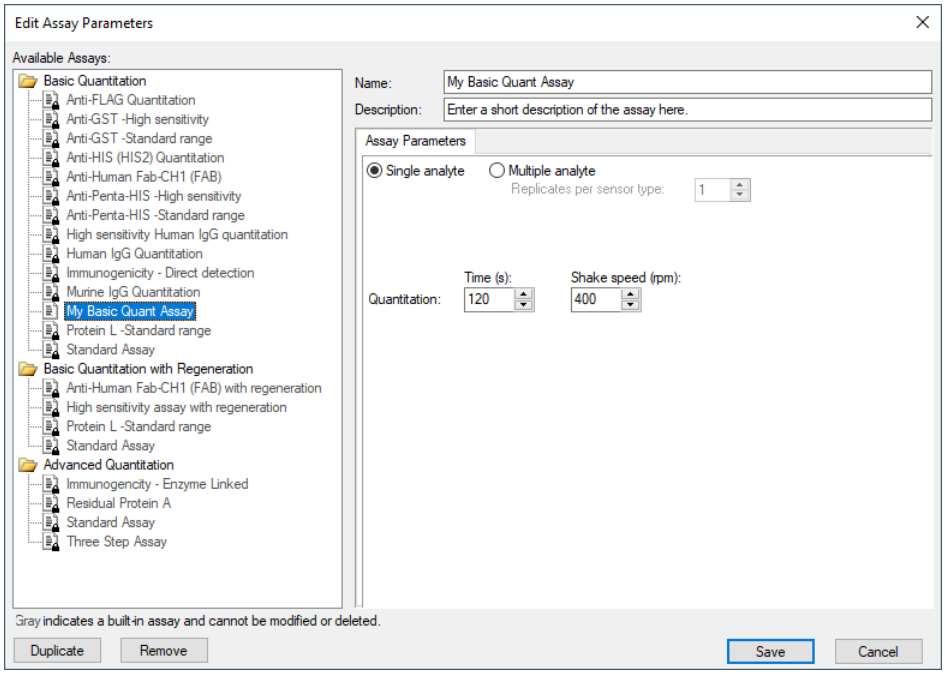


Figure 6-64: Assay Parameters—Basic Quantitation Assay

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 Replicates 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. ***NOTICE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

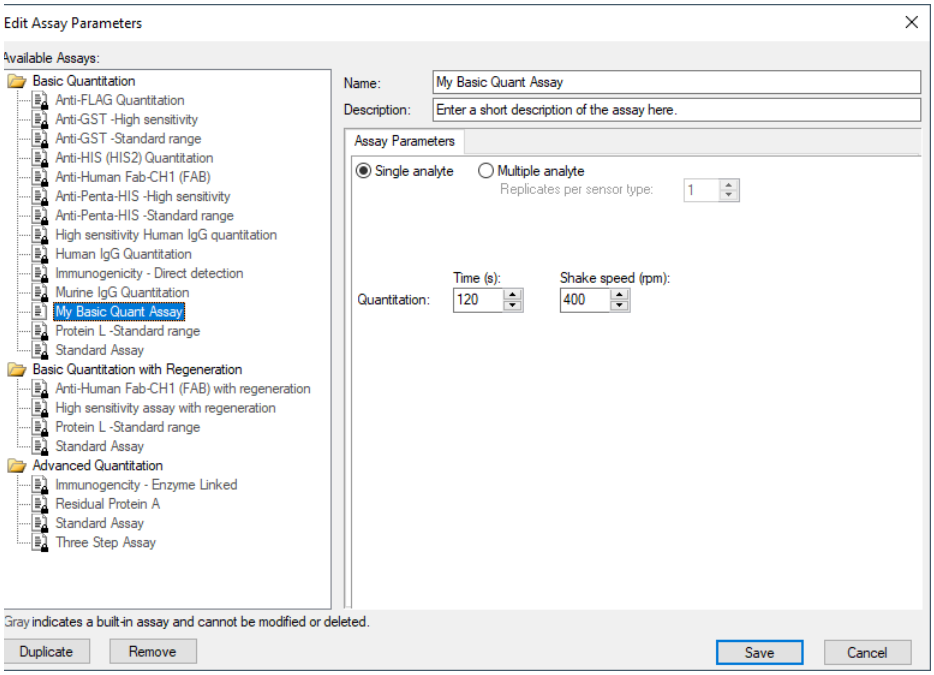


Figure 6-65: Assay Parameters—Basic Quantitation with Regeneration

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 Replicates 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). ***NOTICE:*** *A subset of data points may be selected for processing during data analysis.*

Table 6-15: Assay Parameters—Basic Quantitation with Regeneration (Sheet 2 of 2)

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 regenerated 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.

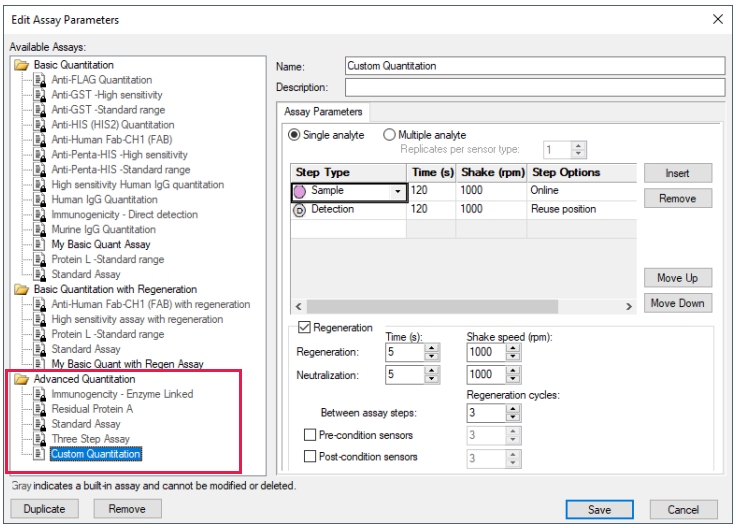


Figure 6-66: Assay Parameters—Advanced Quantitation

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).

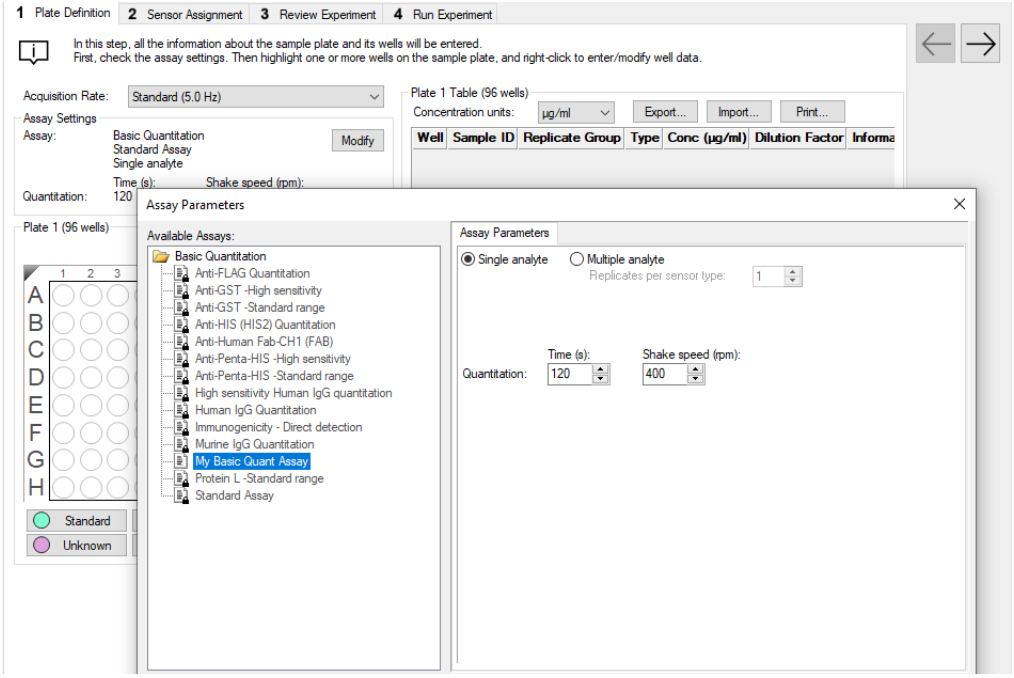


Figure 6-67: Selecting a Custom Assay

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® system and the Octet® 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 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:

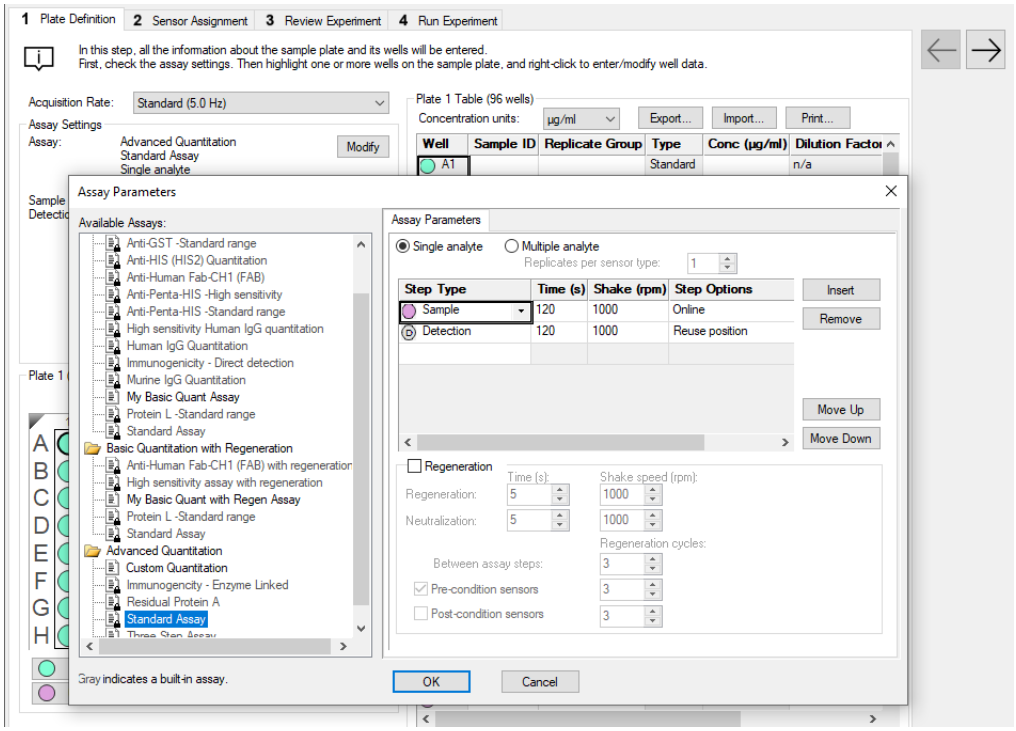


Figure 6-68: Assay Parameters Window.

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.

2. 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:

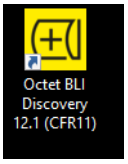
Quantitation Experiments: Octet® RH16, Octet® RH96, and Octet® QK384

Introduction

A quantitation experiment enables you to determine analyte concentration within a sample using a reference set of standards. After starting the Octet® system hardware and the Octet® 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® BLI Discovery software versions 8.0 and higher.*

Table 7-1: Setting Up and Analyzing a Quantitative Experiment

Software	Step	See
Octet® BLI Discovery 	1. Select a quantitation experiment in the **Experiment Wizard** or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 217
	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® Analysis Studio 	7. Analyze the binding data. 8. Generate a report.	*Octet® Analysis Studio Software User Guide*

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® 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® 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  or 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 Regeneration	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two-or three-step quantitation assay that enables signal amplification 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.

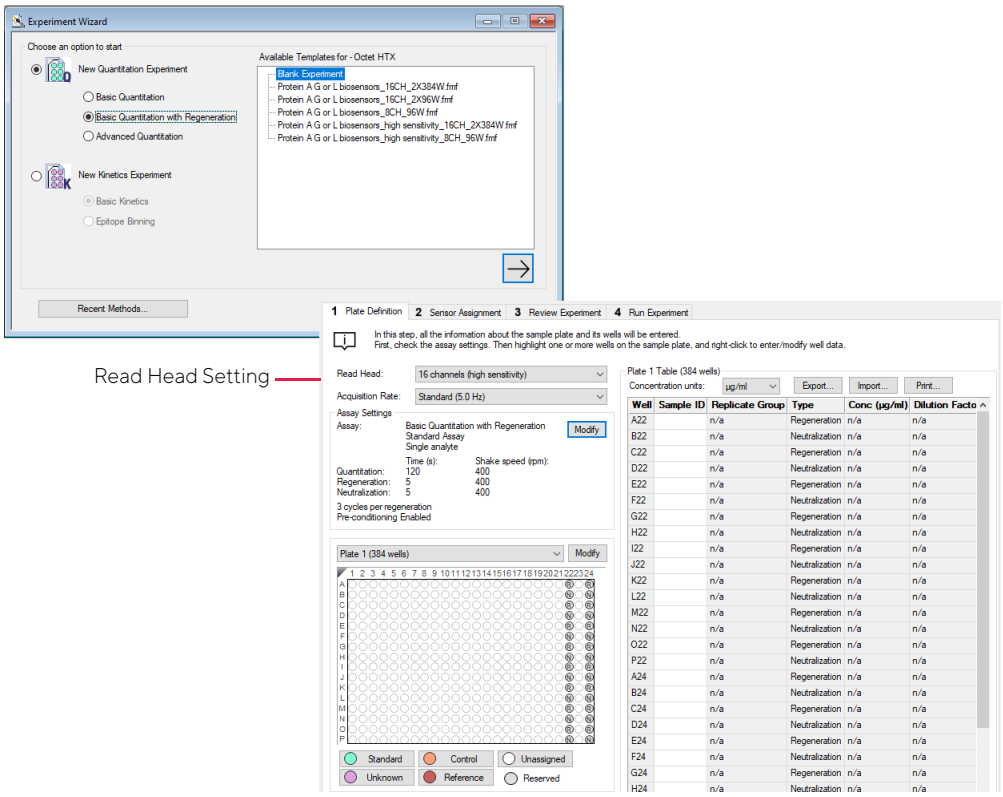


Figure 7-1: Selecting an Experiment Type in the Experiment Wizard (for Octet® RH16)

5. Click the → arrow.

   The **Experiment** window appears (Figure 7-1, right).

6. **Octet® 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.

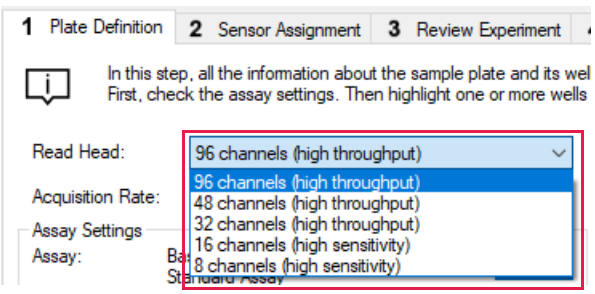


Figure 7-2: Selecting Read Head Channels

Defining the Sample Plate

NOTICE: *Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet® BLI Discovery software versions 8.0 and higher (Figure 7-3).*

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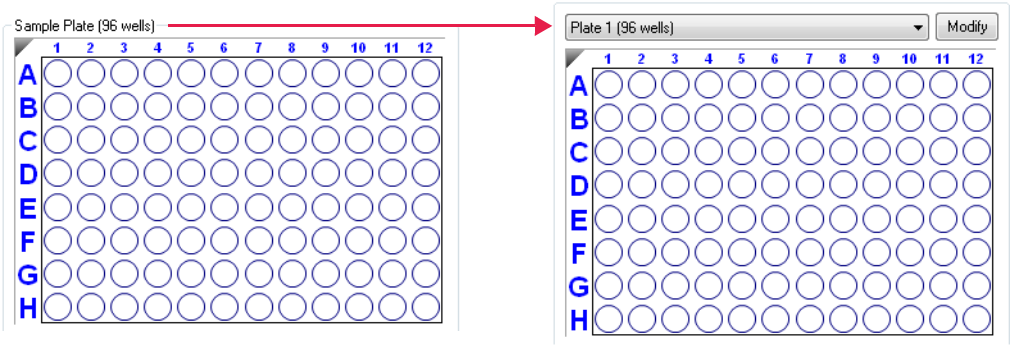


Figure 7-3: Sample Plate Renamed Plate 1 in Software Versions 8.0 and Higher

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).
3.	Designate the samples.
4.	Annotate the samples (optional).
5.	Save the sample plate definition (optional).

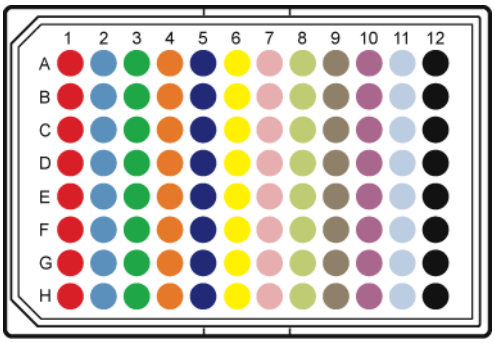
Read Head Configuration and Plate Layout

Octet® RH16 and Octet® QK384

The Octet® 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).

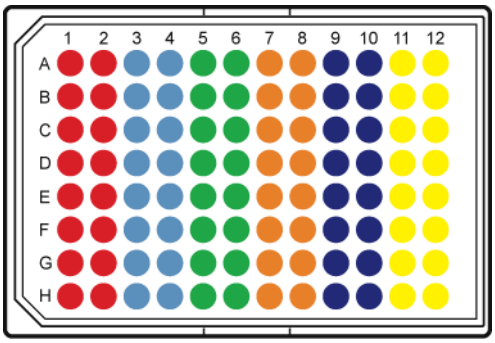
The read head configuration and the plate format (96 or 384 wells) determine the plate layout (Figure 7-4 and Figure 7-5).

8 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

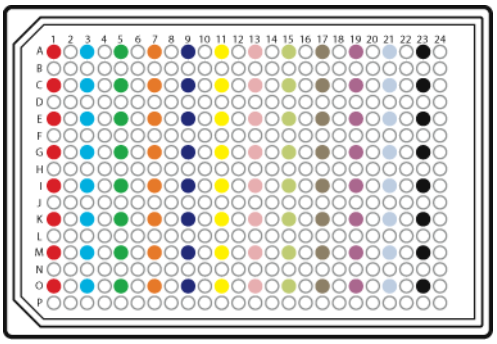
16 Channel Read Head



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.

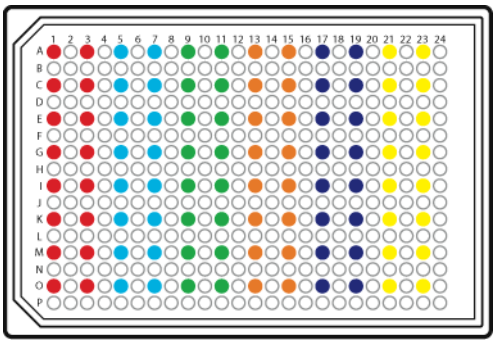
Figure 7-4: Color-Coded Wells: How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

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 7-5: 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® RH96

The Octet® 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® 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.

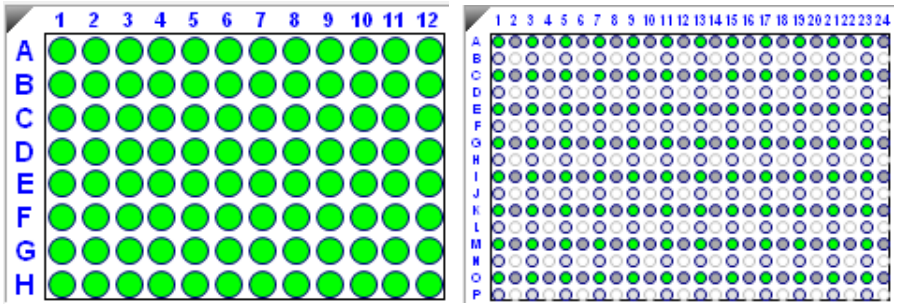


Figure 7-6: Biosensor Layout in 96- and 384-well Plates Using 96-channels Read Head Setting.

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® 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® 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:

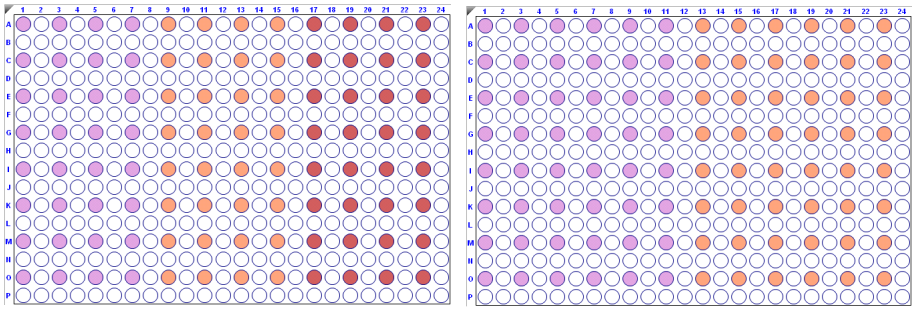


Figure 7-7: Biosensor Layout in 384-well Plates Using 32 (left) and 48 (right) Channels Read Head Setting.

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® RED96e and Octet® 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.

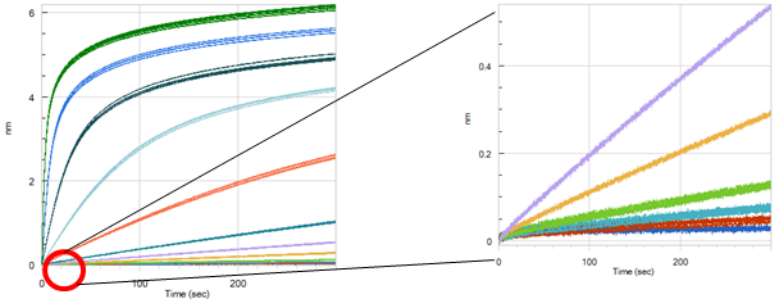
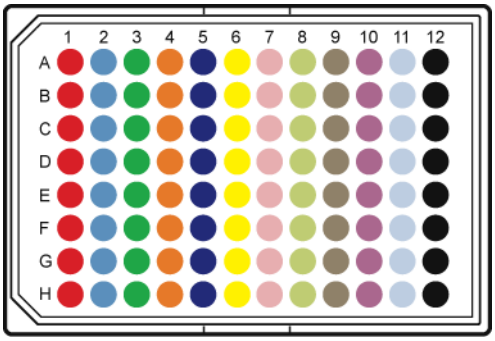


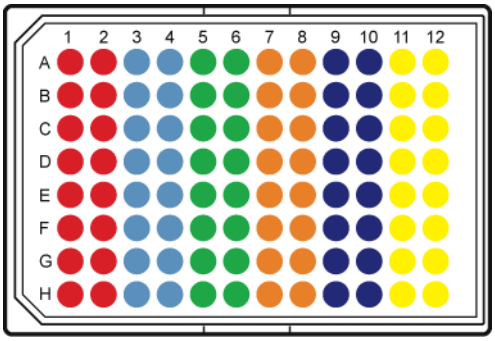
Figure 7-8: Zoomed View of Closely Overlaid Traces Shows Fine Signal Resolution for Human IgG Quantitation Assay with Protein A Biosensors

8 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

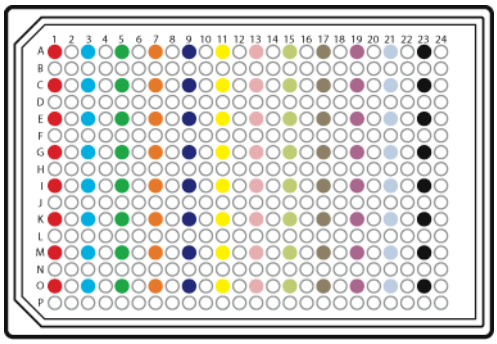
16 Channel Read Head



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.

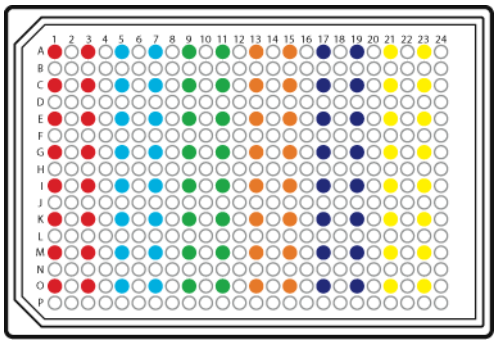
Figure 7-9: Color-Coded Wells: How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

8 Channel Read Head



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 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® 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.

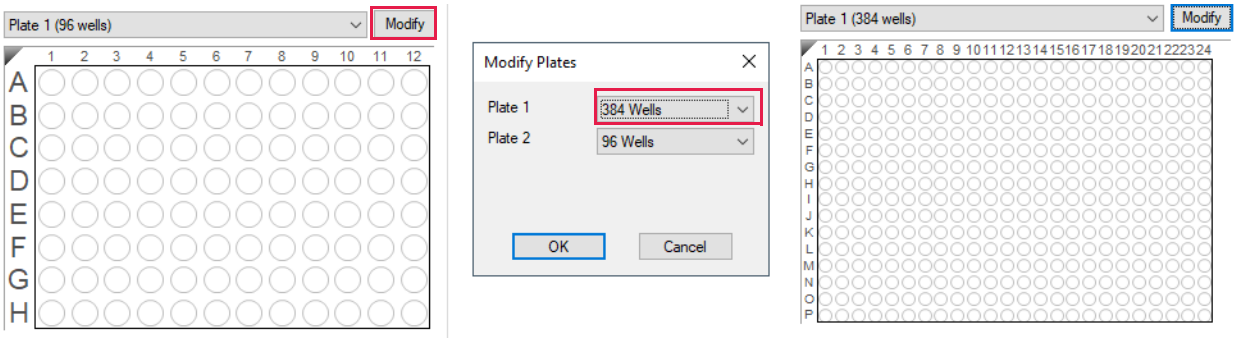


Figure 7-11: Changing the Sample Plate Format

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® 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

Icon	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 **Unknowns**, **Controls**, and **Standards**. 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), **Detection** (D), or **Capture Antibody** (C). Reserved wells are not available for use as **Standards**, **Unknowns**, **Controls**, 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. The default location of the reserved wells depends on the sample plate format (96 or 384-wells) and the Octet® instrument read head configuration (8 or 16 channels).

Reserved samples cannot be removed from the sample plate, but you can change their column location. To change the location of a reserved column (R, N, D, or C) 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
R Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).
N Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.
D 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.
C Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of interest onto the biosensor.

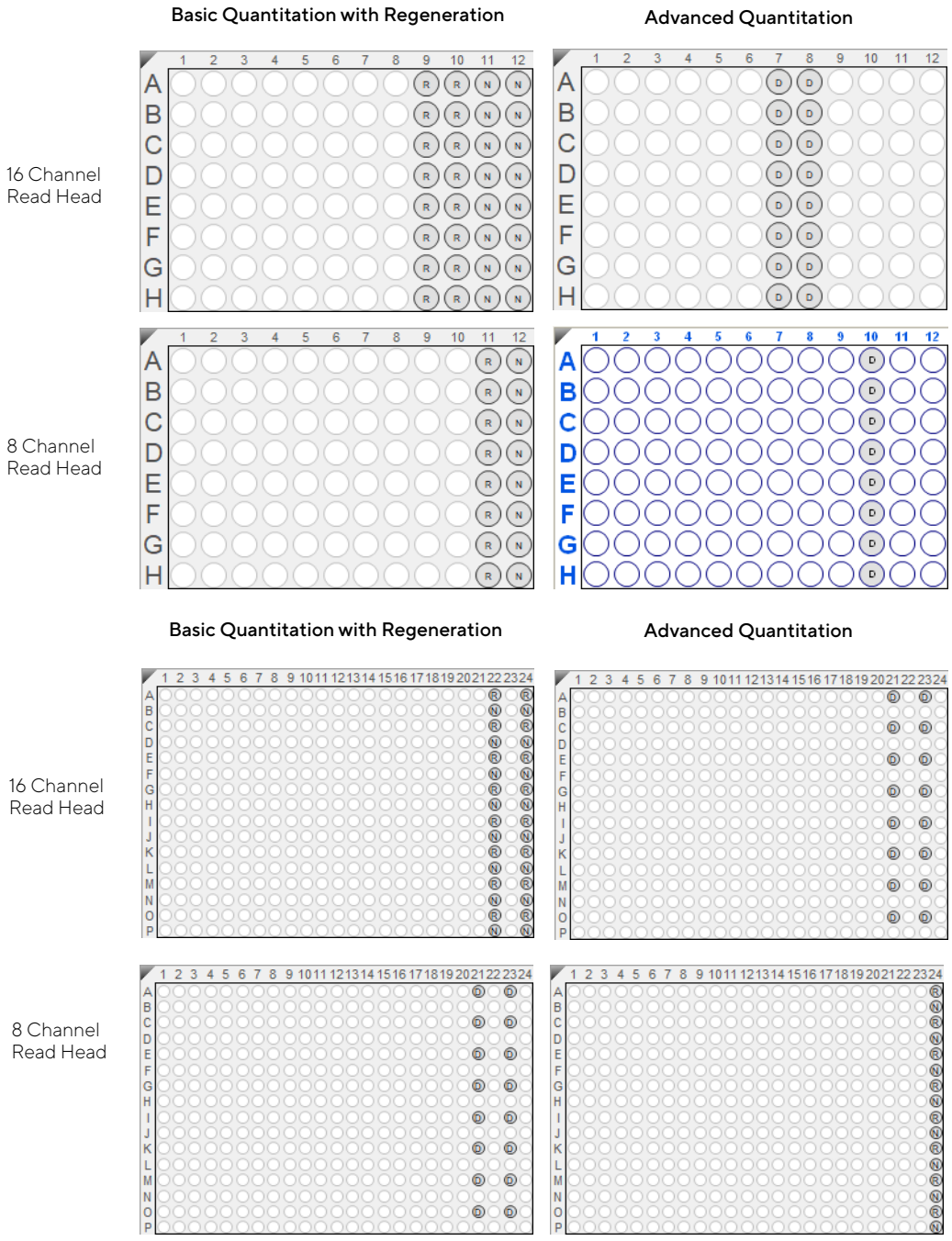


Figure 7-12: Default Locations for Reserved Wells in 96-well (top) and 384-well Sample Plate Maps (bottom)

Selecting Wells in the Sample Plate Map

NOTICE: *Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet® 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 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 (Figure 7-13, center).
- Click a well or draw a box around a group of wells (Figure 7-13, right).

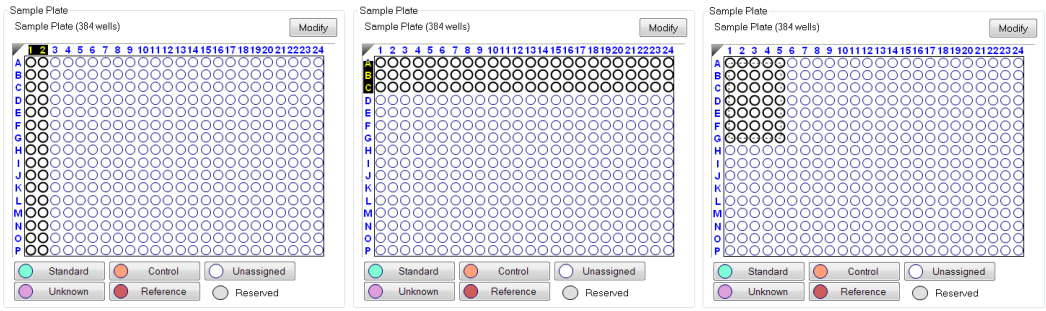


Figure 7-13: Selecting Wells in the Sample Plate Map

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® 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**.

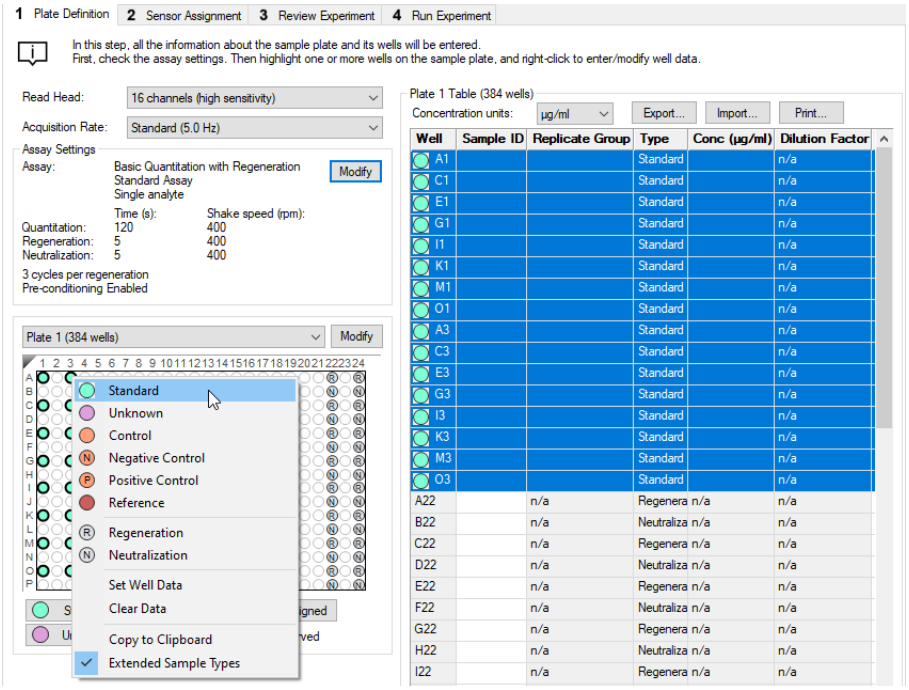


Figure 7-14: Plate Definition Window—Designating Standards

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).

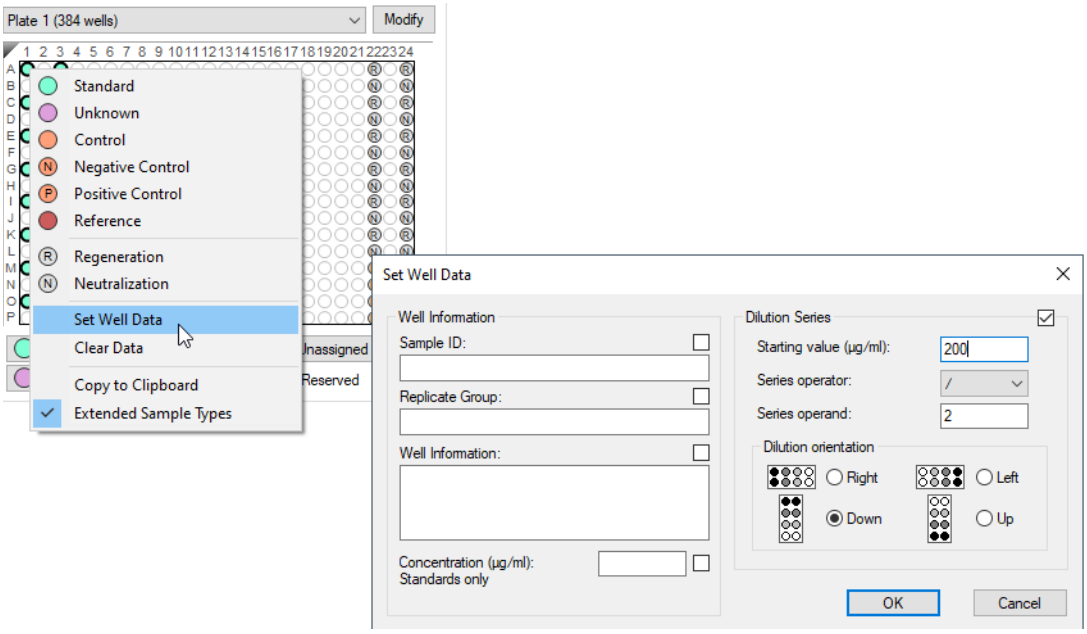


Figure 7-15: Sample Plate Map—Setting a Dilution Series

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 d**isplays 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).

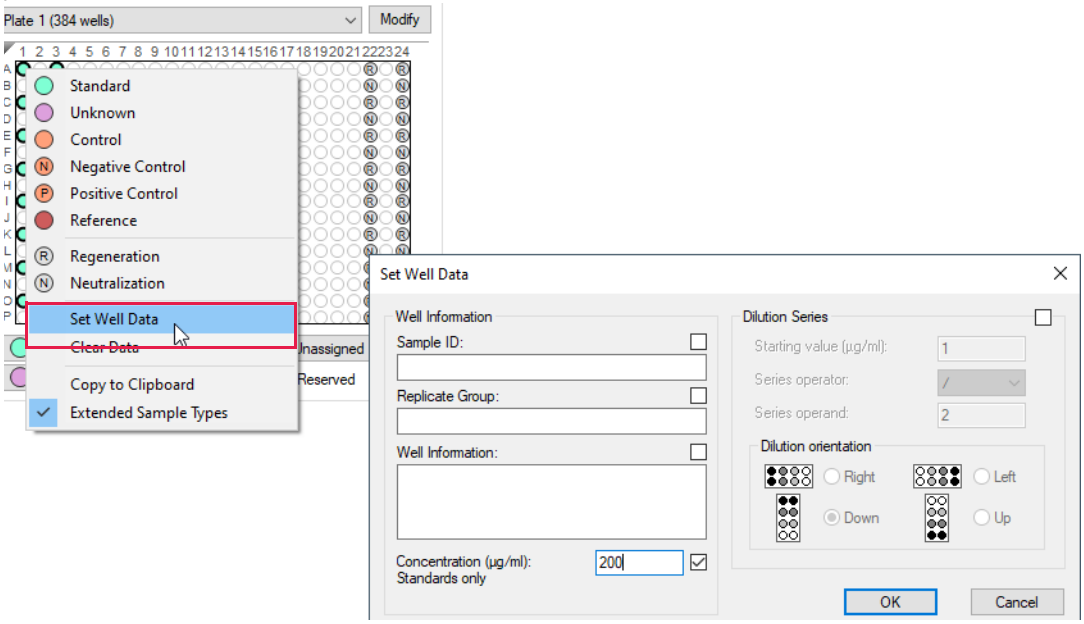


Figure 7-17: Sample Plate Map—Assigning a Standard Concentration

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).

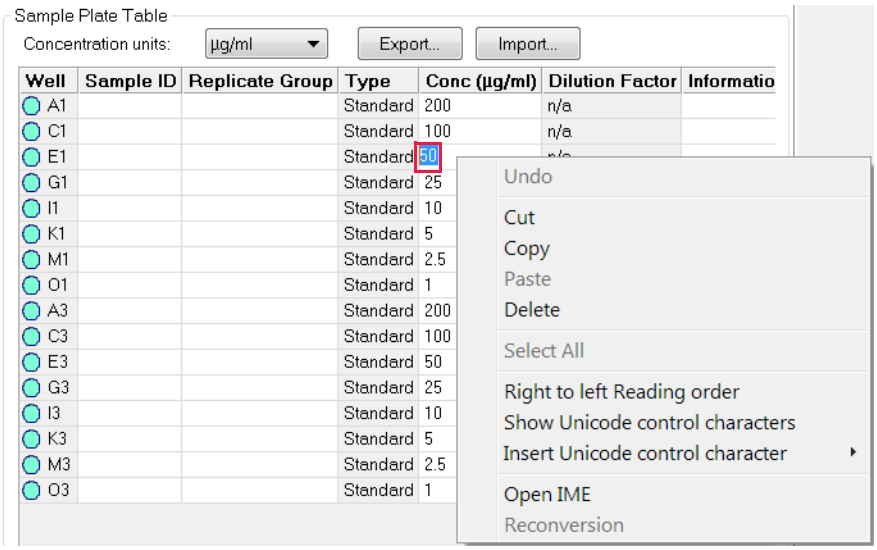


Figure 7-18: Sample Plate Table—Shortcut Menu of Edit Commands

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® 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).

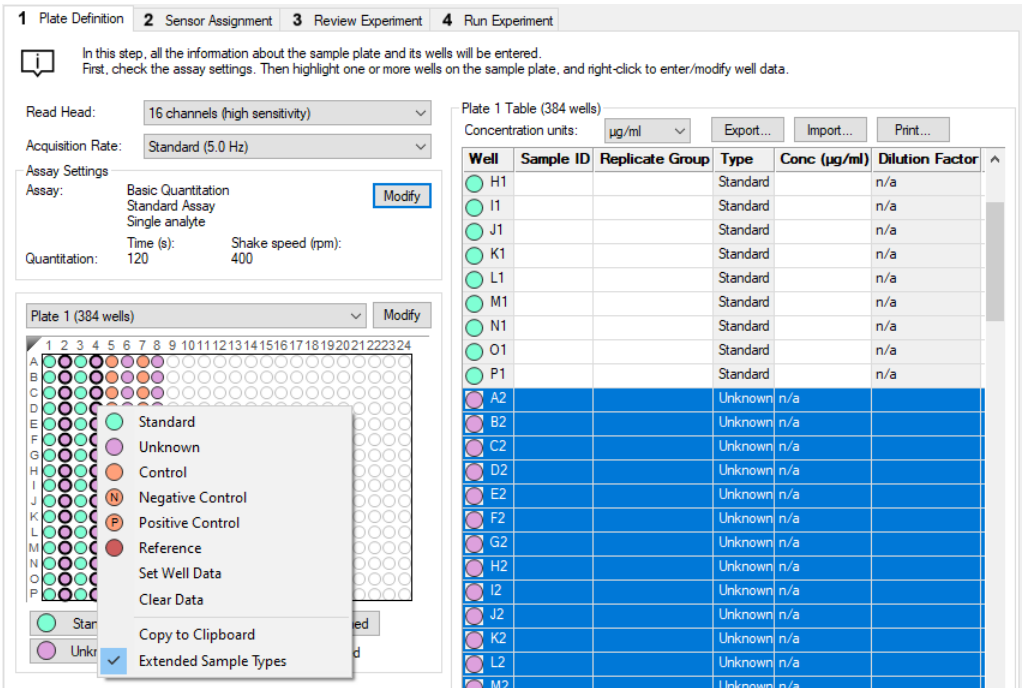


Figure 7-19: Plate Definition Window—Designate Unknown Wells

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).

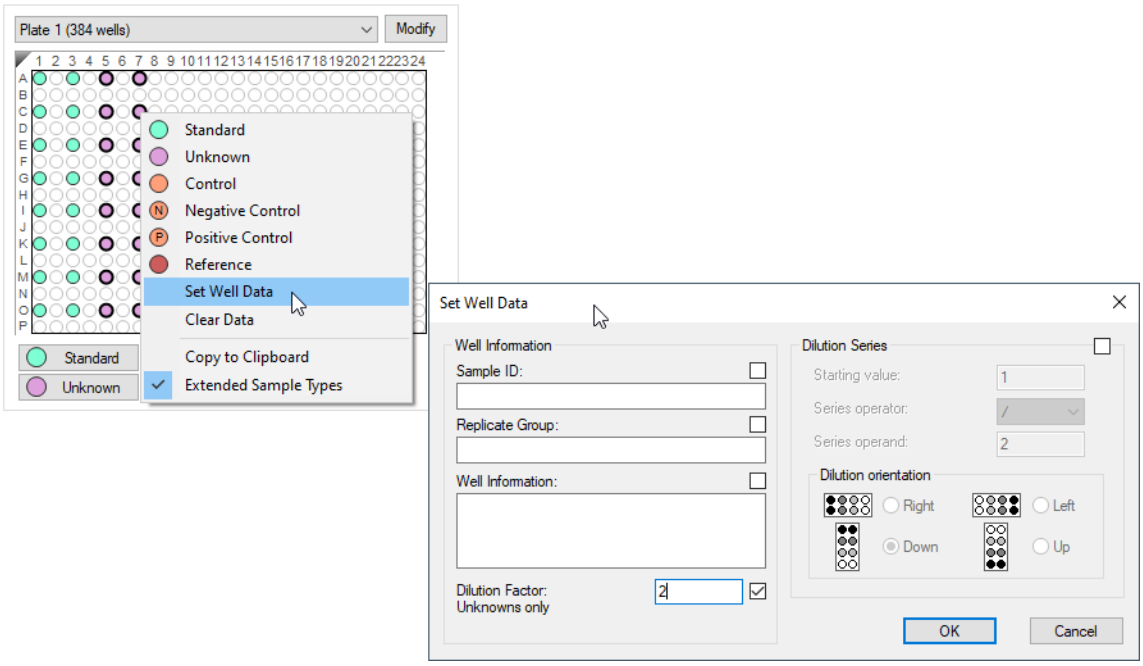


Figure 7-20: Sample Plate Map—Setting a Dilution Factor or a Serial Dilution

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).

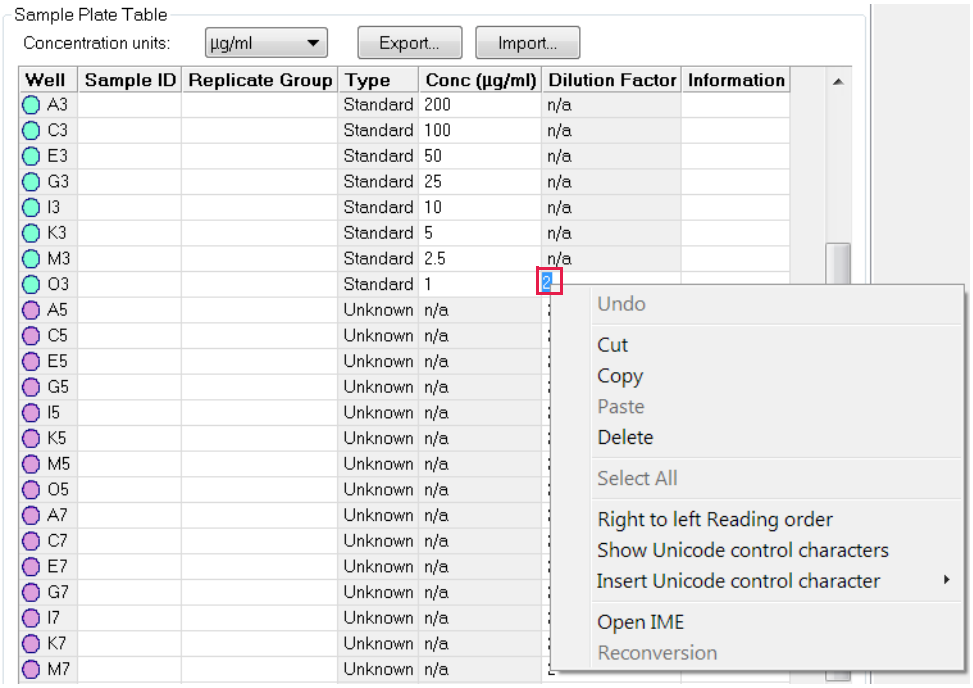


Figure 7-22: Sample Plate Table—Shortcut Menu of Edit Commands

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® 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.

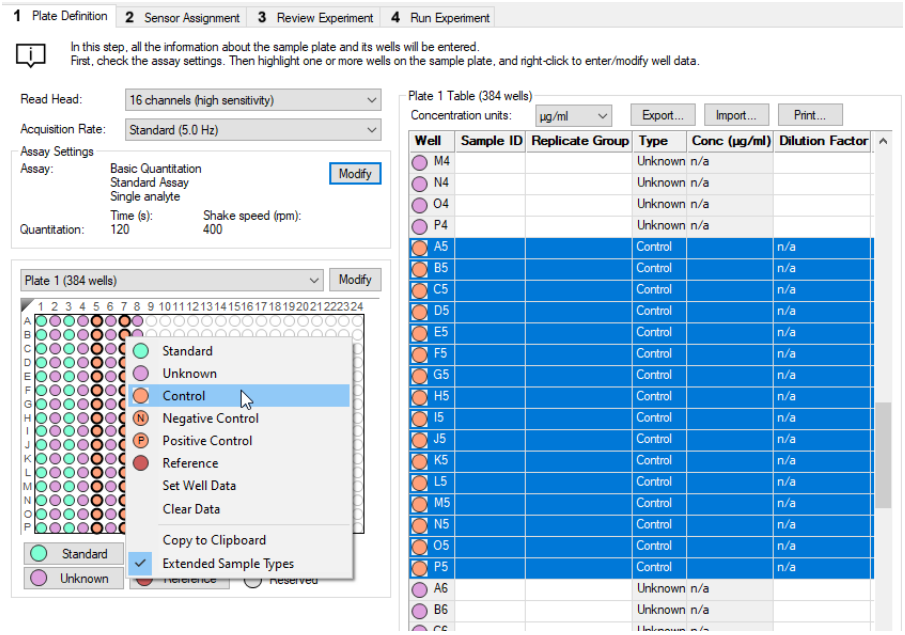


Figure 7-23: Designate Controls or Reference Wells

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**.

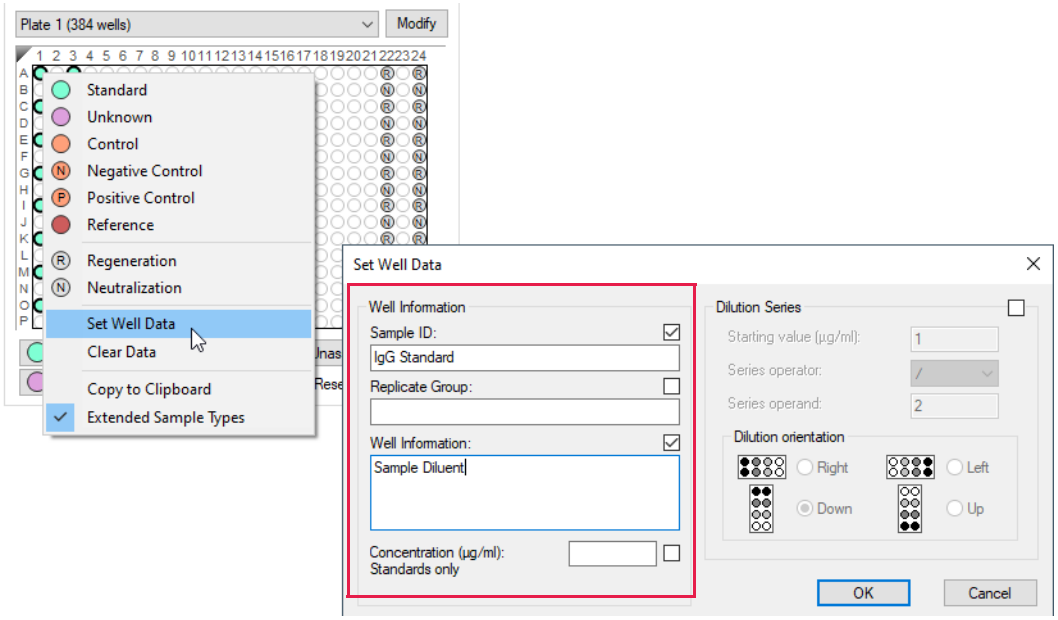


Figure 7-24: Adding Sample Annotations from the Sample Plate Map

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.*

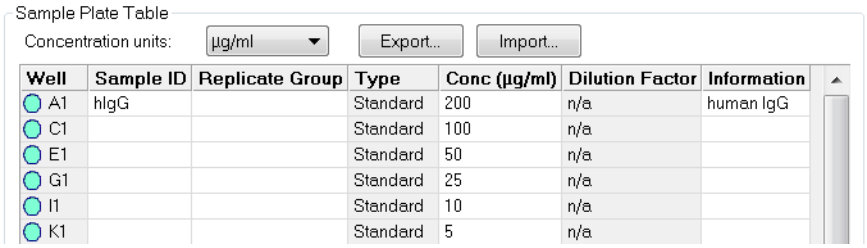


Figure 7-25: Adding Sample Annotations in the Sample Plate Table

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**.

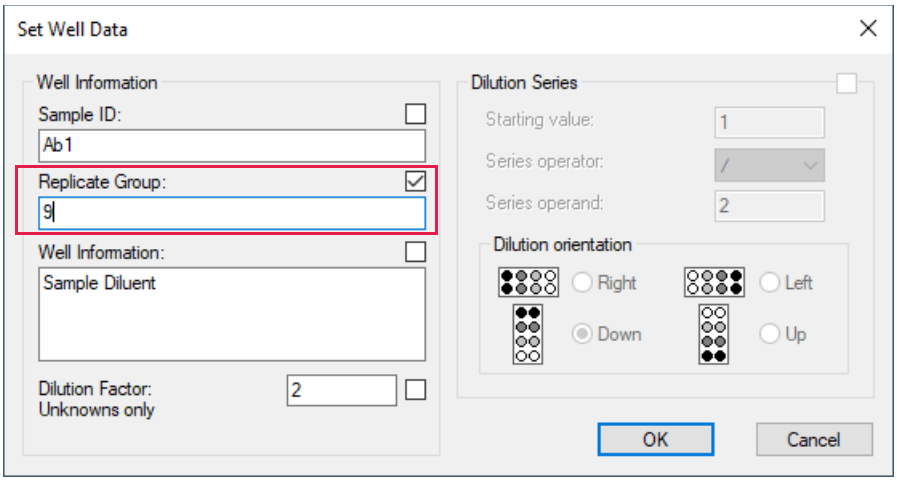


Figure 7-27: Add Replicate Group from the Sample Plate Map

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).

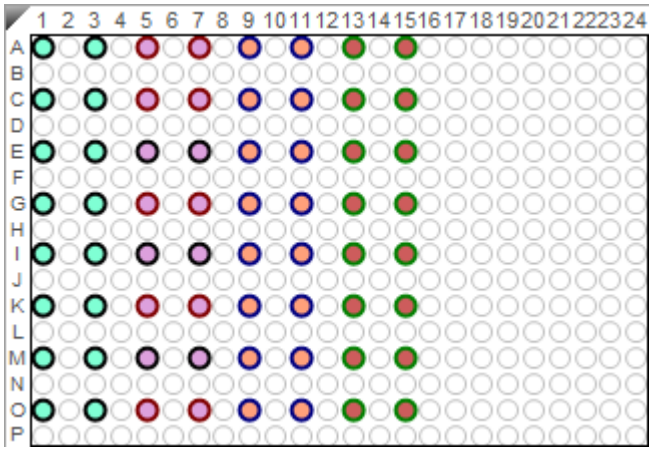
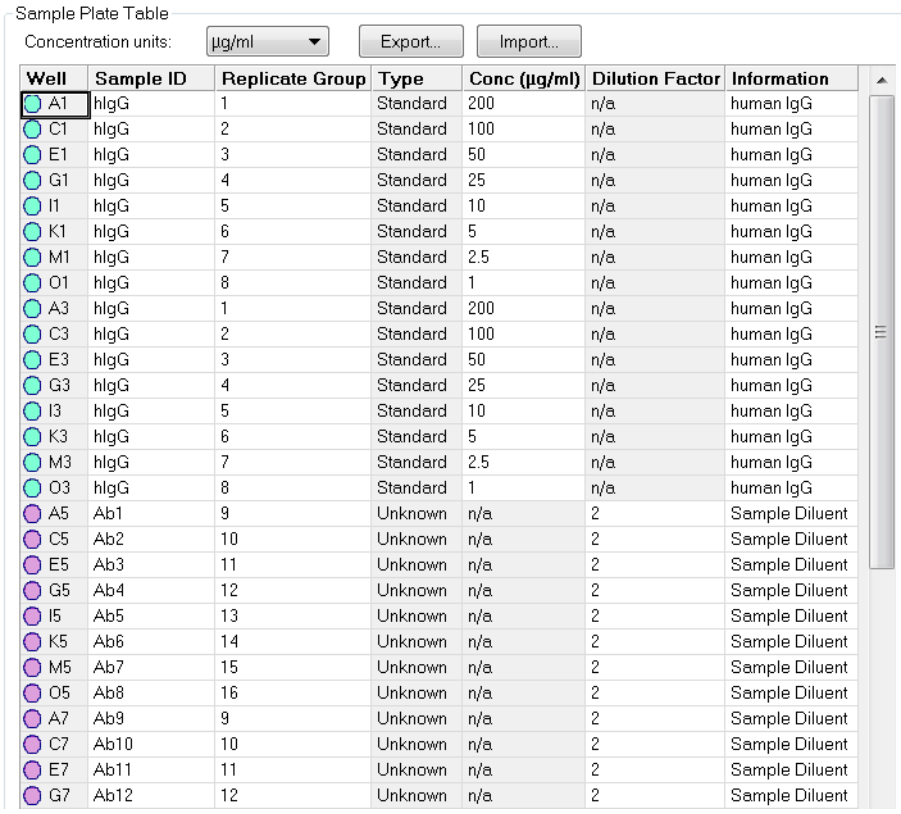


Figure 7-28: Replicate Groups: Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 7-29).


Sample Plate Table

Concentration units: µg/ml Export... Import...

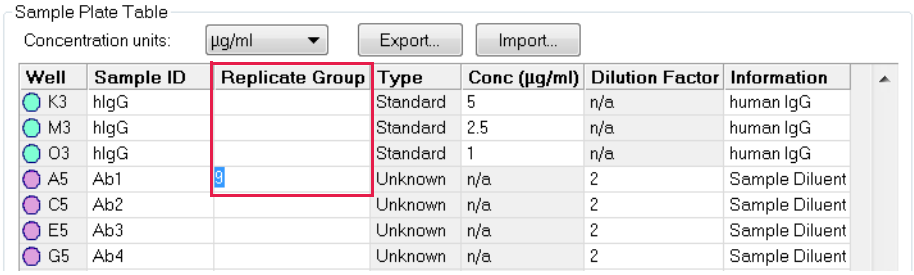
Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hIgG	1	Standard	200	n/a	human IgG
C1	hIgG	2	Standard	100	n/a	human IgG
E1	hIgG	3	Standard	50	n/a	human IgG
G1	hIgG	4	Standard	25	n/a	human IgG
I1	hIgG	5	Standard	10	n/a	human IgG
K1	hIgG	6	Standard	5	n/a	human IgG
M1	hIgG	7	Standard	2.5	n/a	human IgG
O1	hIgG	8	Standard	1	n/a	human IgG
A3	hIgG	1	Standard	200	n/a	human IgG
C3	hIgG	2	Standard	100	n/a	human IgG
E3	hIgG	3	Standard	50	n/a	human IgG
G3	hIgG	4	Standard	25	n/a	human IgG
I3	hIgG	5	Standard	10	n/a	human IgG
K3	hIgG	6	Standard	5	n/a	human IgG
M3	hIgG	7	Standard	2.5	n/a	human IgG
O3	hIgG	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
I5	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
O5	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).


Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
K3	hIgG		Standard	5	n/a	human IgG
M3	hIgG		Standard	2.5	n/a	human IgG
O3	hIgG		Standard	1	n/a	human IgG
A5	Ab1	3	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® 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® 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**.

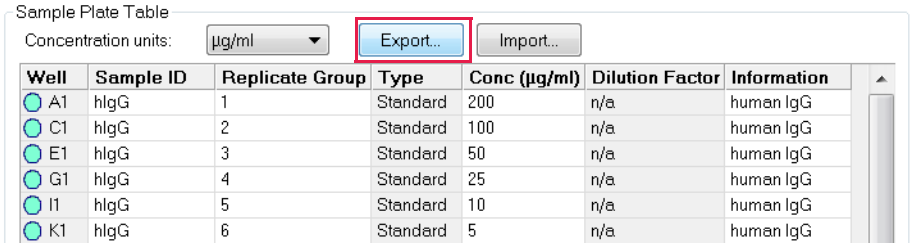


Figure 7-31: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 7-31), select a folder, enter a name for the plate (.csv), and click **Save**.

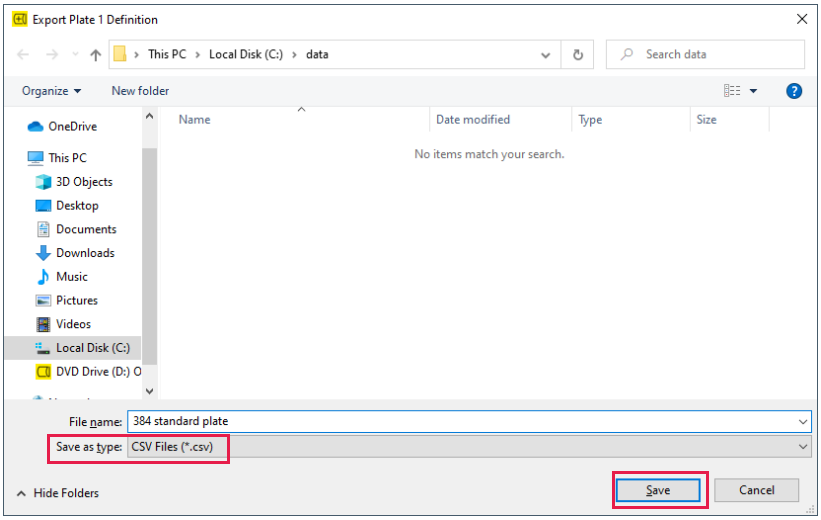


Figure 7-32: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the **Sample Plate Table** (see Figure 7-33), click **Import**.

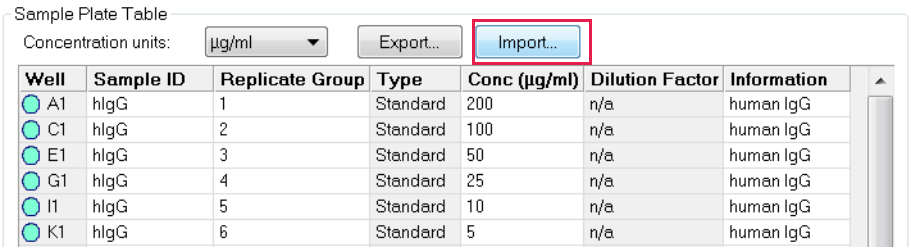


Figure 7-33: Import Button in Sample Plate Table

2. In the **Import Plate Definition** window (see Figure 7-35), select the plate definition (.csv), and click **Open**.

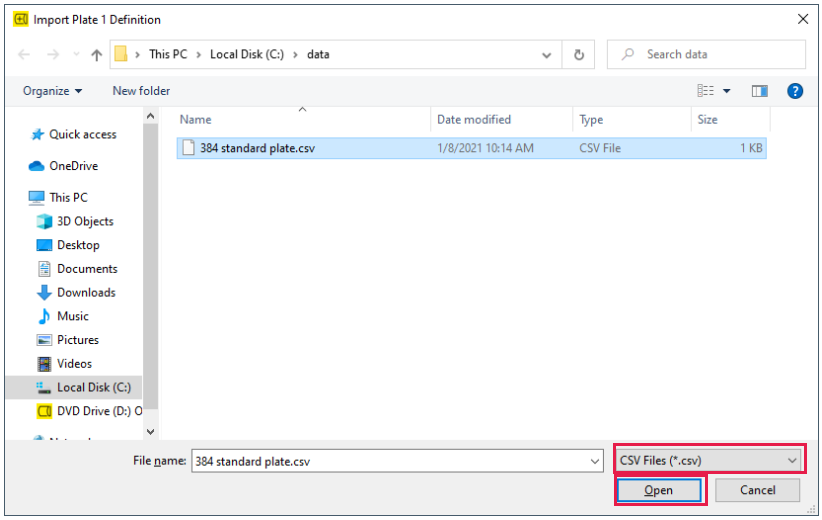


Figure 7-34: Import Plate Definition Window

NOTICE: *You can also create a .csv file for import. Figure 7-35 shows the appropriate column information layout.*

	A	B	C	D	E	F	G
1	PlateWells	384					
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information
3	A1	hIgG	1	Standard	200		human IgG
4	C1	hIgG	2	Standard	100		human IgG
5	E1	hIgG	3	Standard	50		human IgG
6	G1	hIgG	4	Standard	25		human IgG
7	I1	hIgG	5	Standard	10		human IgG
8	K1	hIgG	6	Standard	5		human IgG
9	M1	hIgG	7	Standard	2.5		human IgG
10	O1	hIgG	8	Standard	1		human IgG

384 Q method export

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**.

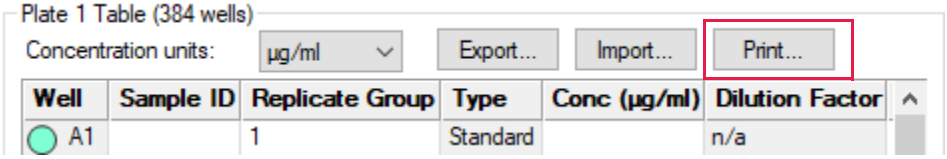


Figure 7-36: Sample Plate/Plate 1 Print Button

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® BLI Discovery software versions 8.0 and higher (Figure 7-37).*

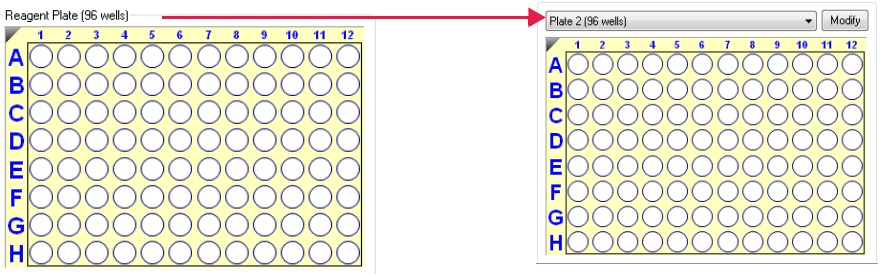


Figure 7-37: Reagent Plate Renamed Plate 2 in Software Versions 8.0 and Higher

Reagent Plate for Octet® RH16 and Octet® 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® RH96

The Octet® 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.

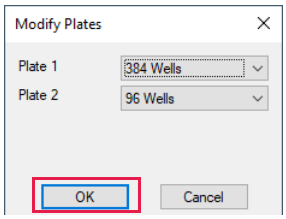
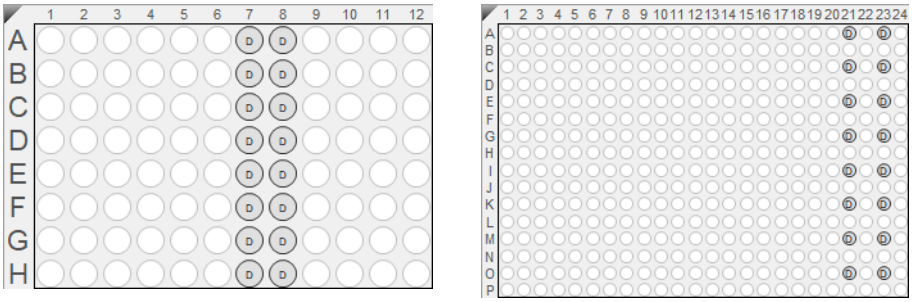


Figure 7-38: Modifying the Reagent Plate

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:

1. In the **Reagent Plate/Plate 2 Map** (see Figure 7-40), click **Print**.

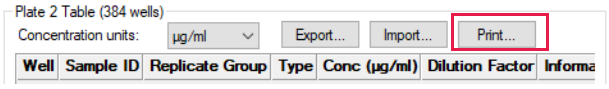


Figure 7-40: Reagent Plate/Plate 2 Print Button

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.

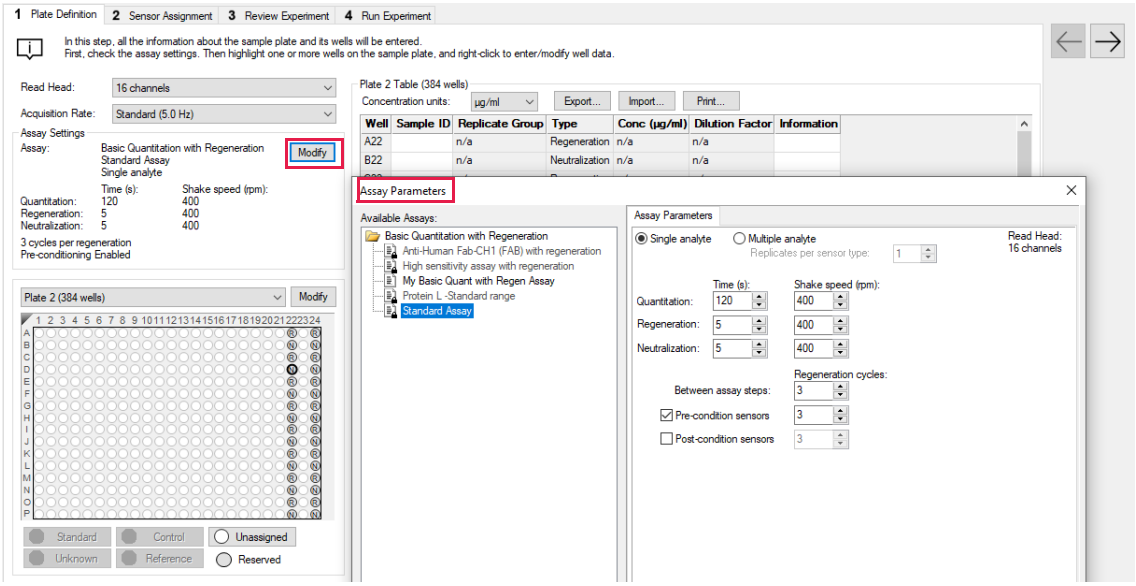


Figure 7-41: Modifying Assay Parameters.

Basic Quantitation Assay Parameters

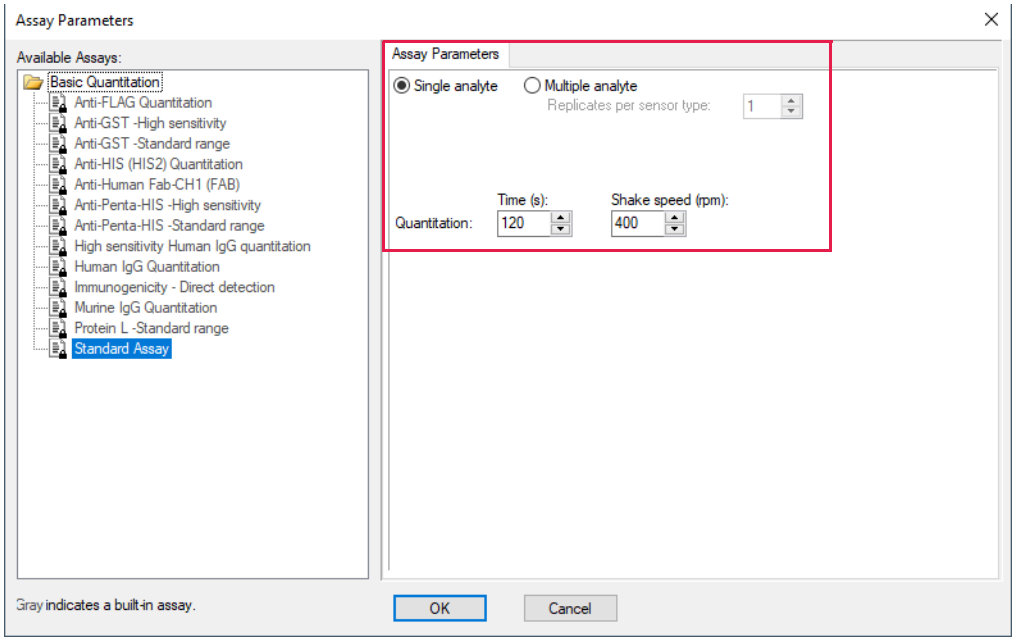


Figure 7-42: Assay Parameters—Basic Quantitation Assay

Table 7-6: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates 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. ***NOTICE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

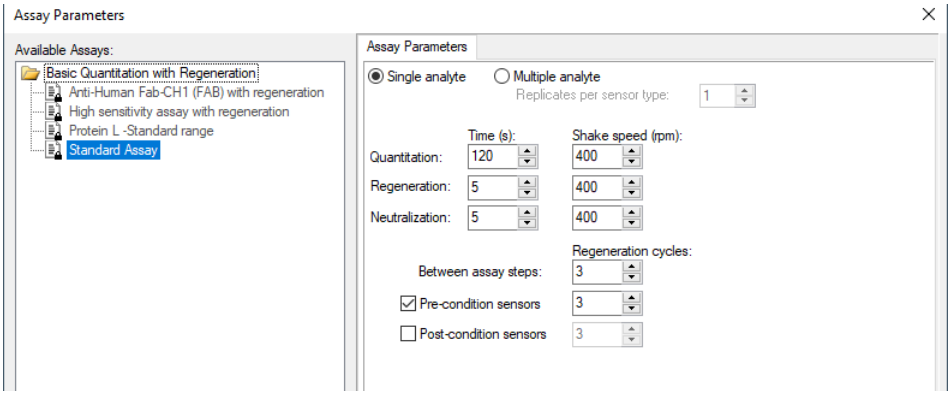


Figure 7-43: Assay Parameters—Basic Quantitation with Regeneration

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 Replicates 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). ***NOTICE:*** *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 regenerated 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.

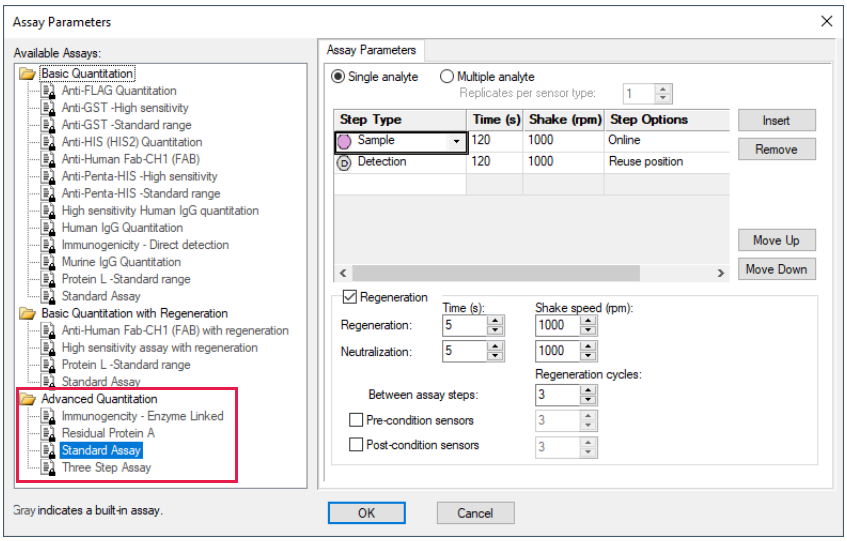


Figure 7-44: Assay Parameters—Advanced Quantitation

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.

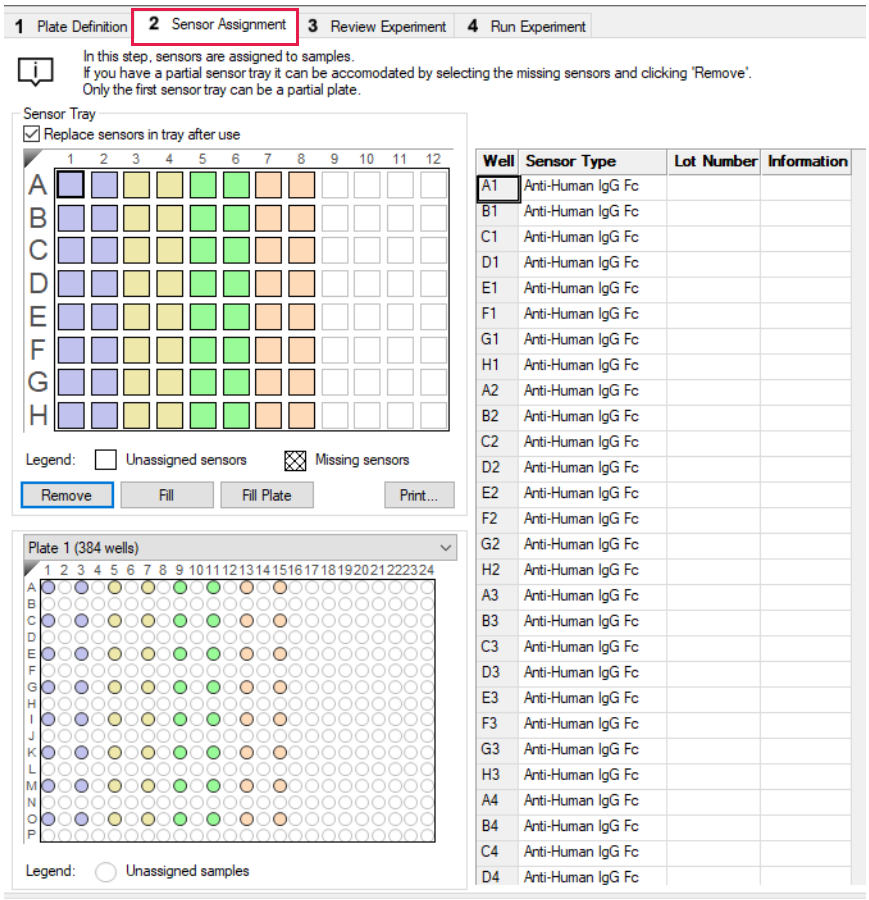


Figure 7-45: Sensor Assignment Window for Basic Quantitation without Regeneration

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.

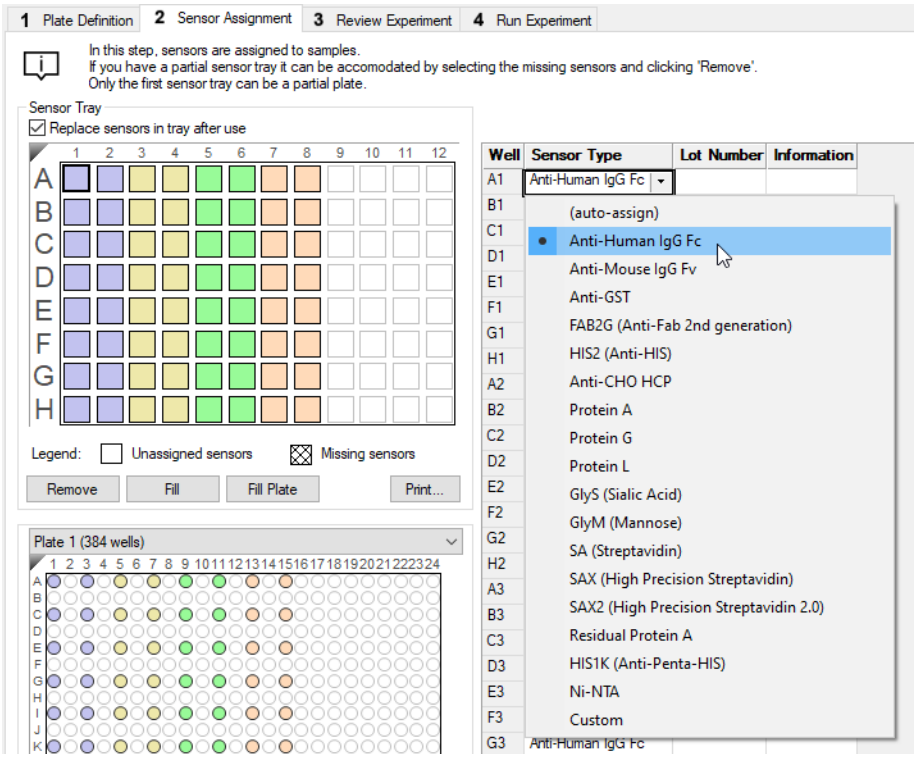


Figure 7-46: Changing Biosensor Types

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.*

5. 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).

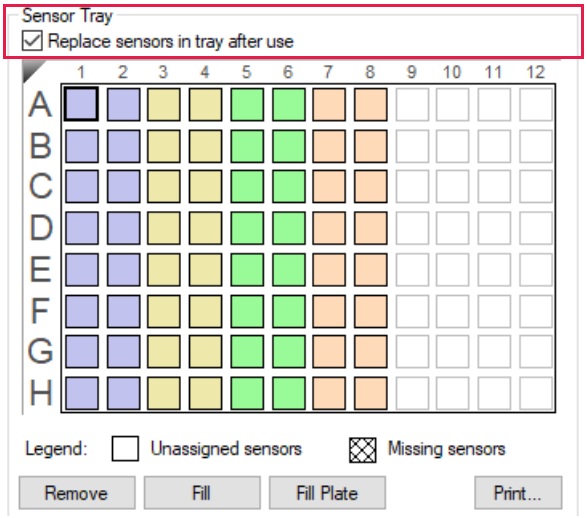


Figure 7-47: Replace Sensors in Tray After Use Check Box

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 → 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.

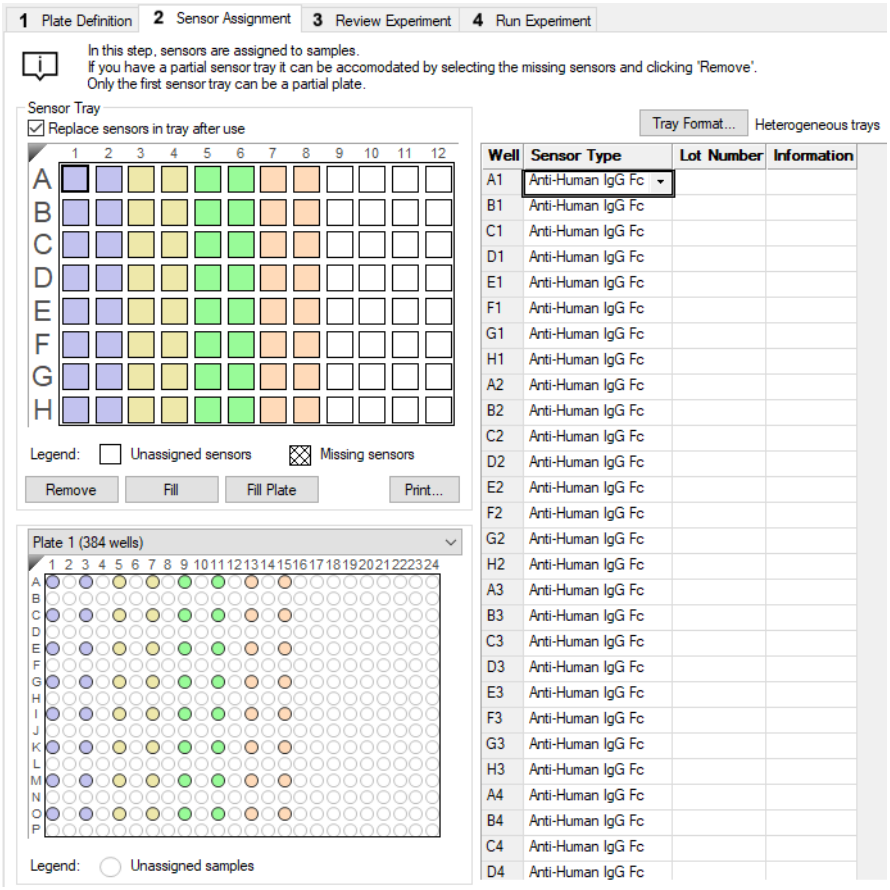


Figure 7-48: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

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 drop-down list (see Figure 7-49).

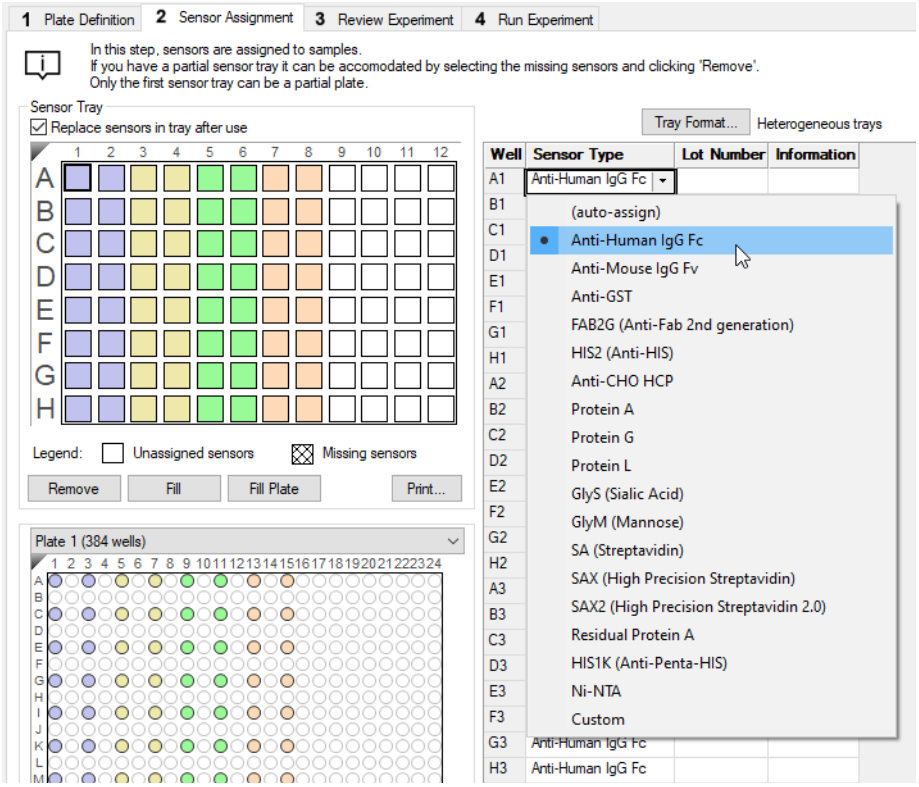


Figure 7-49: Changing Biosensor Types

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.**

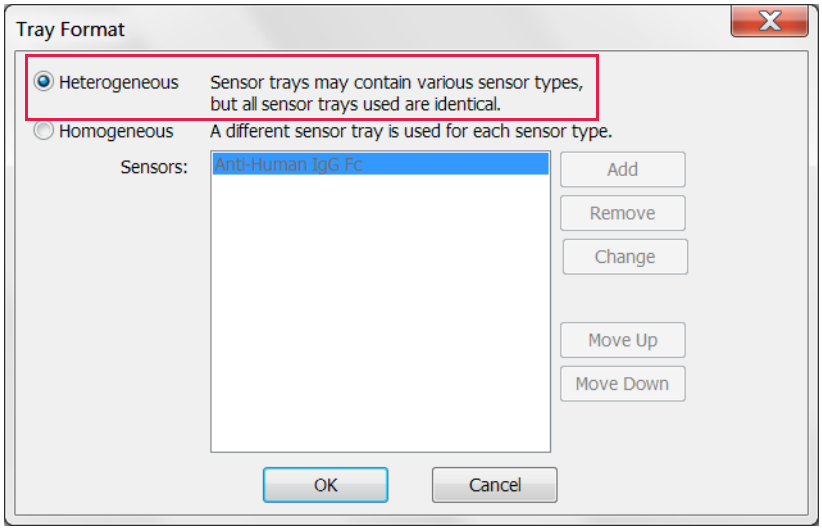


Figure 7-50: Tray Format Dialog Box

   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.

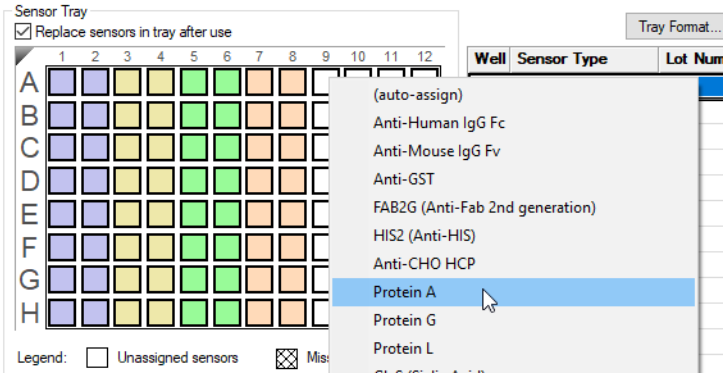


Figure 7-51: Populating the Sensor Tray Map with First Biosensor Type

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.

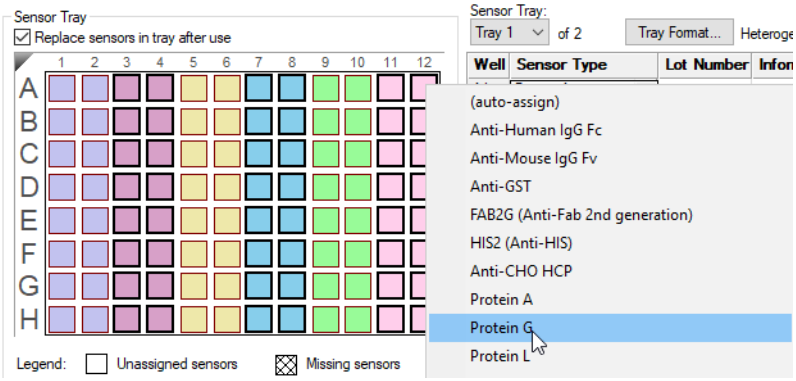


Figure 7-52: Populating the Sensor Tray Map with Second Biosensor Type

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.

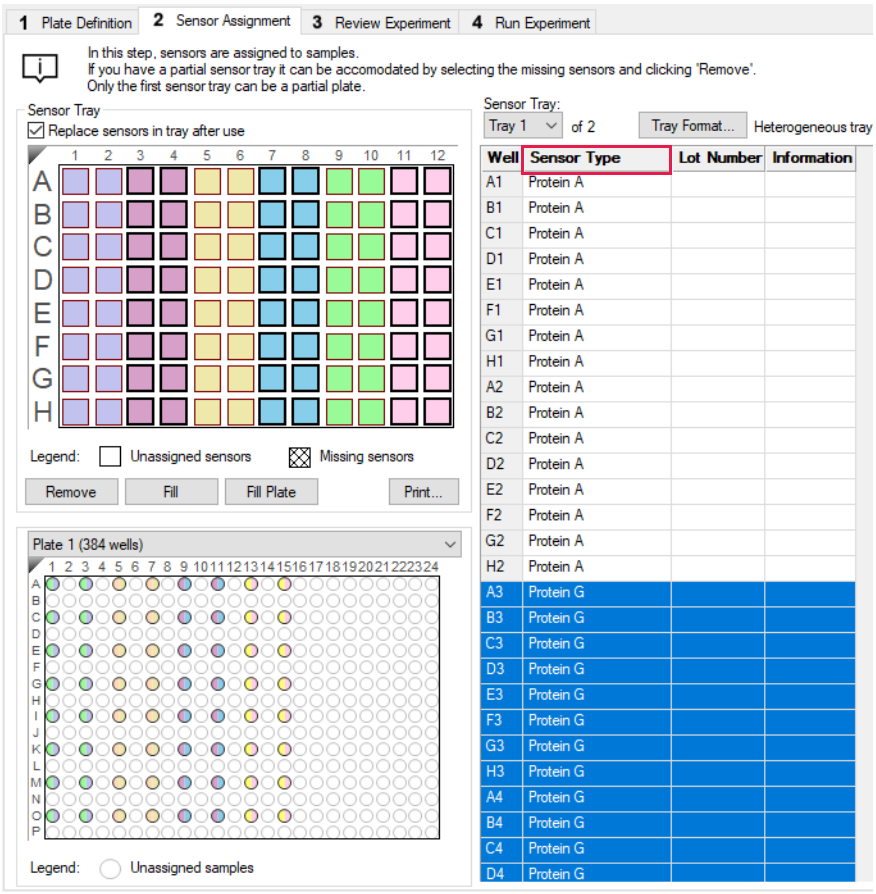


Figure 7-53: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

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).

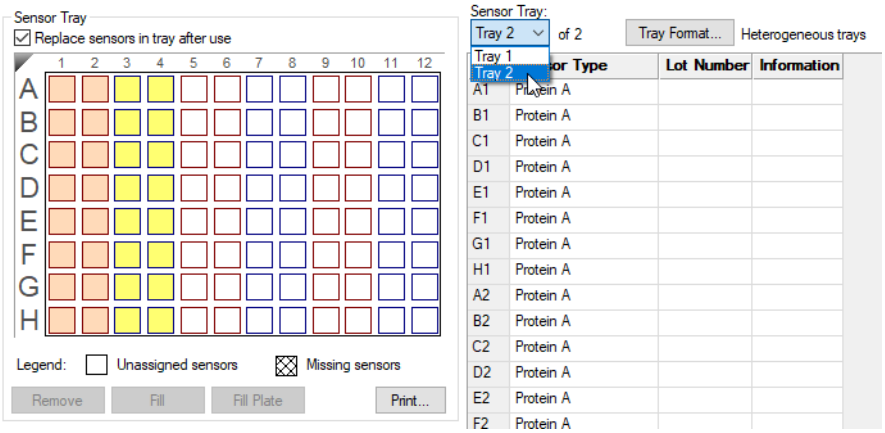


Figure 7-54: Tray Selection

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).

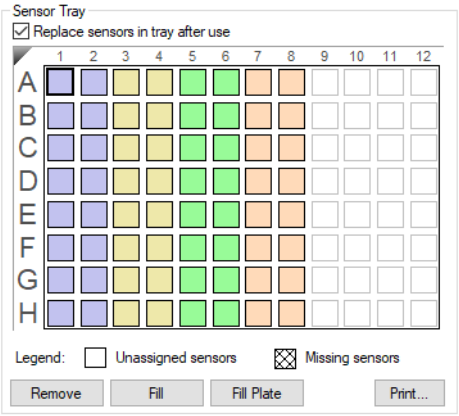


Figure 7-55: Replace Sensors in Tray After Use Check Box

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.

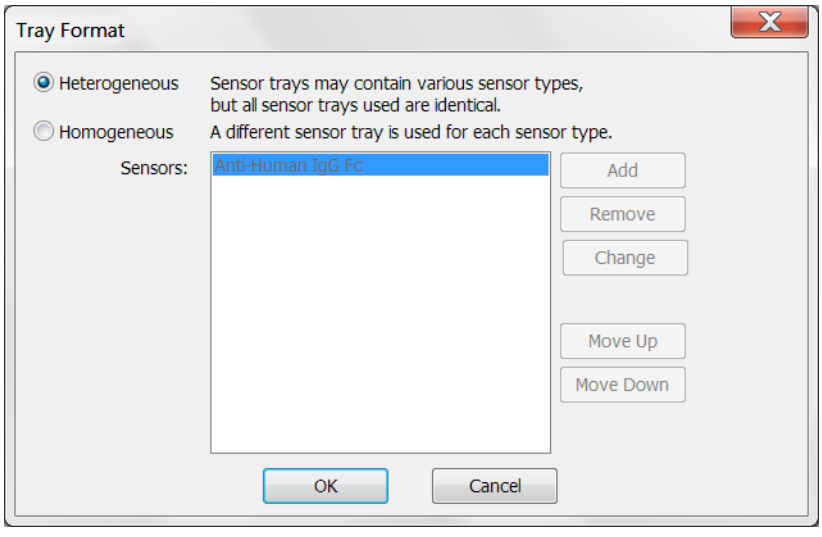


Figure 7-56: Tray Format Dialog Box

2. Select **Homogeneous**. Click **Add** to select the first biosensor type (see Figure 7-57).

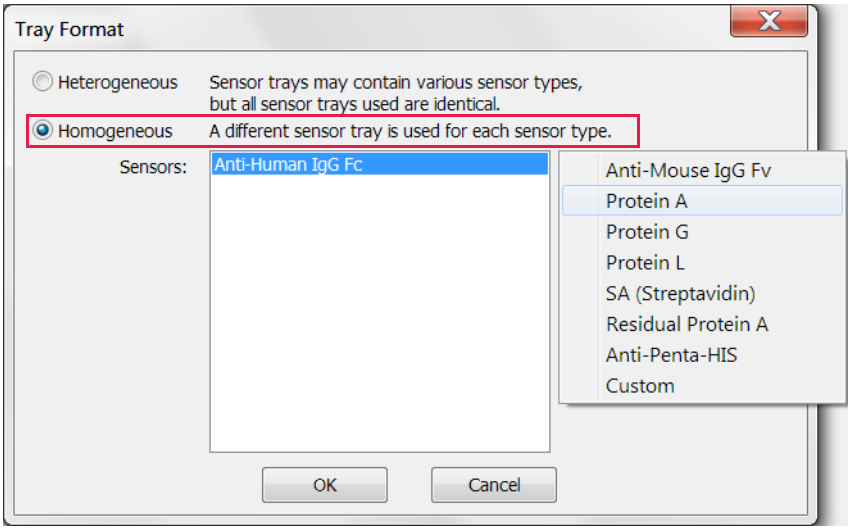


Figure 7-57: Selecting a Biosensor Type in the Tray Format Dialog Box

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).

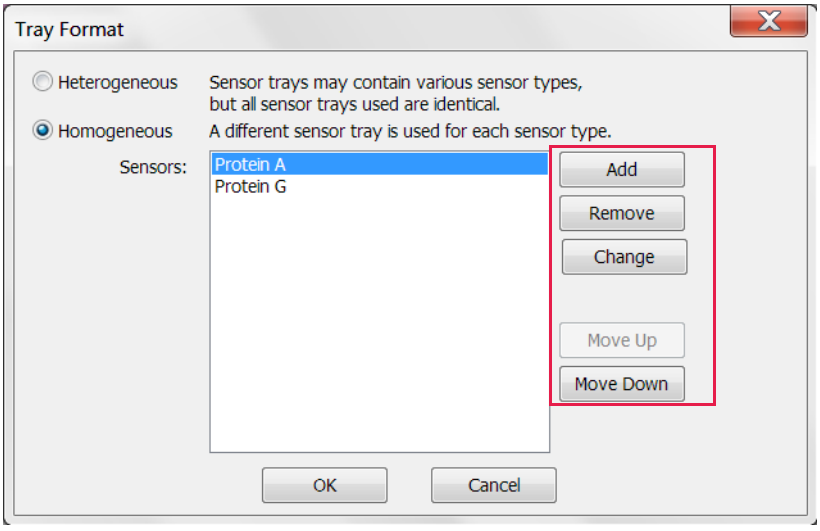


Figure 7-58: Biosensor Types List Order in Sensor Box

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.

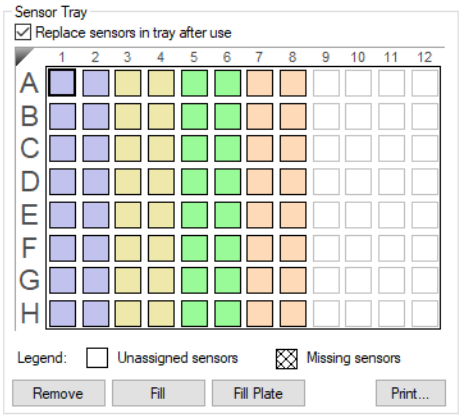


Figure 7-59: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

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 selected tray appear (see Figure 7-54).

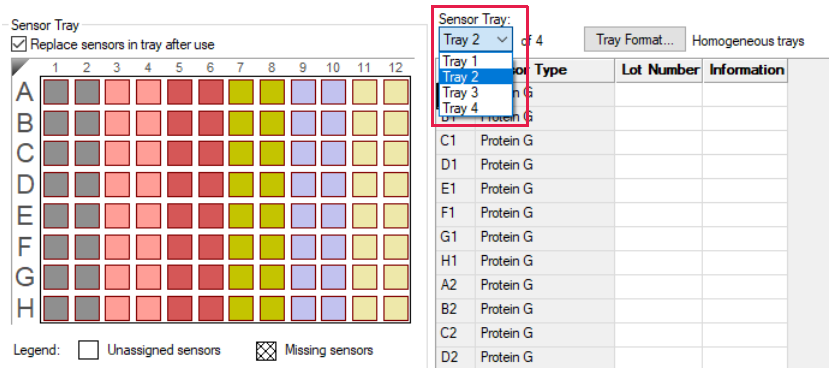


Figure 7-60: Tray Selection

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).

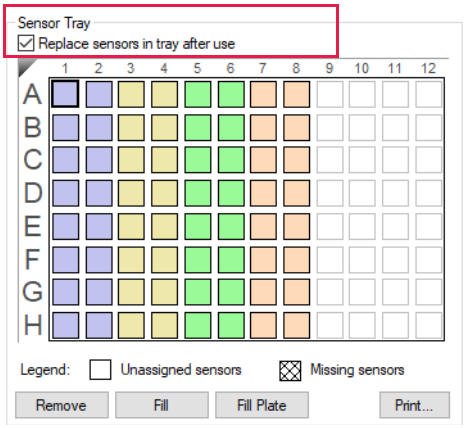


Figure 7-61: Replace Sensors in Tray After Use Check Box

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)

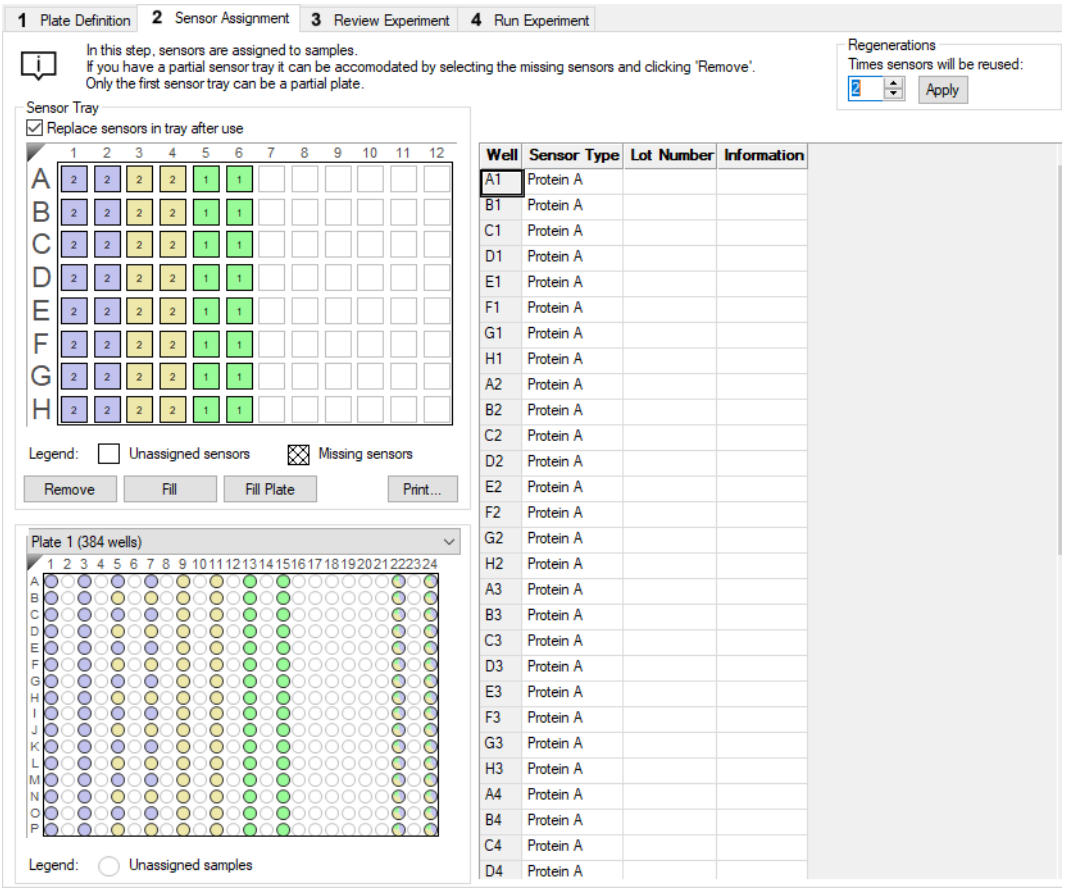


Figure 7-62: Fractional Use of Regeneration and Neutralization Wells

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).

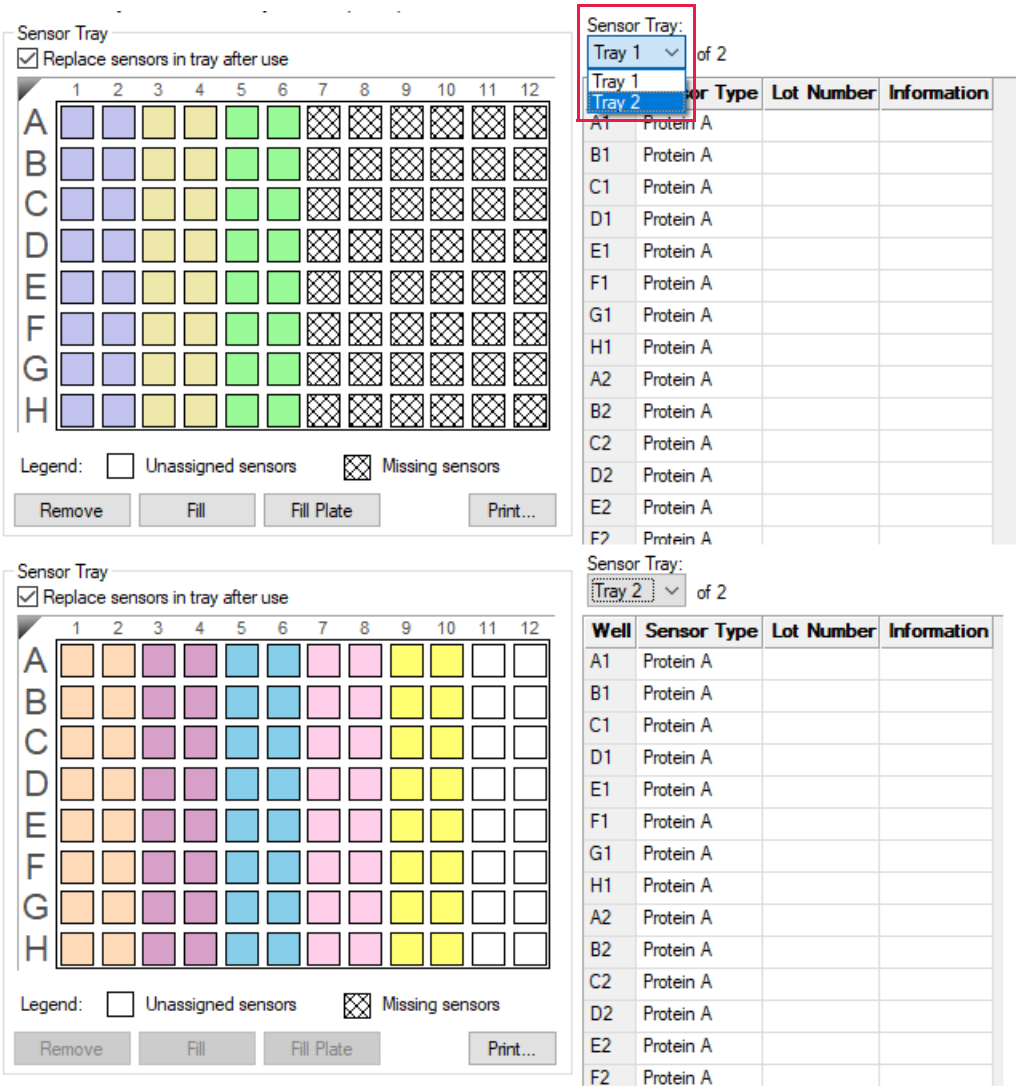


Figure 7-63: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray

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® 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 ← → arrows to select an assay.

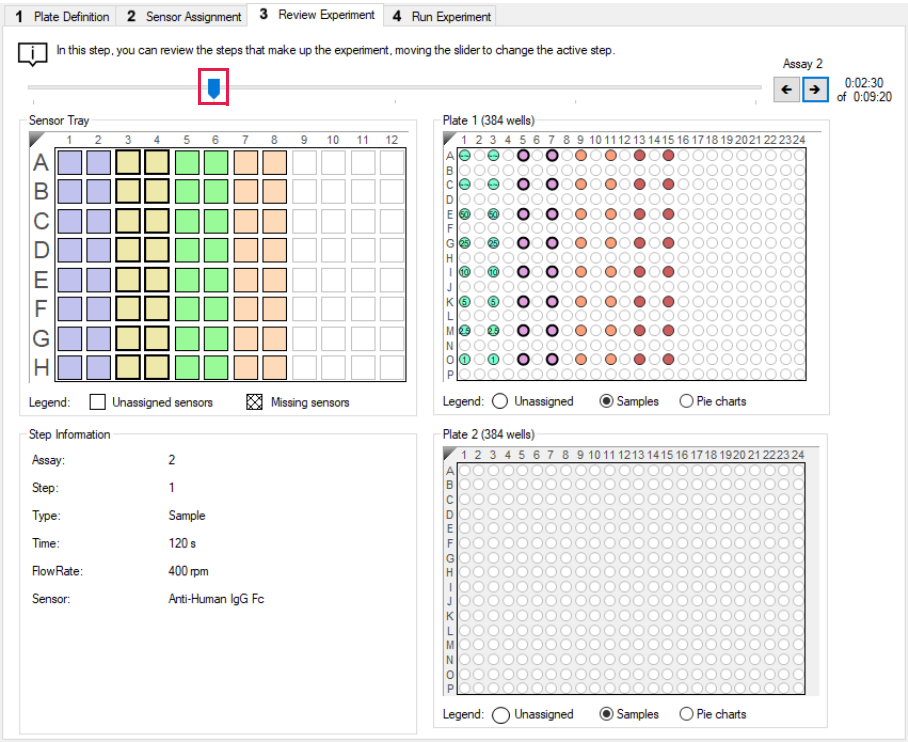


Figure 7-64: Review Experiment Window

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.*

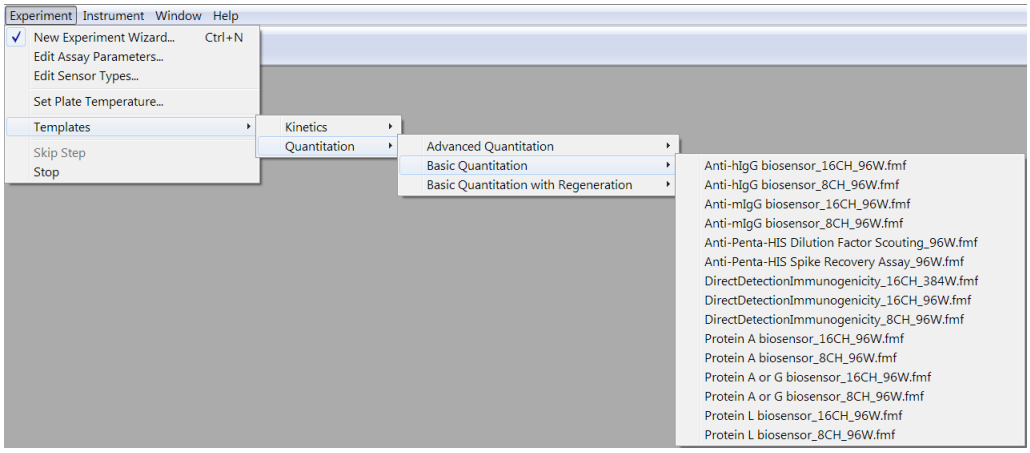


Figure 7-65: Experiments in the Template Folder

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.

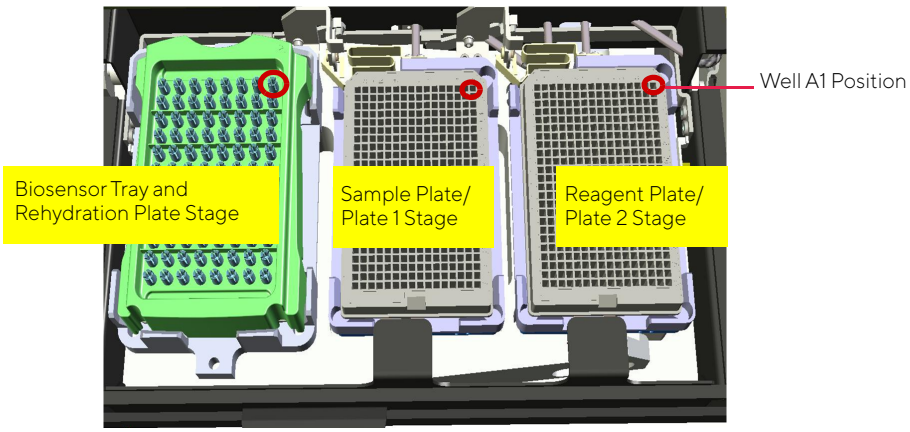


Figure 7-66: Octet® Instrument Stage Platform

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

3. Click ▲ to close the Octet® 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 → to access the Run Experiment window (see Figure 7-67).

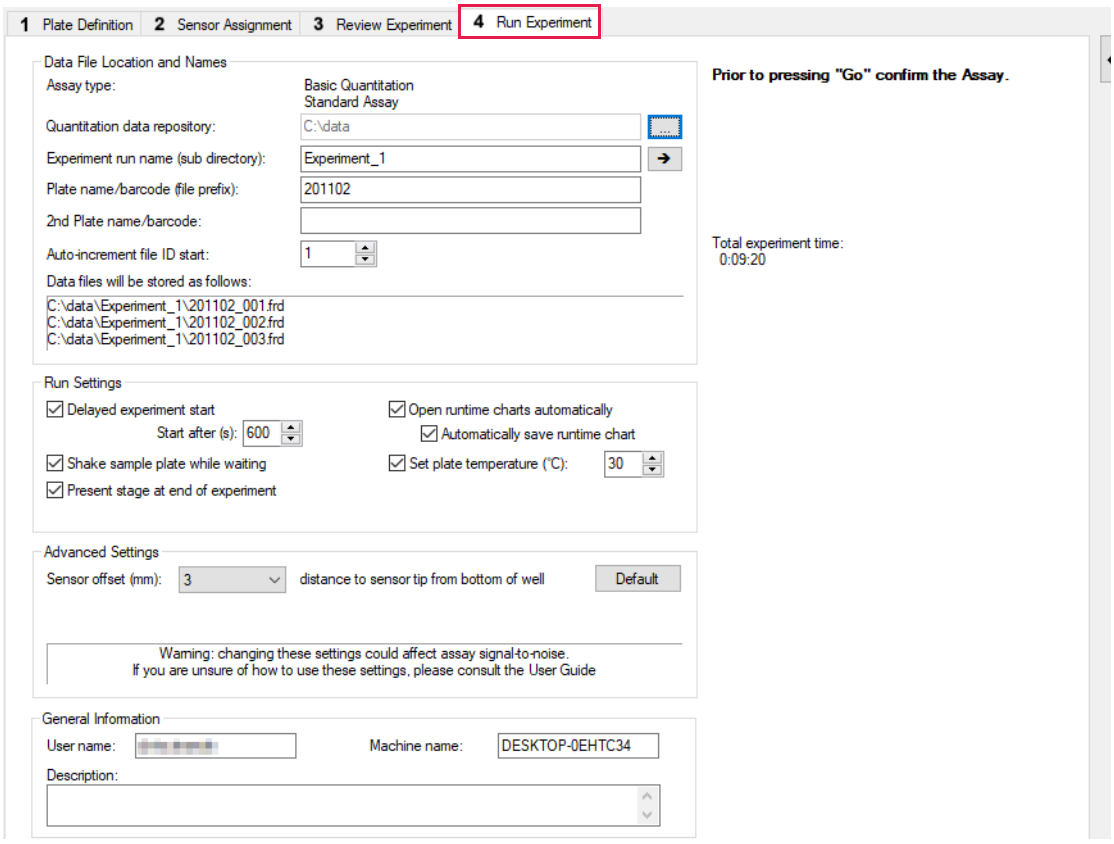


Figure 7-67: Run Experiment Window—Octet® RH16

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.*

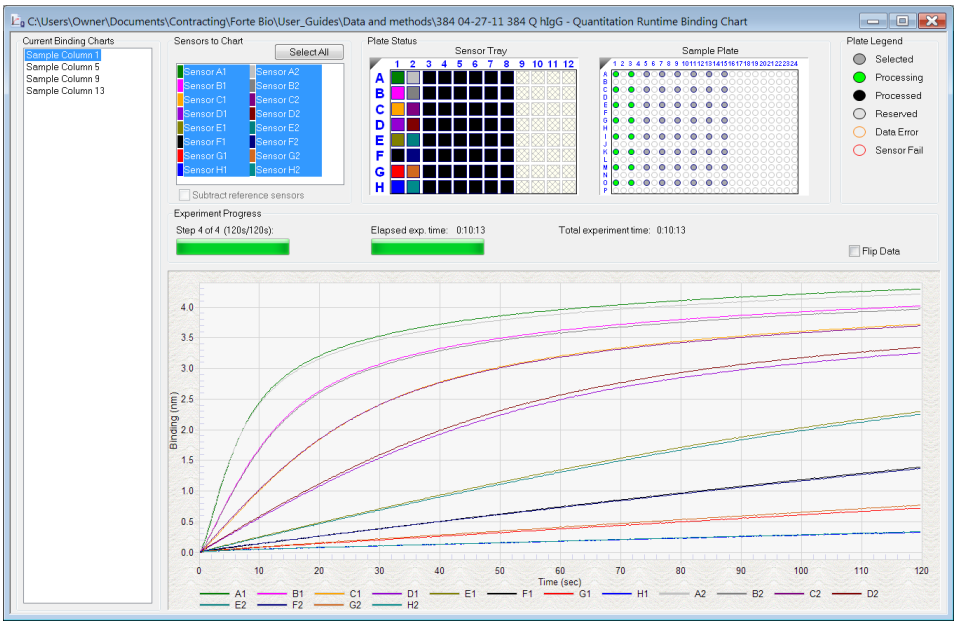


Figure 7-68: Runtime Binding Chart

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® 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® 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.*

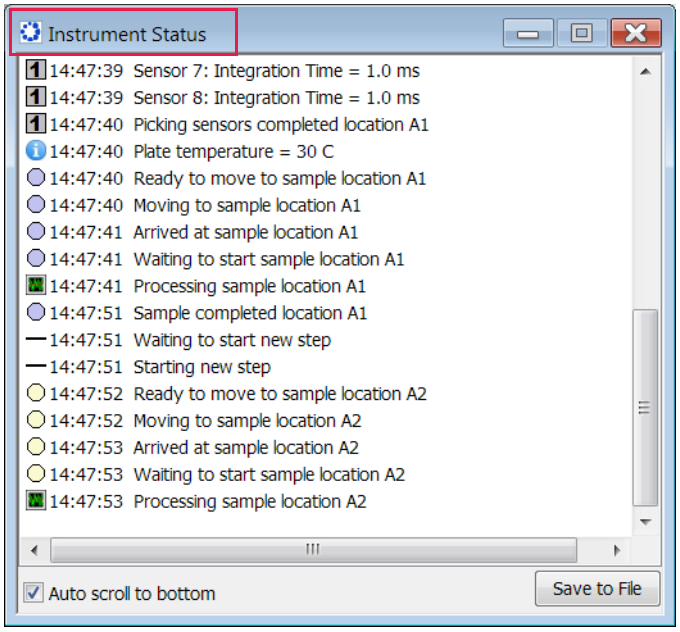


Figure 7-69: Instrument Status Log

Run Experiment Window Settings

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

Table 7-8: 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. ***NOTICE:*** *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 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 **GO**.
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 **Runtime Binding Chart** for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the **Runtime Binding Chart**. 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 **File > Options**. The factory set default temperature is 30 °C. ***NOTICE:*** *If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet® 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® 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® RH96 system:

Table 7-10: Advanced Settings Octet® RH96

Item	Description
Acquisition rate	***NOTICE:*** *For the Octet® RH96 system, acquisition rate settings are available on the Plate Definition Tab.* • 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. • 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. • 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.
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® QK384 system:

Table 7-11: Advanced Settings Octet® QK384

Item	Description
Acquisition rate	• 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. • 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.
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® 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 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 color-coded (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® 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.*

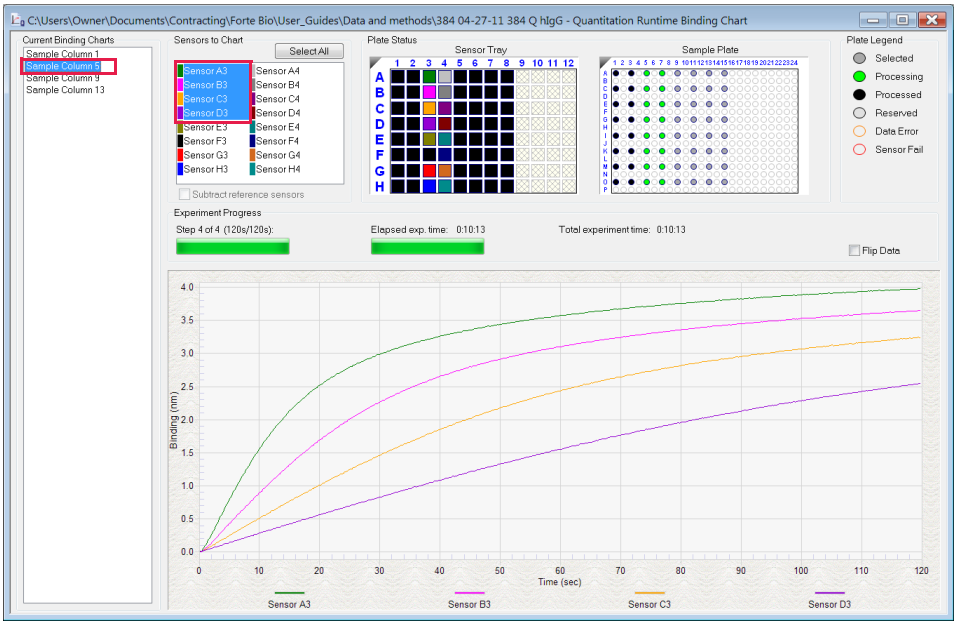


Figure 7-70: Runtime Binding Chart Window

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).

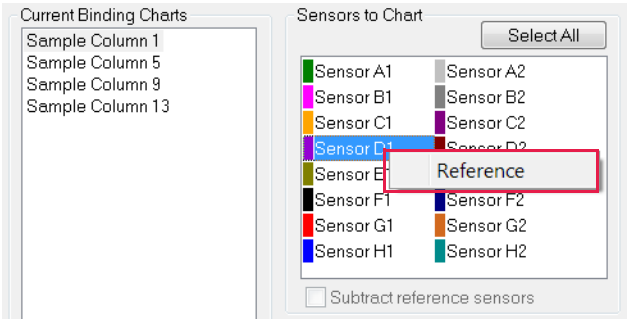


Figure 7-71: Designating a Reference Biosensor in the Runtime Binding Chart

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).

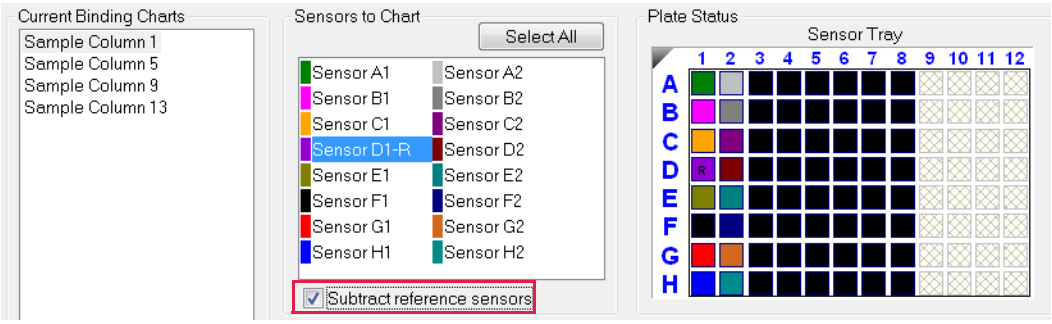


Figure 7-72: Subtract Reference Sensors check box in the Runtime Binding Chart

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.

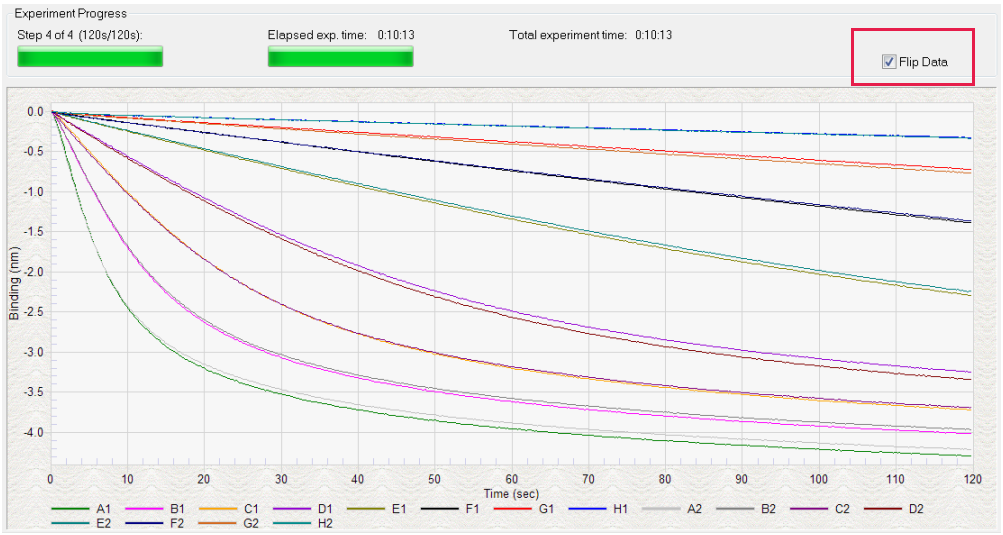


Figure 7-73: Data Inverted Using Flip Data Function

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

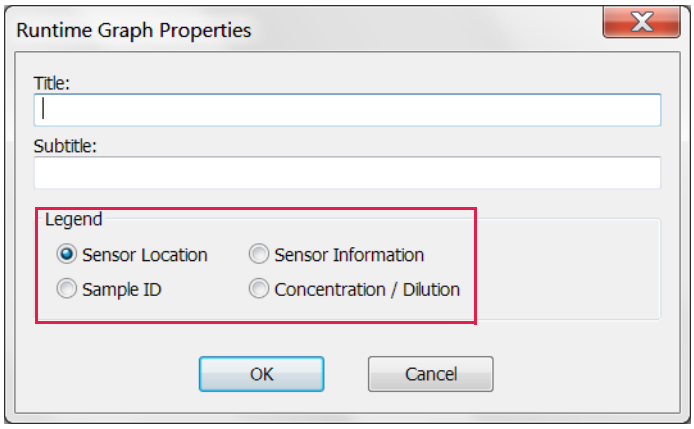


Figure 7-74: Selecting a Runtime Binding Chart Legend

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**.

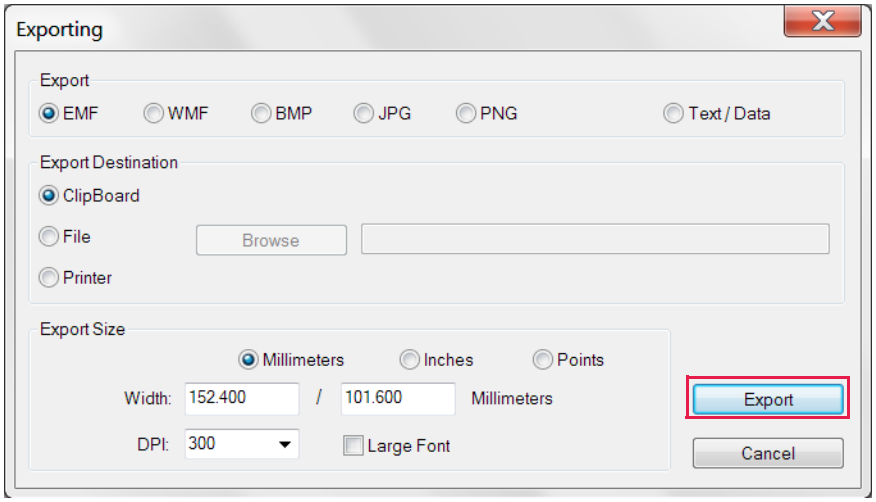


Figure 7-75: Exporting Dialog Box

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 **File** > **Browse** 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 **File** > **Browse** to select a folder and enter a file name.	Creates a graphic image.
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

Managing Experiment Method Files

After you run an experiment, the Octet® 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: W*hen 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 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).

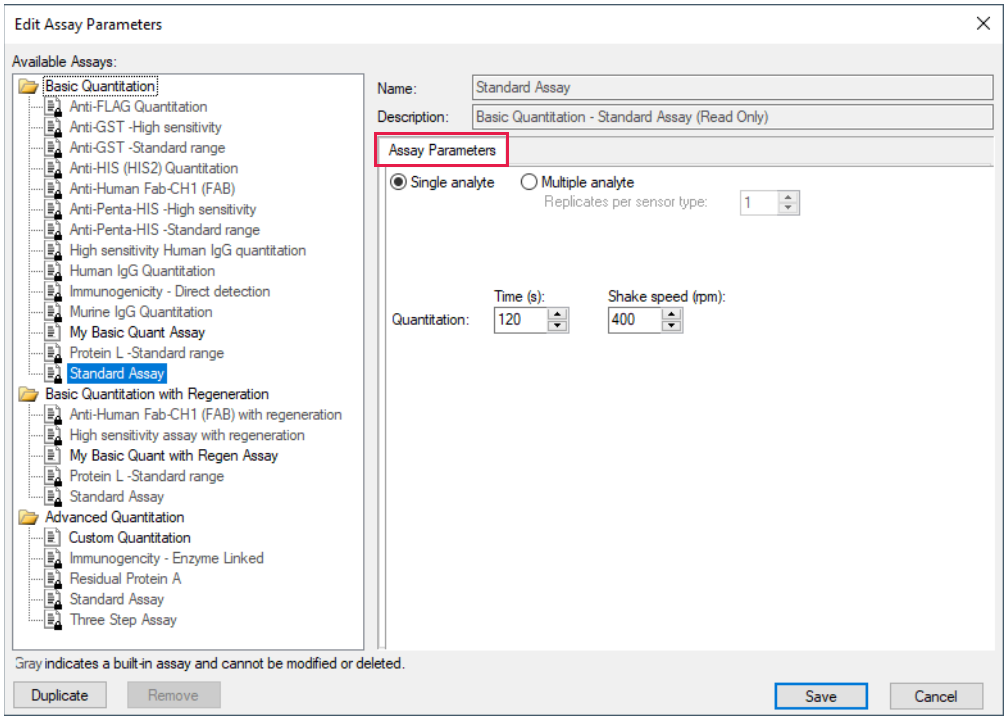


Figure 7-76: Edit Assay Parameters Dialog Box

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).

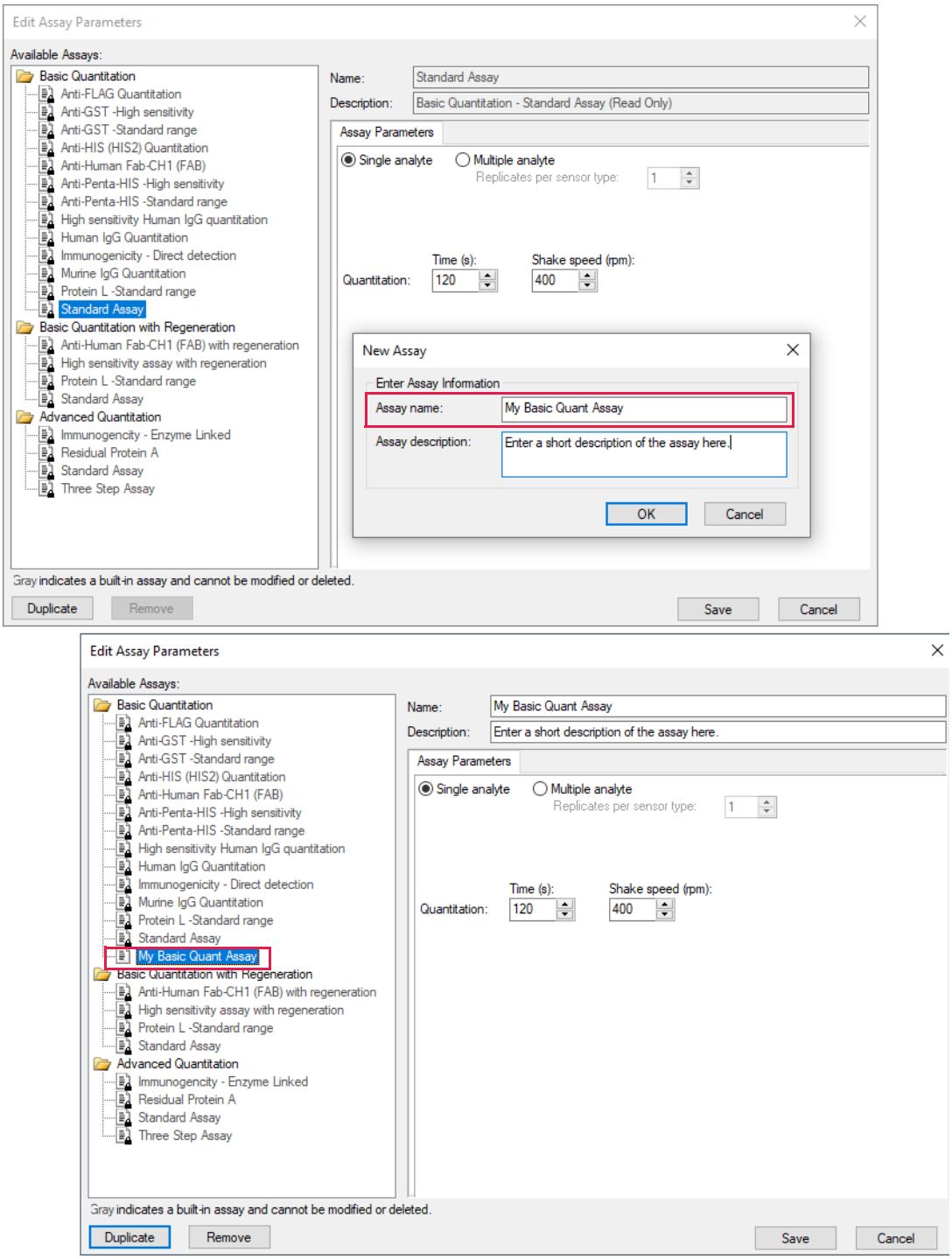


Figure 7-77: Defining a New Assay

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

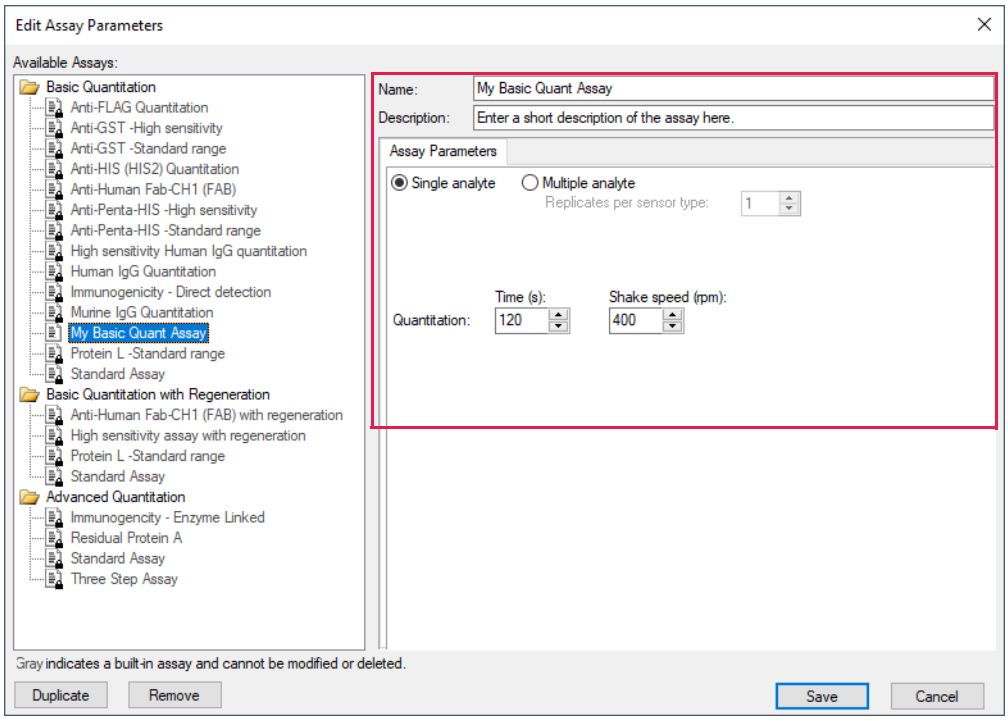


Figure 7-78: Assay Parameters—Basic Quantitation Assay

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 Replicates 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. ***NOTICE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

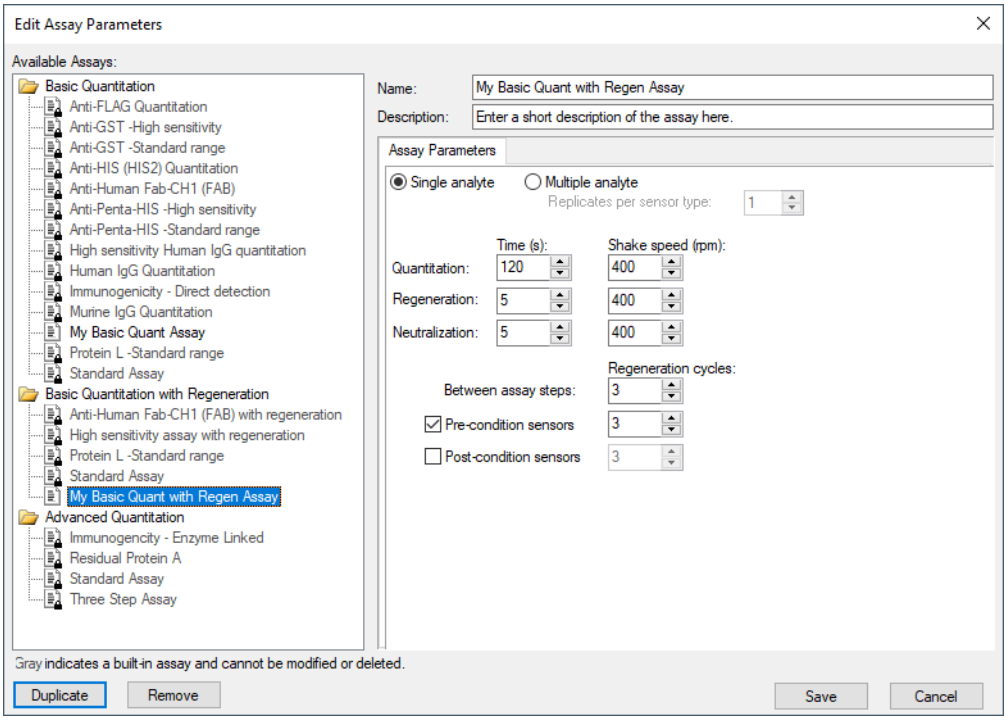


Figure 7-79: Assay Parameters—Basic Quantitation with Regeneration

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 Replicates 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). ***NOTICE:*** *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 regenerated 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.

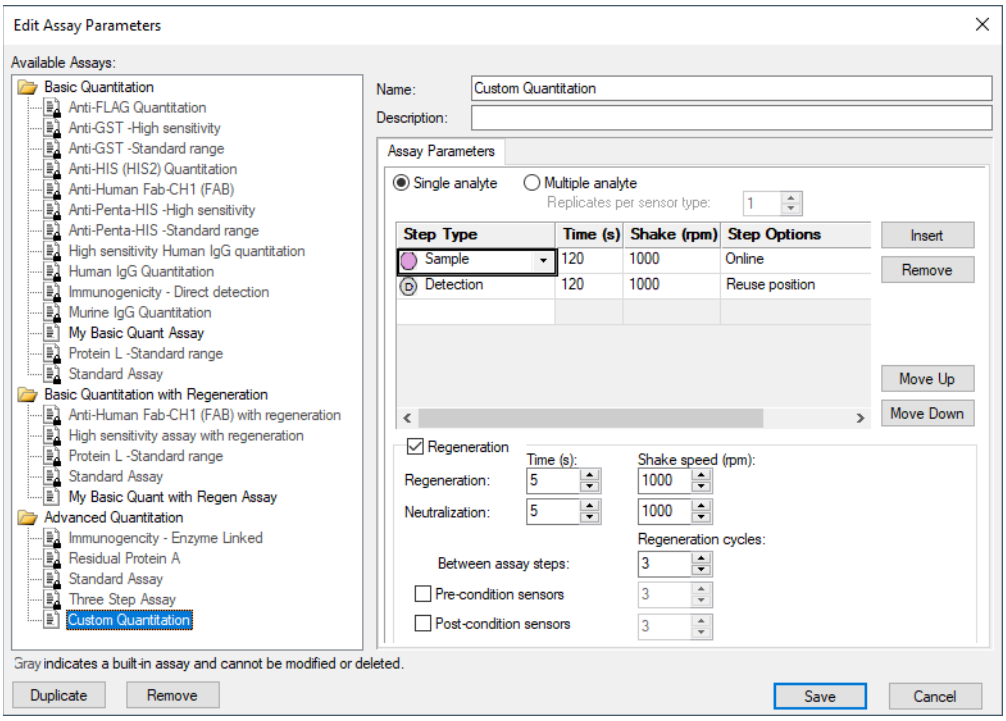


Figure 7-80: Assay Parameters—Advanced Quantitation

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).

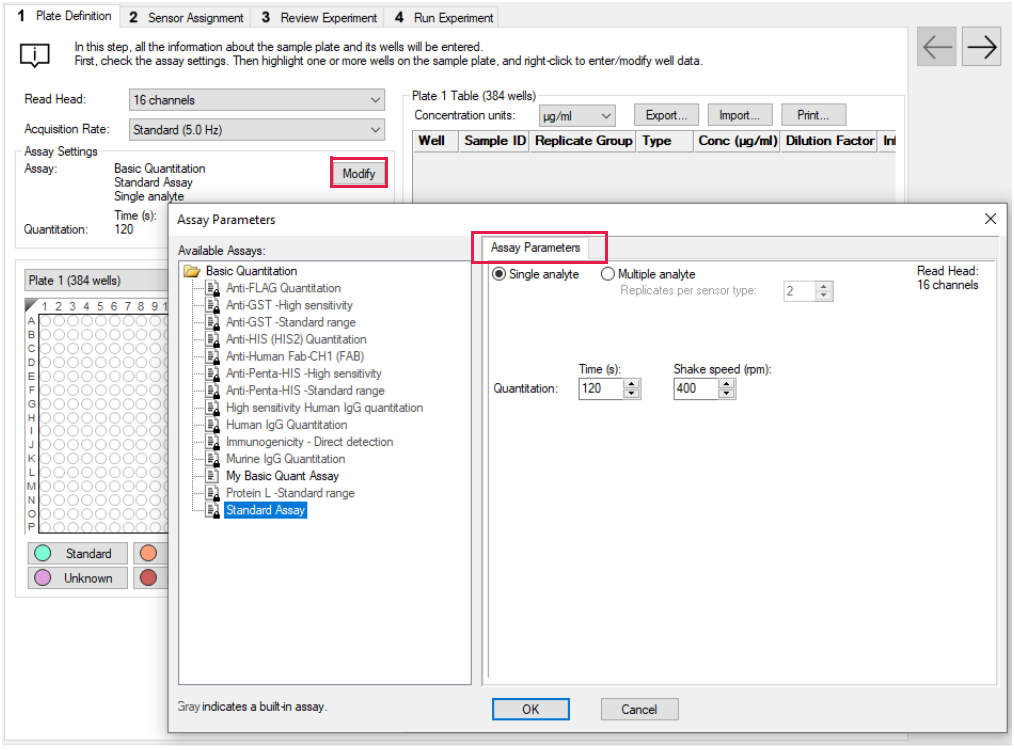


Figure 7-81: Selecting a Custom Assay

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

Multi-Step Advanced Quantitation Experiments

Octet® RH16 and Octet QK® 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® Analysis Studio software.

After starting the Octet® system and the Octet® 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:

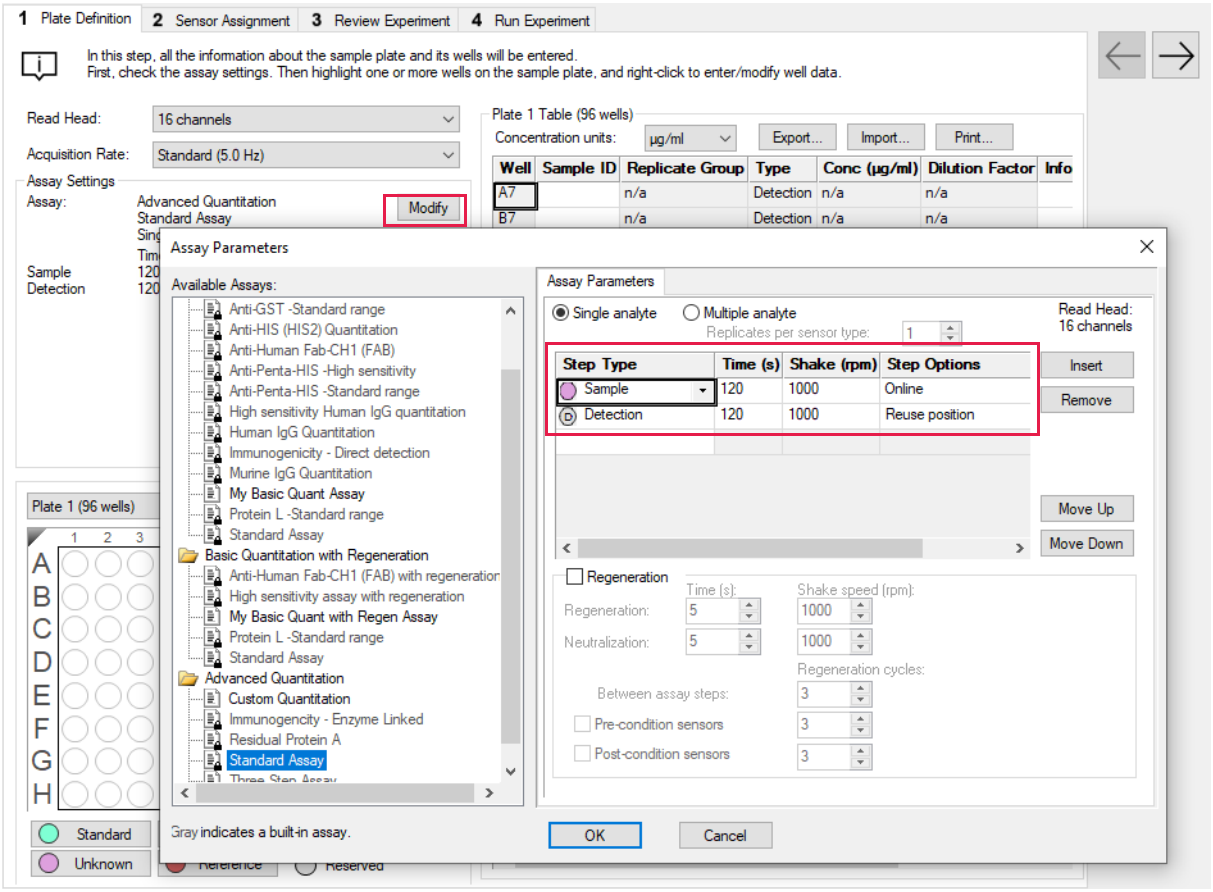


Figure 7-82: Assay Parameters Window.

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.

2. 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® 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® RH96 system and the Octet® 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® 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).

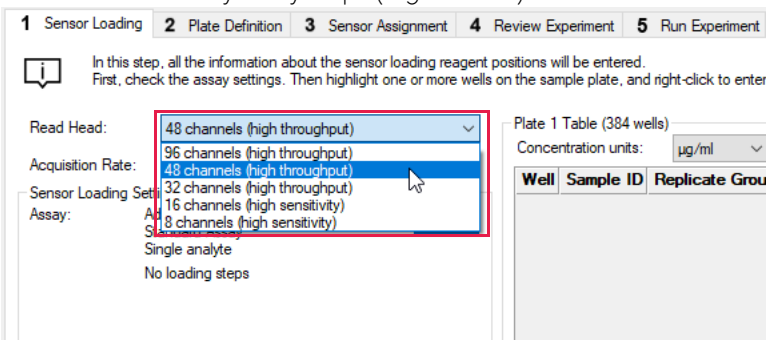


Figure 7-83: Selecting a New Read Head Setting

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).

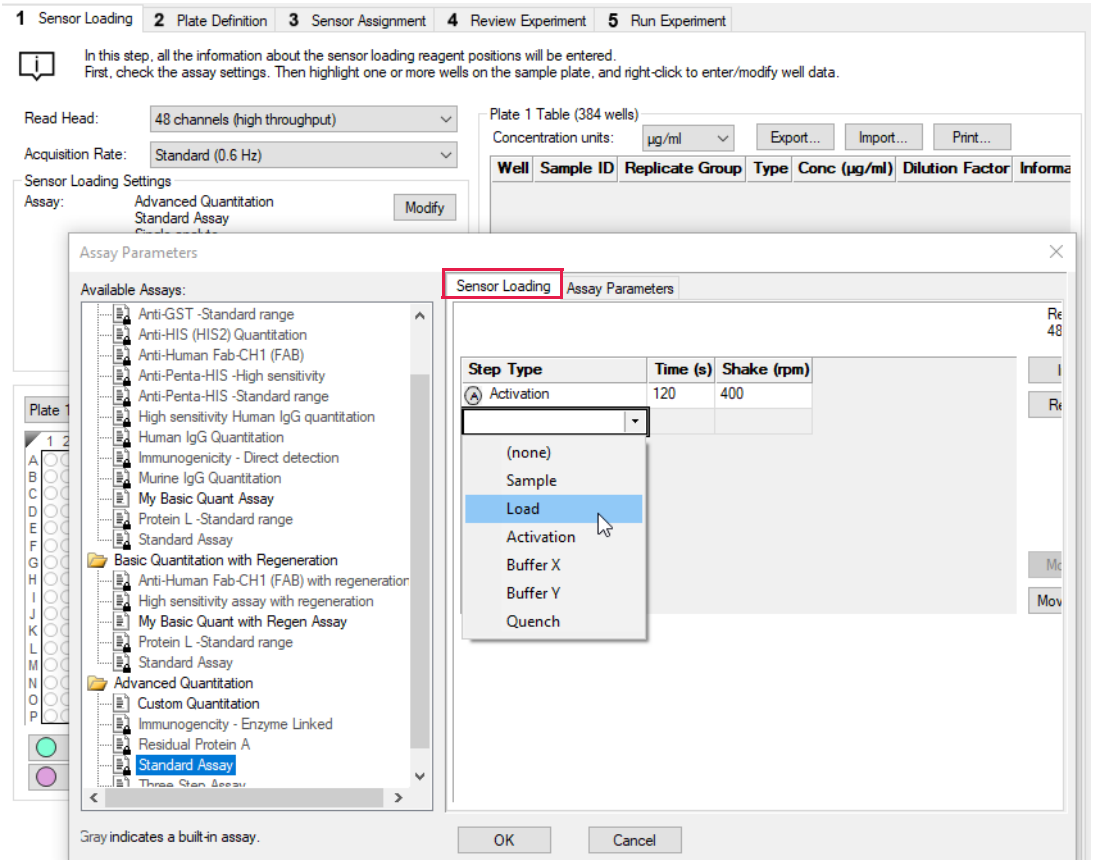


Figure 7-84: Modifying Sensor Loading Parameters

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:

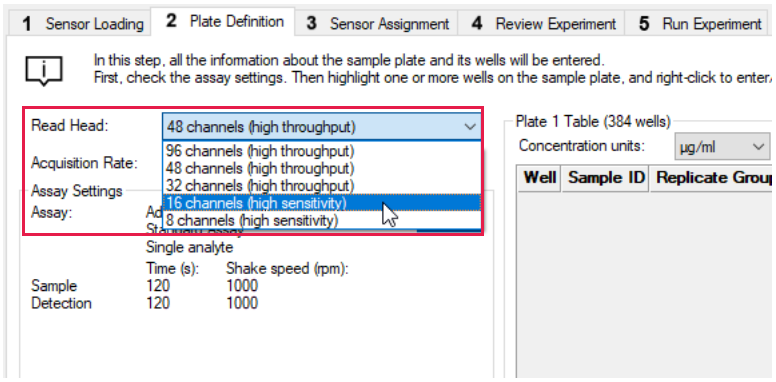


Figure 7-85: Selecting a New Read Head Setting

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:

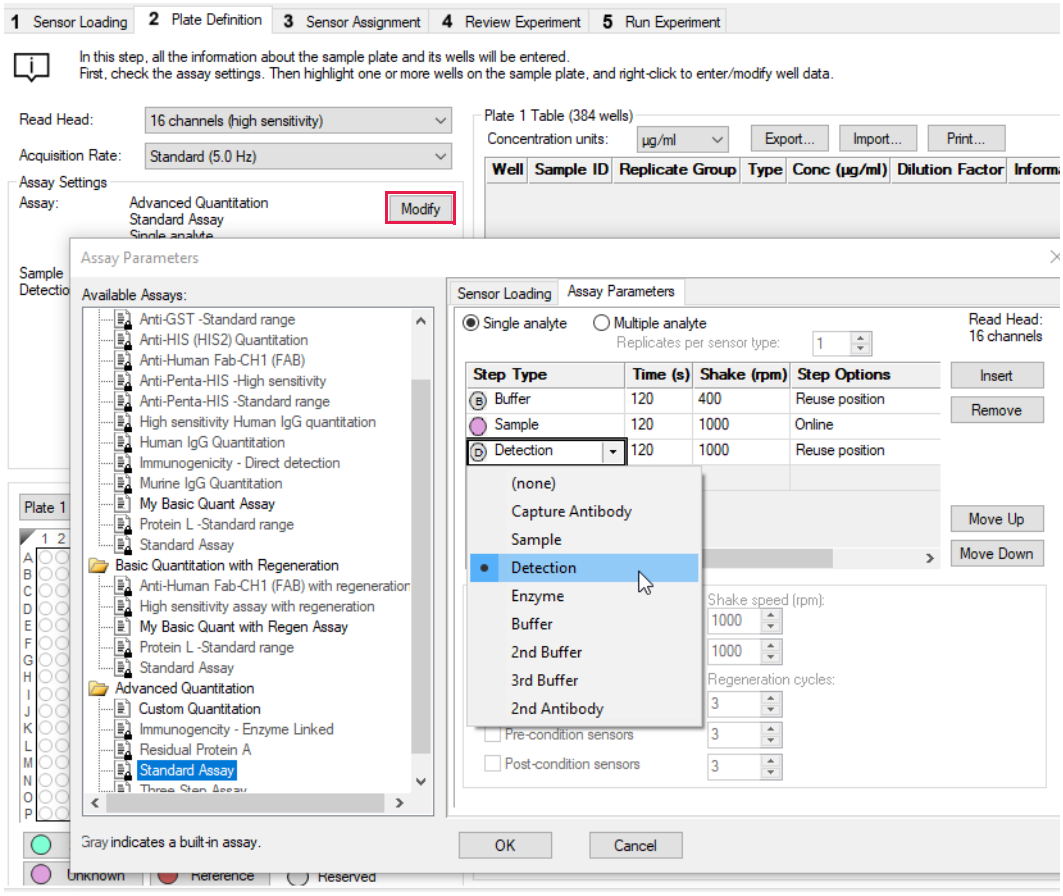


Figure 7-86: Modifying Assay Parameters

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® R2, Octet® R4, and Octet® K2

Introduction

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet® system hardware and the Octet® 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® Analysis Studio Software.*

Table 8-1: Setting Up and Analyzing a Kinetic Experiment

Software	Step	See
Octet® BLI Discovery 	1. Select a kinetics experiment in the **Experiment Wizard** 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® Analysis Studio 	6. View and process the raw data. 7. Analyze the data.	*Octet® Analysis Studio Software User Guide*

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® 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® R2, Octet® R4, or Octet® 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.*

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 ® 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  , 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 ® R2, Octet® R4, or Octet® K2 method templates are not compatible with other Octet® 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® 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(). The Basic Kinetics Experiment window appears (Figure 8-1, right).

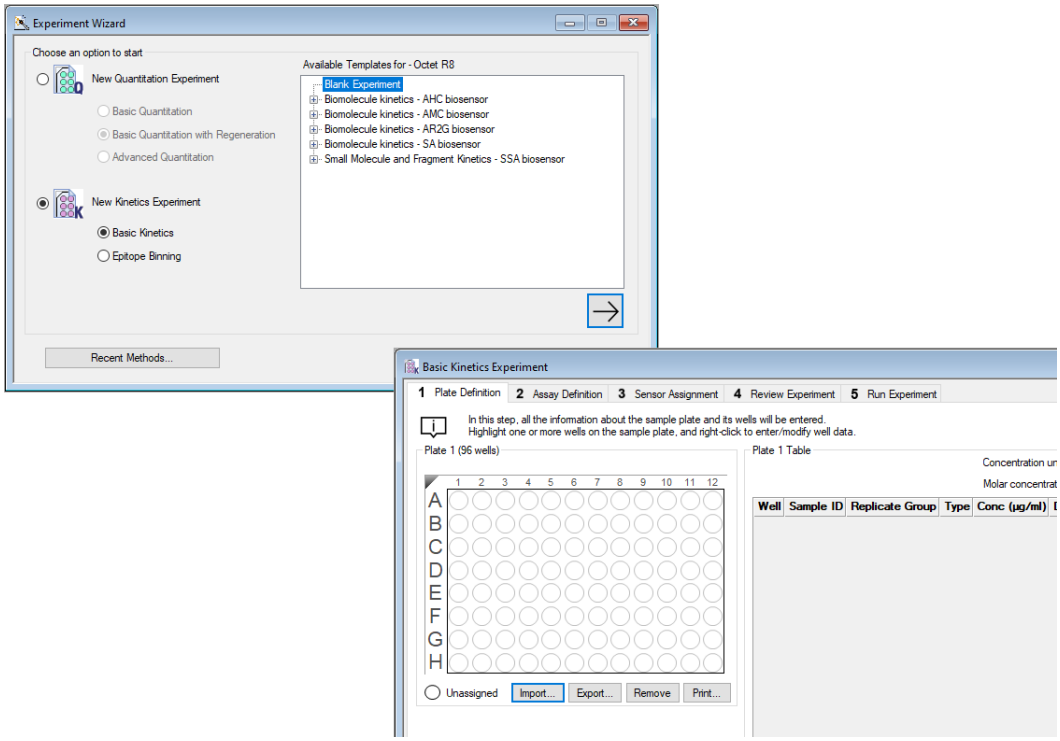


Figure 8-1: Starting a Kinetics Experiment with the Experiment Wizard

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: Types of Sample Wells

Icon	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. • Positive Control: A control sample that contains analyte of known concentration • Negative Control: A control sample known not to contain analyte
(B) 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.
(Q) Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
(L) Load	Ligand to be immobilized (loaded) on the biosensor surface.
(W) Wash	Wash buffer.
(R) Regeneration	Regeneration reagents dissociate the analyte from the ligand.
(N) 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® R2, Octet® R4, or Octet® 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).

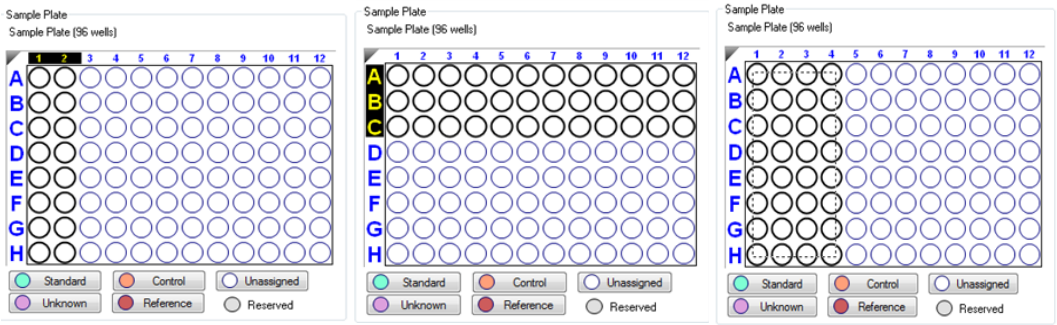


Figure 8-2: Selecting Wells in the Sample Plate Map

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).

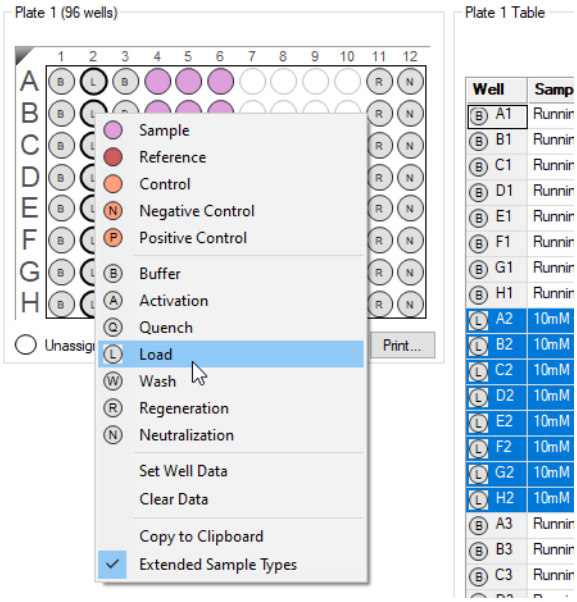


Figure 8-3: Designating a Well Type in the Plate Definition Window

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).

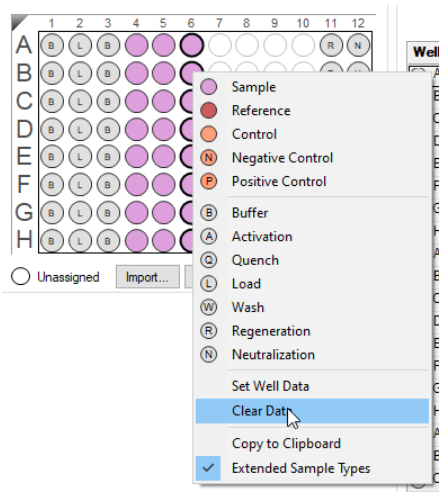


Figure 8-4: Clearing Sample Data from a Sample Plate

Entering Sample Information

NOTICE: *You must specify sample (analyte) concentration and molecular weight, otherwise the Octet® BLI Discovery software cannot 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.*

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).

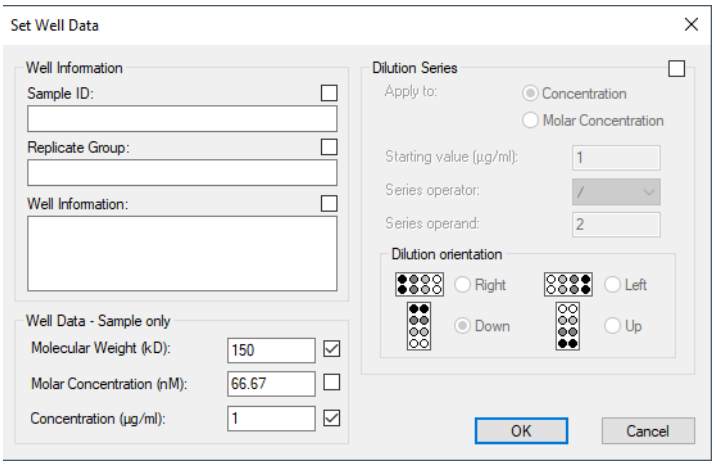
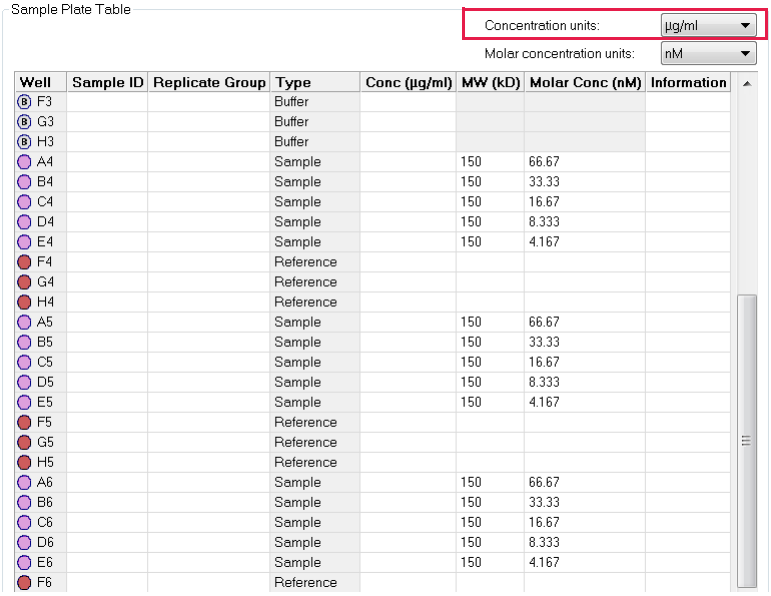


Figure 8-5: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

The information displays in the **Sample Plate Table** (see Figure 8-6).

3. In the **Sample Plate Table**, select the sample concentration units and the molar concentration units.



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
F3			Buffer				
G3			Buffer				
H3			Buffer				
A4			Sample		150	66.67	
B4			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				
A5			Sample		150	66.67	
B5			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	
B6			Sample		150	33.33	
C6			Sample		150	16.67	
D6			Sample		150	8.333	
E6			Sample		150	4.167	
F6			Reference				

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.

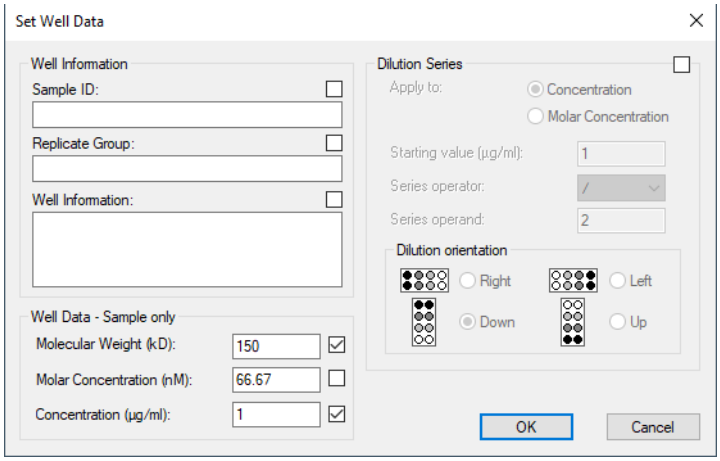


Figure 8-7: Sample Plate Map—Assigning Sample Concentrations by Value

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:

1. 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.

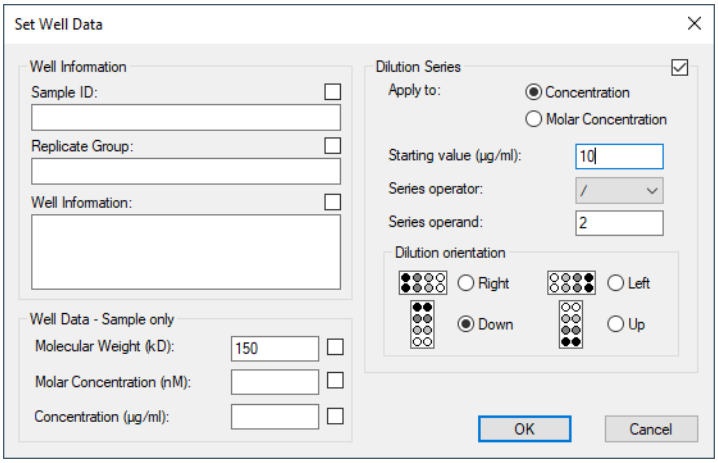


Figure 8-8: Sample Plate Map—Assigning Sample Concentrations Using Dilution Series

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**.

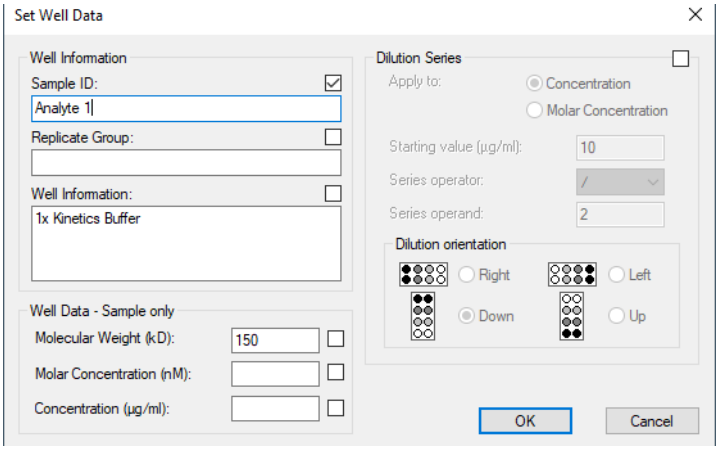


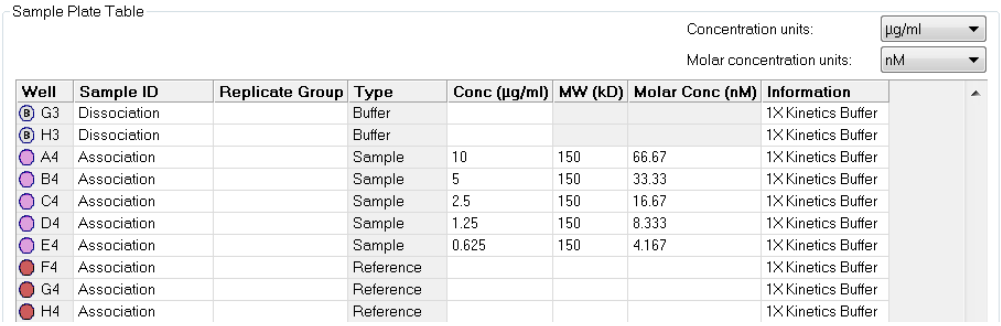
Figure 8-10: Add Sample Annotations from the Sample Plate Map

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.*



Well	Sample ID	Replicate Group	Type	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
B4	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 k*eys (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
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	x		D3	SA (Streptavidin)		4	D3

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**.

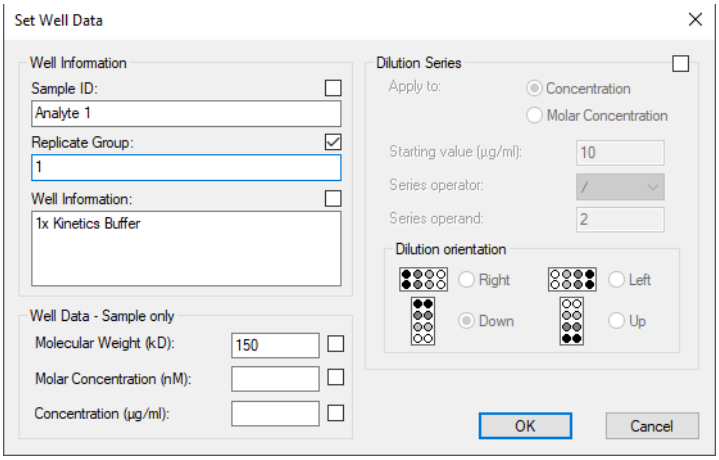


Figure 8-13: Add Replicate Group from the Sample Plate Map

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).

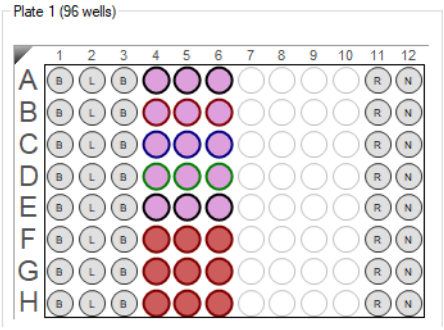


Figure 8-14: Replicate Group in the Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 8-15)

Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

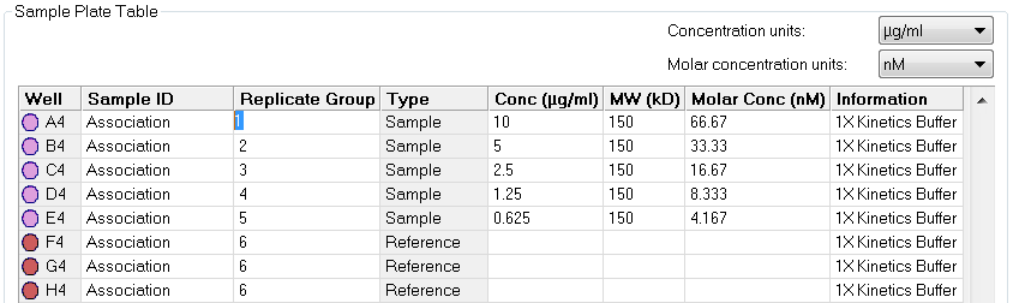
Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association	4	Sample	1.25	150	8.333	1X Kinetics 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
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).



Well	Sample ID	Replicate Group	Type	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).

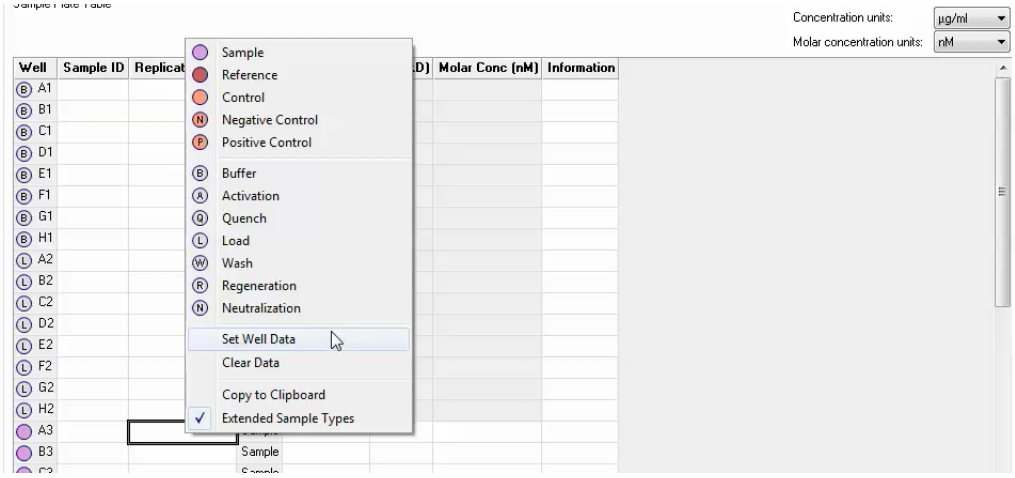


Figure 8-17: Sample Plate Table—Well Designation

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).

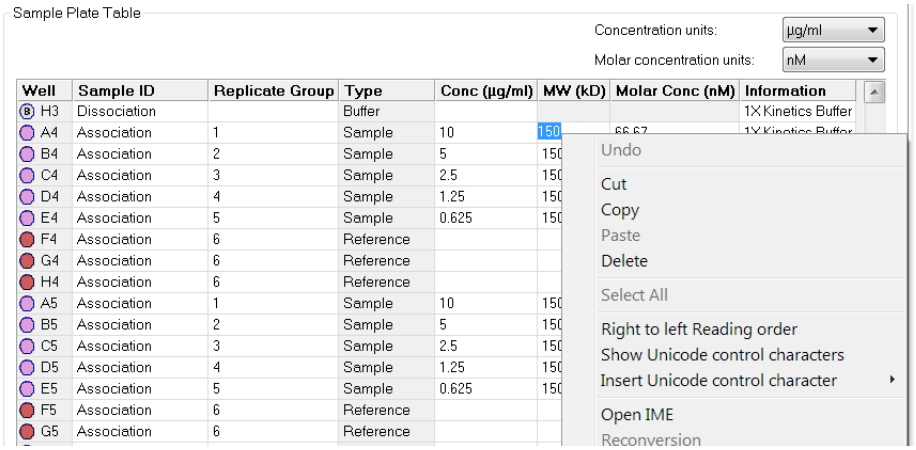


Figure 8-18: Sample Plate Table—Editing Sample Data

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.*

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).

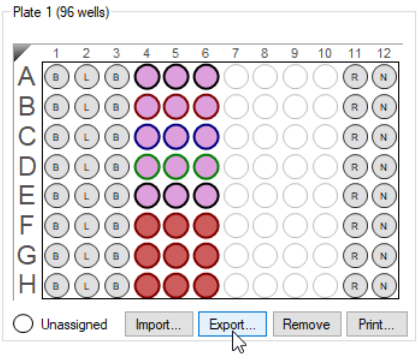


Figure 8-19: Sample Plate Map— Export Button

2. In the **Export Plate Definition** window (see Figure 8-20), select a folder, enter a name for the plate (.csv), and click **Save**.

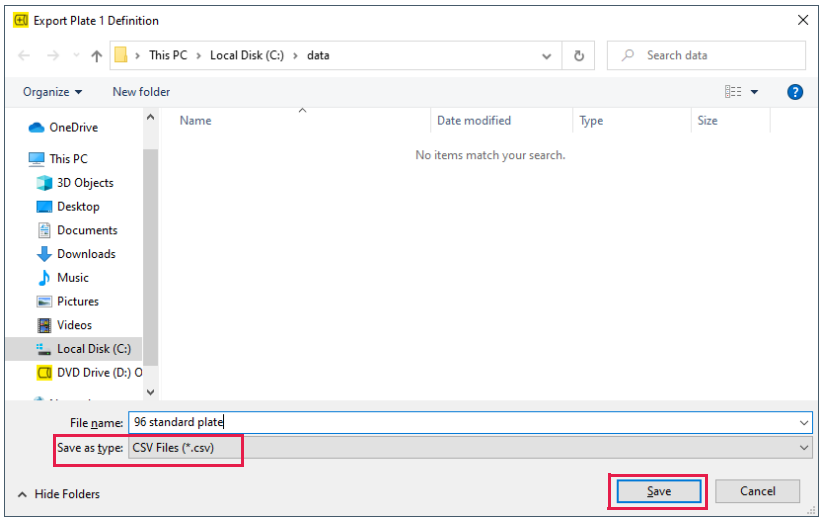


Figure 8-20: Export Plate Definition Window

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**.

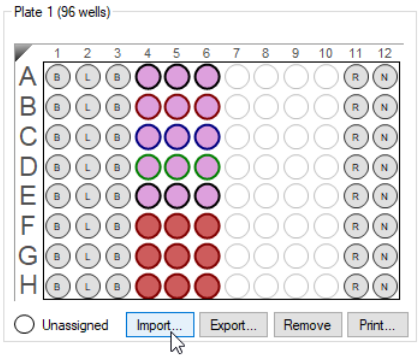


Figure 8-21: Sample Plate Map– Import Button

2. In the **Import Plate Definition** window (see Figure 8-22), select the plate definition (.csv), and click **Open**.

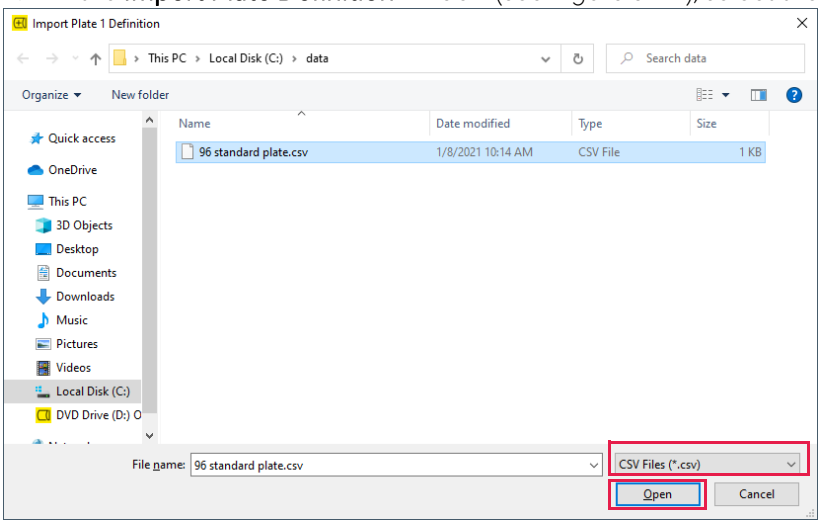


Figure 8-22: Import Plate Definition Window

NOTICE: *You can also create a .csv file for import. Figure 8-23 shows the appropriate column information layout.*

	A	B	C	D	E	F	G	H
1	PlateWells	96						
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information
3	A1	Kinetics Buffer		Buffer				1X Kinetics Buffer
4	B1	Kinetics Buffer		Buffer				1X Kinetics Buffer
5	C1	Kinetics Buffer		Buffer				1X Kinetics Buffer
6	D1	Kinetics Buffer		Buffer				1X Kinetics Buffer
7	E1	Kinetics Buffer		Buffer				1X Kinetics Buffer
8	F1	Kinetics Buffer		Buffer				1X Kinetics Buffer
9	G1	Kinetics Buffer		Buffer				1X Kinetics Buffer
10	H1	Kinetics Buffer		Buffer				1X Kinetics Buffer
11	A2	Loading		Load				12.5 ug/ml ProA
12	B2	Loading		Load				12.5 ug/ml ProA

96 standard plate

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**.

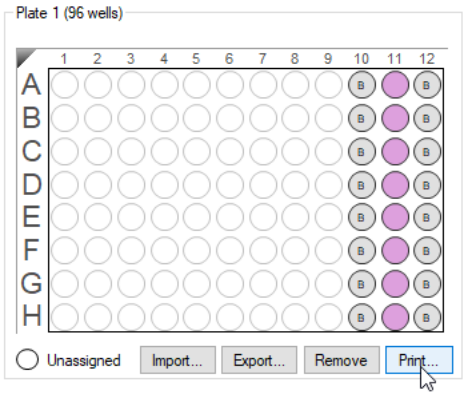


Figure 8-24: Sample Plate Print Button

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_{obs} or 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 baseline step directly precedes an association step, perform the baseline step at 1,000 rpm. ***IMPORTANT:*** *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/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. ***NOTICE:*** *This step may be performed offline (outside the Octet® 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 → 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.

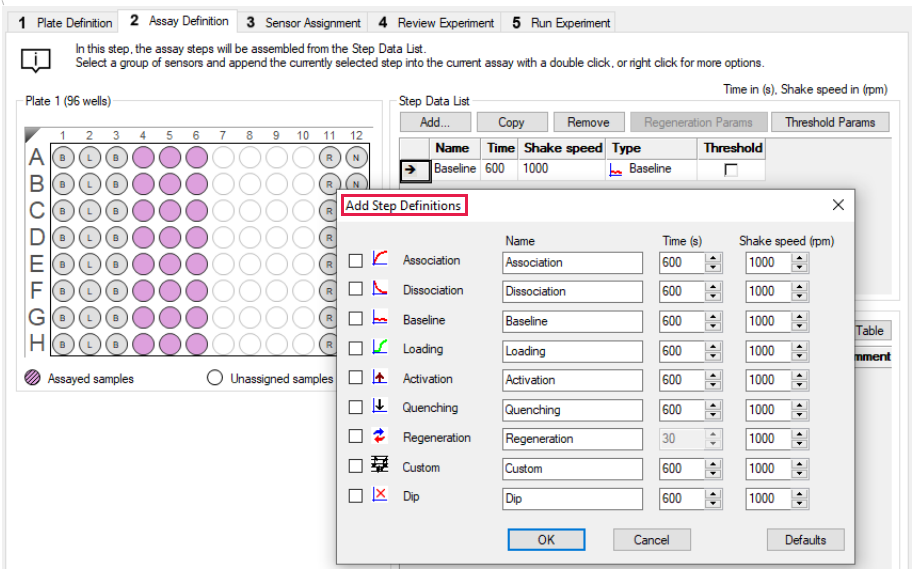


Figure 8-25: Creating an Assay Step Type

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).

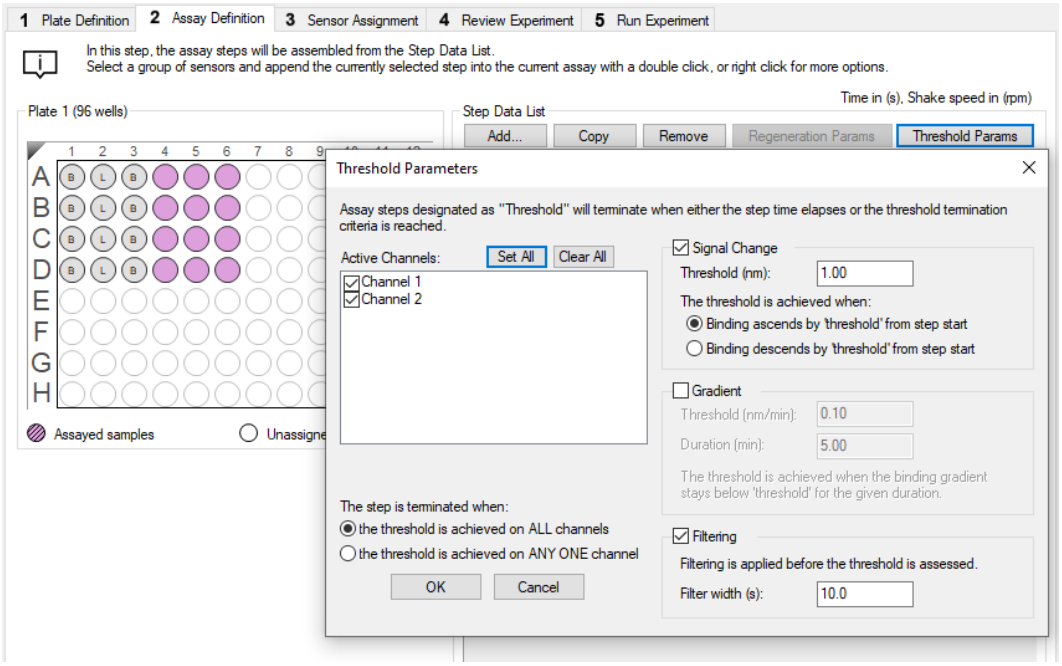


Figure 8-26: Setting Assay Step Threshold Parameters

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

Item	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).

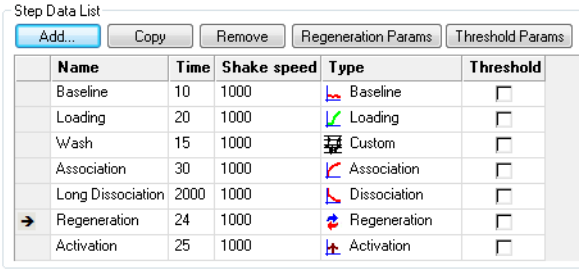


Figure 8-27: Step Data List with Step Types

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)

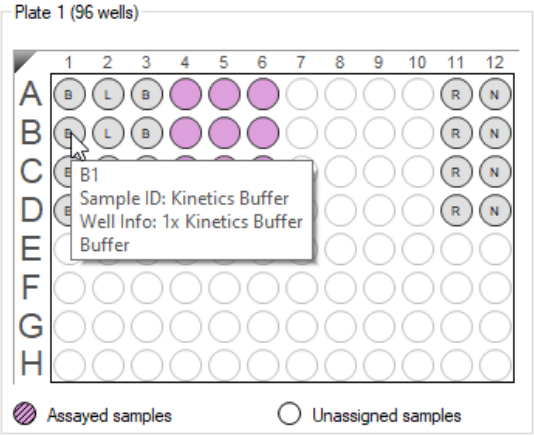


Figure 8-28: Editing a Step Value (left) or Step Type (right)

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).

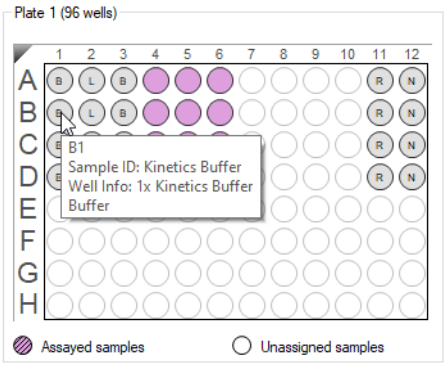


Figure 8-29: Tool Tip of Well Information

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**.

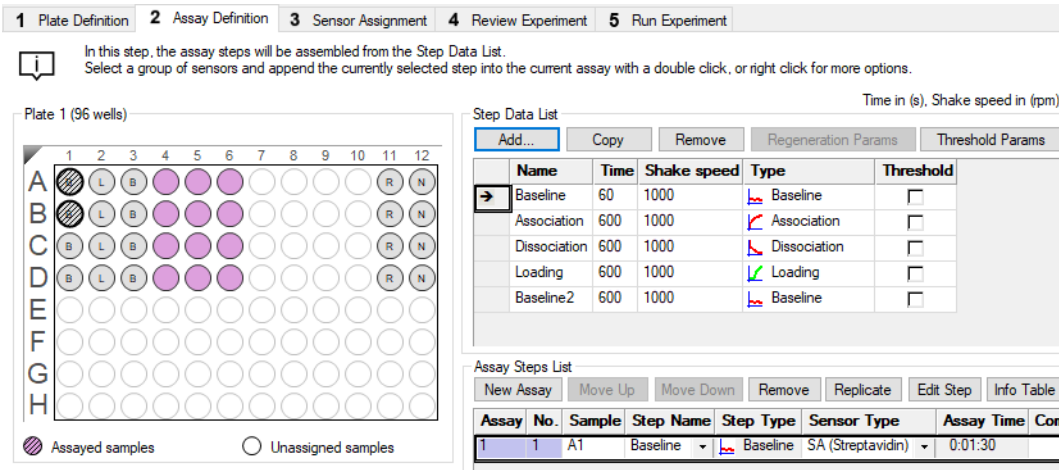


Figure 8-30: Assigning a Step Type to a Column in the Sample Plate

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® R2, or Octet® 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® 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).

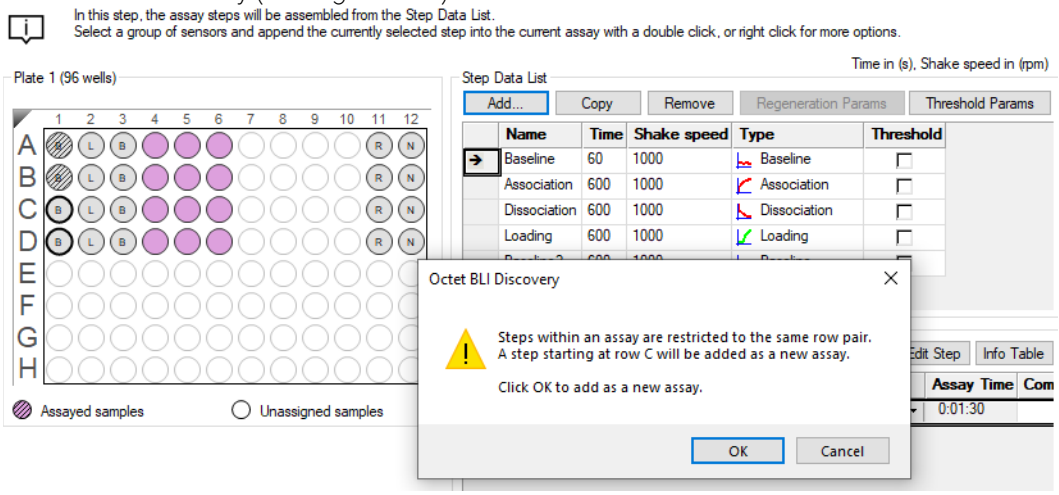


Figure 8-31: Adding a Step Outside a Pair Adds it as a New Assay for Octet® R2, Octet® R4, or Octet® K2;

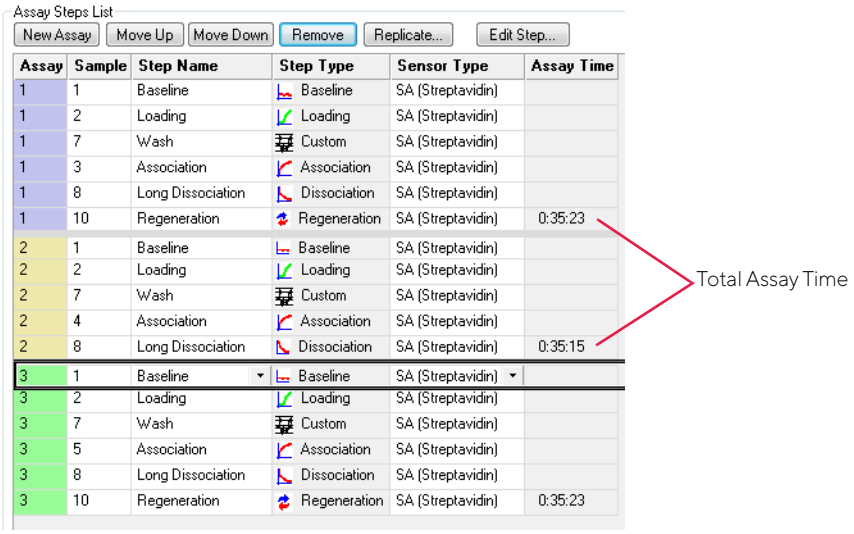


Figure 8-32: Experiment and Assay Time Updates as Steps Are Added to the Assay

IMPORTANT: *If you intend to analyze the data from a sample using the Inter-step correction feature in the Octet® 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).

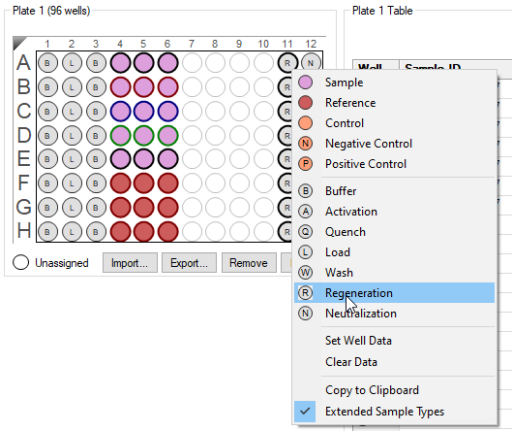


Figure 8-33: Regeneration Step

2. Click **Add** (Figure 8-34) to display the Add Step Definition dialog box (Figure 8-35).

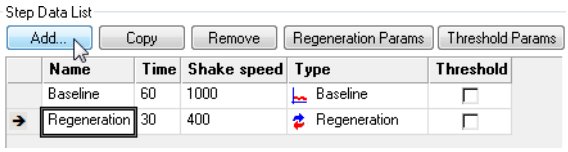


Figure 8-34: Add Button

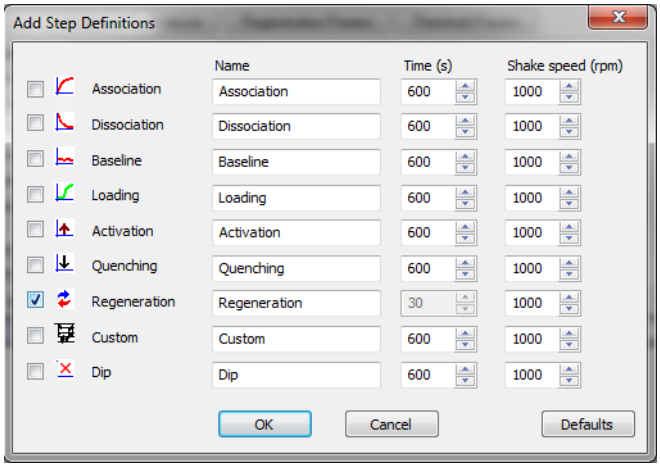


Figure 8-35: Add Step Definition Dialog Box

3. Select **Regeneration** and click **OK**.
4. Click **Regeneration Params** (Figure 8-36).

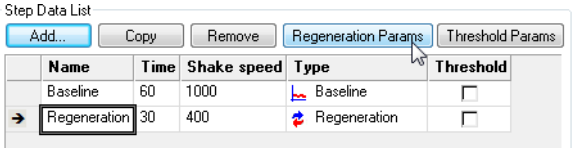


Figure 8-36: Regeneration Params Button

The **Regeneration Parameters** dialog box (Figure 8-37) appears, where you can edit Regeneration parameters as needed.

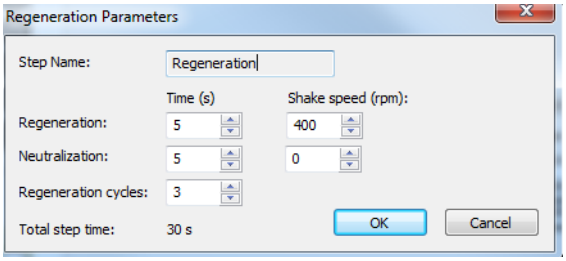


Figure 8-37: Regeneration Parameters Dialog Box

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® R2, or Octet® 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.)

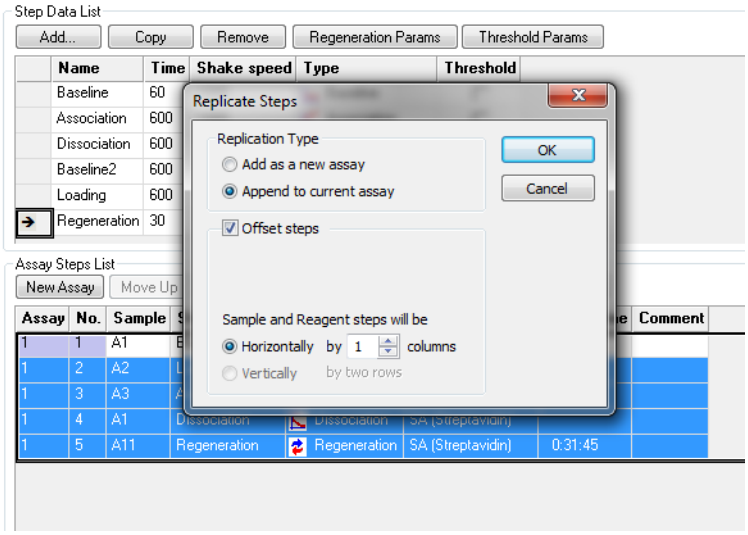


Figure 8-38: Replicating Assay Steps by Appending

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.

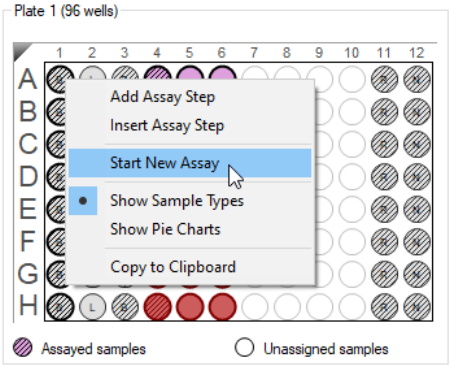


Figure 8-39: Start New Assay

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.
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 ( ) 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.

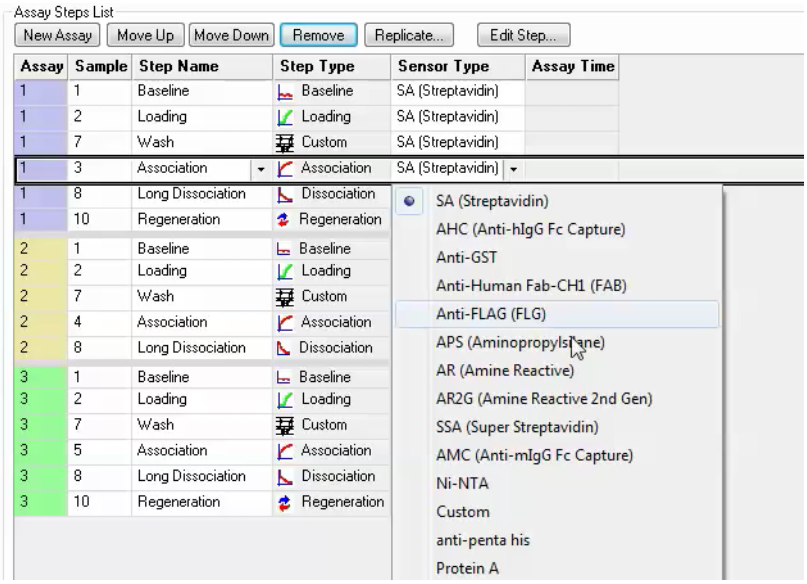


Figure 8-40: Selecting an Assay Sensor Type

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.*

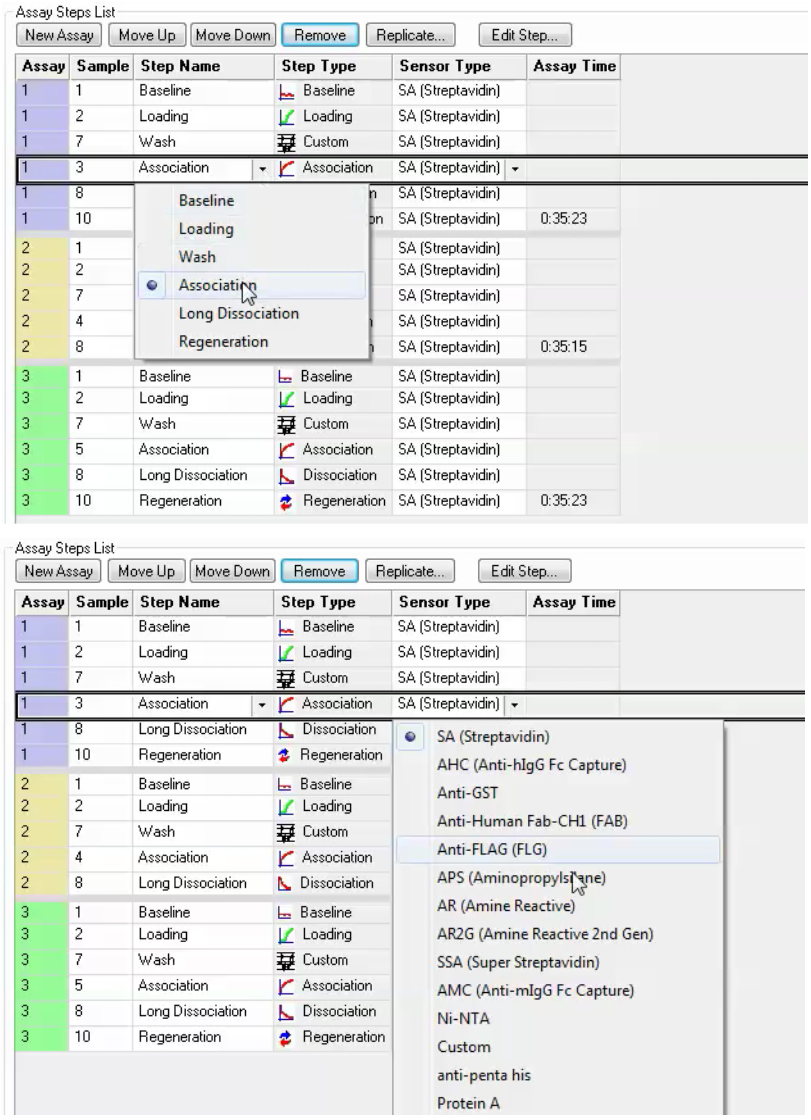


Figure 8-41: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

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® 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).

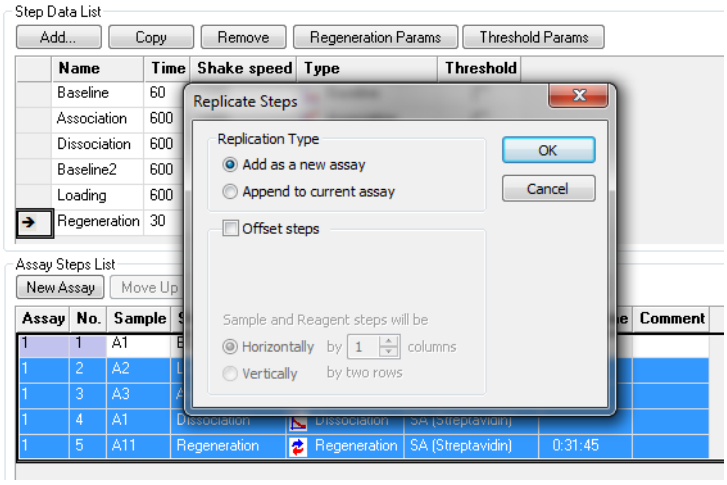


Figure 8-42: Adding a Replicate Assay to a Plate

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 → 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.*

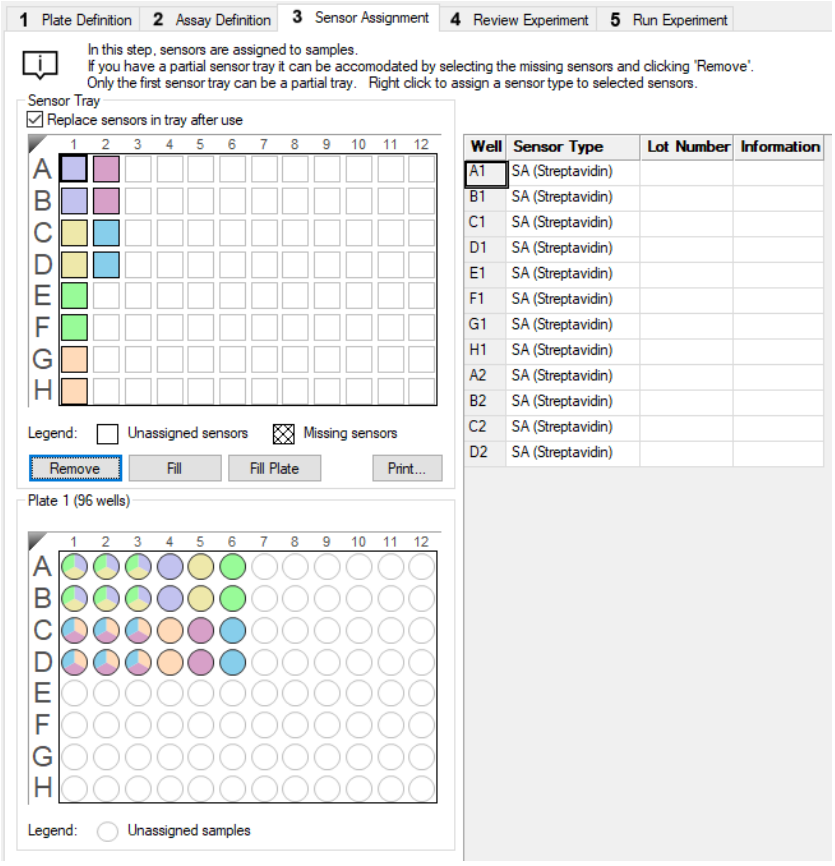


Figure 8-43: Sensor Assignment Window

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).

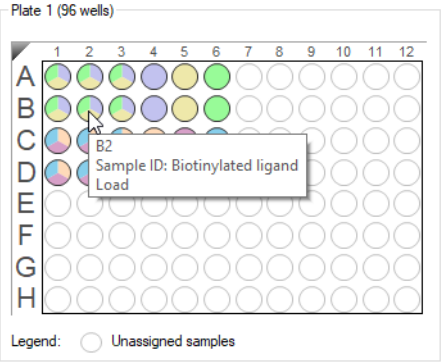


Figure 8-44: Tool Tip of Well Information

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).

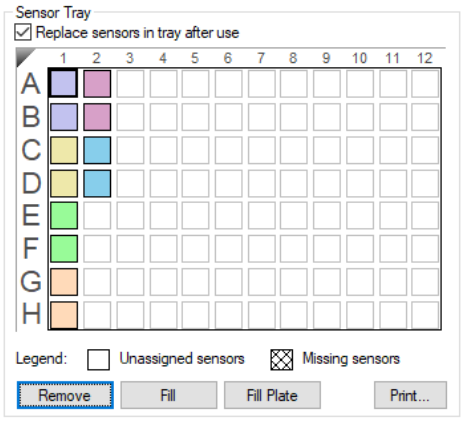


Figure 8-45: Replace Sensors in Tray After Use Check Box

NOTICE: *Do not regenerate biosensors more than 11 times per experiment.*

Entering Biosensor Information

To enter information about a biosensor:

1. 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.*

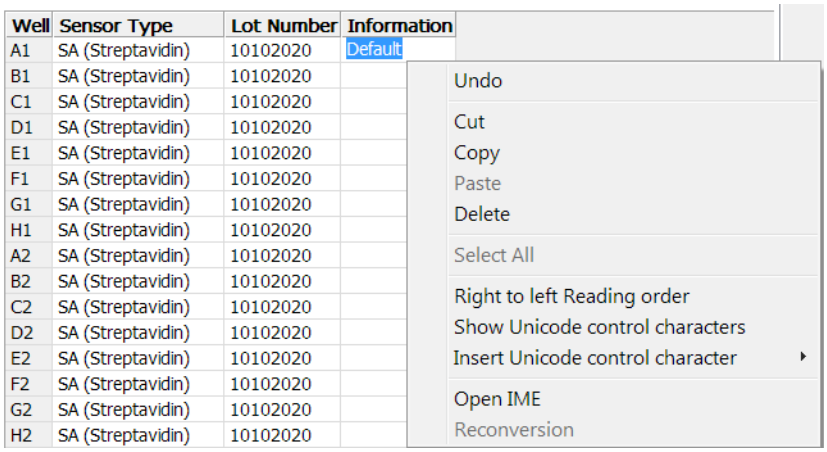


Figure 8-46: Entering or Editing Biosensor Information

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.

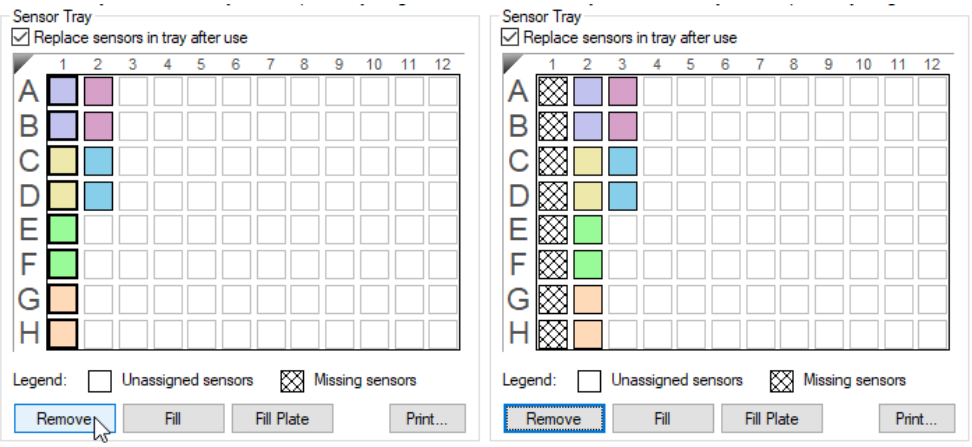


Figure 8-47: Changing Biosensor Location (Method 1)

Method 2

1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 2 top left). Or, right-click the selection 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).

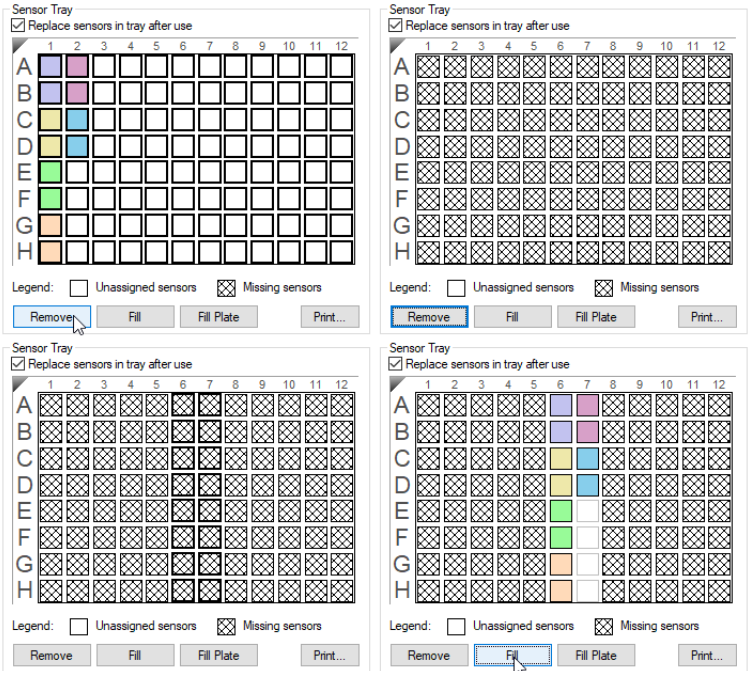


Figure 8-48: Changing Biosensor Location (Method 2)

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 auto-assign 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).

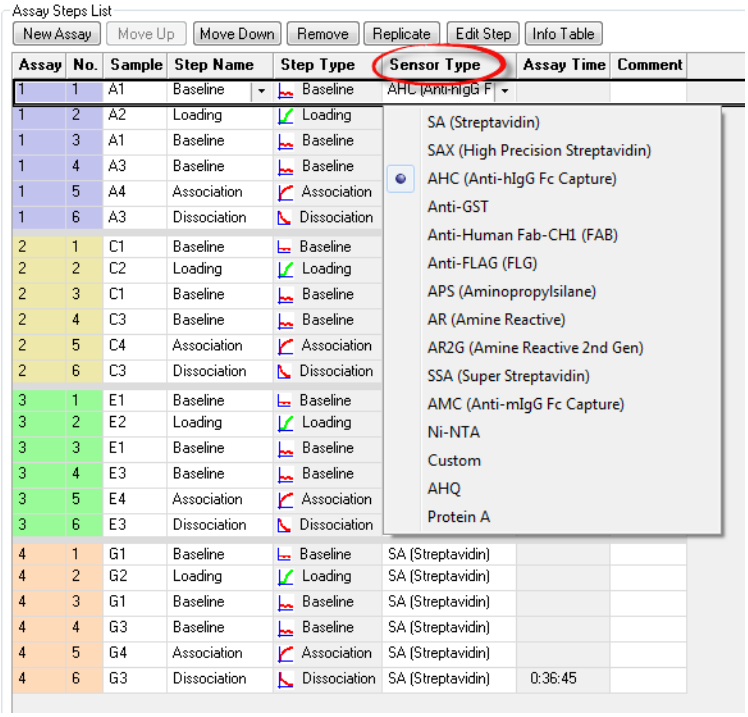


Figure 8-49: Assay Steps List —Changing the Biosensor Type

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.

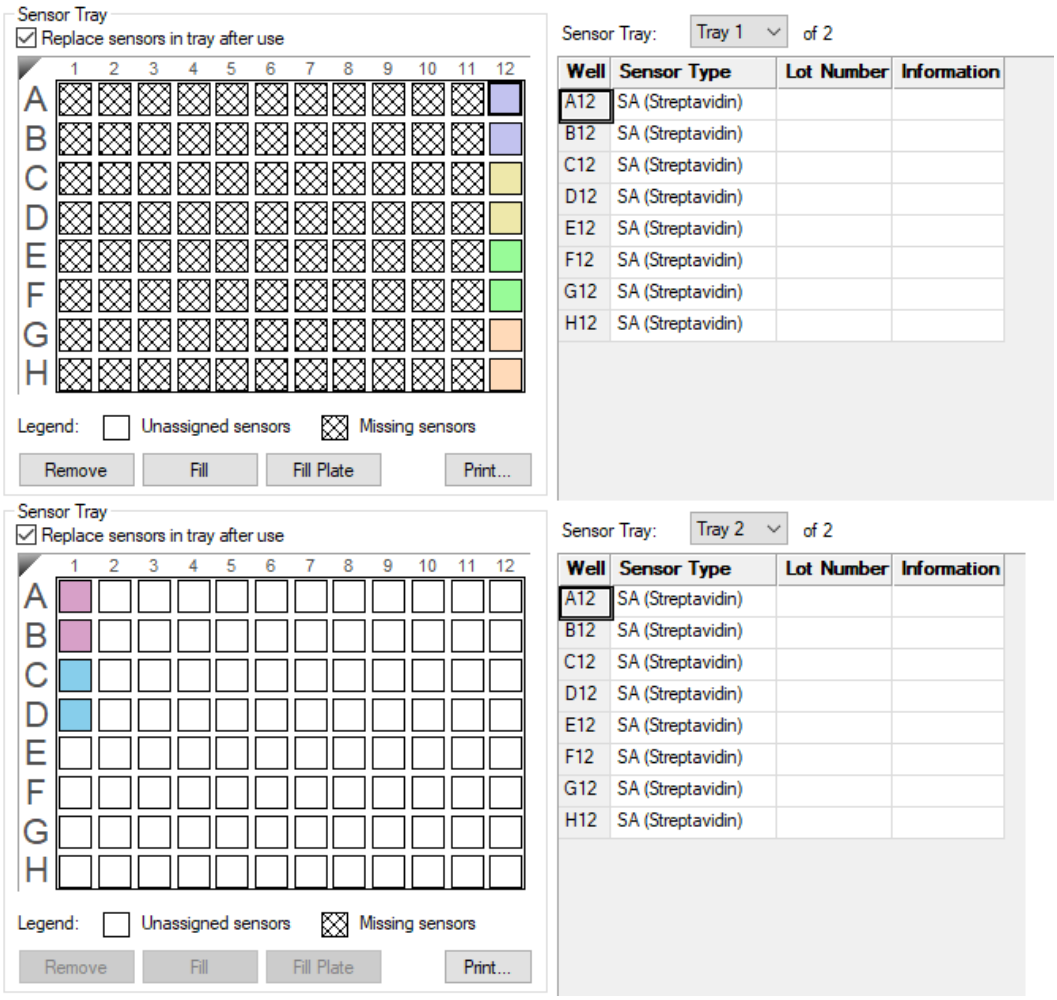


Figure 8-50: Example Experiment Using Two Biosensor Trays

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® 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 ← → arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

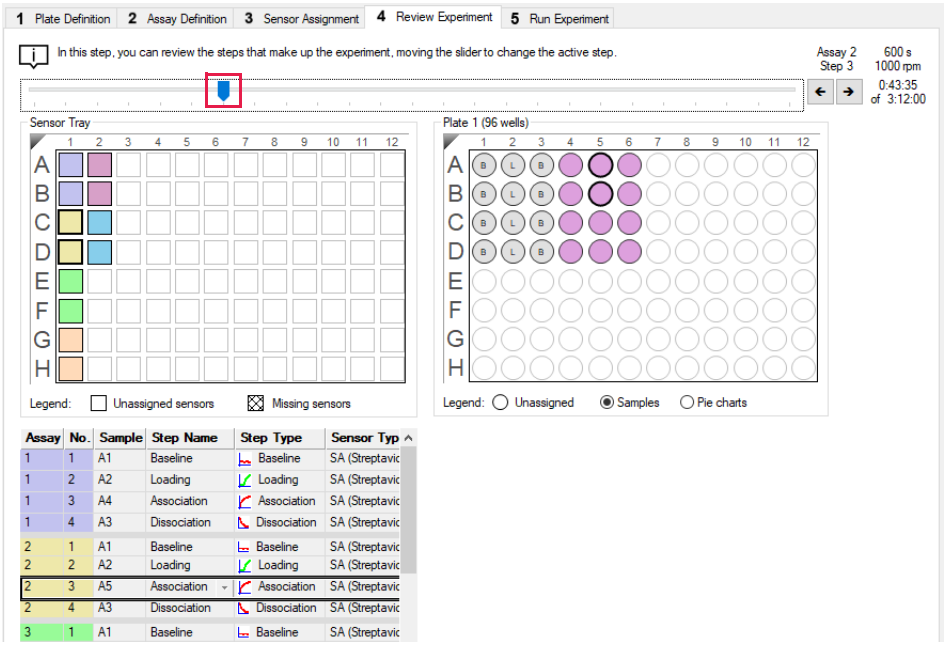


Figure 8-51: Review Experiment Window

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 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 factory-set location, the software may not function properly.*

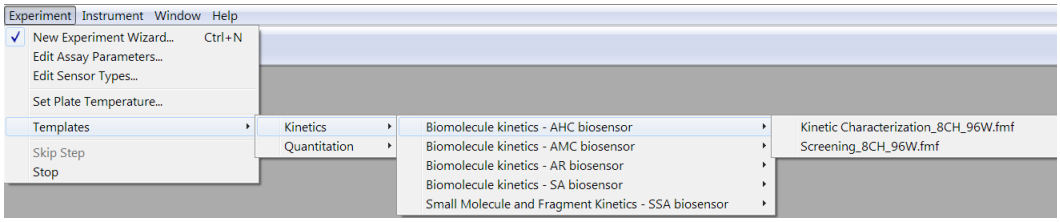


Figure 8-52: Saved Experiments in the Template Folder

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®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).

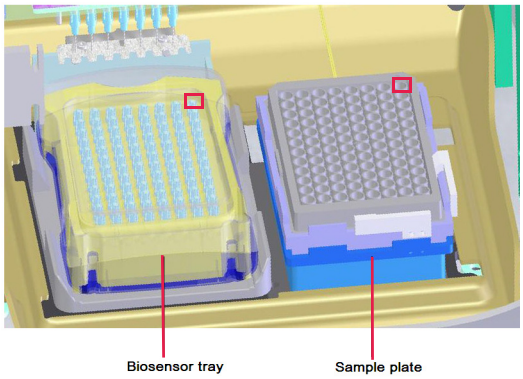


Figure 8-53: Biosensor Stage (left) and Sample Stage (right)

IMPORTANT: *Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.*

4. Close the Octet® 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 (→) to access the Run Experiment window (see Figure 8-54).

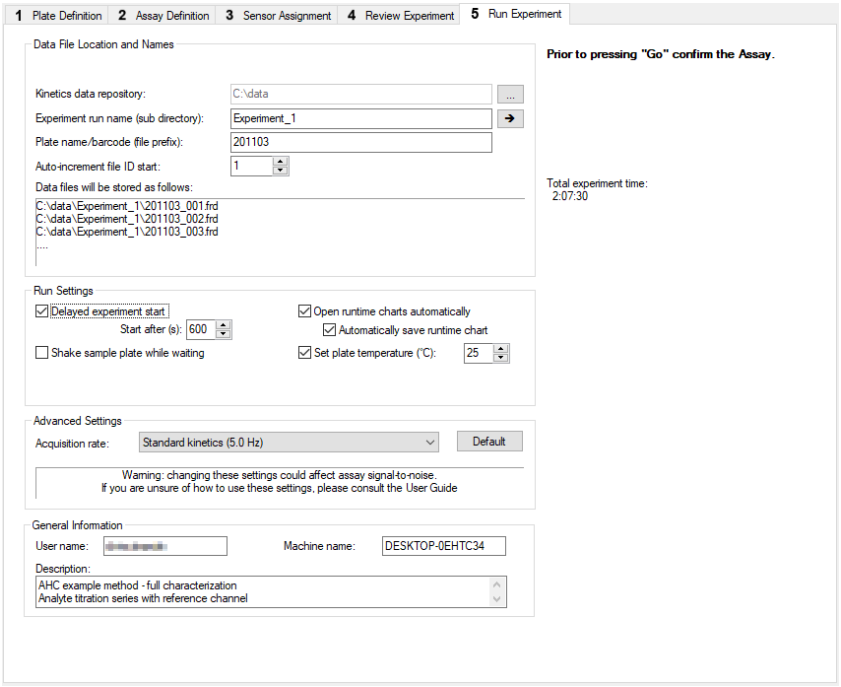


Figure 8-54: Run Experiment Window

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.*

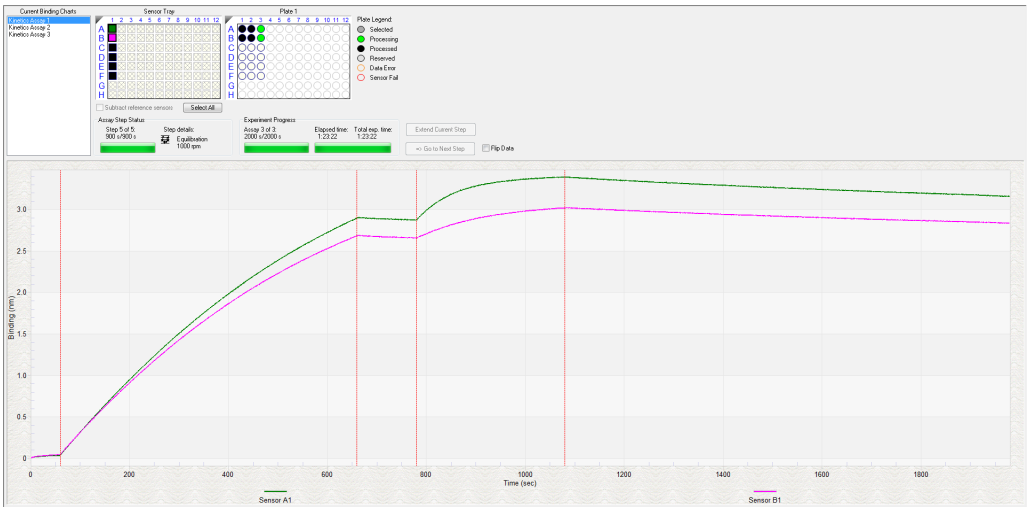


Figure 8-55: Runtime Binding Chart

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.

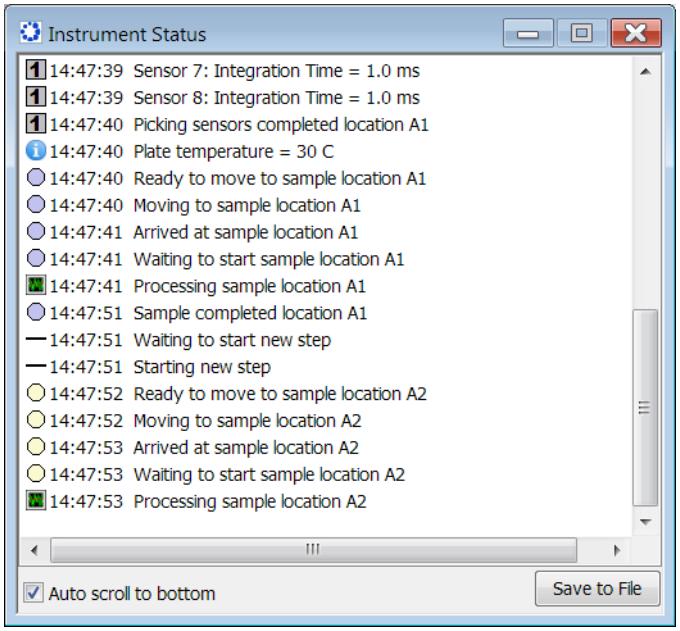


Figure 8-56: Instrument Status Log

WARNING: *Do not open the Octet® 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® 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 **Browse** to select another data location. ***NOTICE:*** *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/barcode	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 GO.
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 **Runtime Binding Chart** for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the **Runtime Binding Chart**. 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 **File > Options**. The factory set default temperature is 30 °C. ***NOTICE:*** *If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet® 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® 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® R2, Octet® R4, or Octet® K2 System

Item	Description
Acquisition rate	• 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. • Standard kinetics (5 Hz, averaging by 20 - The average of 20 data frames is reported as one data point.
Default	Sets acquisition rate and sensor offset to the defaults.

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 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 color-coded (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® 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.*

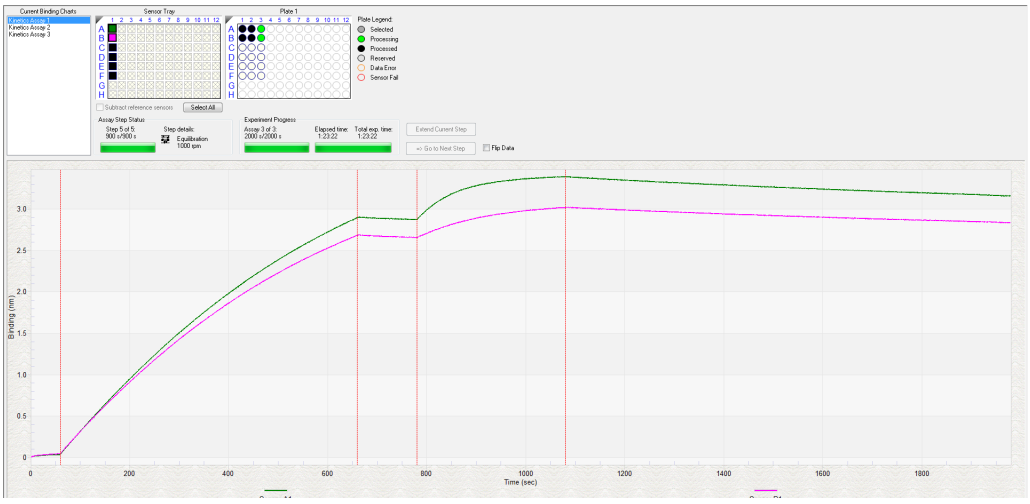


Figure 8-57: Runtime Binding Chart Window

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).

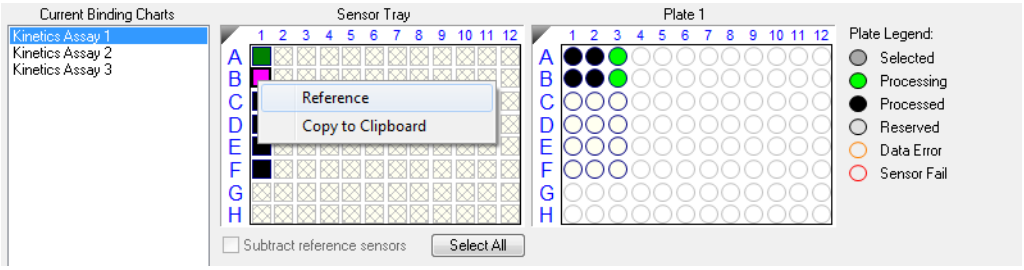


Figure 8-58: Designating a Reference Biosensor in the Runtime Binding Chart

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).

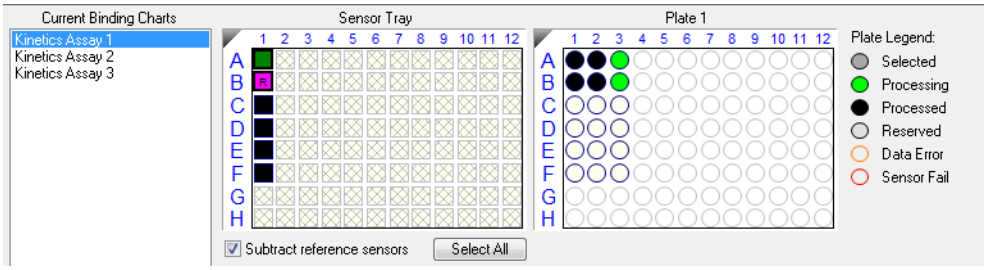


Figure 8-59: Subtract Reference Sensors check box in the Runtime Binding Chart

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.

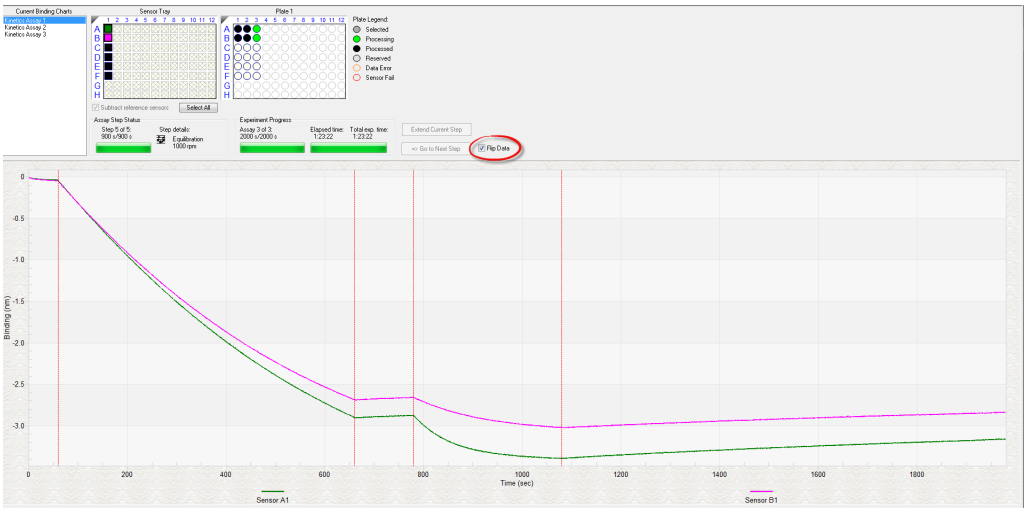


Figure 8-60: Data Inverted Using Flip Data Function

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**.

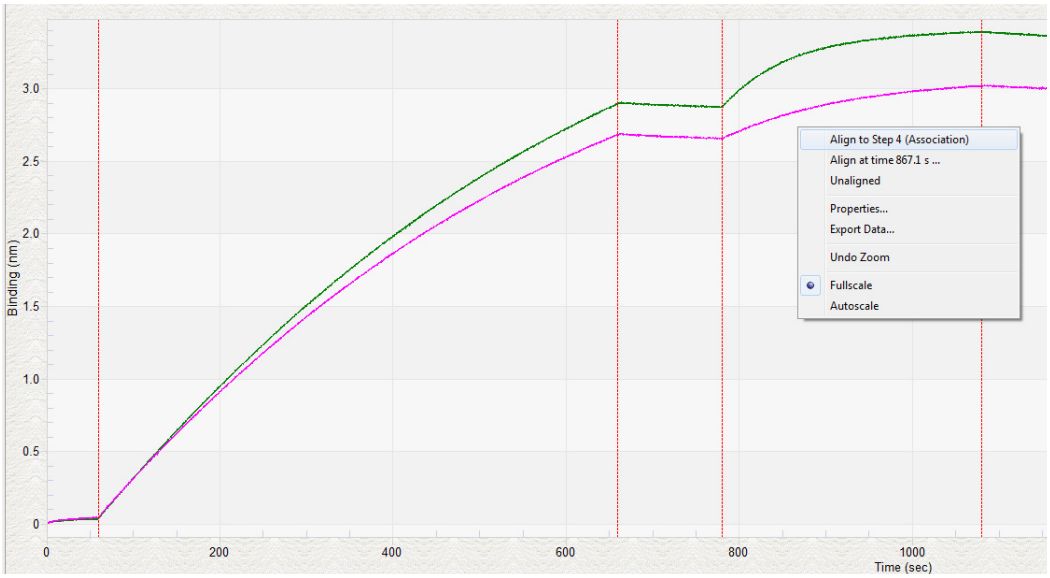


Figure 8-61: Runtime Binding Chart—Aligning the Data to a User-Selected Step

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**.

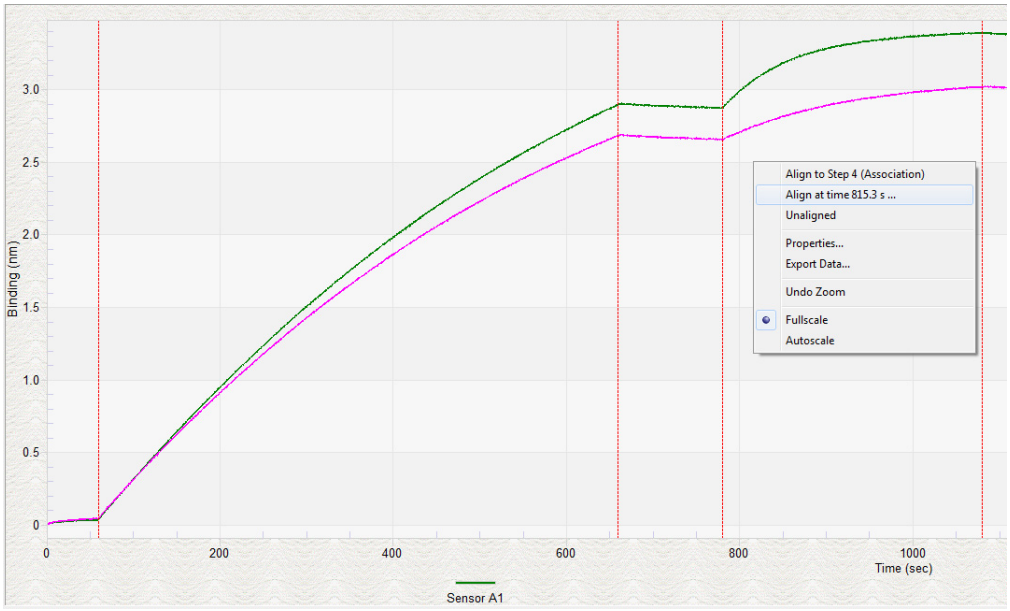


Figure 8-62: Runtime Binding Chart—Aligning the Data to a User-Specified Time

The Align at Time dialog box appears (Figure 8-63).

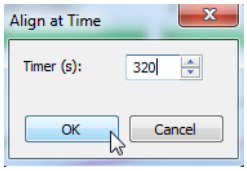


Figure 8-63: Align at Time Dialog Box

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**.

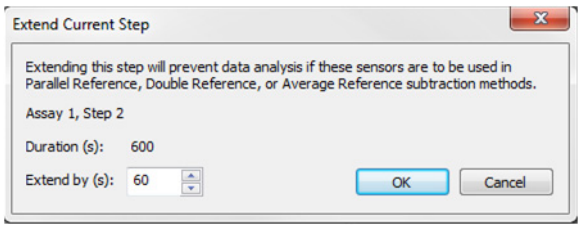


Figure 8-64: Extend Current Step Dialog Box

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

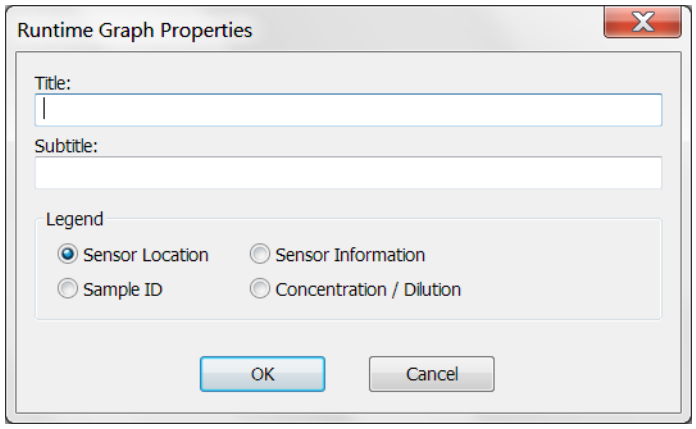


Figure 8-65: Selecting a Runtime Binding Chart Legend

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**.

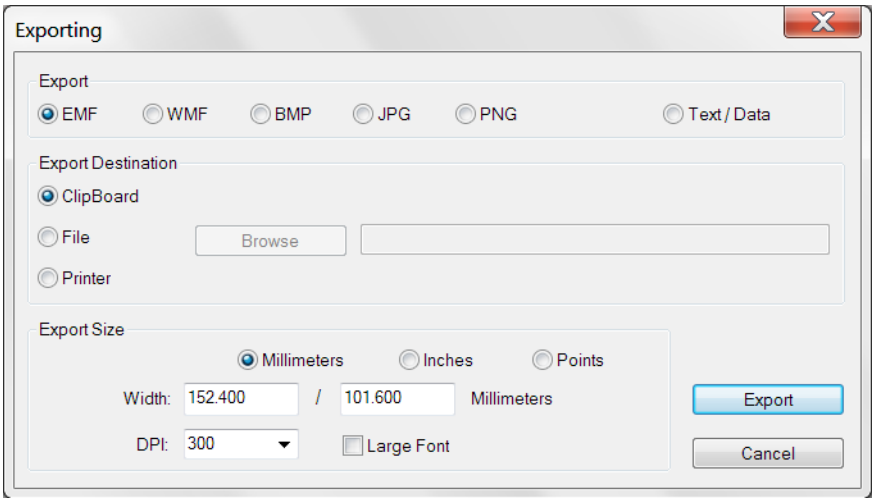


Figure 8-66: Exporting Dialog Box

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	✓		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 biosensor. 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		✓	Clipboard	Copies the chart to the system clip-board

Table 8-9: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Print the Runtime Binding Chart	✓		Printer	Opens the Print dialog box.

Managing Experiment Method Files

After you run an experiment, the Octet® 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 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 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® 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® system and the Octet® BLI Discovery software, follow the steps in Table 8-11 to set up and run an epitope binning experiment.

Table 8-11: Octet® BLI Discovery Steps for Epitope Binning Assays

Octet® Software	Functions
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). ***NOTICE:*** *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** 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.

Tab 1 (Plate Definition)

NOTICE: *For the Octet® 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:

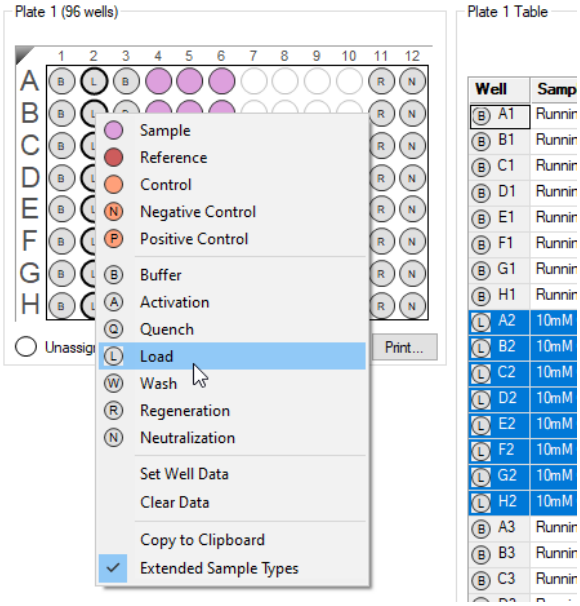


Figure 8-67: Designing well types

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:

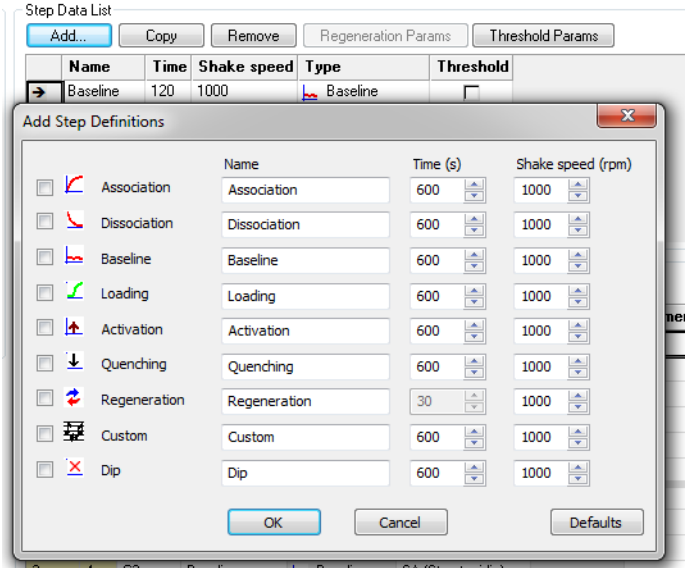


Figure 8-68: Add Step Definition Box

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:

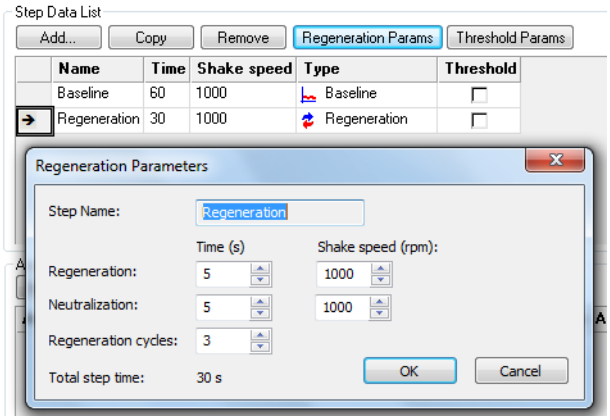


Figure 8-69: Regeneration Parameters Box

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:

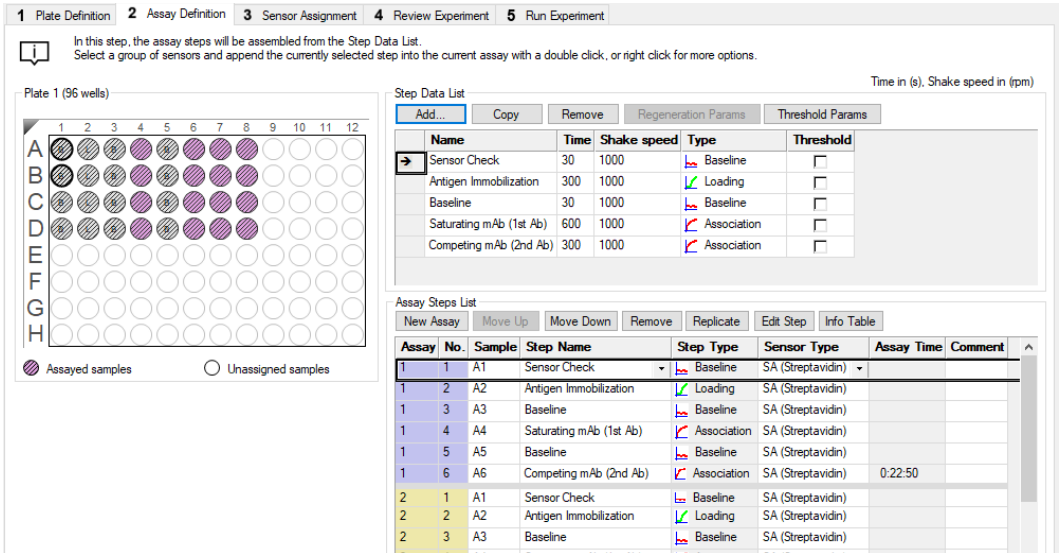


Figure 8-70: Assay Steps List

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:

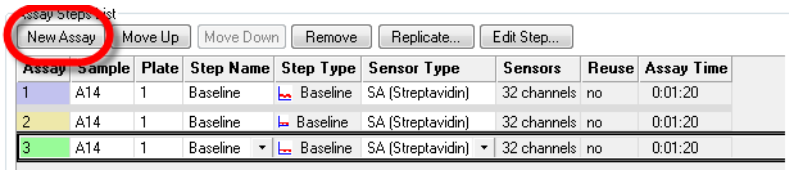


Figure 8-71: New Assay Button

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".

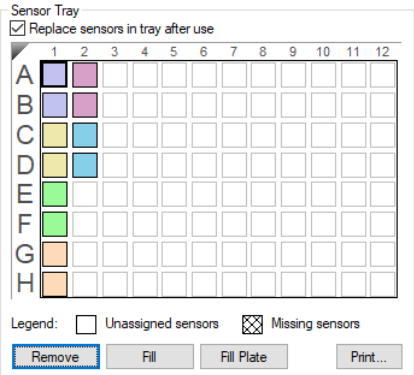


Figure 8-72: Replace Sensors in Tray After Use Check Box

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:

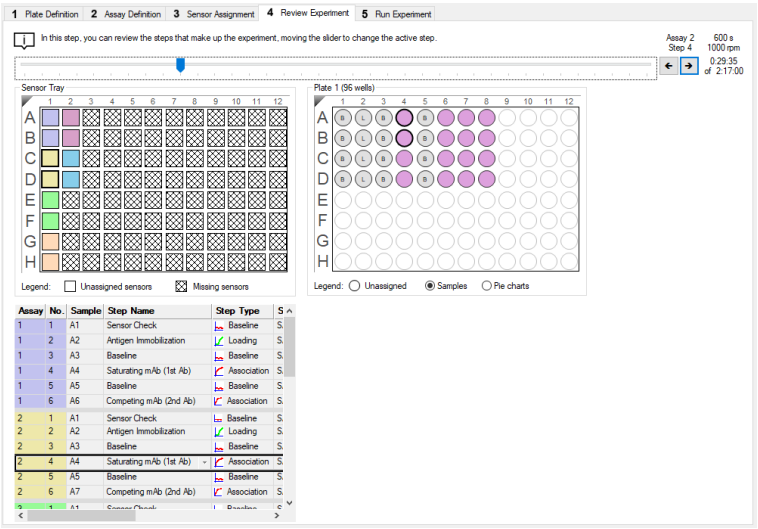


Figure 8-73: Navigating the Review Experiment Tab

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® 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).

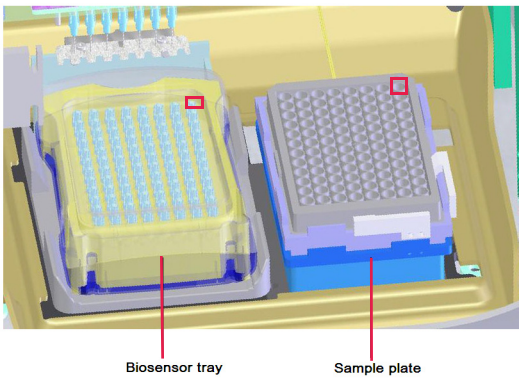


Figure 8-74: Biosensor Stage (left) and Sample Stage (right)

IMPORTANT: *Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.*

4. Close the Octet® 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:

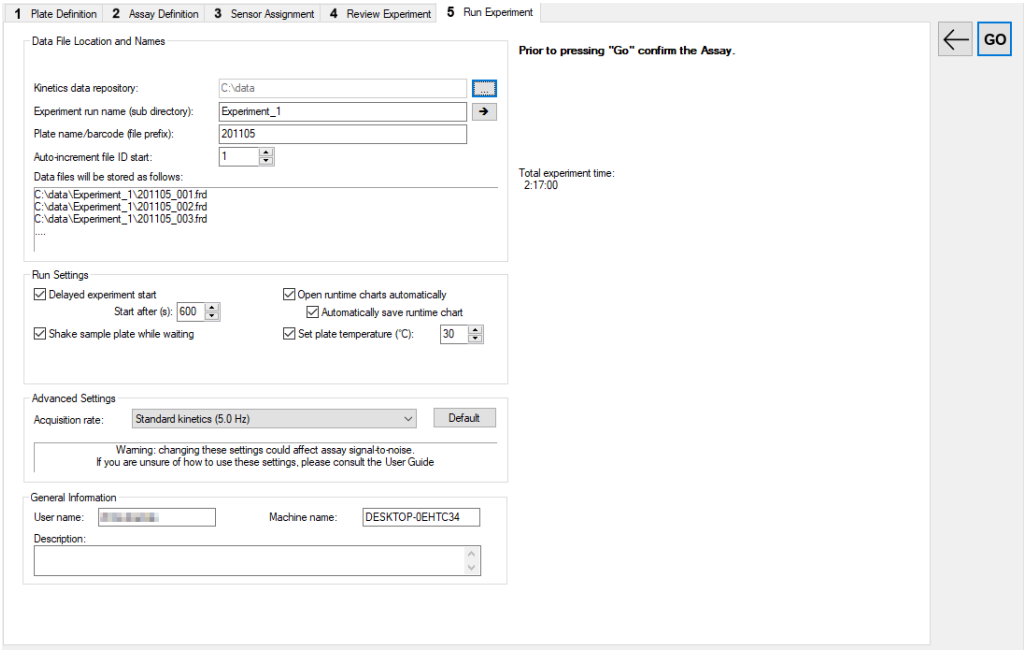


Figure 8-75: GO Button

Chapter 9:

Kinetics Experiments: Octet® R8, Octet® RED96e, and Octet® QKe

Introduction

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet® system hardware and the Octet® 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 that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet® 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.*

Table 9-1: Setting Up and Analyzing a Kinetic Experiment

Software	Step	See
Octet® BLI Discovery 	1. Select a kinetics experiment in the **Experiment Wizard** 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
	3. 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® Analysis Studio 	6. View and process the raw data. 7. Analyze the data.	*Octet® Analysis Studio Software User Guide*

Starting a Basic Kinetics Experiment

IMPORTANT: *Using 96-well half-area plates on the Octet® RED96 and Octet® RED96e, and Octet® 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® 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 , 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( ). The **Basic Kinetics Experiment** window displays (Figure 9-1, right).

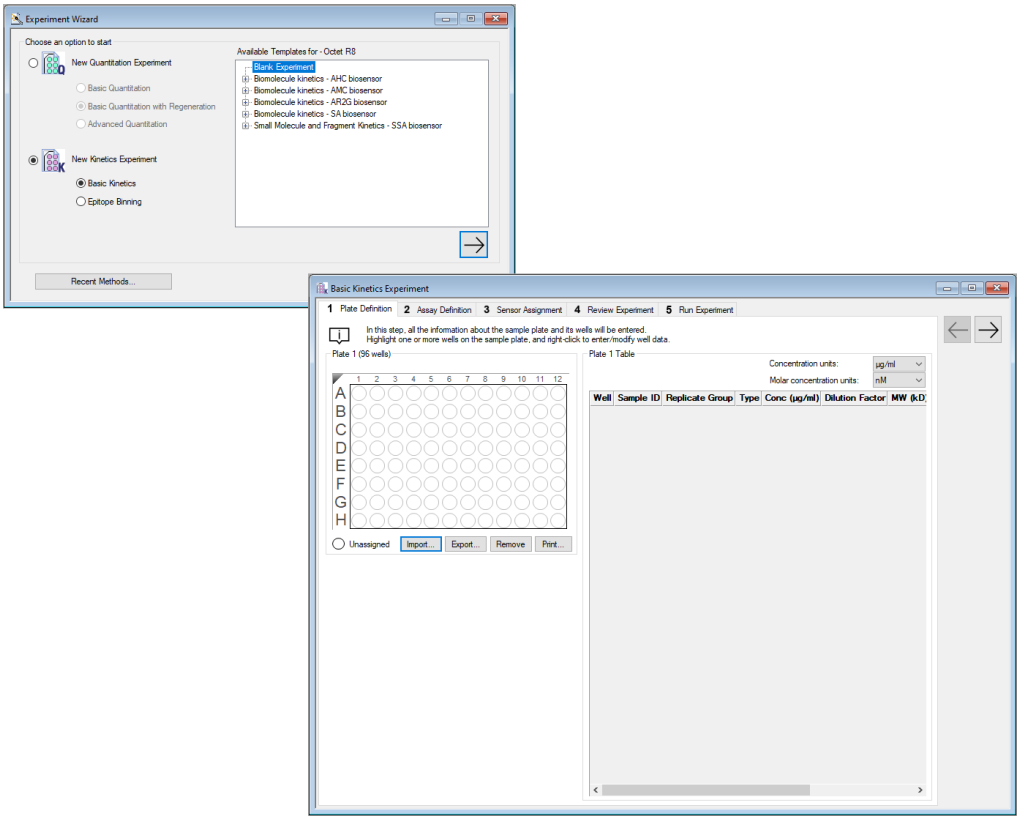


Figure 9-1: Starting a Kinetics Experiment with the Experiment Wizard

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

Icon	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. • Positive Control: A control sample that contains analyte of known concentration • Negative Control: A control sample known not to contain analyte
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

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).

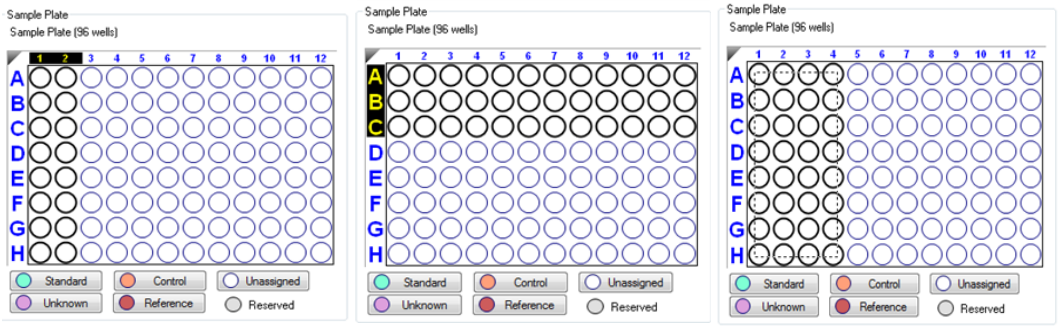


Figure 9-2: Selecting Wells in the Sample Plate Map

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).

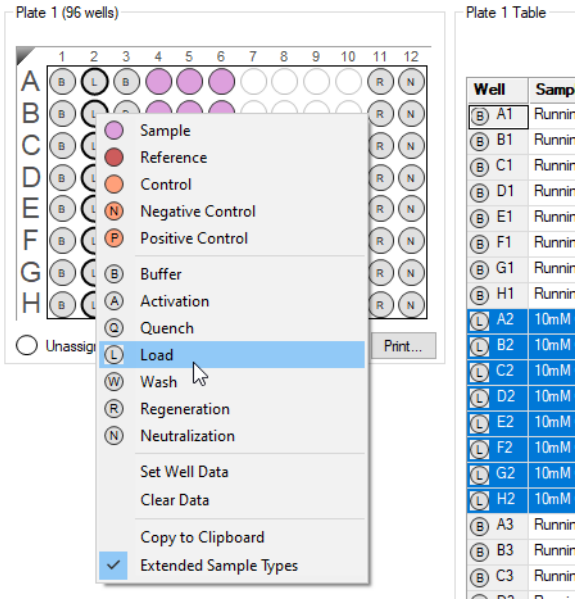


Figure 9-3: Designating a Well Type in the Plate Definition Window

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).

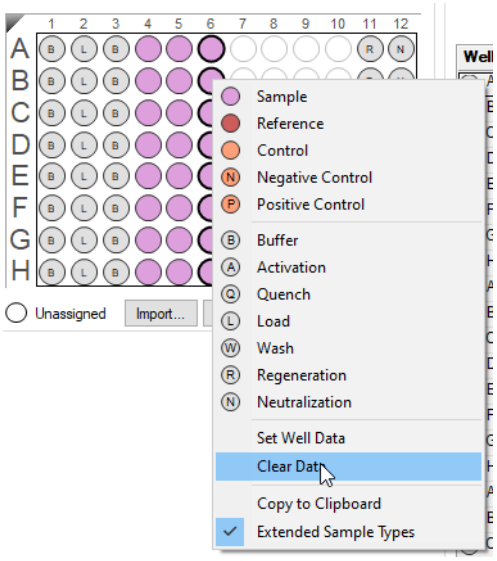


Figure 9-4: Clearing Sample Data from a Sample Plate

Entering Sample Information

NOTICE: *You must specify sample (analyte) concentration and molecular weight, otherwise the Octet® BLI Discovery software cannot 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.*

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).

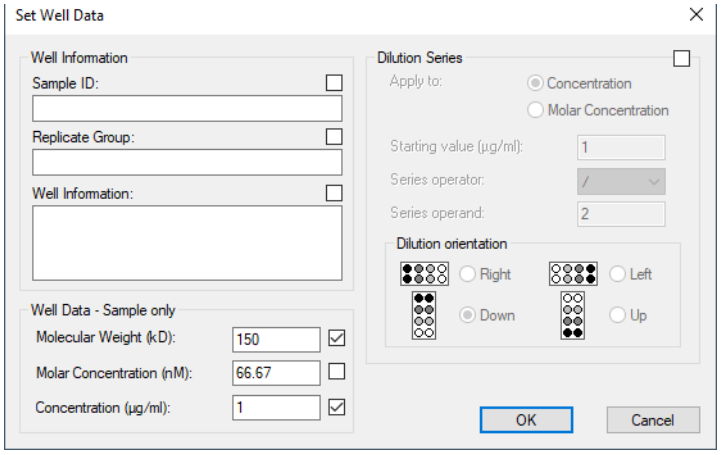


Figure 9-5: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

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.

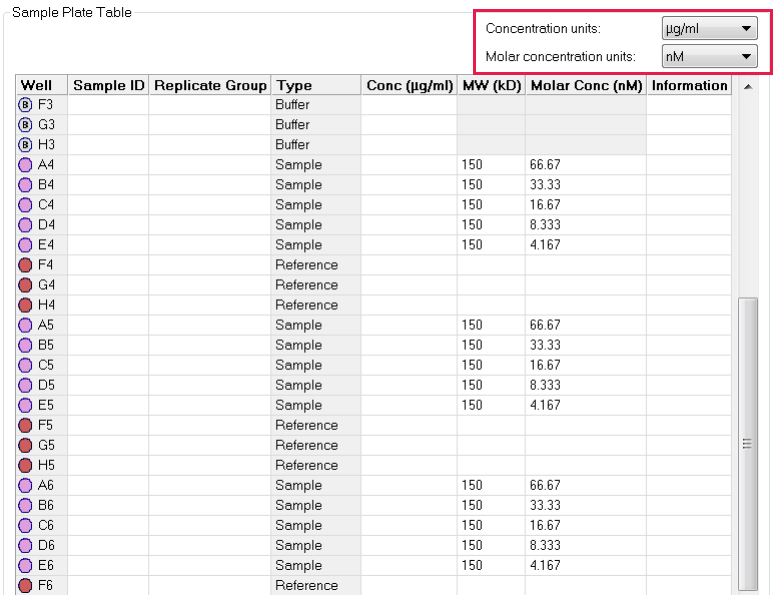


Figure 9-6: Entering Molecular Weight and Molar Concentration from the Plate Table

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.

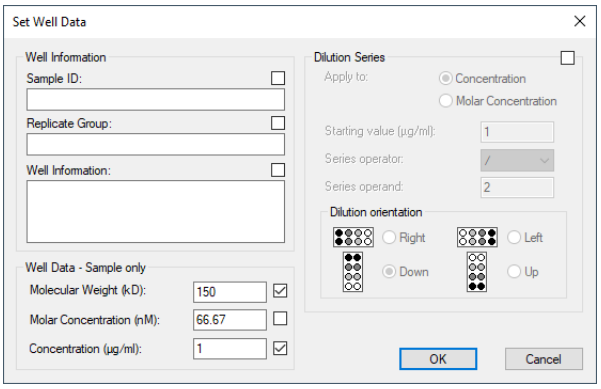


Figure 9-7: Sample Plate Map—Assigning Sample Concentrations by Value

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:

1. 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.

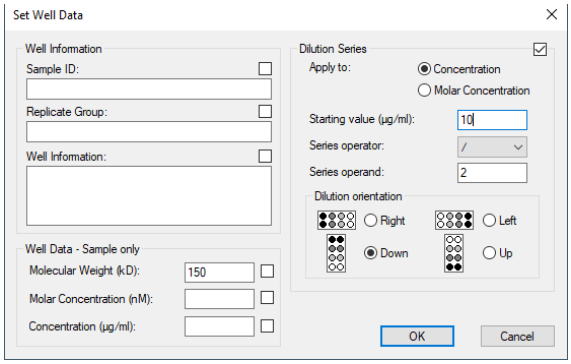


Figure 9-8: Sample Plate Map—Assigning Sample Concentrations Using Dilution Series

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 **Run-time 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**.

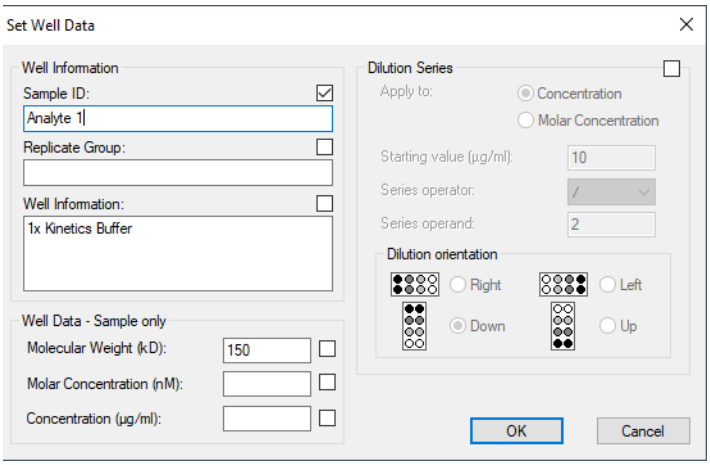


Figure 9-10: Add Sample Annotations from the Sample Plate Map

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.*

Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

Well	Sample ID	Replicate Group	Type	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
B4	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
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	x		D3	SA (Streptavidin)		4	D3

Figure 9-12: Replicate Group Color-Coding

NOTICE: R*eplicate 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**.

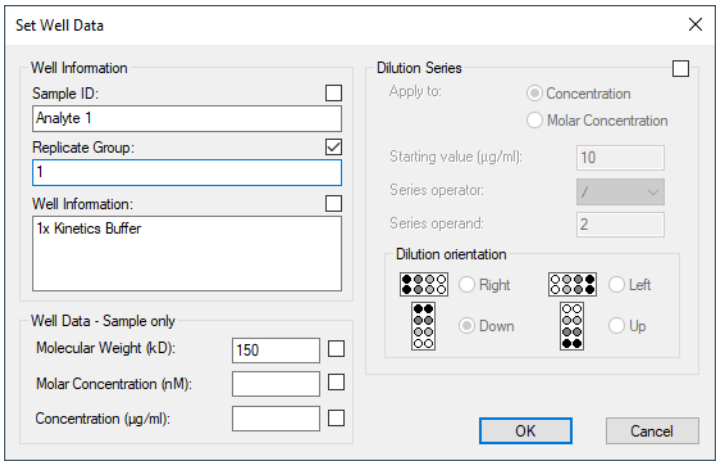


Figure 9-13: Add Replicate Group from the Sample Plate Map

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® 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).

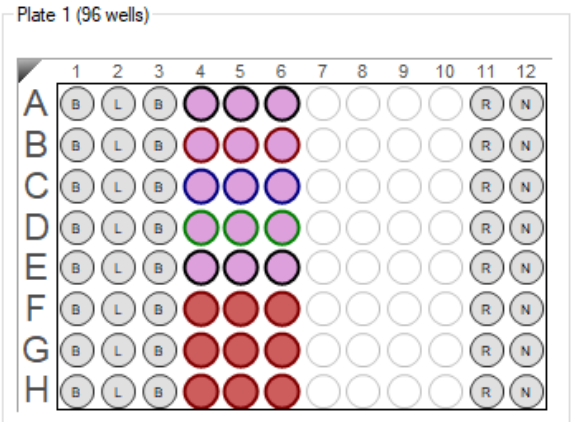


Figure 9-14: Replicate Groups in Sample Plate Map

The **Sample Plate Table u**pdates with the **Replicate Group** names entered (see Figure 9-15).

Well	Sample ID	Replicate Group	Type	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

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

Concentration units: µg/ml

Molar concentration units: nM

Well	Sample ID	Replicate Group	Type	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 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.

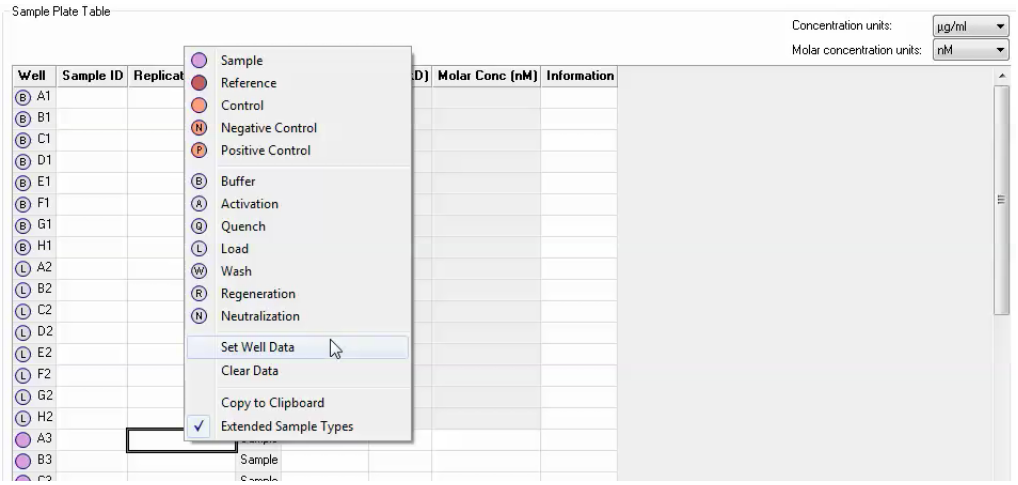


Figure 9-17: Sample Plate Table - Well Designation

Editing Sample Information

To edit sample data in the **Sample Plate Table**, double-click a value and enter a new value.

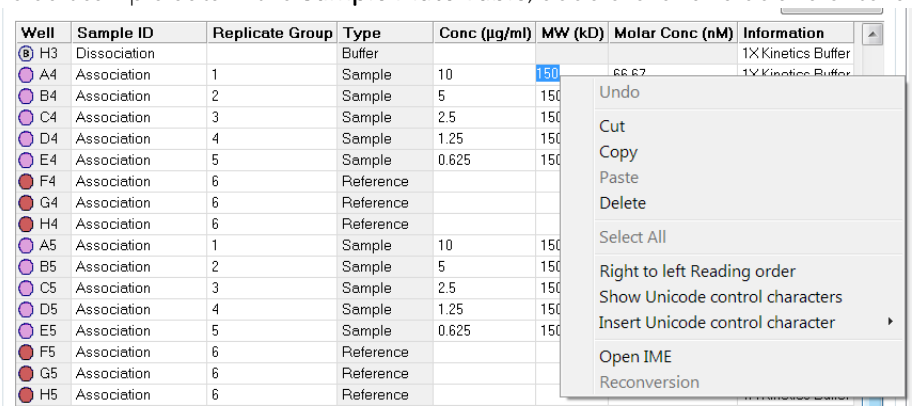


Figure 9-18: Sample Plate Table - Editing Sample Data

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.**

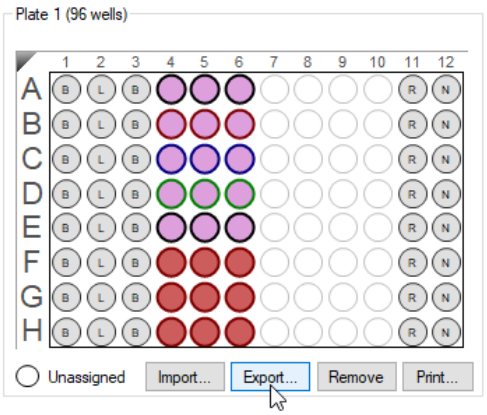


Figure 9-19: Sample Plate Map— Export Button

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

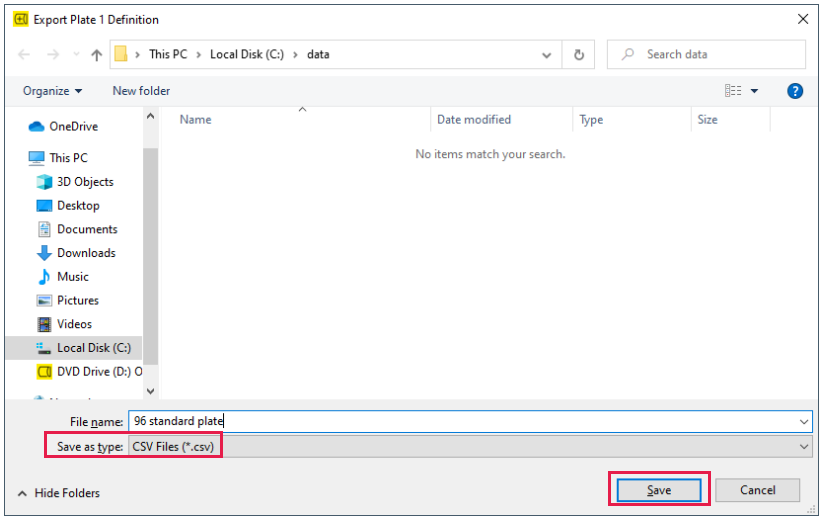


Figure 9-20: Export Plate Definition Window

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**.

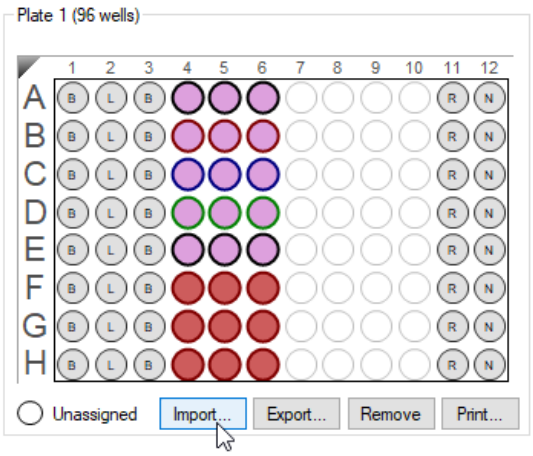


Figure 9-21: Sample Plate Map– **Import** Button

2. In the **Import Plate Definition** window (see Figure 9-22), select the plate definition (.csv), and click **Open**.

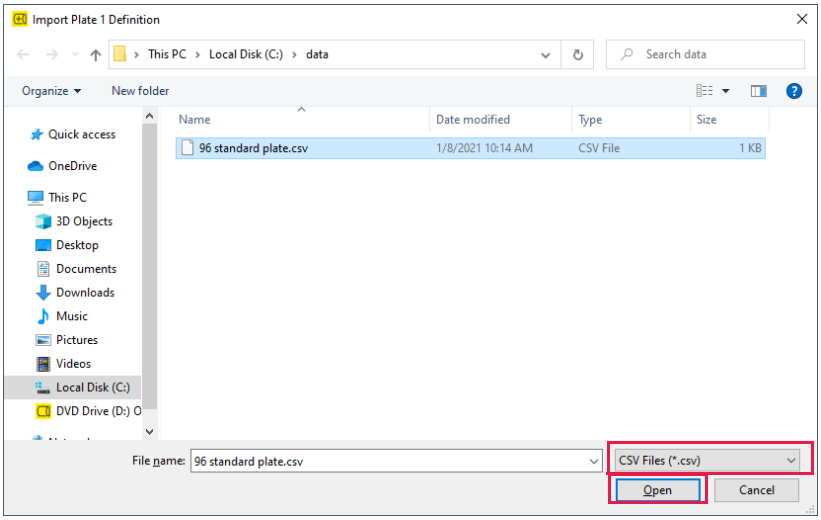


Figure 9-22: Import Plate Definition Window

NOTICE: *You can also create a .csv file for import. Figure 9-23 shows the appropriate column information layout.*

	A	B	C	D	E	F	G	H
1	PlateWells	96						
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information
3	A1	Kinetics Buffer		Buffer				1X Kinetics Buffer
4	B1	Kinetics Buffer		Buffer				1X Kinetics Buffer
5	C1	Kinetics Buffer		Buffer				1X Kinetics Buffer
6	D1	Kinetics Buffer		Buffer				1X Kinetics Buffer
7	E1	Kinetics Buffer		Buffer				1X Kinetics Buffer
8	F1	Kinetics Buffer		Buffer				1X Kinetics Buffer
9	G1	Kinetics Buffer		Buffer				1X Kinetics Buffer
10	H1	Kinetics Buffer		Buffer				1X Kinetics Buffer
11	A2	Loading		Load				12.5 ug/ml ProA
12	B2	Loading		Load				12.5 ug/ml ProA

96 standard 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**.

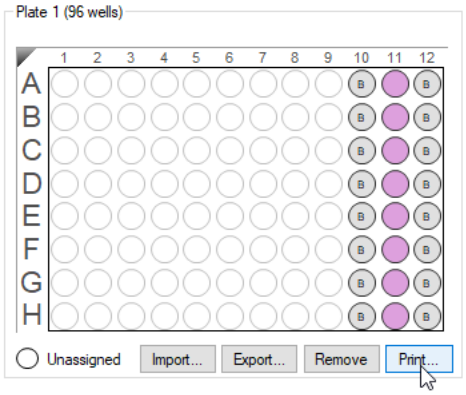


Figure 9-24: Sample Plate Print Button

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 baseline step directly precedes an association step, perform the baseline step at 1,000 rpm. ***IMPORTANT:*** *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. ***NOTICE:*** *This step may be performed offline (outside the Octet® 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 → 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).

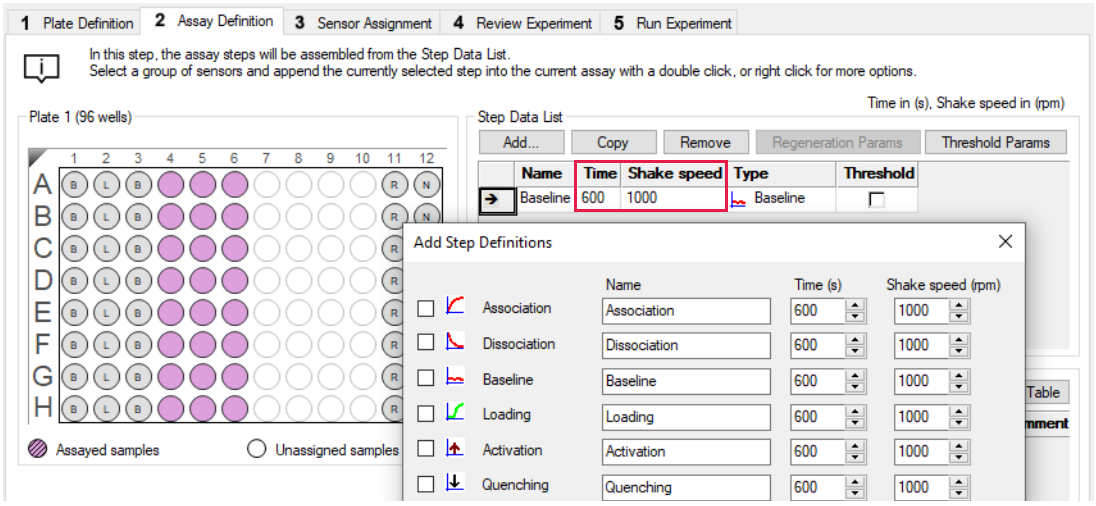


Figure 9-25: Creating an Assay Step Type

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 9-26).
   b. Set the threshold parameters (refer to Table 9-4 for the parameter definitions).

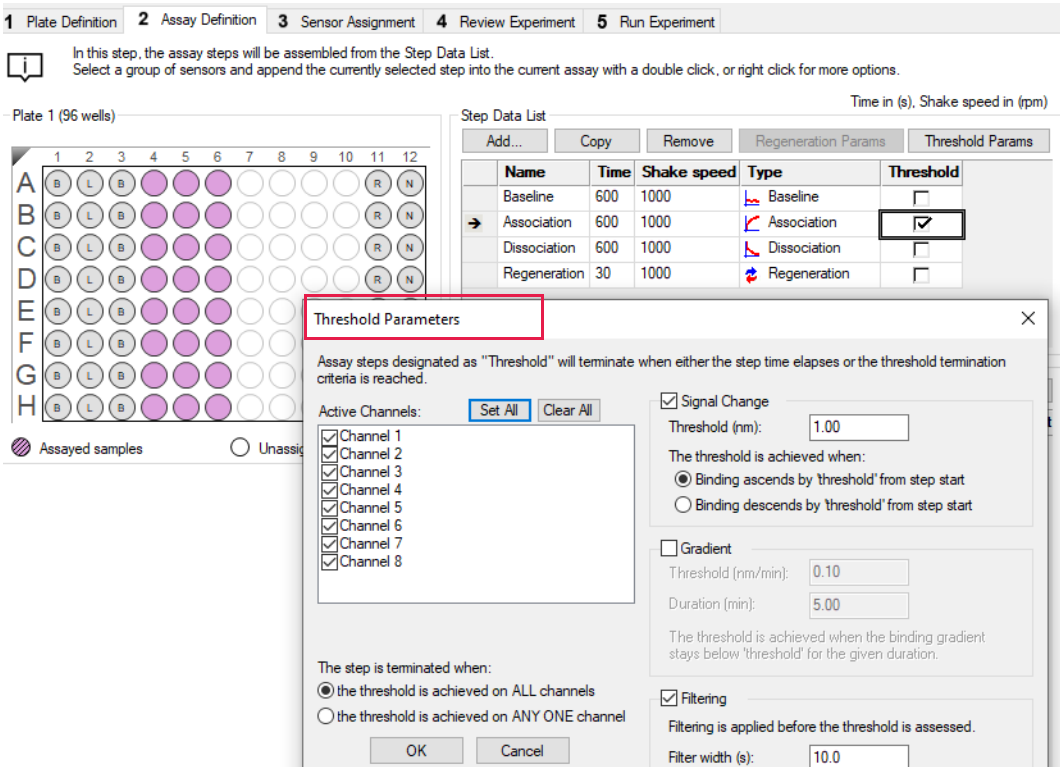


Figure 9-26: Setting Assay Step Threshold Parameters

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

Item	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.

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 9-27).

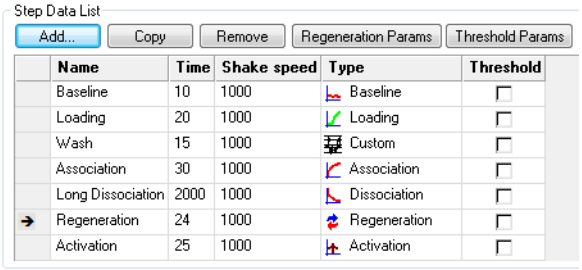


Figure 9-27: Step Data List with Step Types

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).

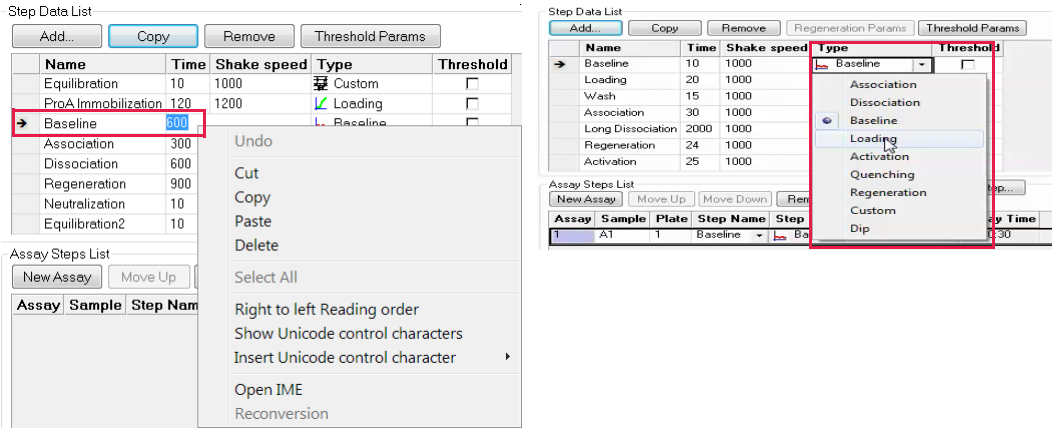


Figure 9-28: Editing a Step Value (left) or Step Type (right)

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).

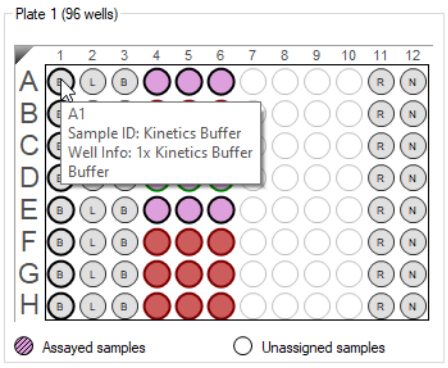


Figure 9-29: Tool Tip of Well Information

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**.

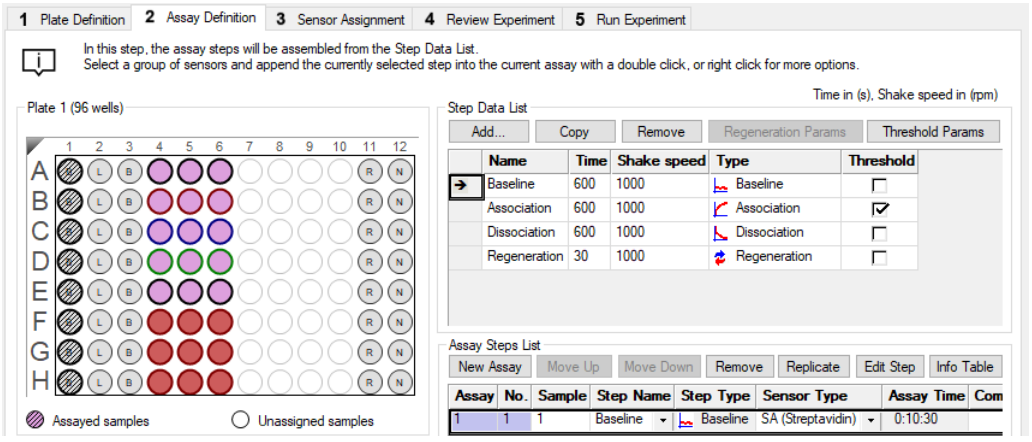
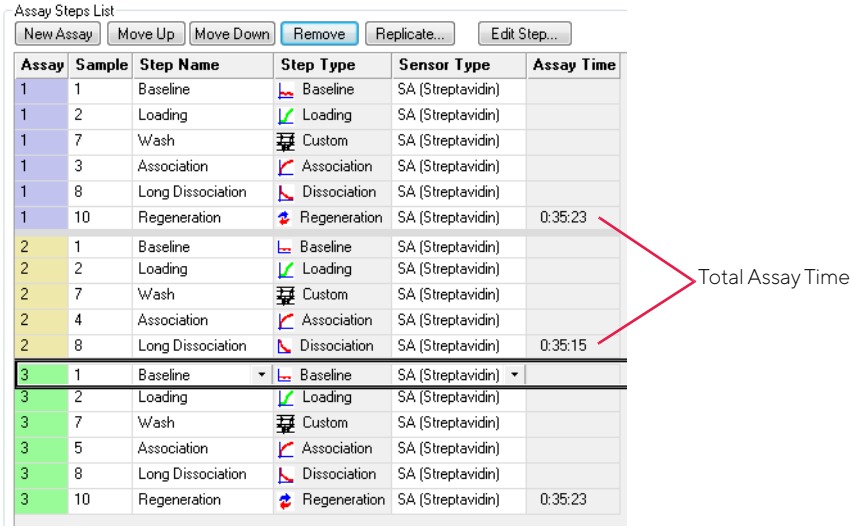


Figure 9-30: Assigning a Step Type to a Column in the Sample Plate

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).



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)	
2	7	Wash	Custom	SA (Streptavidin)	
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® 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).

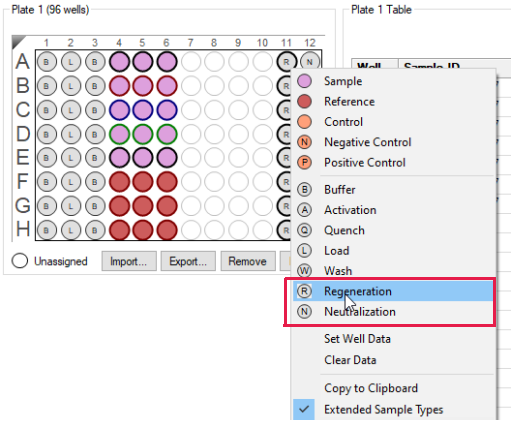


Figure 9-32: Regeneration Step

2. Click **Add** (Figure 9-33) to display the Add Step Definition dialog box (Figure 9-34).

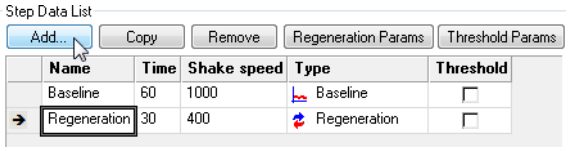


Figure 9-33: Add Button

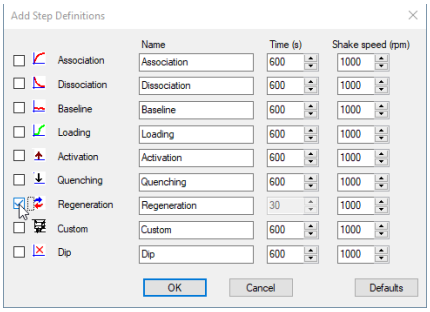


Figure 9-34: Add Step Definition Dialog Box

3. Select **Regeneration** and click **OK**.
4. Click **Regeneration Params** (Figure 9-35).

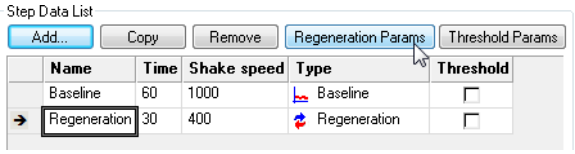


Figure 9-35: Regeneration Params Button

The **Regeneration Parameters** dialog box (Figure 9-36) appears and you can edit Regeneration parameters.

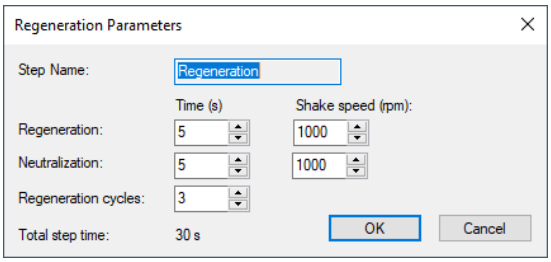


Figure 9-36: Regeneration Parameters Dialog Box

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.)

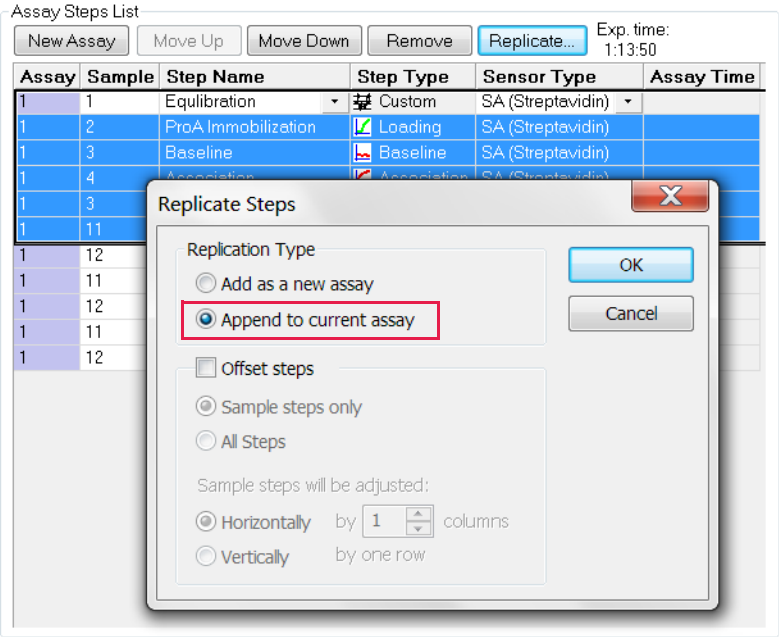


Figure 9-37: Replicating Assay Steps by Appending

4. Click **OK**. The step(s) appear at the end of the assay in the **Assay Steps List**.

Table 9-5: 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.
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 (Continued).

Item	Description
Sample 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.

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.

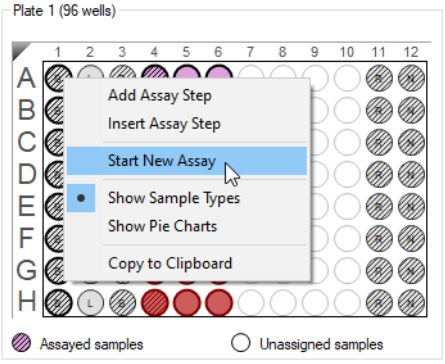


Figure 9-38: Start New Assay

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.
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 ( ) 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.

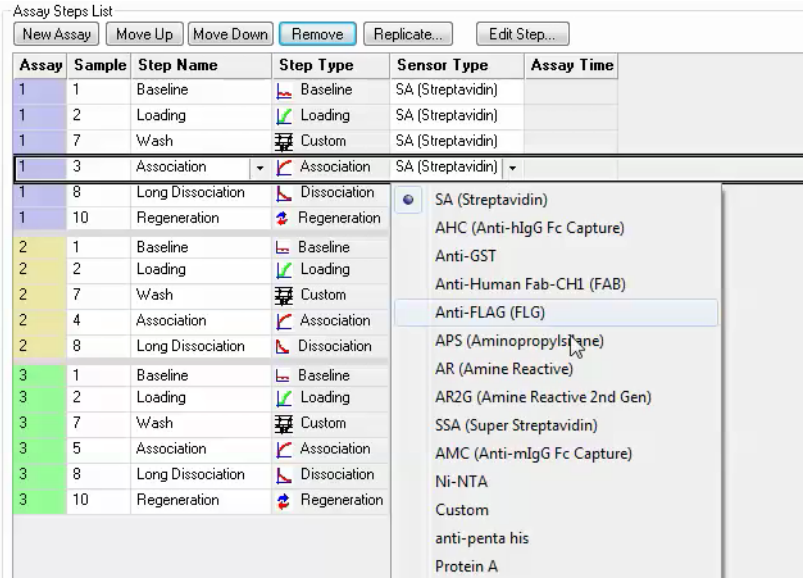


Figure 9-39: Selecting an Assay Sensor Type

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.*

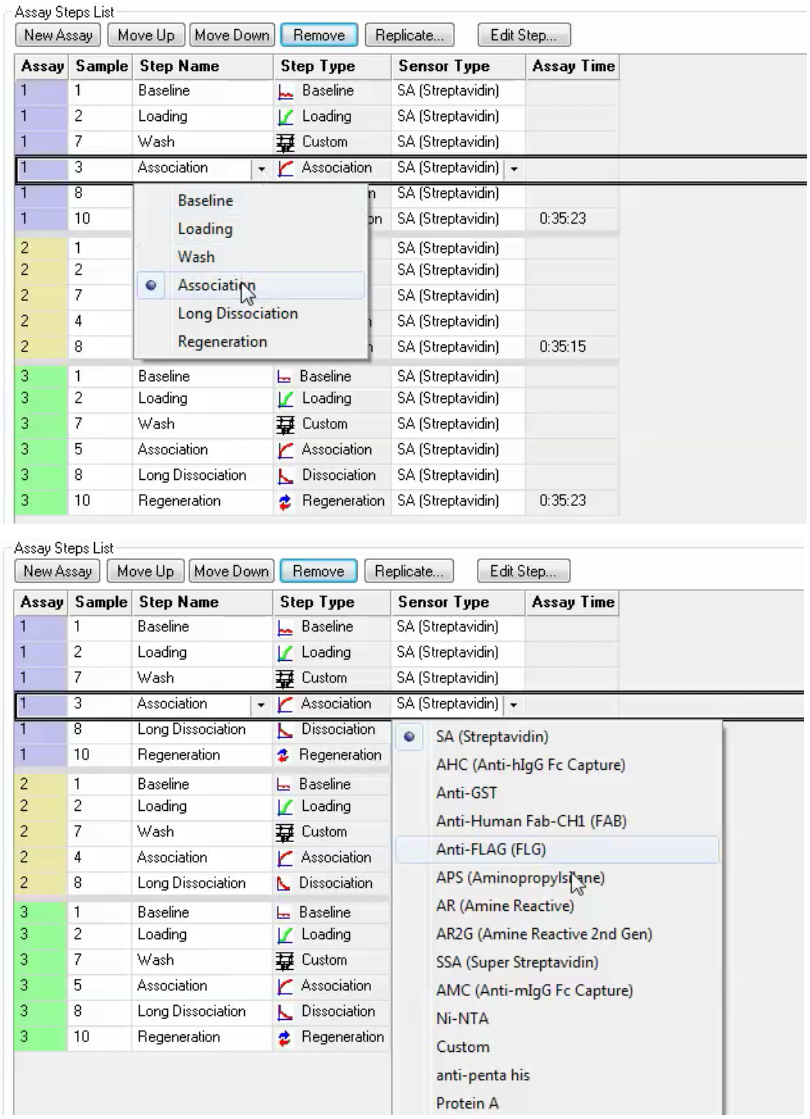


Figure 9-40: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

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).

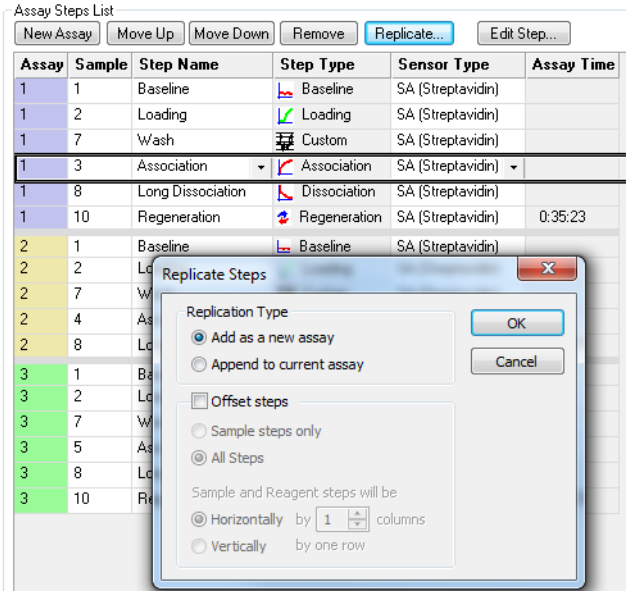


Figure 9-41: Adding a Replicate Assay to a Plate

3. 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 → 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.*

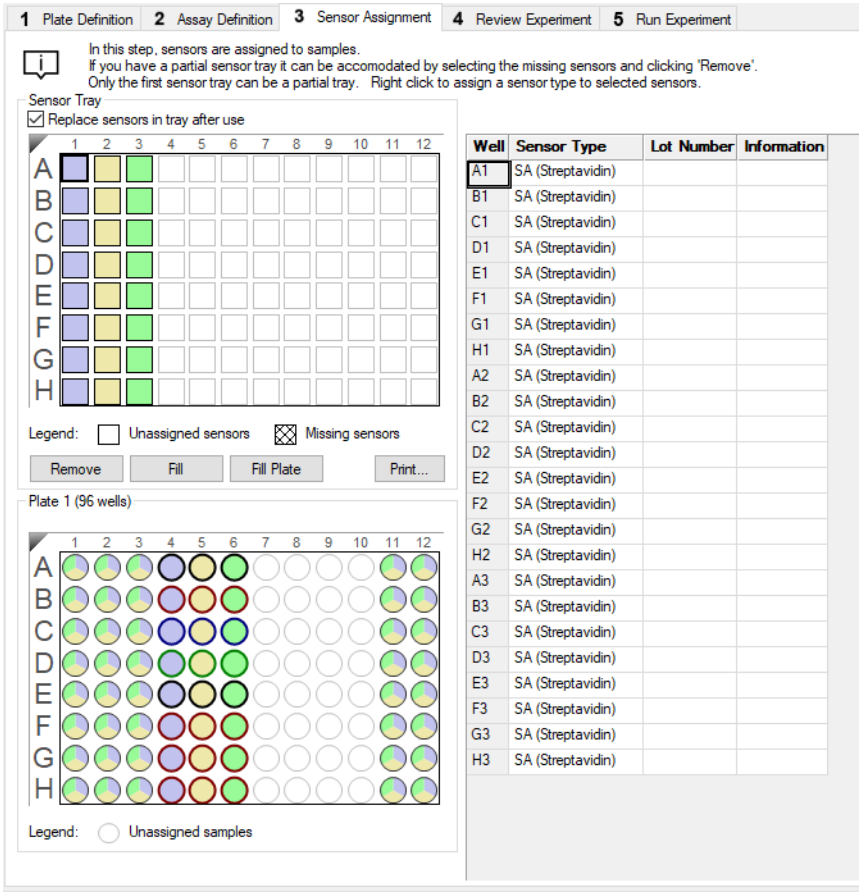


Figure 9-42: Sensor Assignment Window

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).

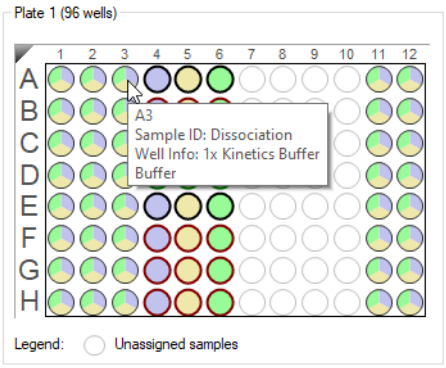


Figure 9-43: Tool Tip of Well Information

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).

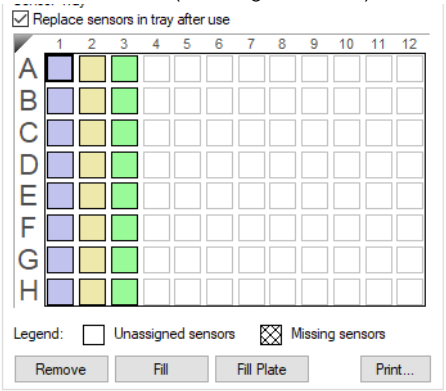


Figure 9-44: Replace Sensors in Tray After Use Check Box

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

Entering Biosensor Information

To enter information about a biosensor:

1. 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.*

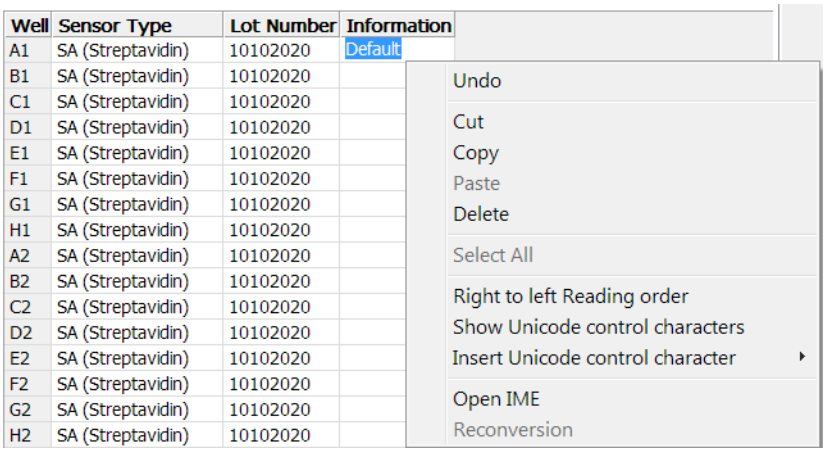


Figure 9-45: Entering or Editing Biosensor Information

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

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.

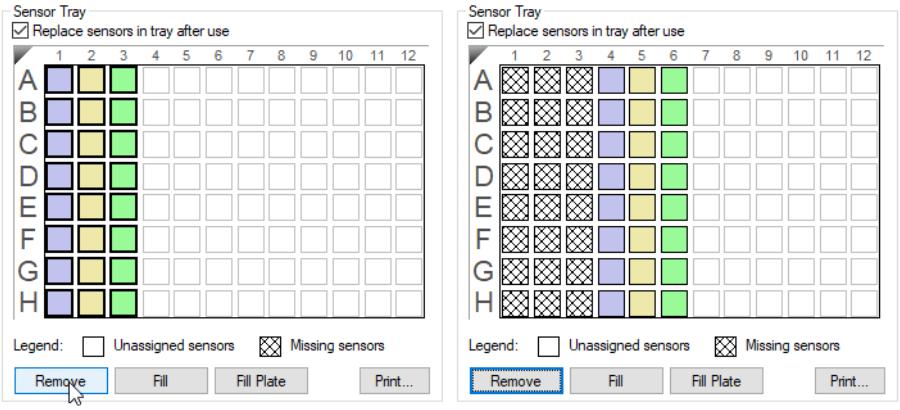


Figure 9-46: Changing Biosensor Location (Method 1)

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).

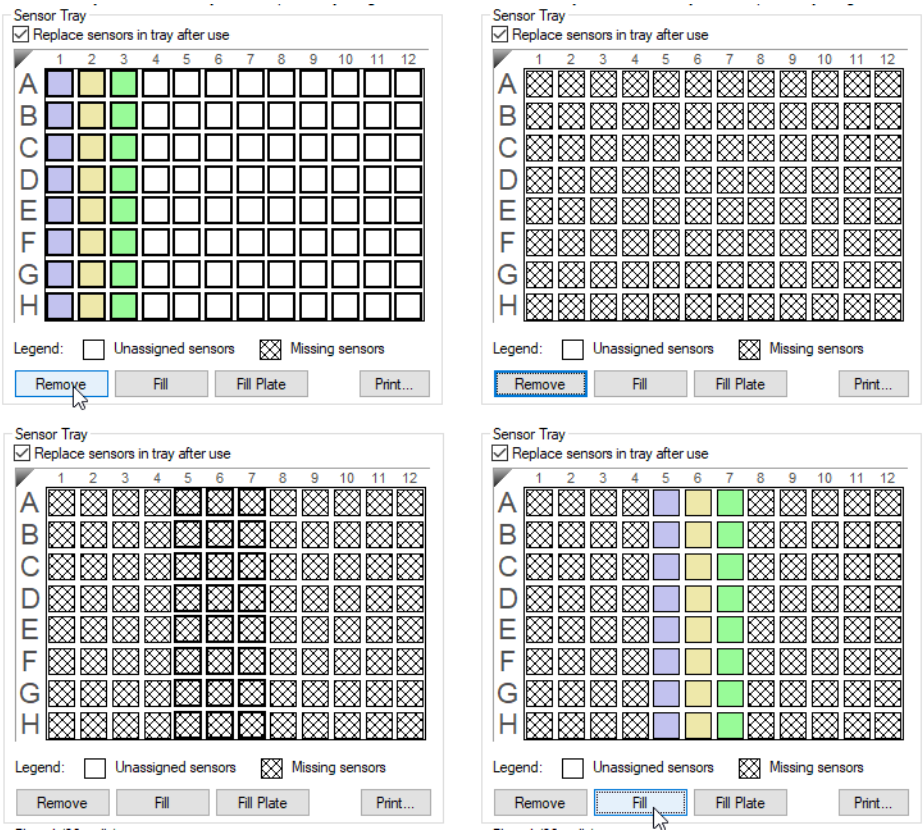


Figure 9-47: Changing Biosensor Location (Method 2)

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 drop-down 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.

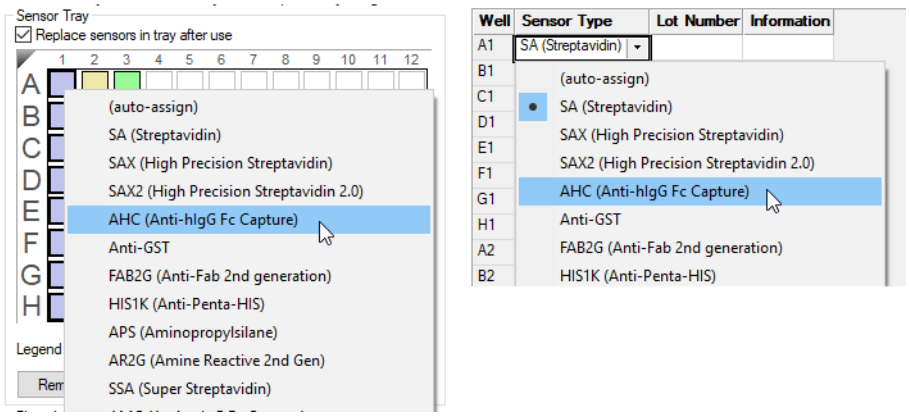


Figure 9-48: Sensor Assignment Window—Changing the Biosensor Type

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).

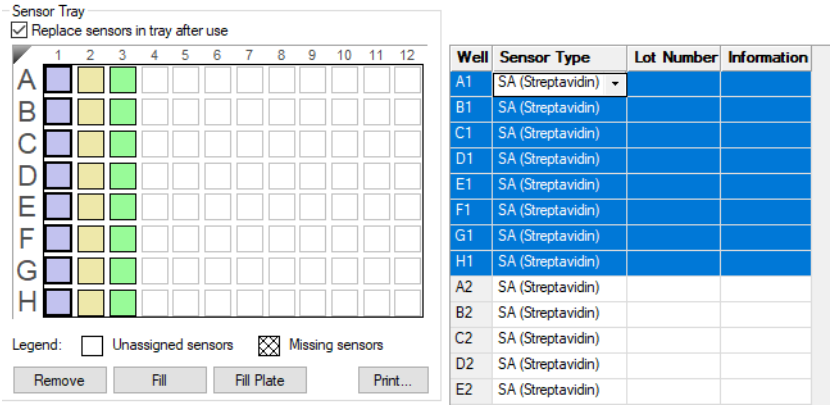


Figure 9-49: Selecting a Sensor Tray Column

2. 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.

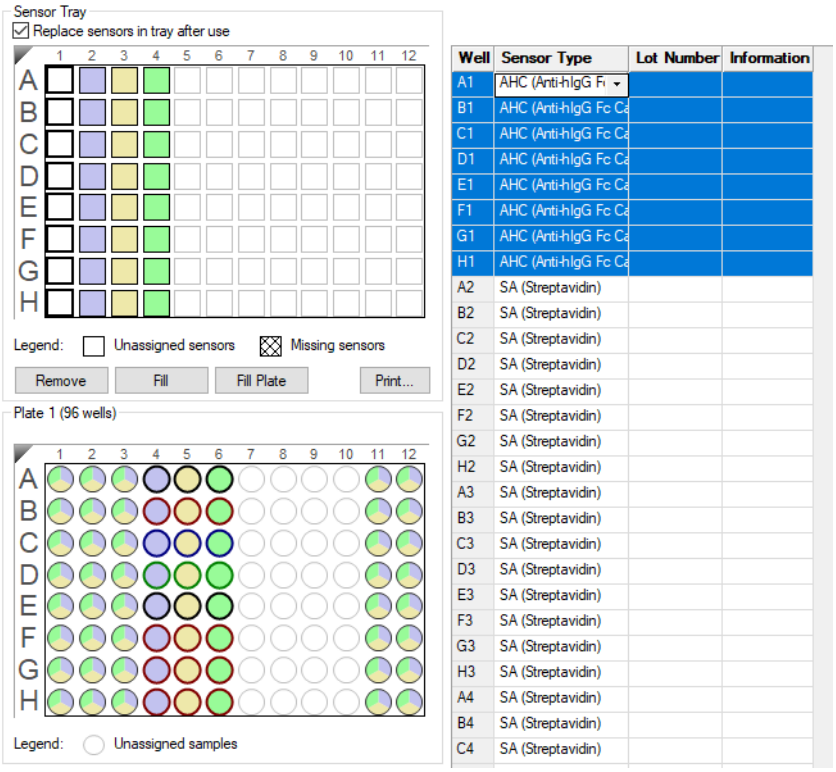


Figure 9-50: Assay Sensor Type Reassignment

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.

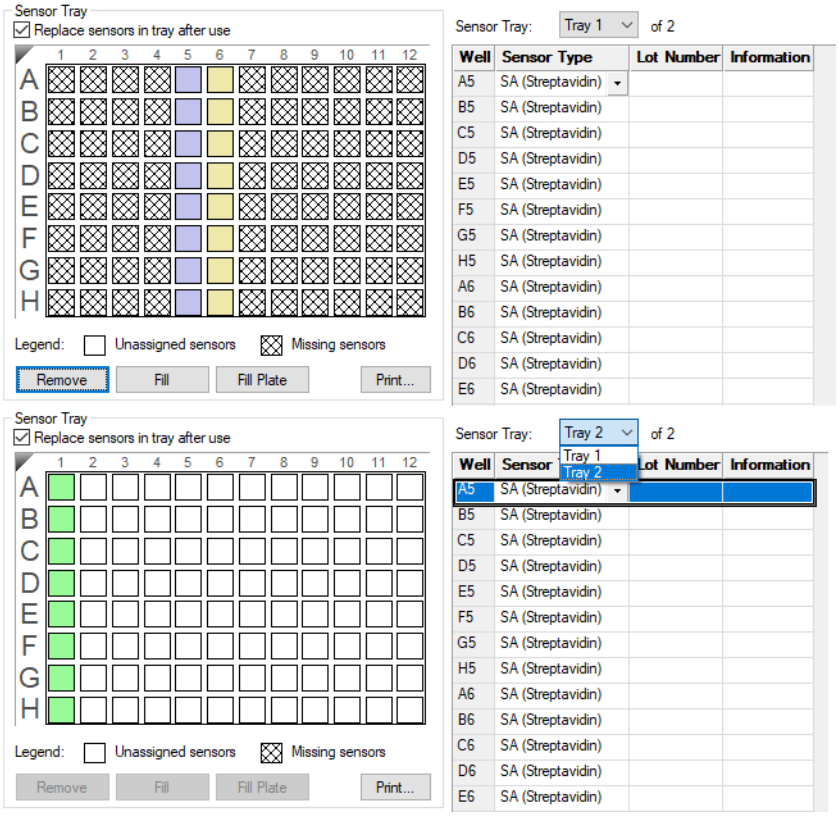


Figure 9-51: Example Experiment Using Two Biosensor Trays

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® 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.*

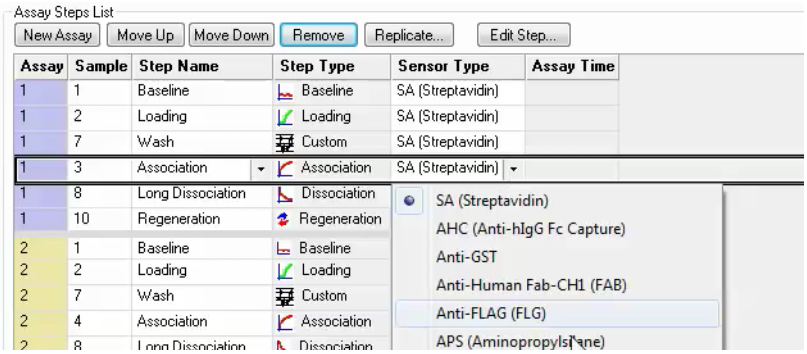


Figure 9-52: Assay Definition Window—Changing the Biosensor Type

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 ← → arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

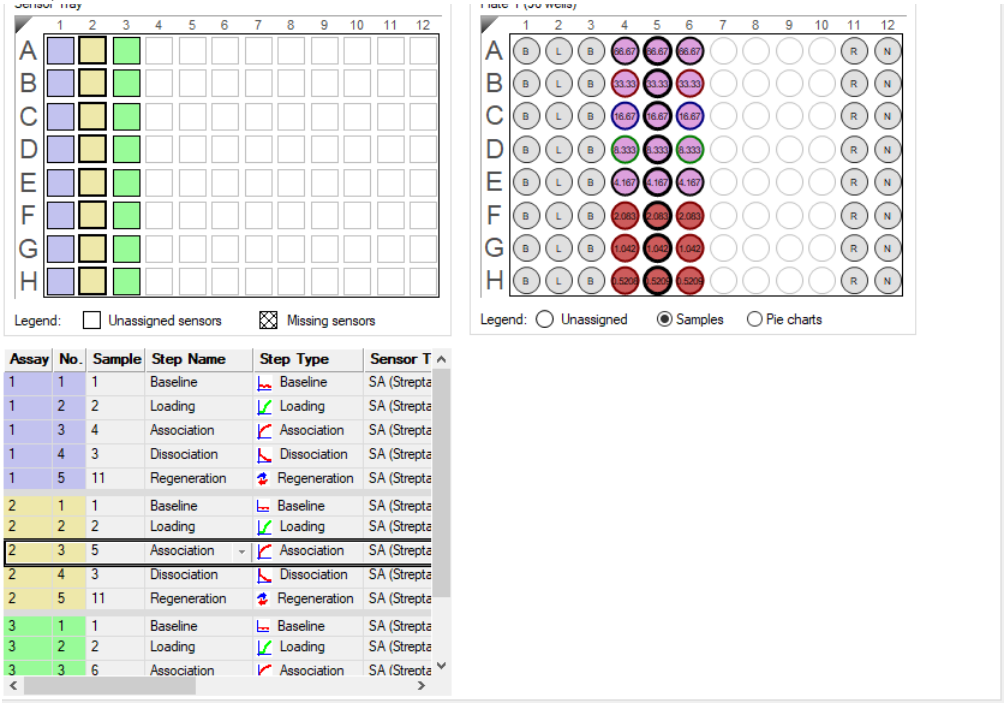


Figure 9-53: Review Experiment Window

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 factory-set location, the software may not function properly.*

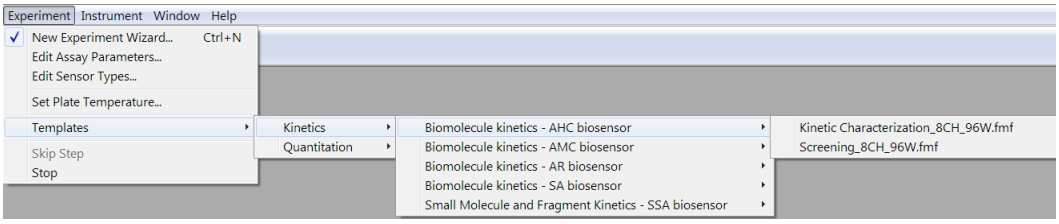


Figure 9-54: Saved Experiments in the Template Folder

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® 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).

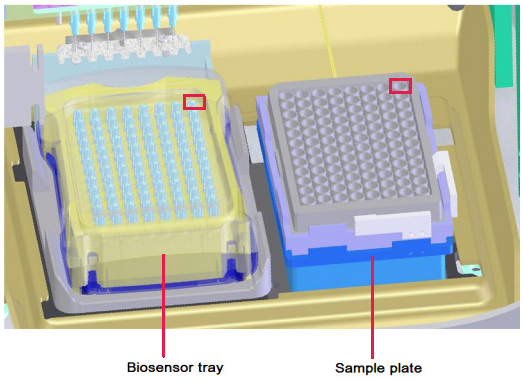


Figure 9-55: Biosensor Stage (left) and Sample Stage (right)

IMPORTANT: *Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.*

4. **Octet® RED96e and Octet®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® R8 instruments). For more information, see "Microplate Evaporation Cover" on page 64.
5. Close the Octet® 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 (→) to access the Run Experiment window (see Figure 9-56).

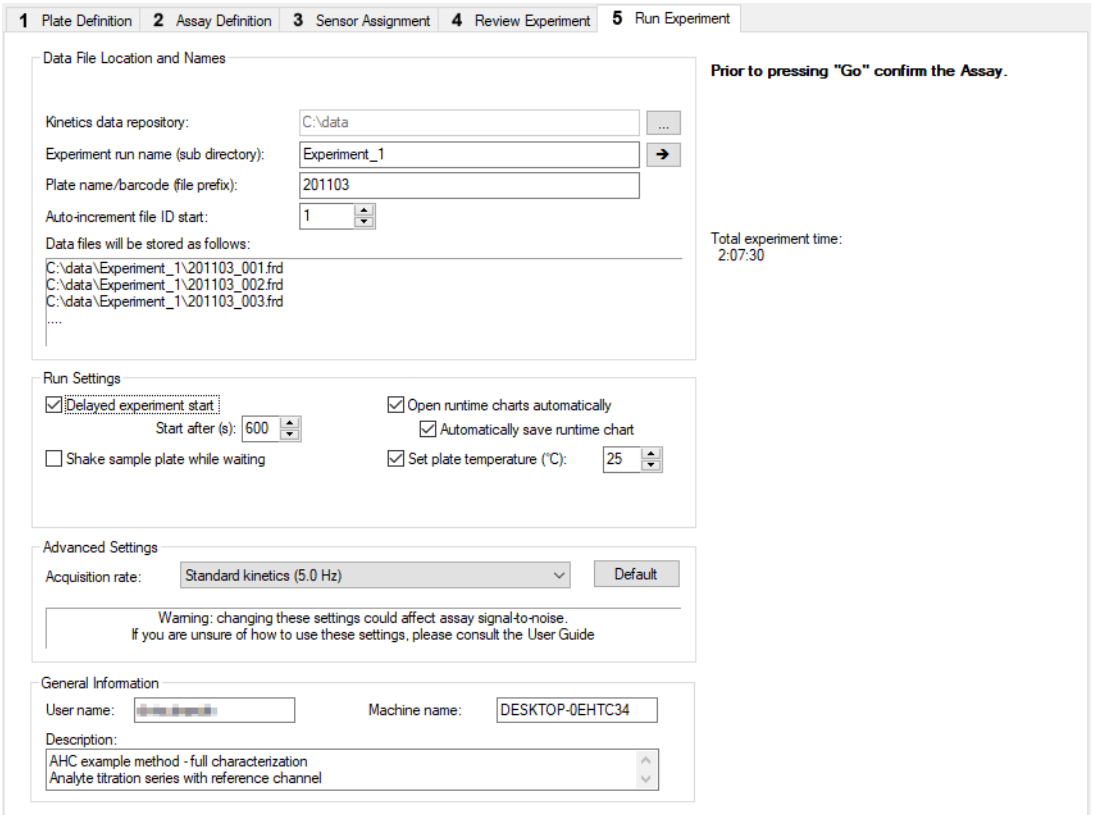


Figure 9-56: Run Experiment Window—Octet RED96

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.*

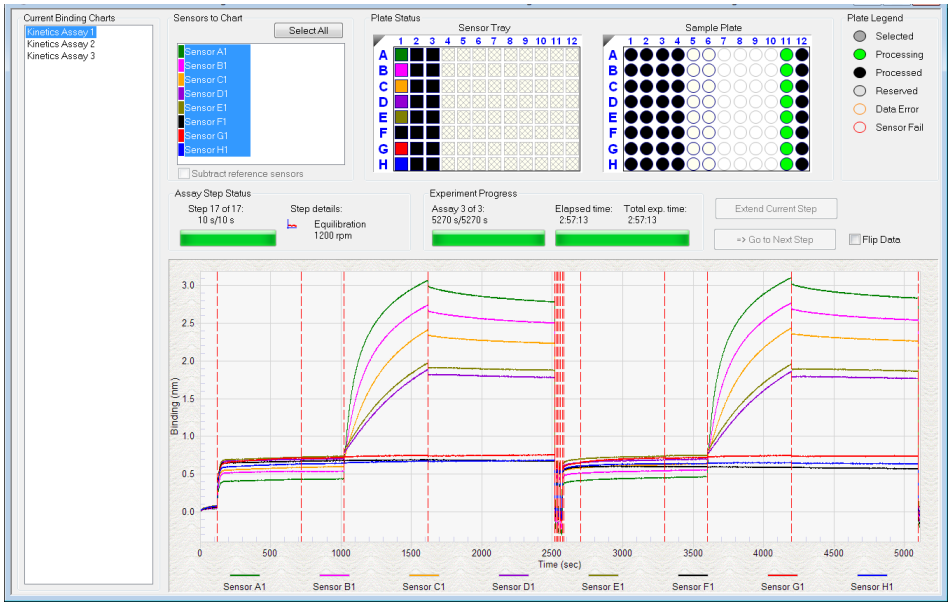


Figure 9-57: Runtime Binding Chart

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.

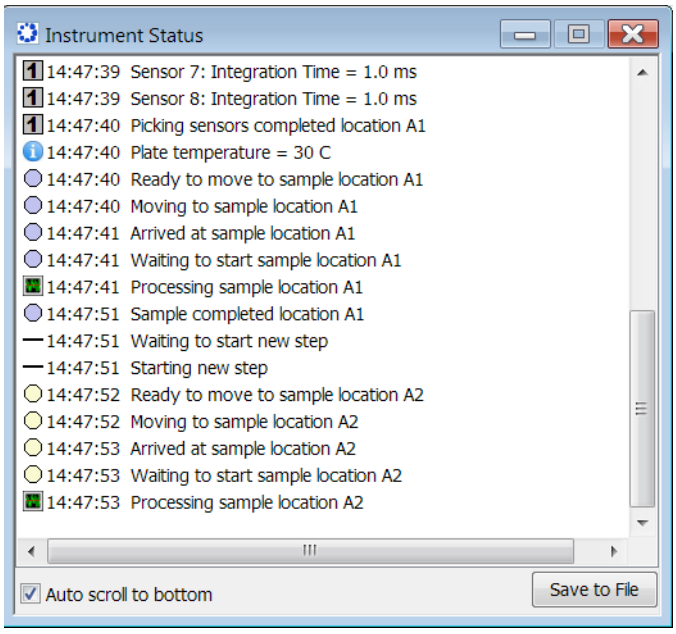


Figure 9-58: Instrument Status Log

WARNING: *Do not open the Octet® 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® 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 repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click **Browse** to select another data location. ***NOTICE:*** *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/barcode	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

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 **GO**.
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 **Runtime Binding Chart** 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 **Runtime Binding Chart**. 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 **File > Options**. The factory set default temperature is 30 °C. ***NOTICE:*** *If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet® 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® 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® QKe	• 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. • 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.
Acquisition rate • Octet® RED96, • Octet® RED96e • Octet® R8	• 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. • Standard kinetics (5 Hz, averaging by 20 - The average of 20 data frames is reported as one data point.
Sensor offset (mm) • Octet® 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 ⊗ 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 color-coded (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® 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.*

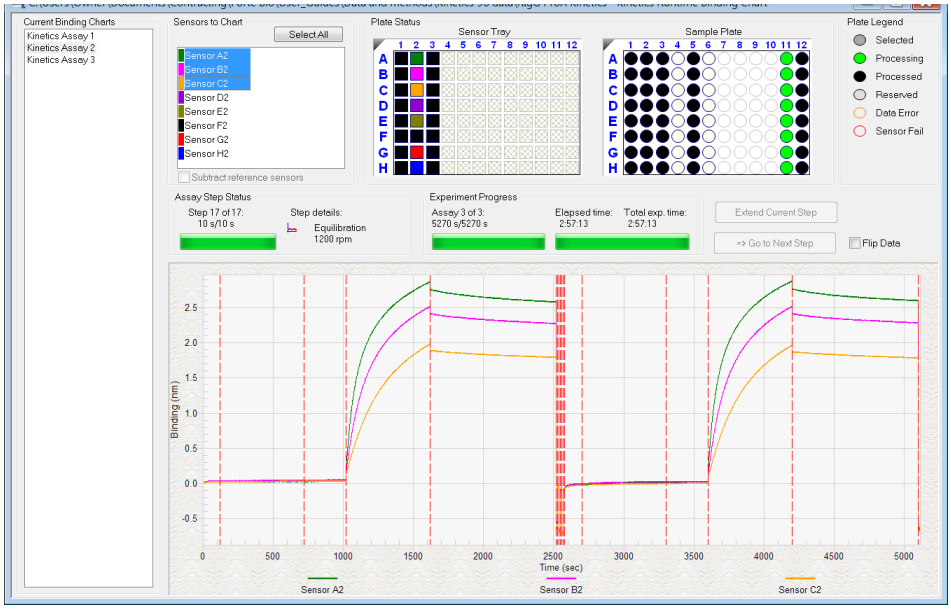


Figure 9-59: Runtime Binding Chart Window

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).

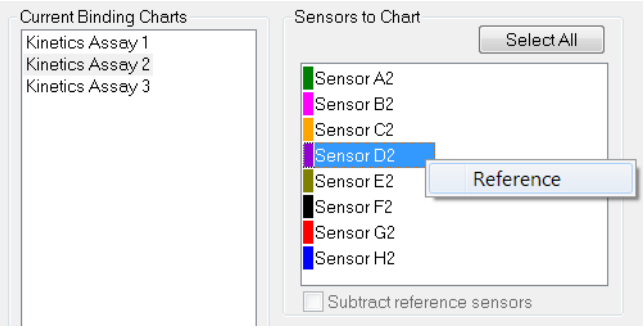


Figure 9-60: Designating a Reference Biosensor in the Runtime Binding Chart

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).

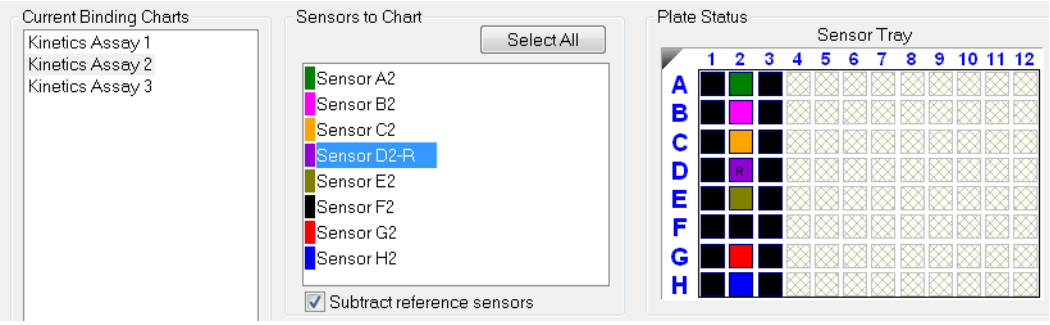


Figure 9-61: Subtract Reference Sensors check box in the Runtime Binding Chart

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.

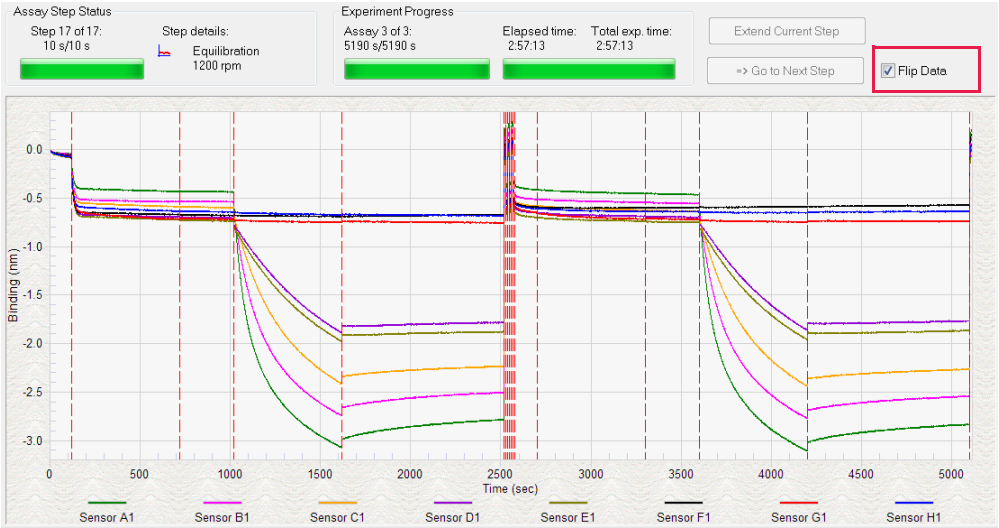


Figure 9-62: Data Inverted Using Flip Data Function

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**.

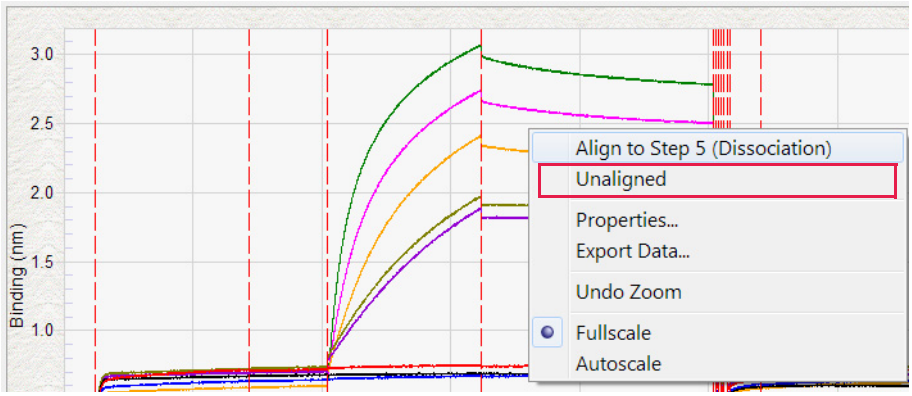


Figure 9-63: Runtime Binding Chart—Aligning the Data to a User-Selected Step

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**.

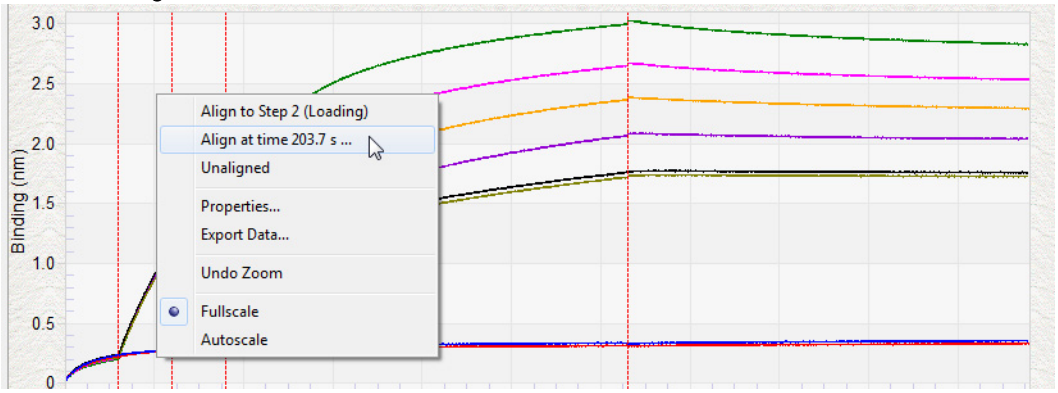


Figure 9-64: Runtime Binding Chart—Aligning the Data to a User-Specified Time

The Align at Time dialog box appears (Figure 9-65).

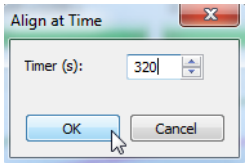


Figure 9-65: Align at Time Dialog Box

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**.

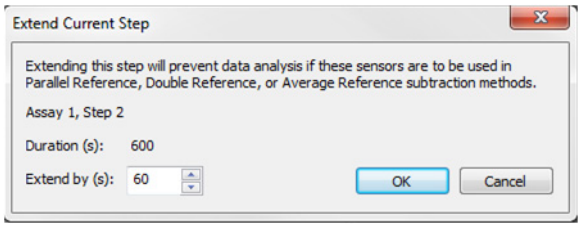


Figure 9-66: Extend Current Step Dialog Box

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

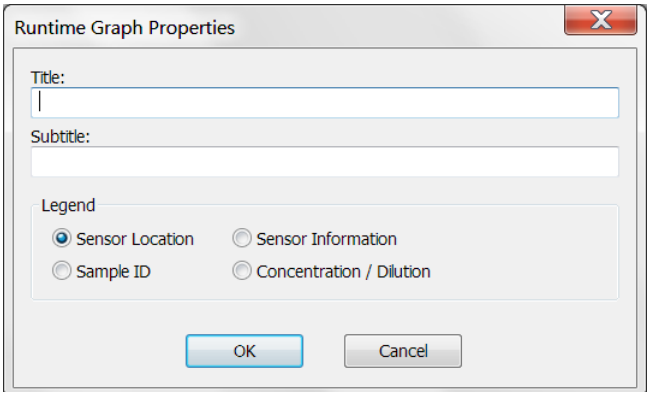


Figure 9-67: Selecting a Runtime Binding Chart Legend

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**.

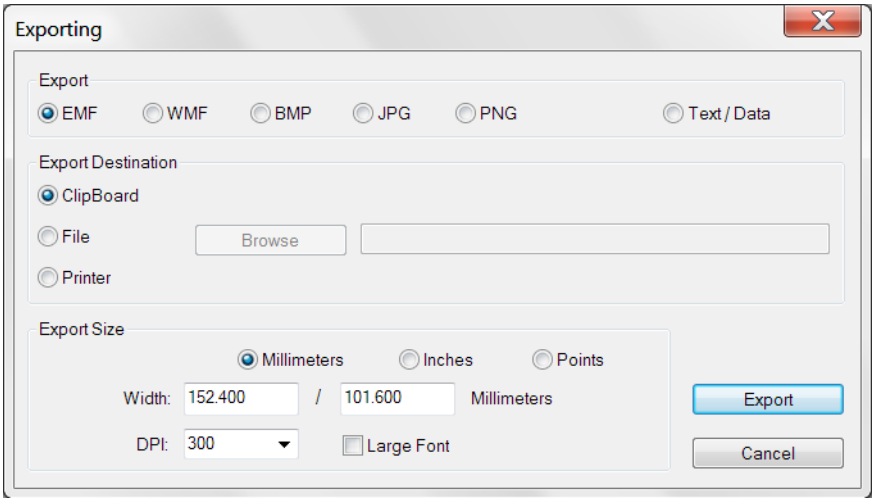


Figure 9-68: Exporting Dialog Box

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	✓		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 biosensor. 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		✓	Clipboard	Copies the chart to the system clip-board
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

Managing Experiment Method Files

After you run an experiment, the Octet® 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® 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 9-10: 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 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® 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® system and the Octet® BLI Discovery software, follow the steps in Table 9-11 to set up and run an epitope binning experiment.

Table 9-11: Octet® BLI Discovery Steps for Epitope Binning Assays

Octet Software	Functions
Octet® 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). ***NOTICE:*** *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** 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.

Tab 1 (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:

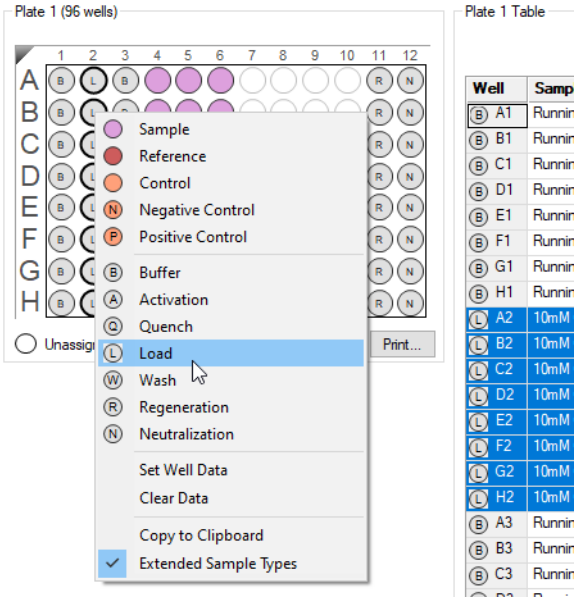


Figure 9-69: Designating Well Types

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:

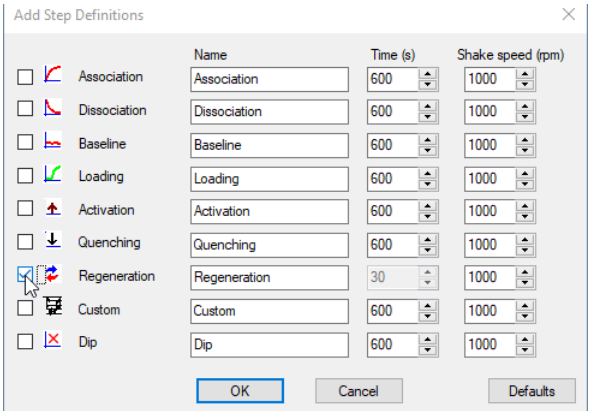


Figure 9-70: Add Step Definition Box

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:

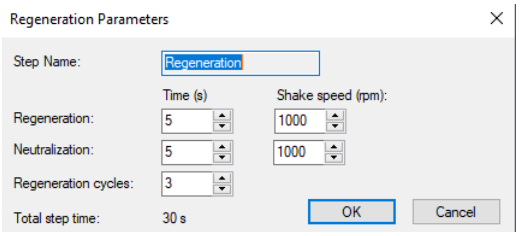


Figure 9-71: Regeneration Parameters Box

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:

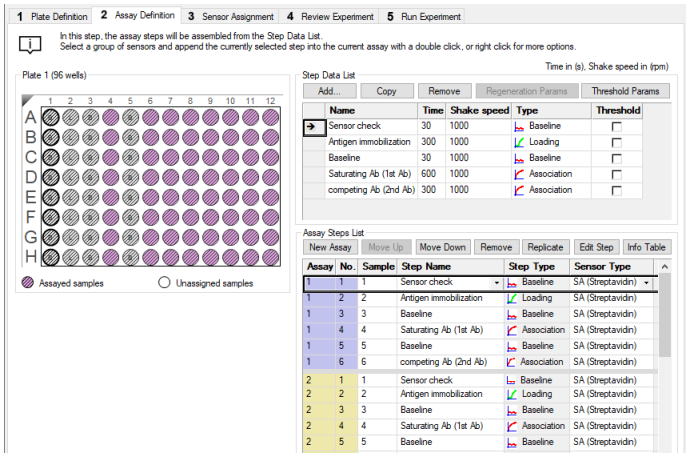


Figure 9-72: Assay Steps List

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:

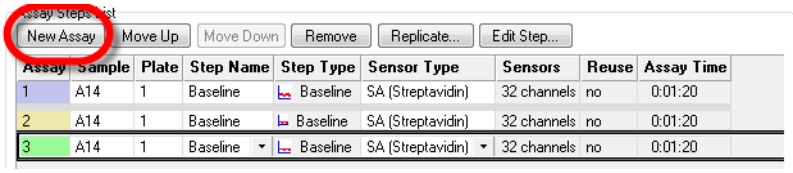


Figure 9-73: New Assay Button

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:

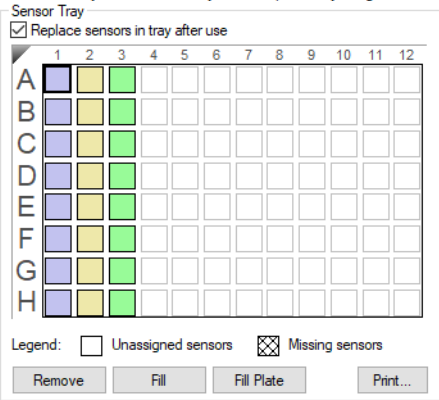


Figure 9-74: Replace Sensors in Tray After Use Check Box

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:

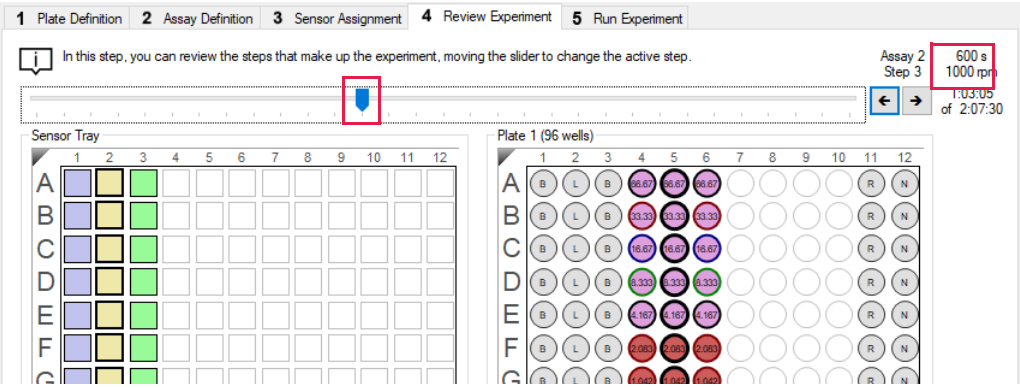


Figure 9-75: Navigating the Review Experiment Tab

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® 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).

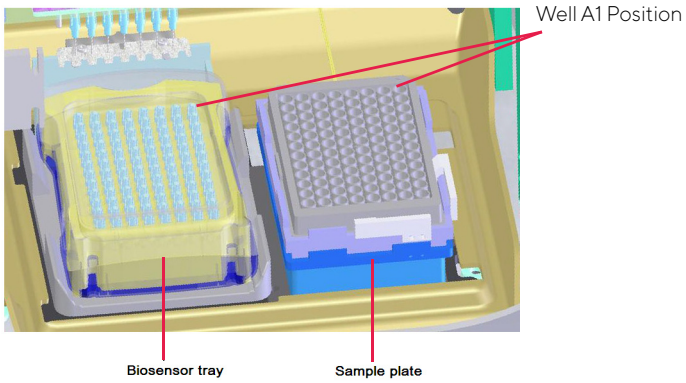


Figure 9-76: Biosensor Stage (left) and Sample Stage (right)

IMPORTANT: *Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.*

4. **Octet® RED96e and Octet® 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® RED96e and Octet®R8 instruments). For more information, see "Microplate Evaporation Cover" on page 64.
5. Close the Octet® 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:

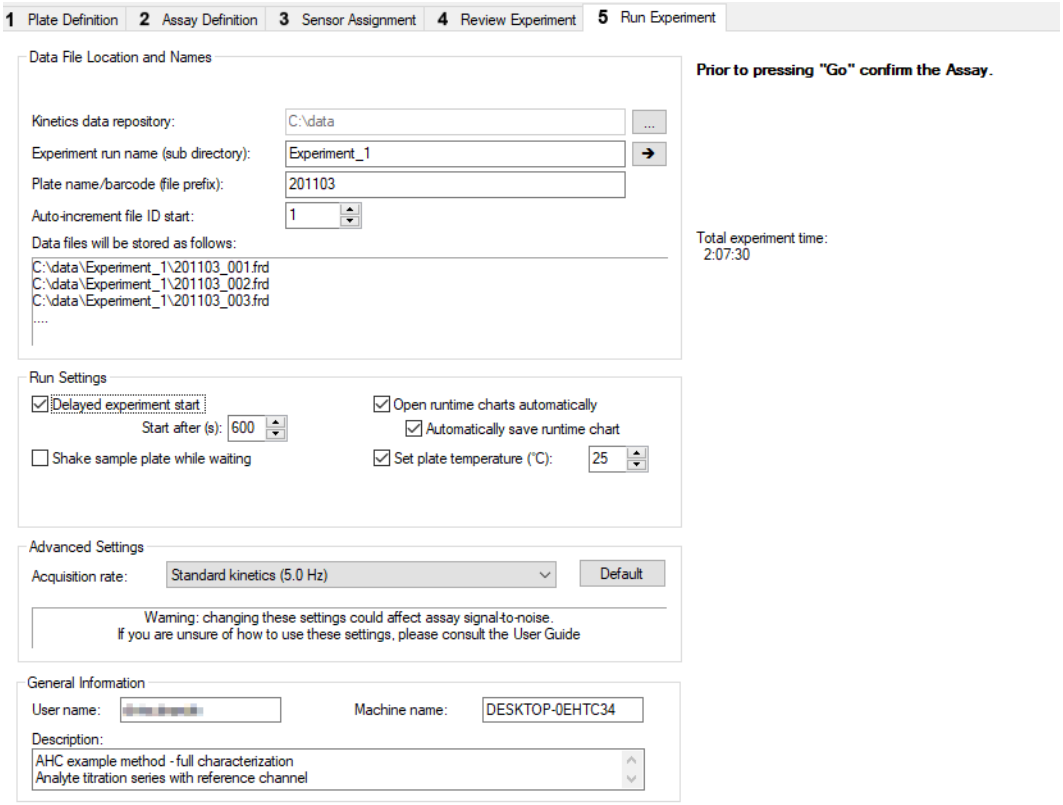


Figure 9-77: GO Button

Chapter 10:

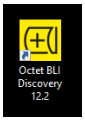
Kinetics Experiments: Octet QK® 384, Octet RH® 96 and Octet RH® 16

Introduction

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet® system hardware and the Octet® 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® BLI Discovery software versions 8.0 and higher.*

Table 10-1: Setting Up and Analyzing a Kinetic Experiment

Octet® Software		Step	See
BLI Discovery 	1.	Select a kinetics experiment in the **Experiment Wizard** or open a method file (.fmf).	"Starting a Basic Kinetics Experiment: Octet® RH16 and Octet® 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® Analysis Studio Software User Guide*
	8.	Analyze the data.	

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® 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® RH16 and Octet® 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® 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 , 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( ). The **Basic Kinetics Experiment** window displays (Figure 10-1, right).

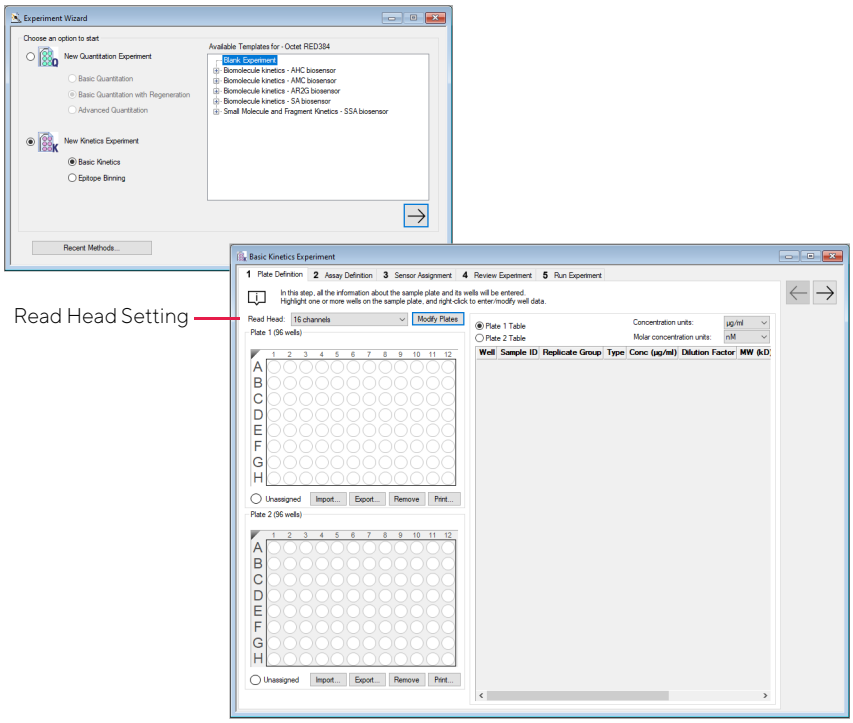


Figure 10-1: Starting a Kinetics Experiment with the Experiment Wizard

Defining the Sample Plate

NOTICE: *Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet® BLI Discovery software versions 8.0 and higher (Figure 10-2).*

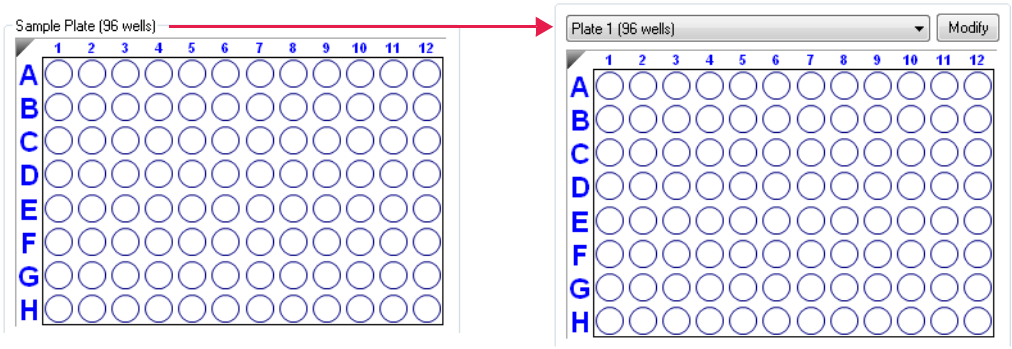


Figure 10-2: Sample Plate Renamed Plate 1 in Software Versions 8.0 and Higher

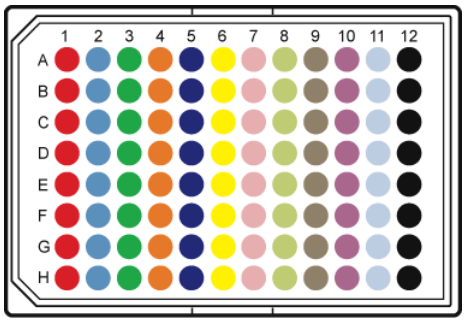
The steps to define a sample plate include:

	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

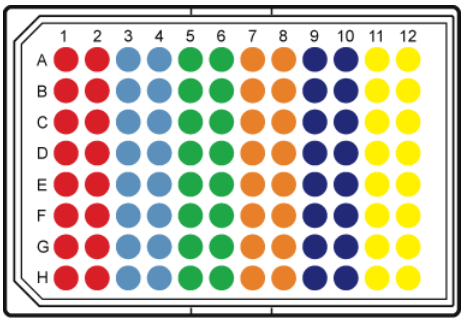
The Octet® read head contains the collection optics. If the read head is set to 8 channels, one column of 8 biosensors interrogate 8 plate wells. If the read head is set to 16 channels, two columns of biosensors interrogate 16 wells (see Figure 10-3). The read head configuration and the plate format (96 or 384 wells) determine the plate layout (see example Figure 10-4).

8 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

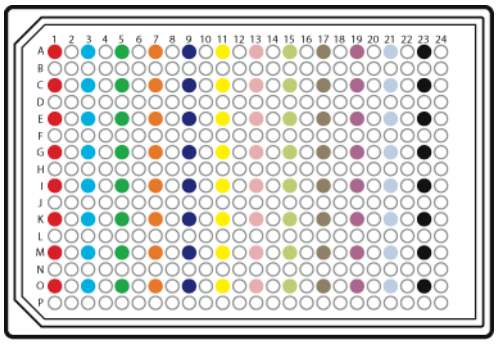
16 Channel Read Head



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 o

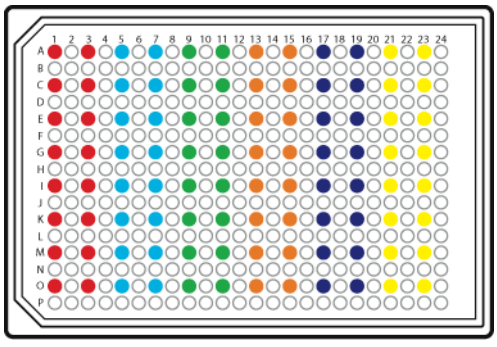
Figure 10-3: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

8 Channel Read Head



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 10-4: Color-Coded Wells Display 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.*

Changing the Sample Plate Format

NOTICE:

- Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet® 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.

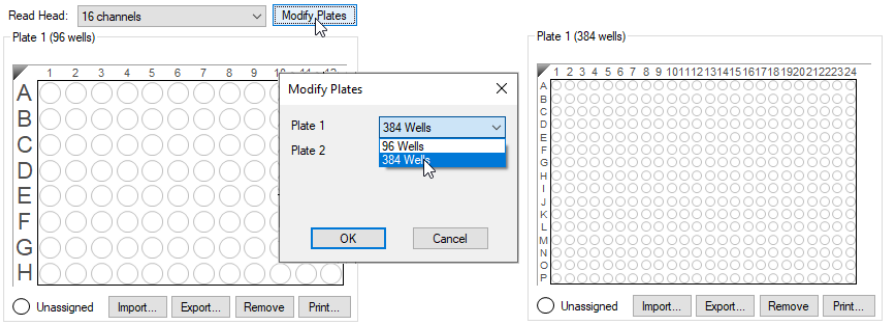


Figure 10-5: Changing the Sample Plate Format

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® 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

Icon	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 • Negative Control: A control sample known not to contain analyte
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® 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 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 (Figure 10-6, center).
- Click a well or draw a box around a group of wells(Figure 10-6, right).

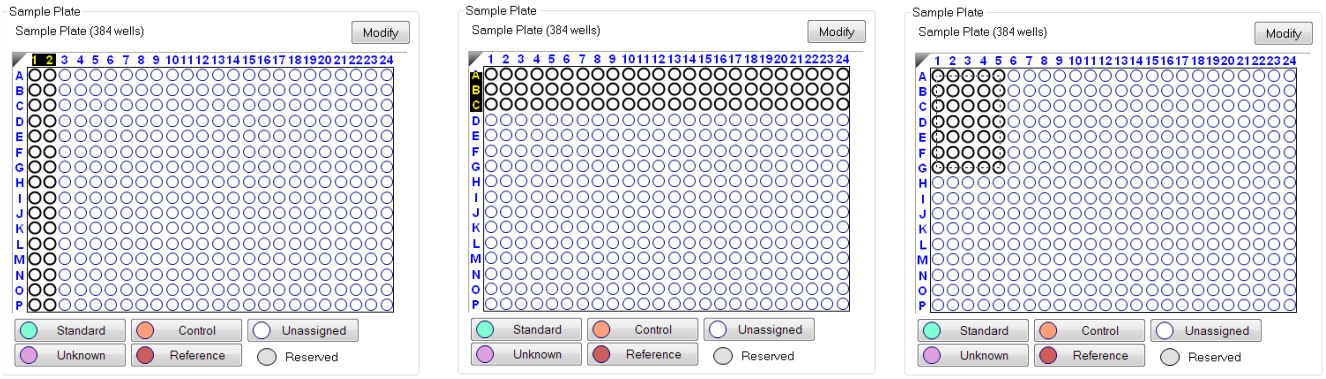


Figure 10-6: Selecting Wells in the Sample Plate Map

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® 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).

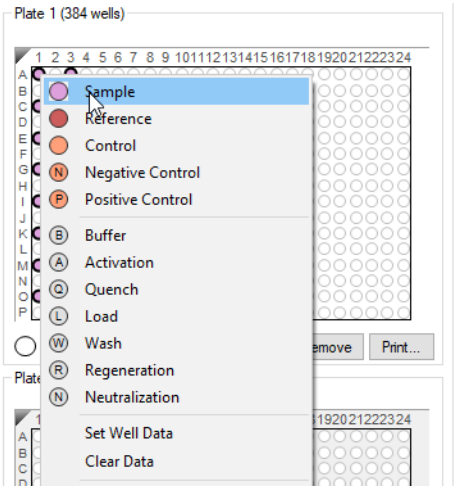


Figure 10-7: Designating a Well Type in the Plate Definition Window

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).

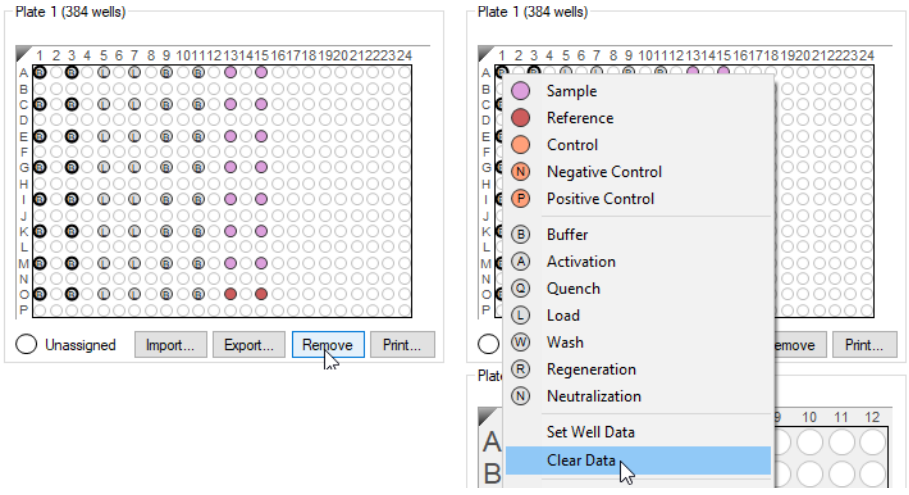


Figure 10-8: Clearing Sample Data from a Sample Plate

Entering Sample Information

NOTICE: *You must specify sample (analyte) concentration and molecular weight; otherwise, the Octet® BLI Discovery software cannot 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.*

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).

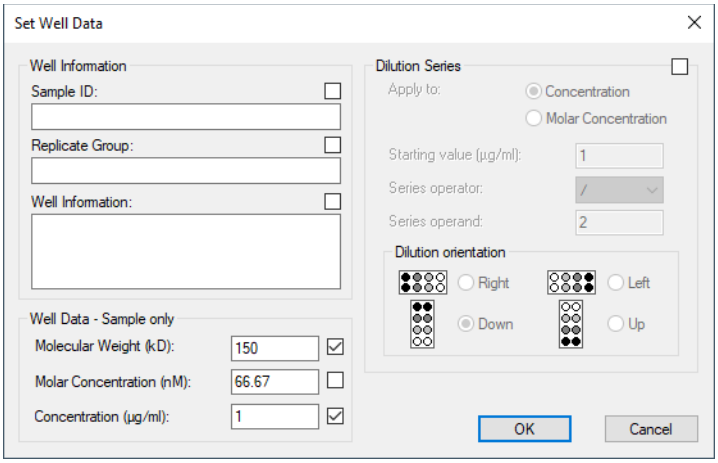
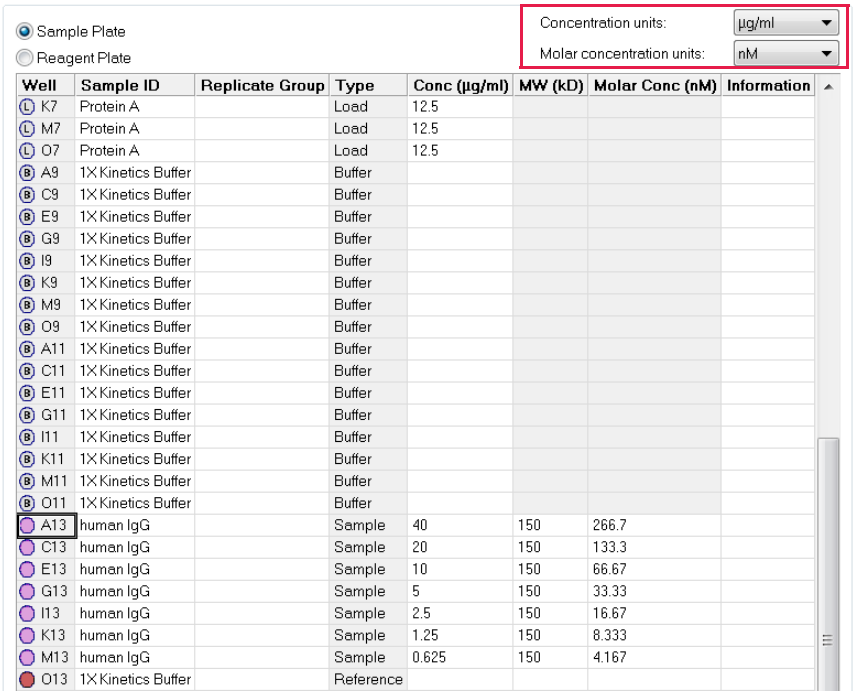


Figure 10-9: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

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.



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
K7	Protein A		Load	12.5			
M7	Protein A		Load	12.5			
O7	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				
I9	1X Kinetics Buffer		Buffer				
K9	1X Kinetics Buffer		Buffer				
M9	1X Kinetics Buffer		Buffer				
O9	1X Kinetics Buffer		Buffer				
A11	1X Kinetics Buffer		Buffer				
C11	1X Kinetics Buffer		Buffer				
E11	1X Kinetics Buffer		Buffer				
G11	1X Kinetics Buffer		Buffer				
I11	1X Kinetics Buffer		Buffer				
K11	1X Kinetics Buffer		Buffer				
M11	1X Kinetics Buffer		Buffer				
O11	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	
I13	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	
O13	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.

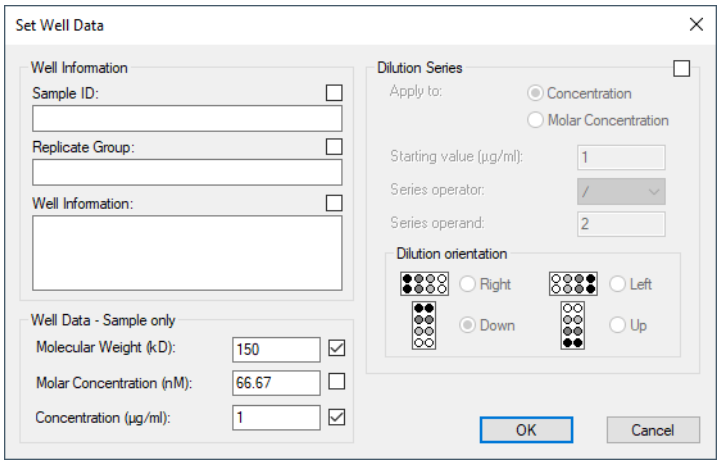


Figure 10-11: Sample Plate Map—Assigning Sample Concentrations by Value

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:

1. 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.

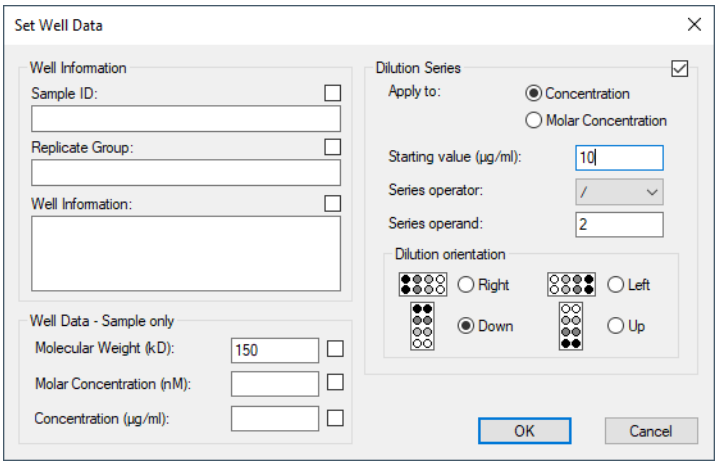


Figure 10-12: Sample Plate Map—Assigning Sample Concentrations Using Dilution Series

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 **Run-time 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**.

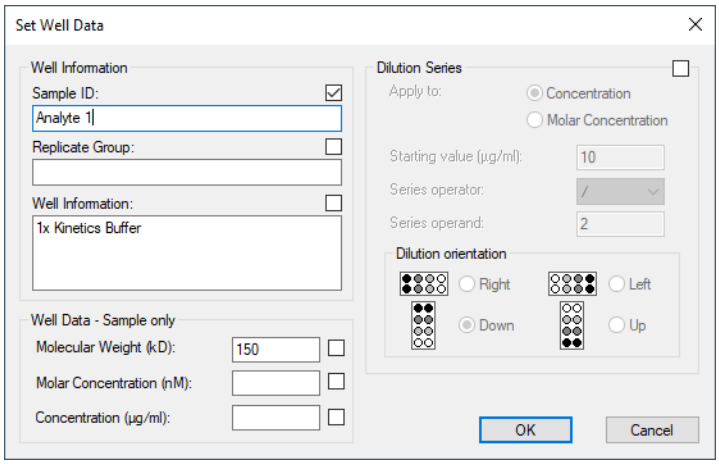


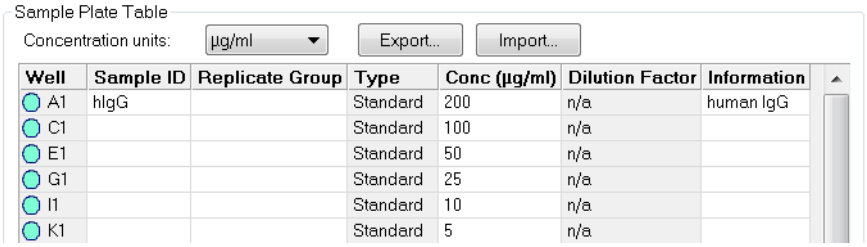
Figure 10-14: Add Sample Annotations from the Sample Plate Map

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 10-15).

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



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hIgG		Standard	200	n/a	human IgG
C1			Standard	100	n/a	
E1			Standard	50	n/a	
G1			Standard	25	n/a	
I1			Standard	10	n/a	
K1			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
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	x		D3	SA (Streptavidin)		4	D3

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**.

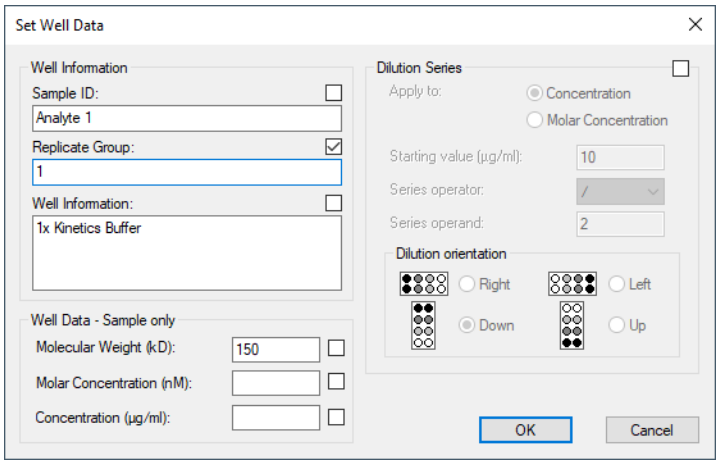


Figure 10-17: Add Replicate Group from the Sample Plate Map

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).

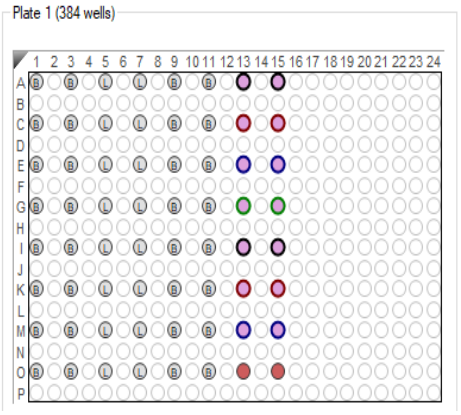
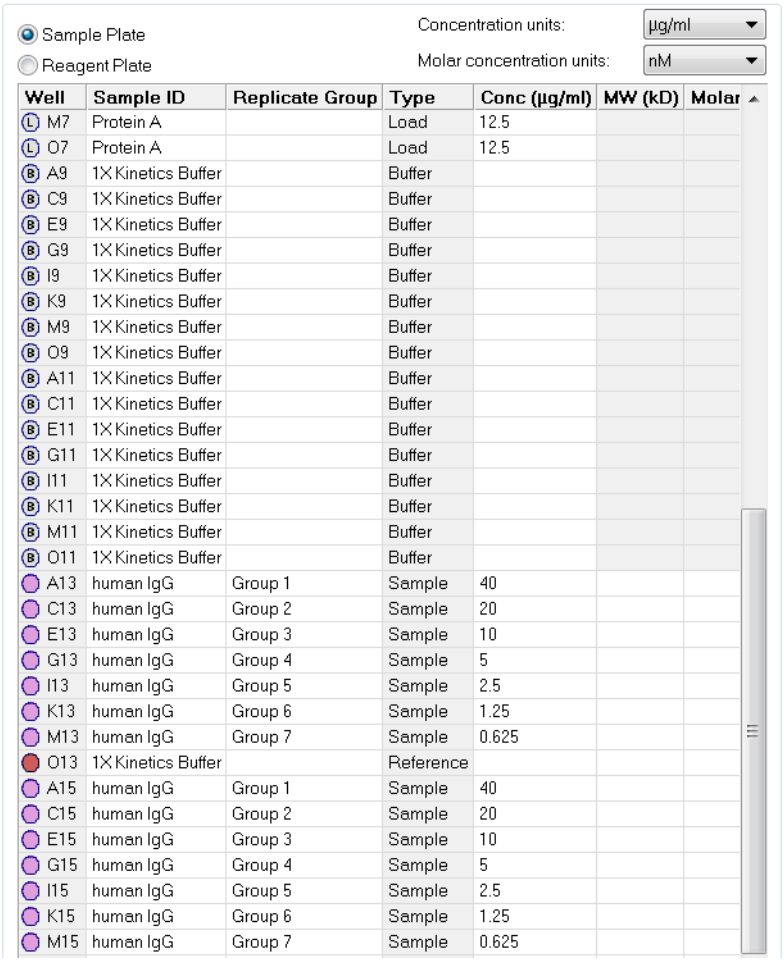


Figure 10-18: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 10-19)



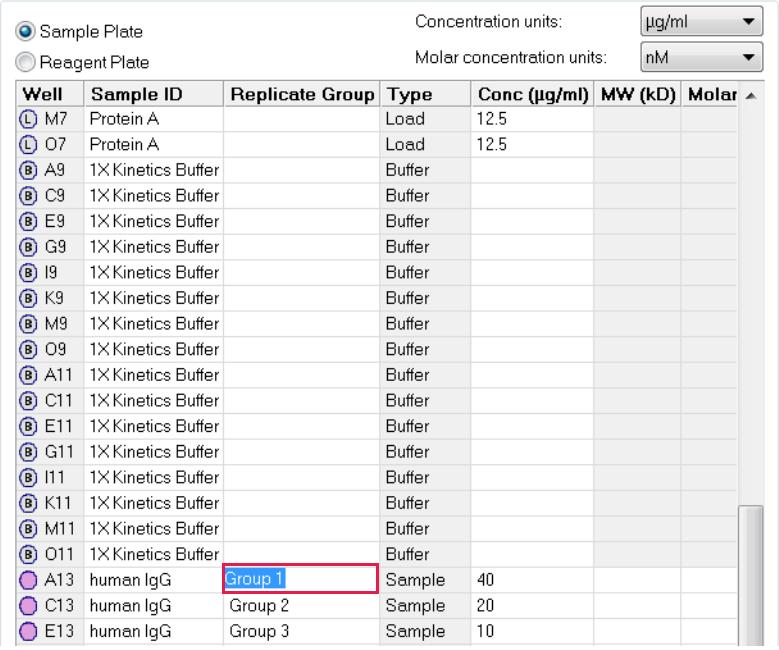
Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar
M7	Protein A		Load	12.5		
O7	Protein A		Load	12.5		
A9	1X Kinetics Buffer		Buffer			
C9	1X Kinetics Buffer		Buffer			
E9	1X Kinetics Buffer		Buffer			
G9	1X Kinetics Buffer		Buffer			
I9	1X Kinetics Buffer		Buffer			
K9	1X Kinetics Buffer		Buffer			
M9	1X Kinetics Buffer		Buffer			
O9	1X Kinetics Buffer		Buffer			
A11	1X Kinetics Buffer		Buffer			
C11	1X Kinetics Buffer		Buffer			
E11	1X Kinetics Buffer		Buffer			
G11	1X Kinetics Buffer		Buffer			
I11	1X Kinetics Buffer		Buffer			
K11	1X Kinetics Buffer		Buffer			
M11	1X Kinetics Buffer		Buffer			
O11	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		
I13	human IgG	Group 5	Sample	2.5		
K13	human IgG	Group 6	Sample	1.25		
M13	human IgG	Group 7	Sample	0.625		
O13	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		
I15	human IgG	Group 5	Sample	2.5		
K15	human IgG	Group 6	Sample	1.25		
M15	human IgG	Group 7	Sample	0.625		

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).



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar
M7	Protein A		Load	12.5		
O7	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			
I9	1X Kinetics Buffer		Buffer			
K9	1X Kinetics Buffer		Buffer			
M9	1X Kinetics Buffer		Buffer			
O9	1X Kinetics Buffer		Buffer			
A11	1X Kinetics Buffer		Buffer			
C11	1X Kinetics Buffer		Buffer			
E11	1X Kinetics Buffer		Buffer			
G11	1X Kinetics Buffer		Buffer			
I11	1X Kinetics Buffer		Buffer			
K11	1X Kinetics Buffer		Buffer			
M11	1X Kinetics Buffer		Buffer			
O11	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 group2 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).

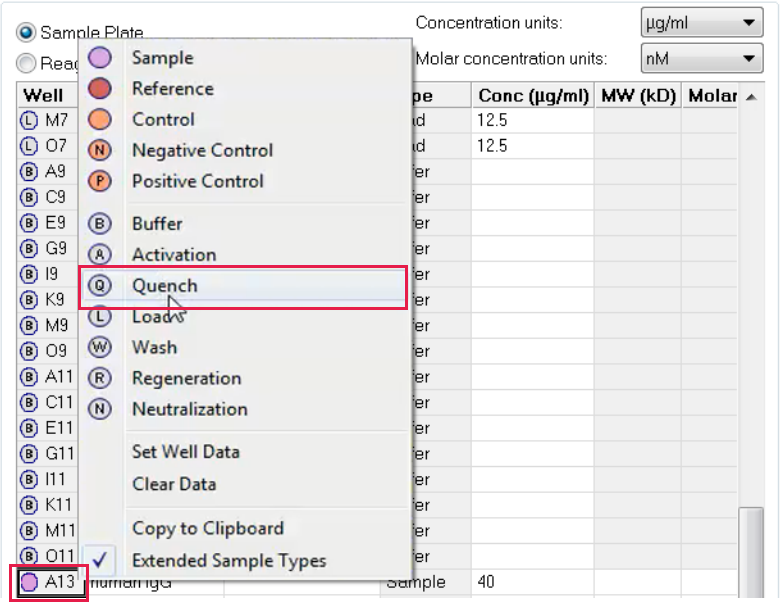


Figure 10-21: Sample Plate Table—Well Designation

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).

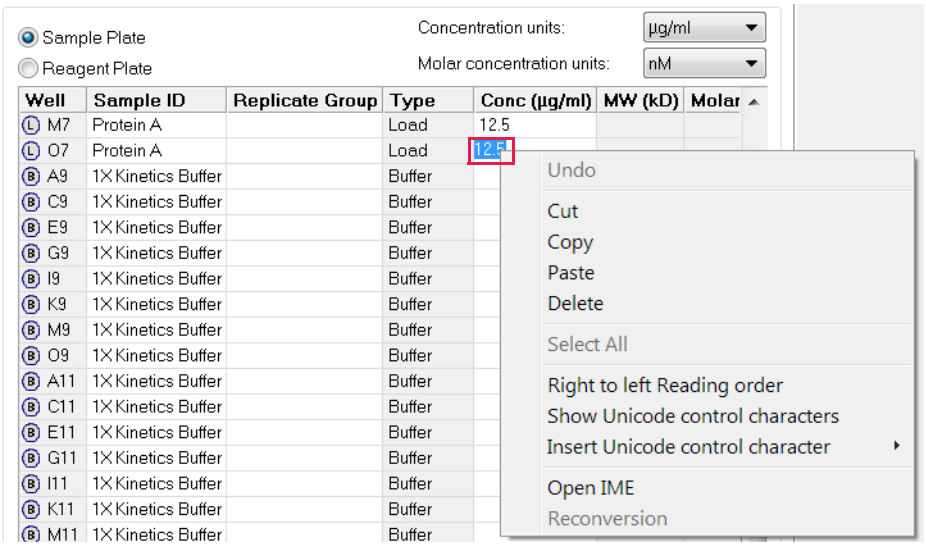


Figure 10-22: Sample Plate Table—Editing Sample Data

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® 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).

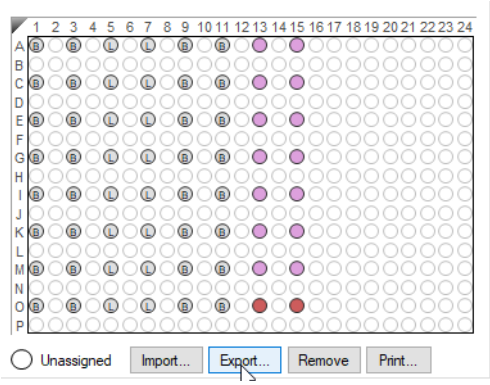


Figure 10-23: Sample Plate Map — Export Button

2. In the **Export Plate Definition** window (see Figure 10-24), select a folder, enter a name for the plate (.csv), and click **Save**.

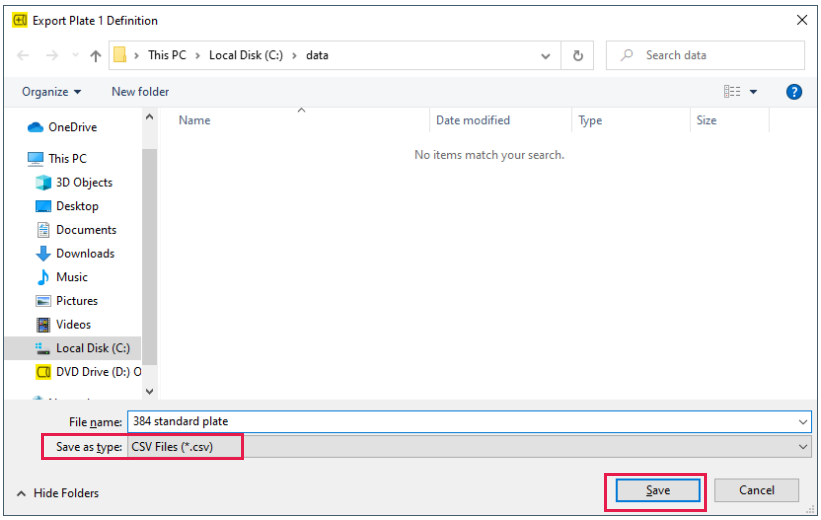


Figure 10-24: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the Plate Definition window (see Figure 10-23: on page 430), click **Import**.

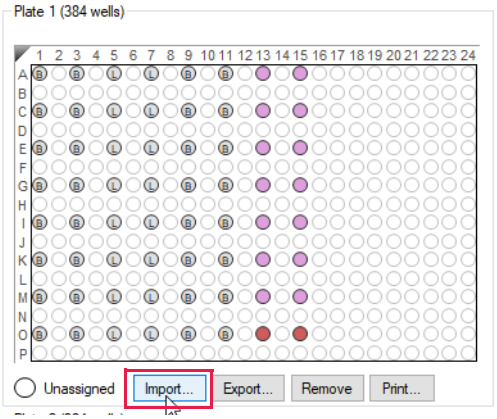


Figure 10-25: Sample Plate Map– **Import** Button

2. In the **Import Plate Definition** window (see Figure 10-26), select the plate definition (.csv), and click **Open**.

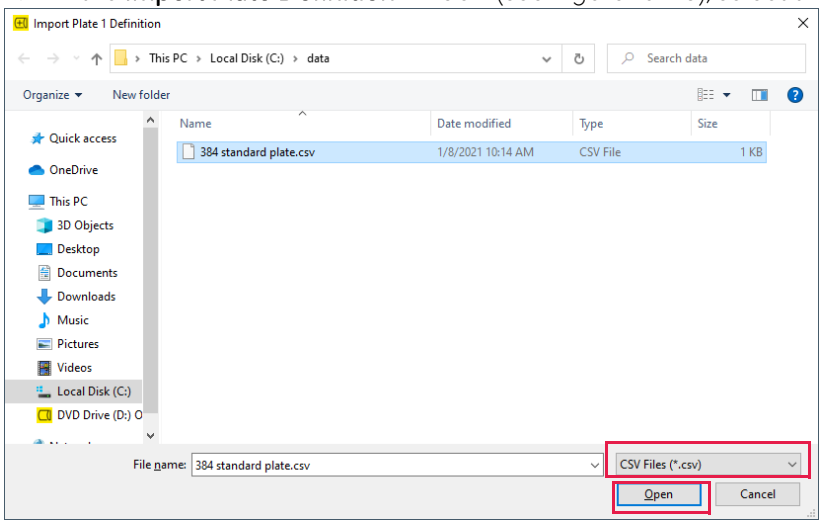


Figure 10-26: Import Plate Definition Window

NOTICE: *You can also create a .csv file for import. Figure 10-27 shows the appropriate column information layout.*

	A	B	C	D	E	F	G	H
1	PlateWells	384						
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information
3	A1	1X Kinetics Buffer		Buffer				
4	C1	1X Kinetics Buffer		Buffer				
5	E1	1X Kinetics Buffer		Buffer				
6	G1	1X Kinetics Buffer		Buffer				
7	I1	1X Kinetics Buffer		Buffer				
8	K1	1X Kinetics Buffer		Buffer				
9	M1	1X Kinetics Buffer		Buffer				
10	O1	1X Kinetics Buffer		Buffer				
11	A3	1X Kinetics Buffer		Buffer				
12	C3	1X Kinetics Buffer		Buffer				
13	E3	1X Kinetics Buffer		Buffer				
14	G3	1X Kinetics Buffer		Buffer				
15	I3	1X Kinetics Buffer		Buffer				
16	K3	1X Kinetics Buffer		Buffer				
17	M3	1X Kinetics Buffer		Buffer				
18	O3	1X Kinetics Buffer		Buffer				
19	A5	Protein A		Load	12.5			
20	C5	Protein A		Load	12.5			

384 K method 4 export

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**.

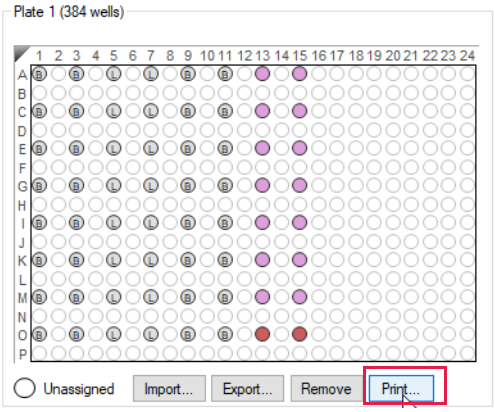


Figure 10-28: Sample Plate/Plate 1 Print Button

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® BLI Discovery software versions 8.0 and higher (Figure 10-29).*

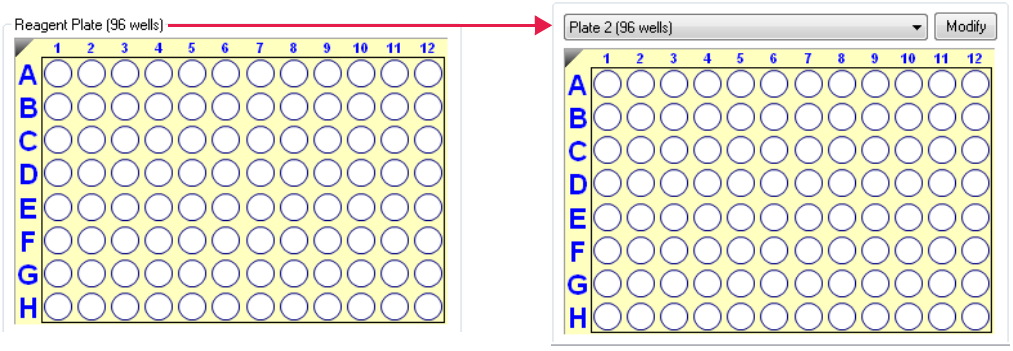


Figure 10-29: Reagent Plate Renamed Plate 2 in Software Versions 8.0 and Higher

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).

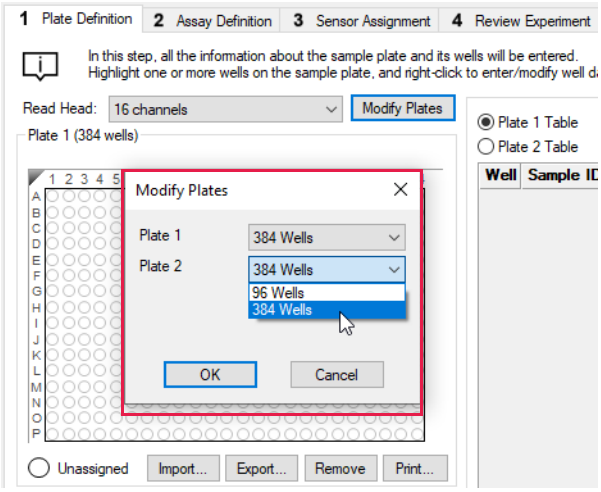


Figure 10-30: Modifying the Reagent Plate

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**.
5. 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.

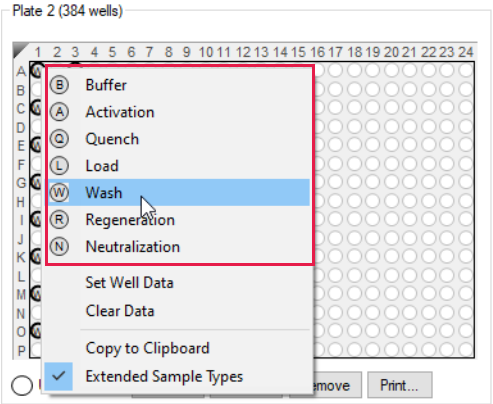


Figure 10-31: Defining Wells in the Reagent Plate

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**.

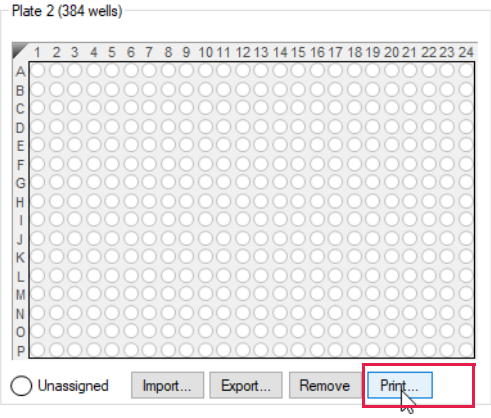


Figure 10-32: Reagent Plate/Plate 2 Print Button

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 k_{obs}. 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 baseline step directly precedes an association step, perform the baseline step at 1,000 rpm. ***IMPORTANT:*** *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. ***NOTICE:*** *This step may be performed offline (outside the Octet® 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 → 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).

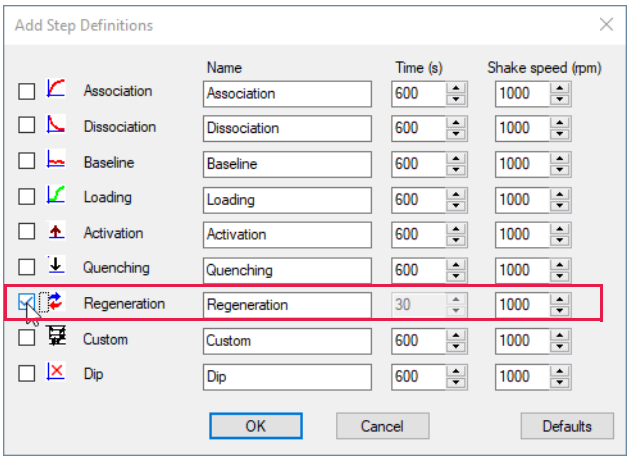


Figure 10-33: Creating an Assay Step Type

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).

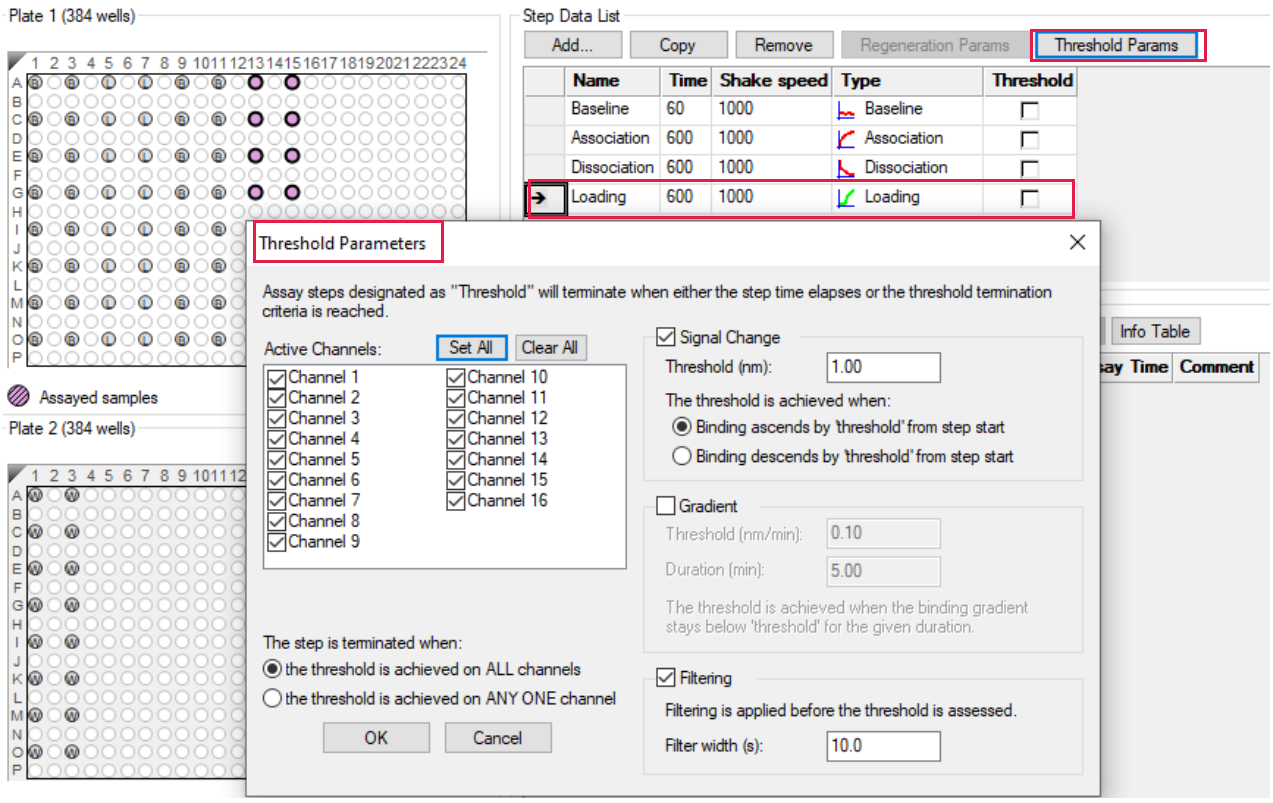


Figure 10-34: Setting Assay Step Threshold Parameters

NOTICE: *If thresholds are applied, the step is terminated when either the step time elapses or the threshold termination criteria is reached.*

Table 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: • **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.

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).

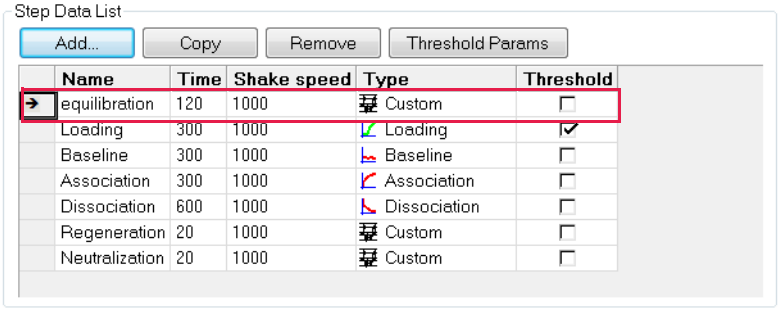


Figure 10-35: Step Data List—Displaying Step Types

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, right-click 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).

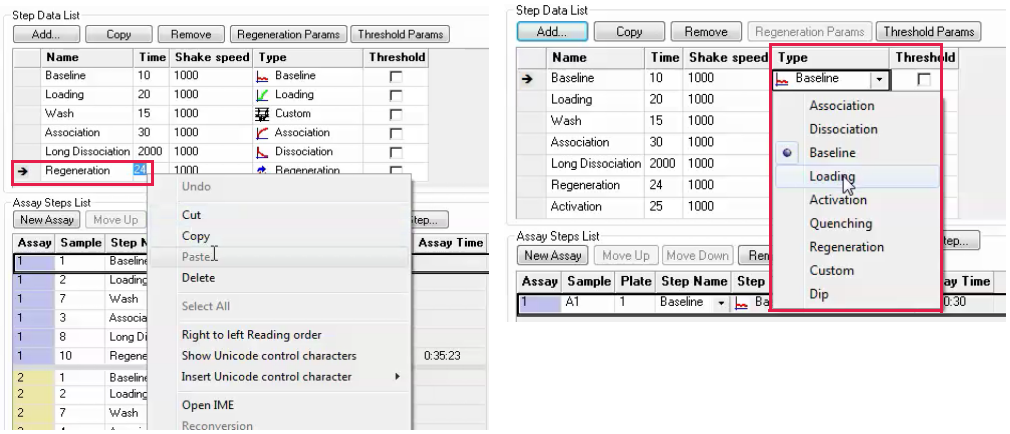


Figure 10-36: Editing a Step Value (left) or Step Type (right)

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).

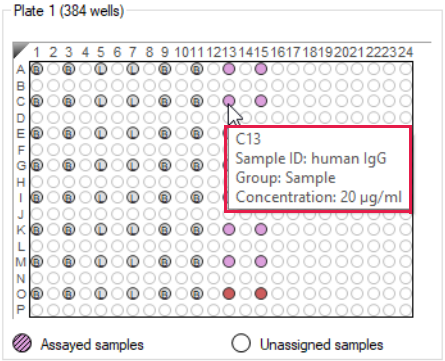


Figure 10-37: Tool Tip of Well Information

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.*

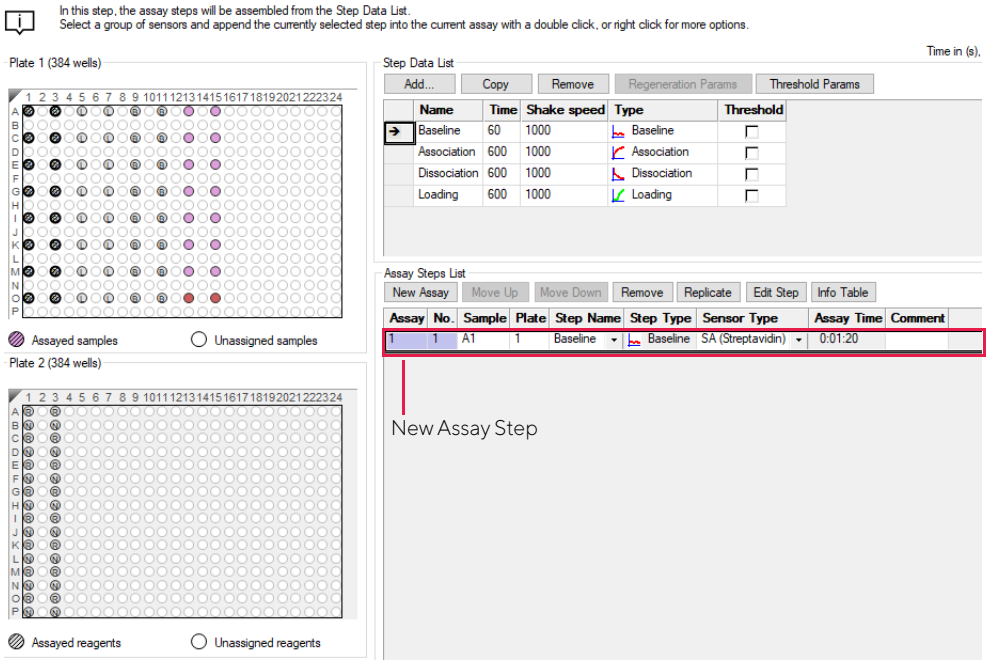


Figure 10-38: Assigning a Step Type to a Column in the Sample Plate

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).

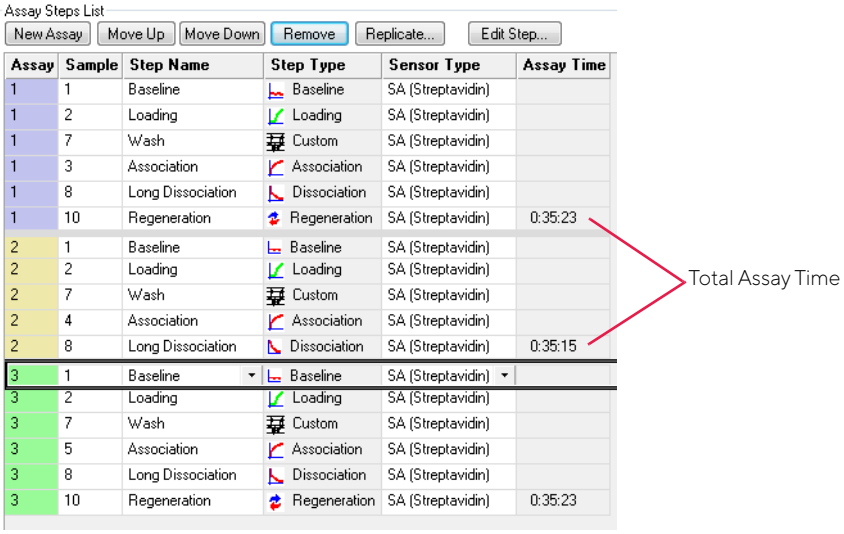


Figure 10-39: Experiment and Assay Time Updates as Steps Are Added to the Assay

IMPORTANT: *If you intend to analyze the data from a sample using the Inter-step correction feature in the Octet® 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).

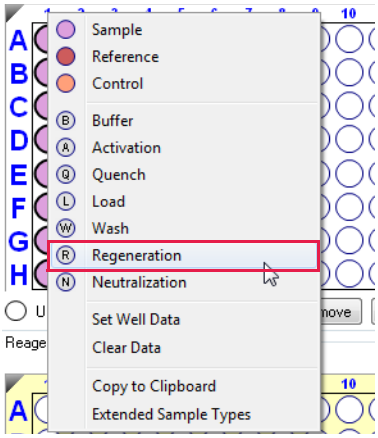


Figure 10-40: Regeneration Step

2. Click **Add t**o display the Add Step Definition dialog box (Figure 10-41).

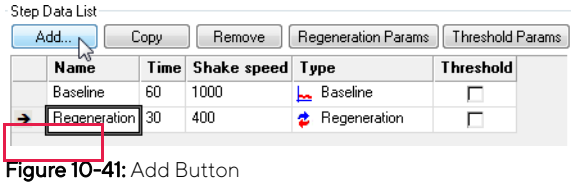


Figure 10-41: Add Button

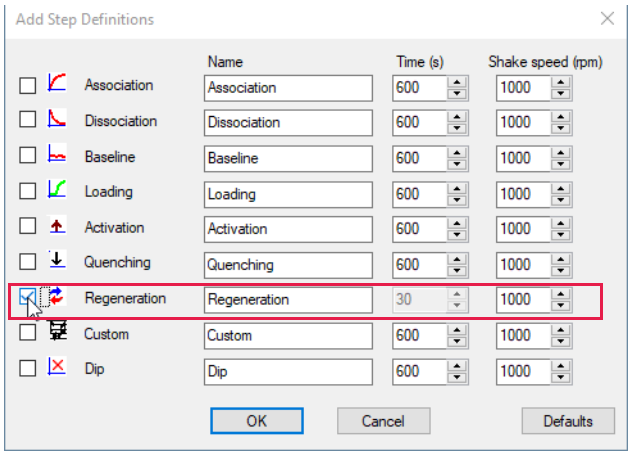


Figure 10-42: Add Step Definition Dialog Box

3. Select **Regeneration** and click **OK**.
4. Click **Regeneration Params** (Figure 10-43).

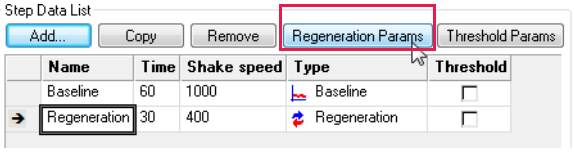


Figure 10-43: Regeneration Params Button

The **Regeneration Parameters** dialog box appears, and you can edit Regeneration parameters as necessary.

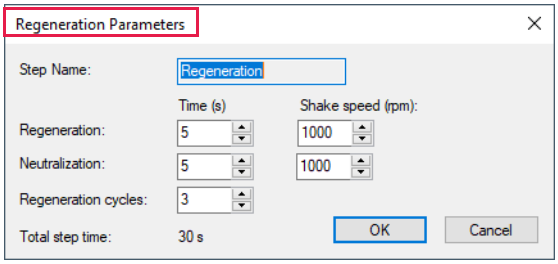


Figure 10-44: Regeneration Parameters Dialog Box

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.)

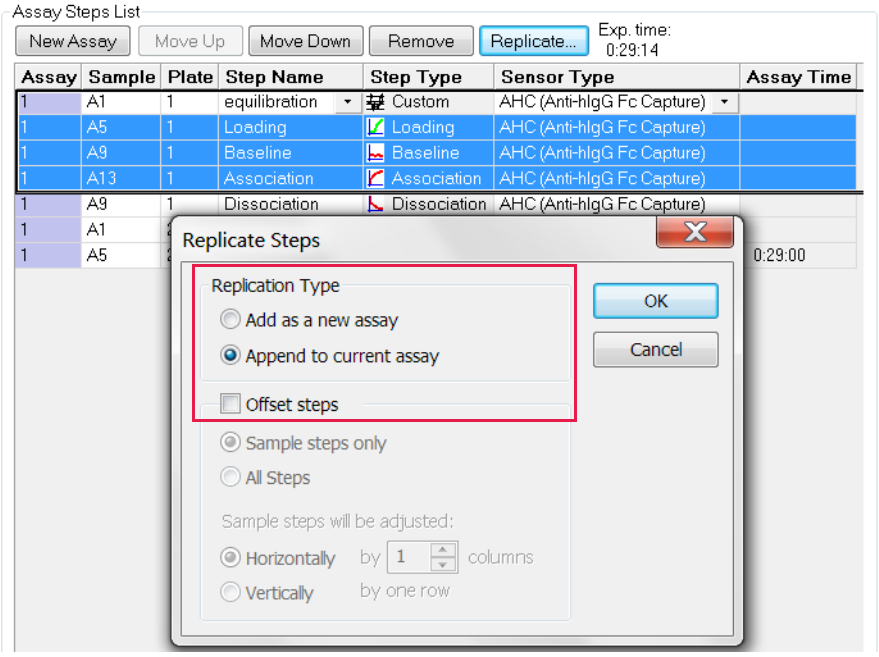


Figure 10-45: Replicating Assay Steps by Appending

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 **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.
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 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.
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.

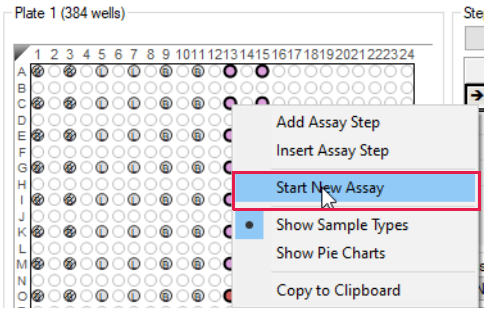


Figure 10-46: Start New Assay

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.

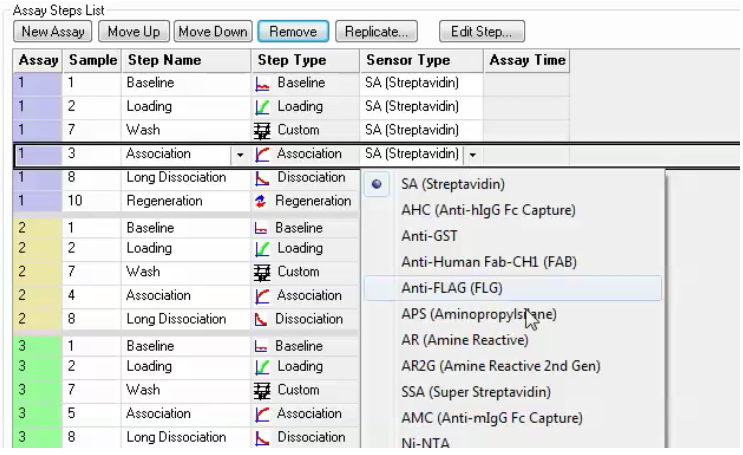


Figure 10-47: Selecting an Assay Sensor Type

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.*

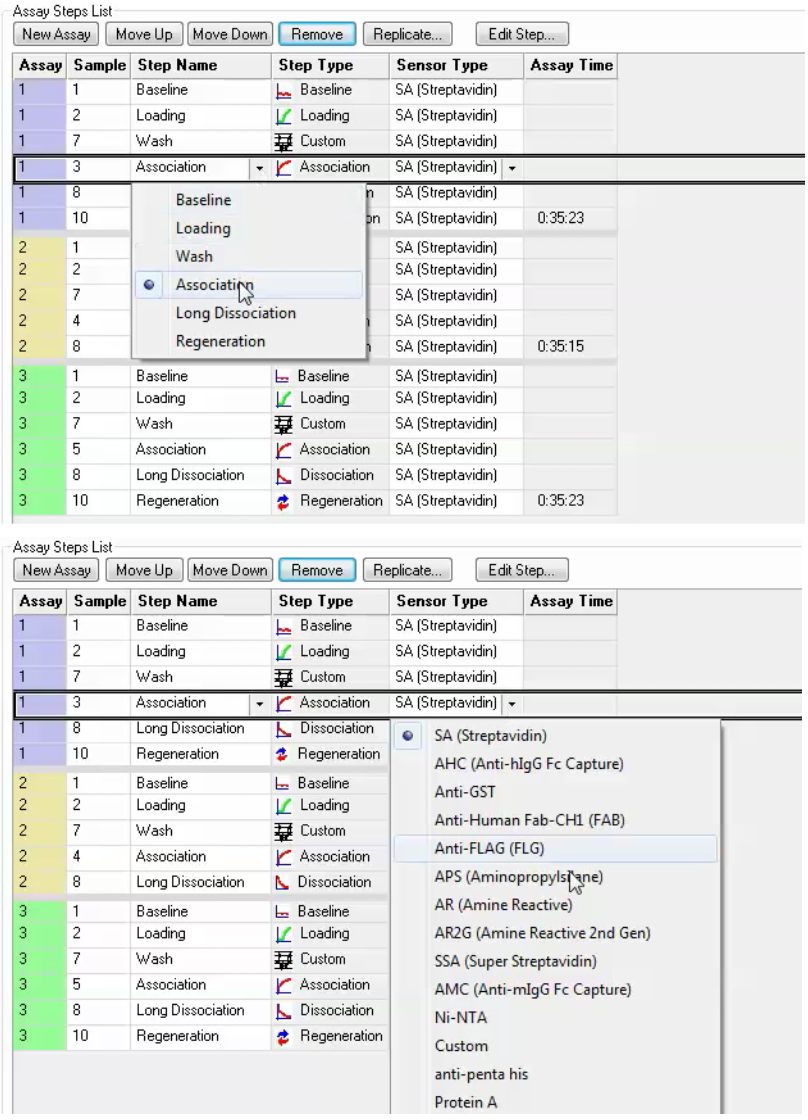


Figure 10-48: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

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).

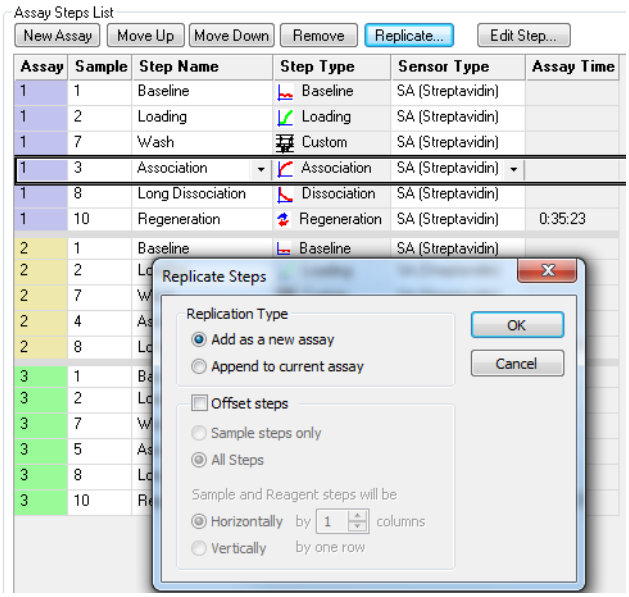


Figure 10-49: Adding a Replicate Assay to a Plate

3. 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 → 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.*

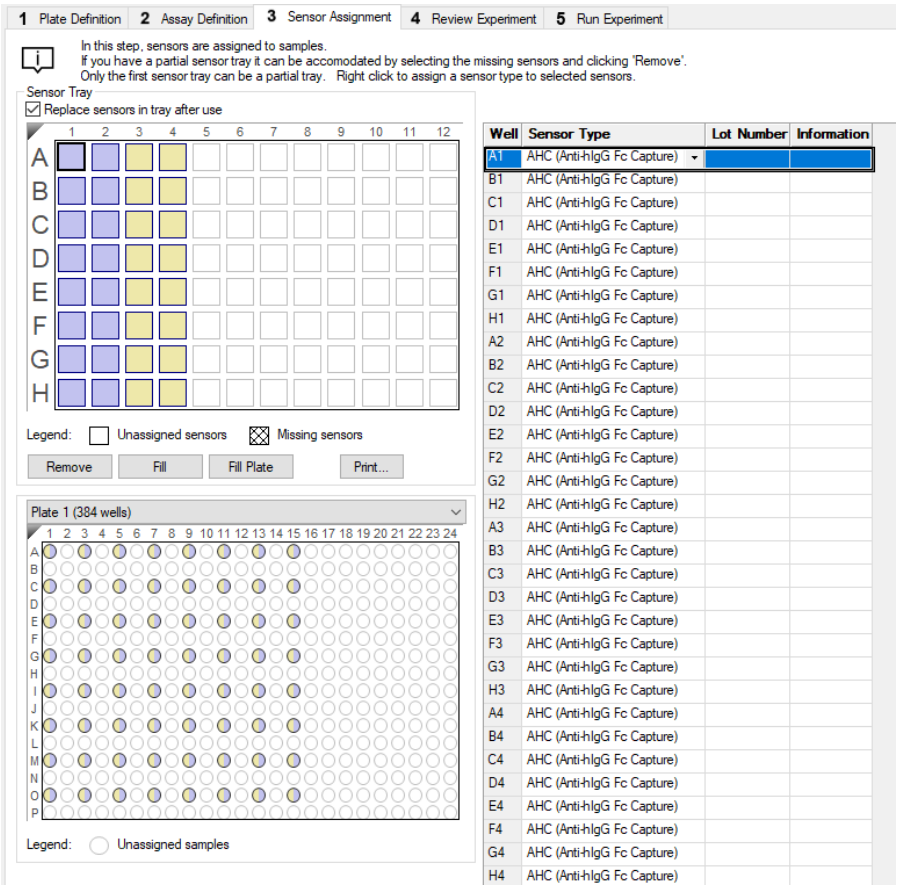


Figure 10-50: Sensor Assignment Window

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).

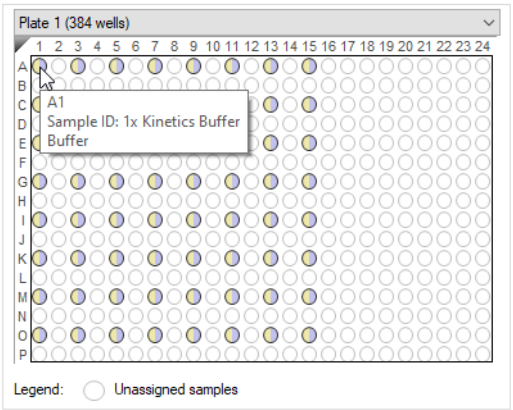


Figure 10-51: Tool Tip of Well Information

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).

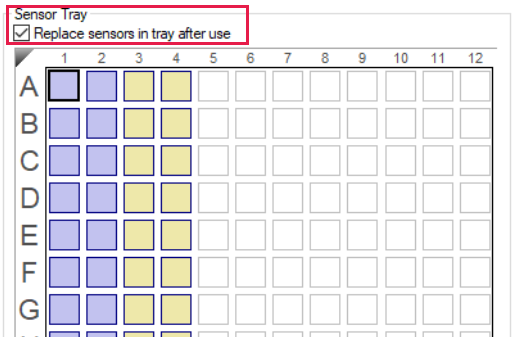


Figure 10-52: Replace Sensors in Tray After Use Check Box

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

Entering Biosensor Information

To enter information about a biosensor:

1. 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.*

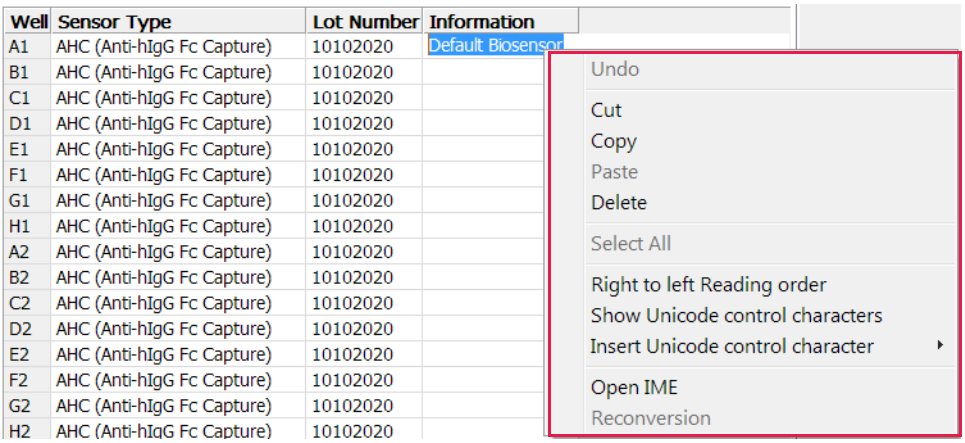


Figure 10-53: Entering or Editing Biosensor Information

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).

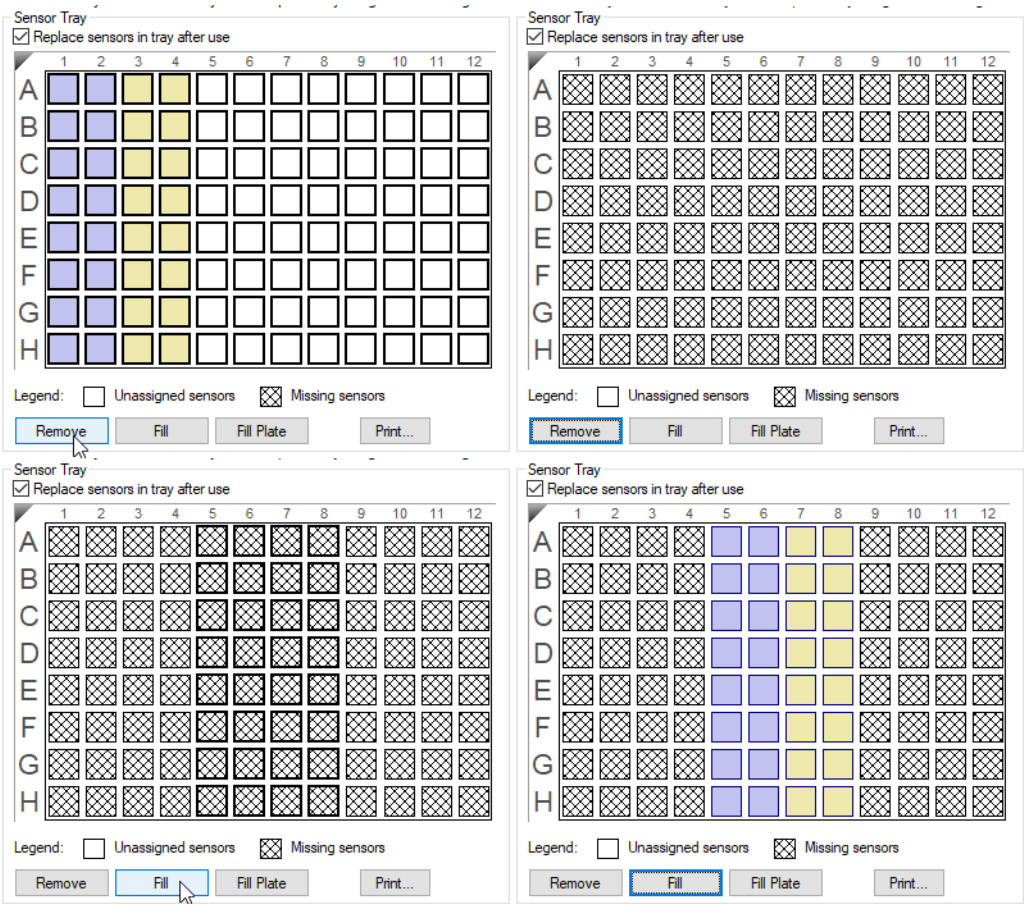


Figure 10-55: Changing Biosensor Location (Method 2)

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 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.

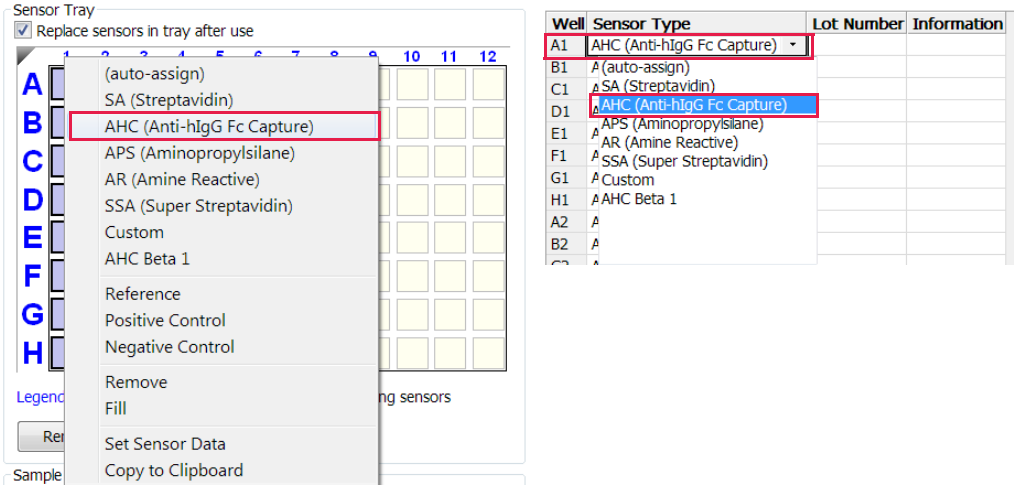


Figure 10-56: Sensor Assignment Window—Changing the Biosensor Type

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).

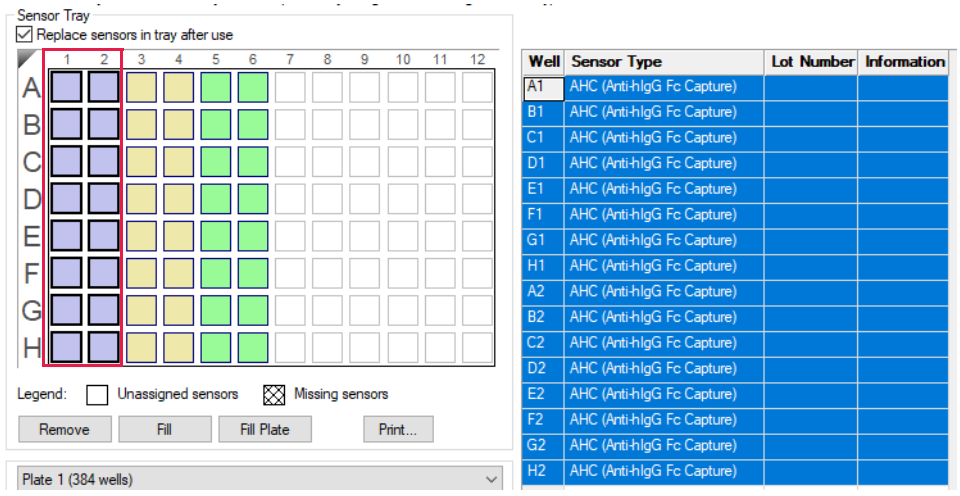


Figure 10-57: Selecting a Sensor Tray Column

2. 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.

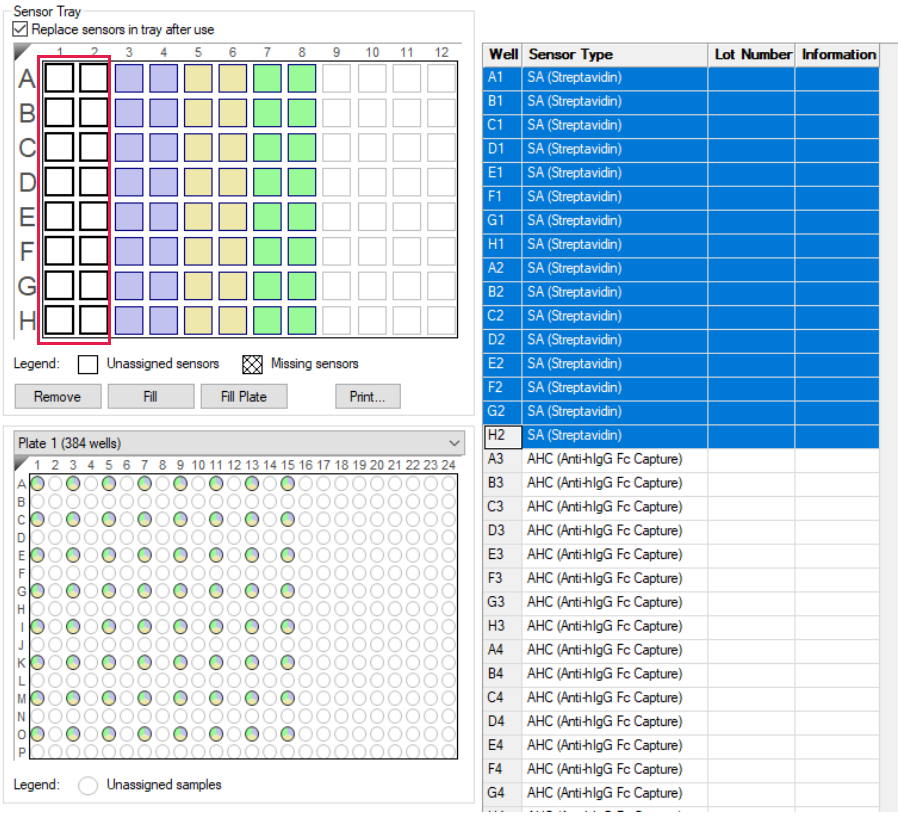


Figure 10-58: Assay Sensor Type Reassignment

3. Repeat the previous steps to assign locations for the remaining biosensor types in the tray.

*I**MPORTANT:** 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 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.

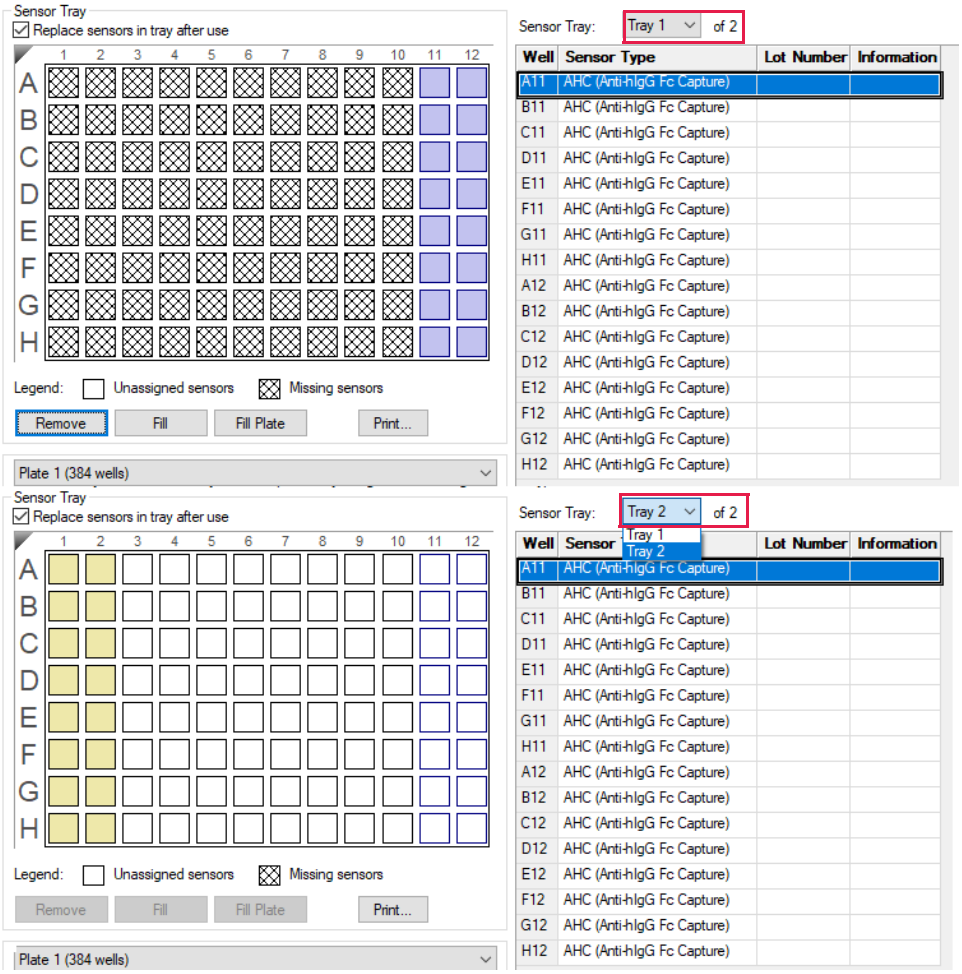


Figure 10-59: Example Experiment Using Two Biosensor Trays

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® 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.*

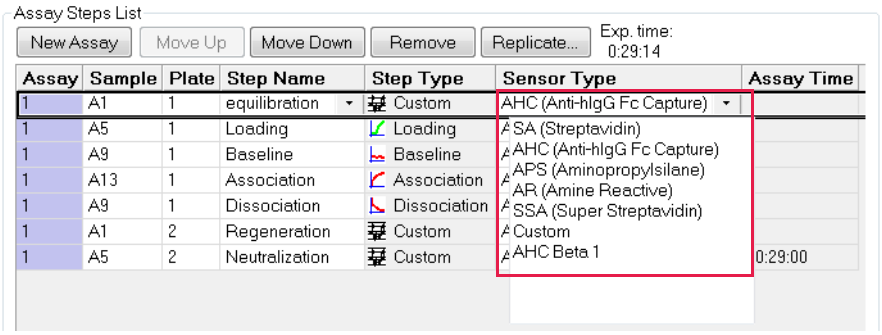


Figure 10-60: Assay Definition Window—Changing the Biosensor Type

Starting a Basic Kinetics Experiment: Octet® 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® RH96 system and the Octet® BLI Discovery software, follow the steps below to set up and run a kinetic experiment with multiple Read Head configurations

Table 10-6: Octet® BLI Discovery Steps for Kinetic Assays

Octet® 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). ***NOTICE:*** *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® 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® 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.

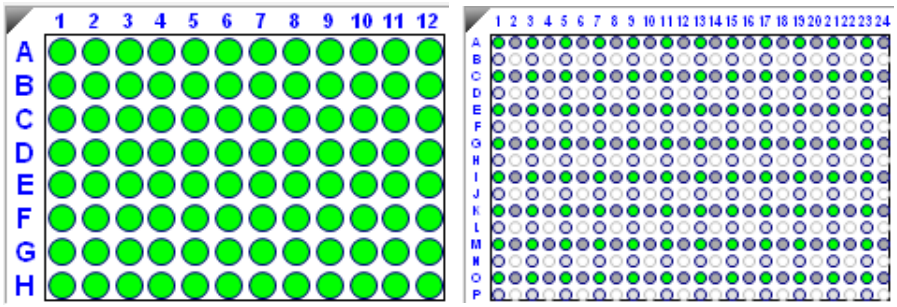


Figure 10-61: Biosensor Layout in 96- and 384-well Plates Using 96-channels Read Head Setting.

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® 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® 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:

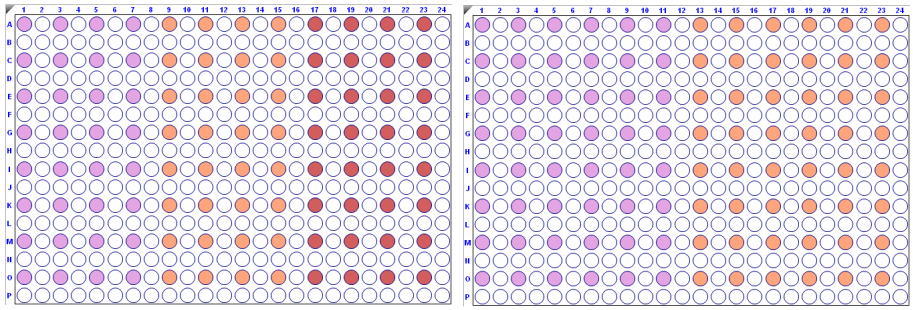


Figure 10-62: Biosensor Layout in 384-well Plates Using 32 (left) and 48 (right) Channels Read Head Setting.'

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® RED96e and Octet® 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

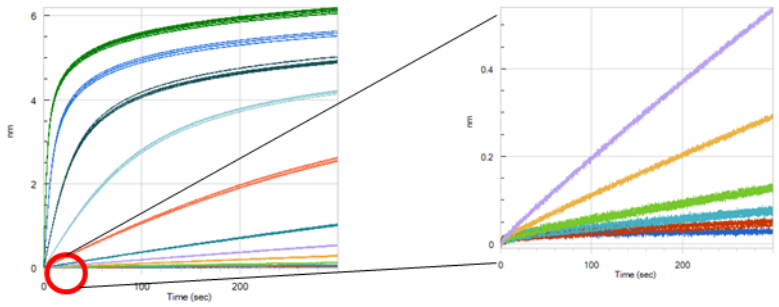
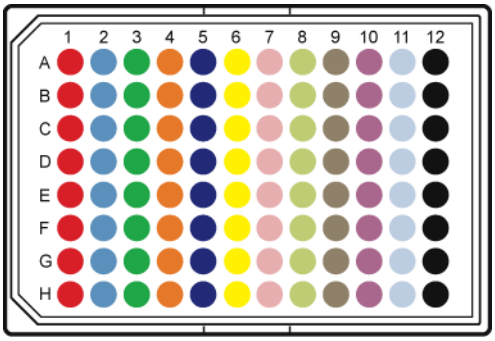


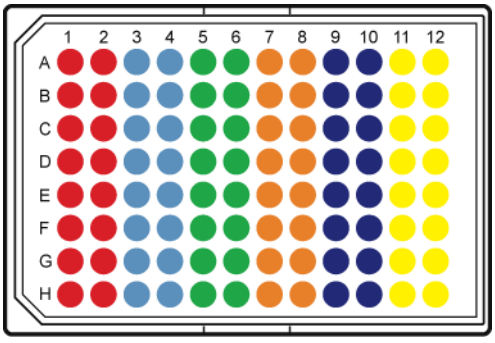
Figure 10-63: Zoomed View of Closely Overlaid Traces Shows Fine Signal Resolution for Human IgG Quantitation Assay with Protein A Biosensors

8 Channel Read Head



Biosensors interrogate 8 wells in a column, one column is interrogated at a time.

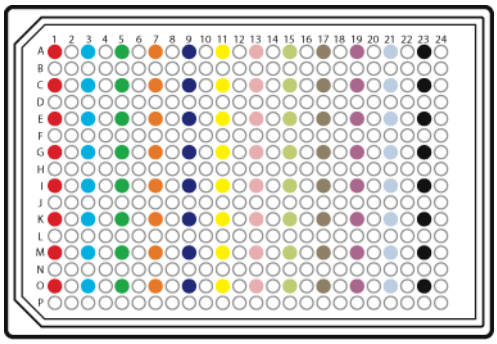
16 Channel Read Head



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

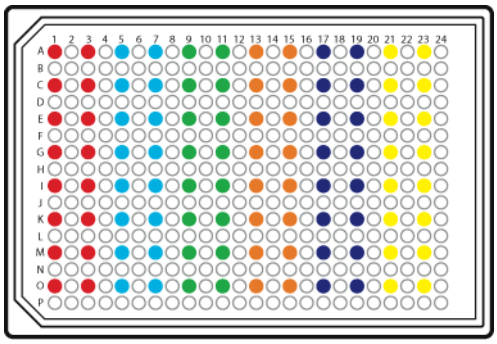
Figure 10-64: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

8 Channel Read Head



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 10-65: Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head

Tab 1 (Plate Definition)

1. Choose the number of simultaneous wells to be read from the **Read Head** drop down list:

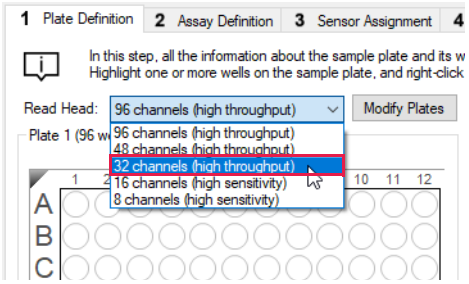


Figure 10-66: Select Wells to be Read

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).*

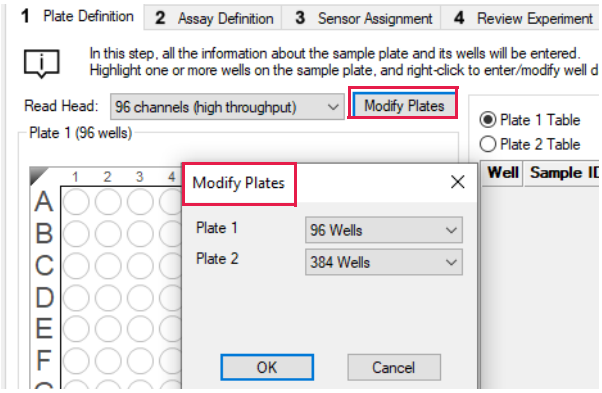


Figure 10-67: Select Plate 1 and Plate 2 Formats

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.*

Table 10-7: Types of Sample Wells

Icon	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 • Negative Control: A control sample known not to contain analyte
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.

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).

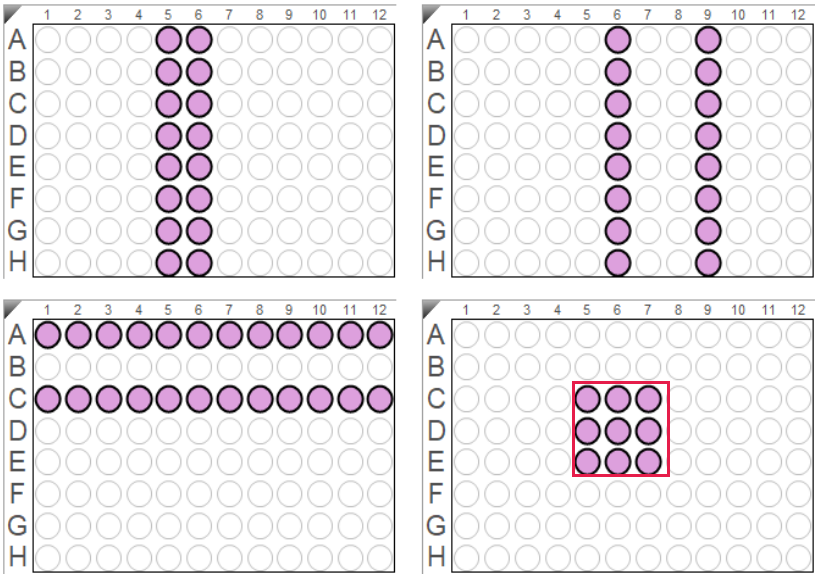


Figure 10-68: Selecting Sample Wells in a Plate Map

4. Designate well types by right-clicking on selected wells and assigning a sample type:

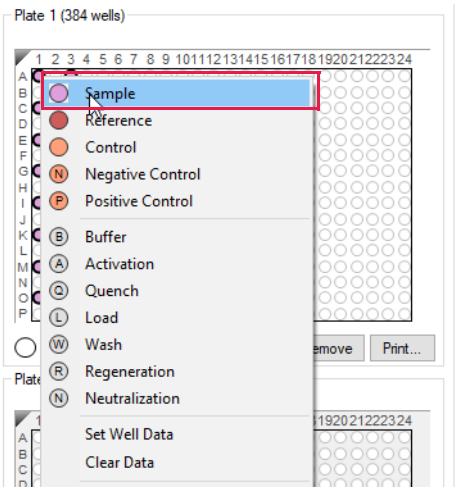


Figure 10-69: Designating Well Types

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**:

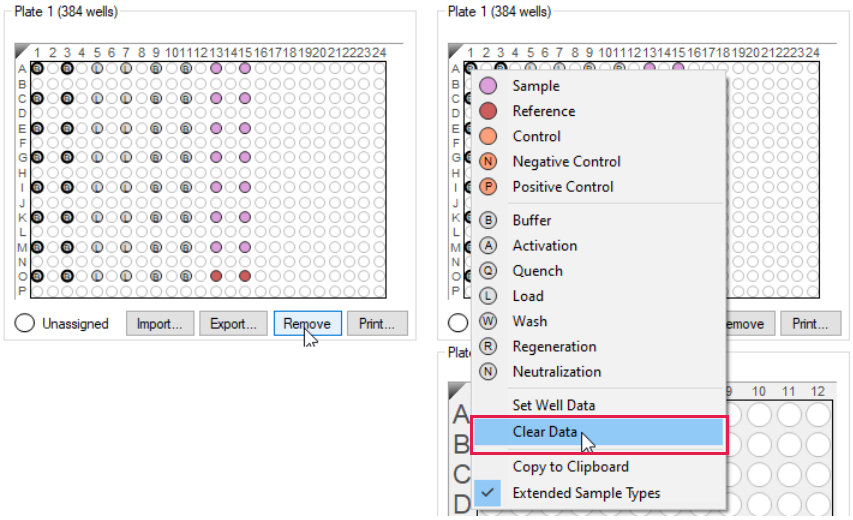


Figure 10-70: Clearing Sample Well Designations from the Plate Map

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:**

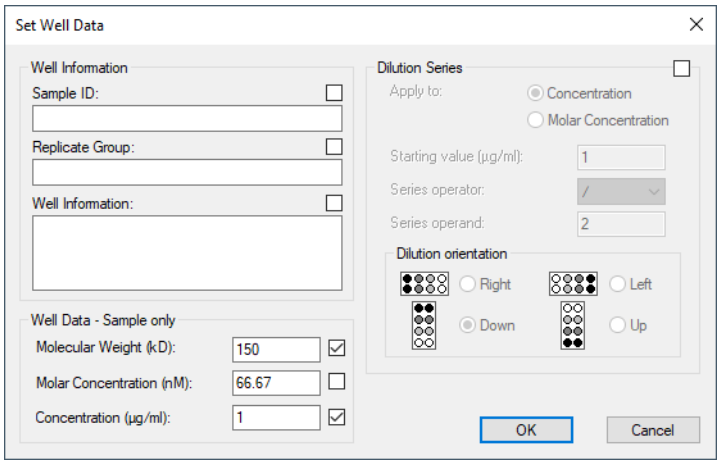


Figure 10-71: Entering Molecular Weight and Molar Concentration

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:

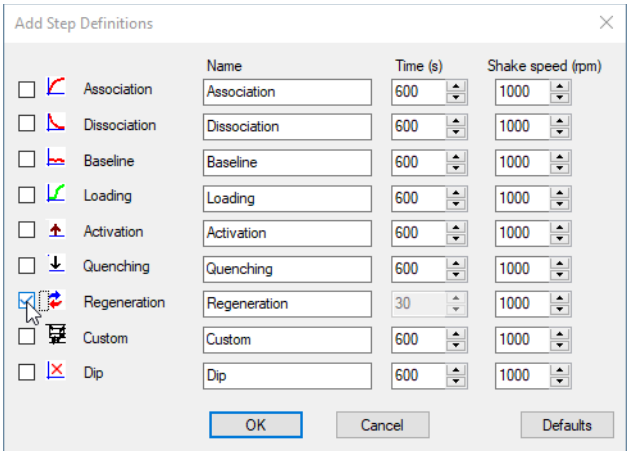


Figure 10-72: Add Step Definition Box

   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:

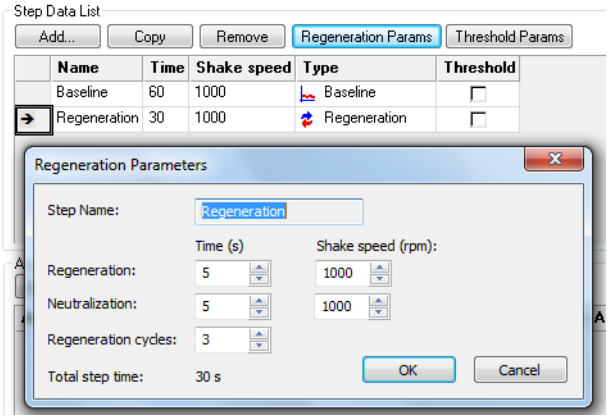


Figure 10-73: Regeneration Parameters Box

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:

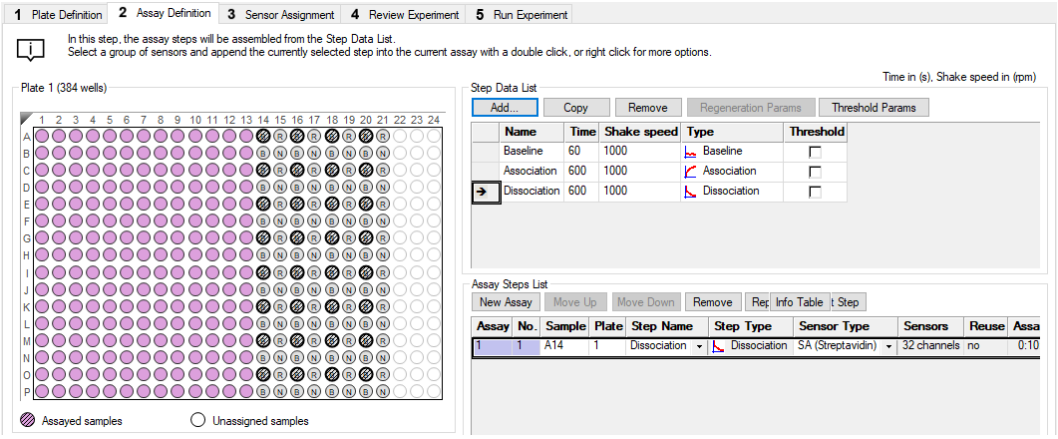


Figure 10-74: Assay Steps List

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:

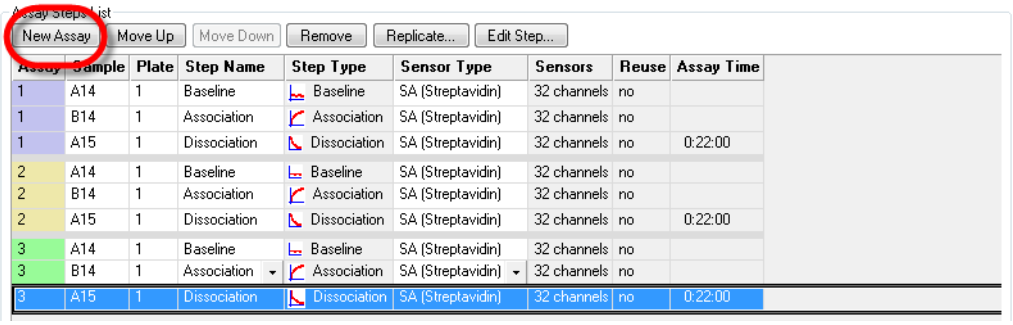


Figure 10-75: New Assay Button

4. Change the Read Head setting for an individual assay by clicking the **Edit Step** button to bring up the edit step dialogue:

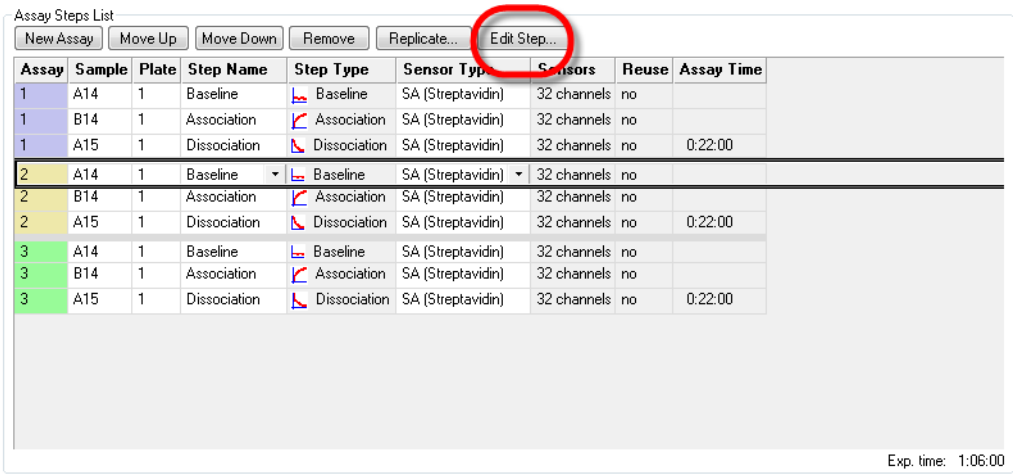


Figure 10-76: Edit Step Button

5. Choose a new setting from the Read Head drop-down list, then click **OK**:

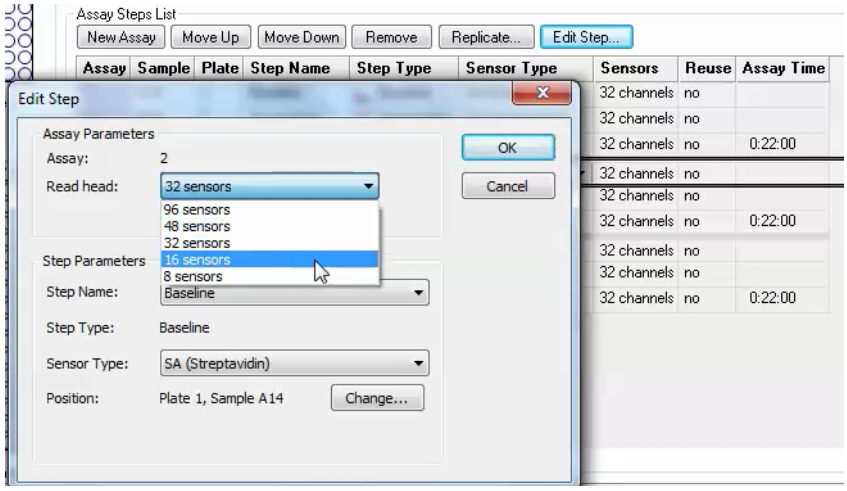


Figure 10-77: Setting Read Head Sensors

6. Repeat for new Read Head settings for other assays in the experiment:

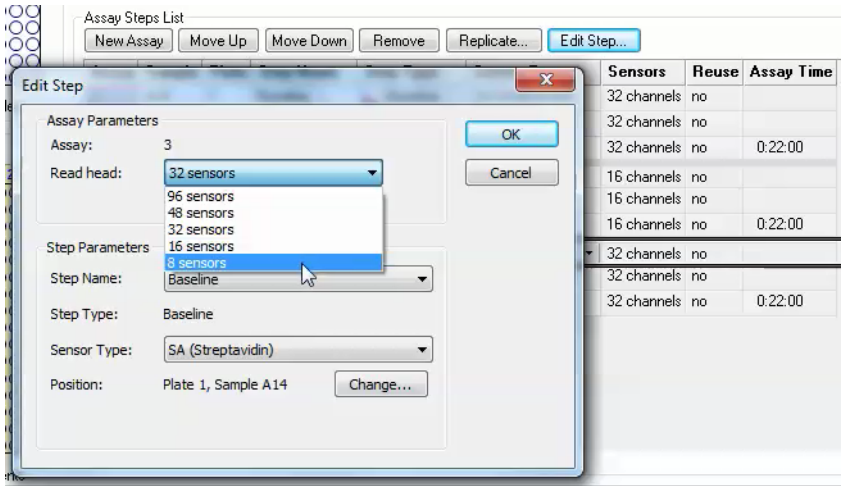


Figure 10-78: Setting Read Head Sensors for Experiment Assays

7. Edit or change the columns associated with a step type by selecting the individual step and clicking the **Edit Step** button:

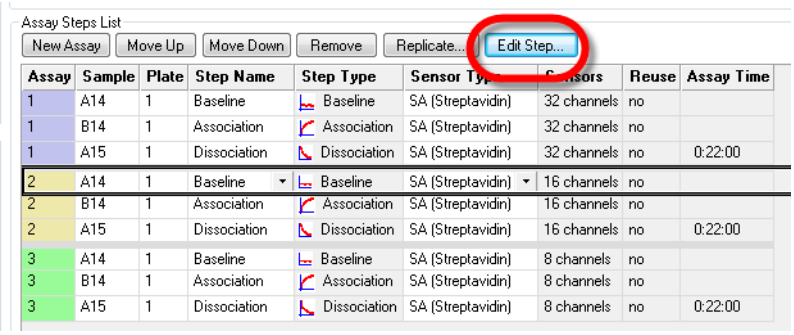


Figure 10-79: Edit Step Button

8. Click the **Change** button, then click **OK**.

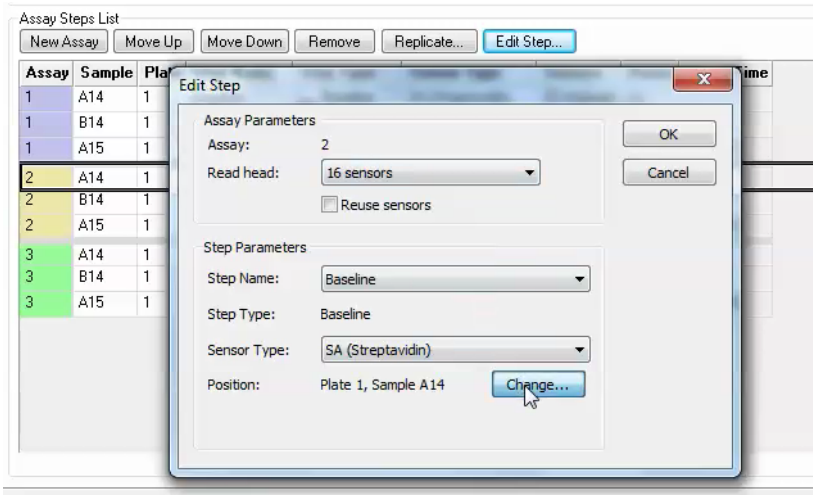


Figure 10-80: Edit Step Change Button

9. This will bring up the Set Position plate map. Click on the new column(s) associated with that step:

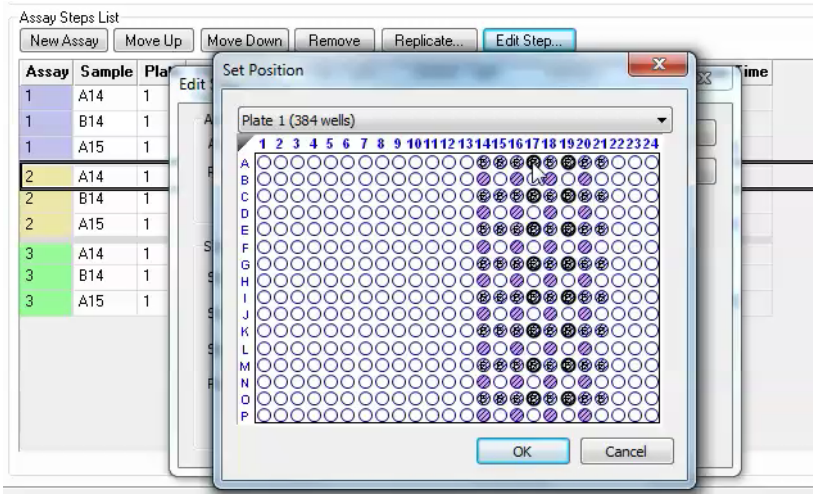


Figure 10-81: Set Position Plate Map

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:

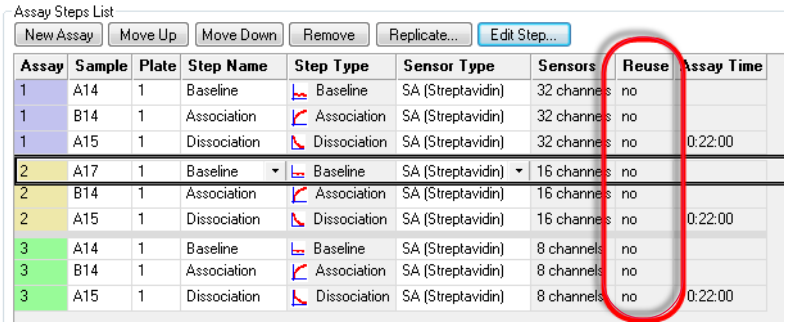


Figure 10-82: Default Biosensor Reuse Selection

NOTICE: *The Reuse option is only available for the Octet® 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.

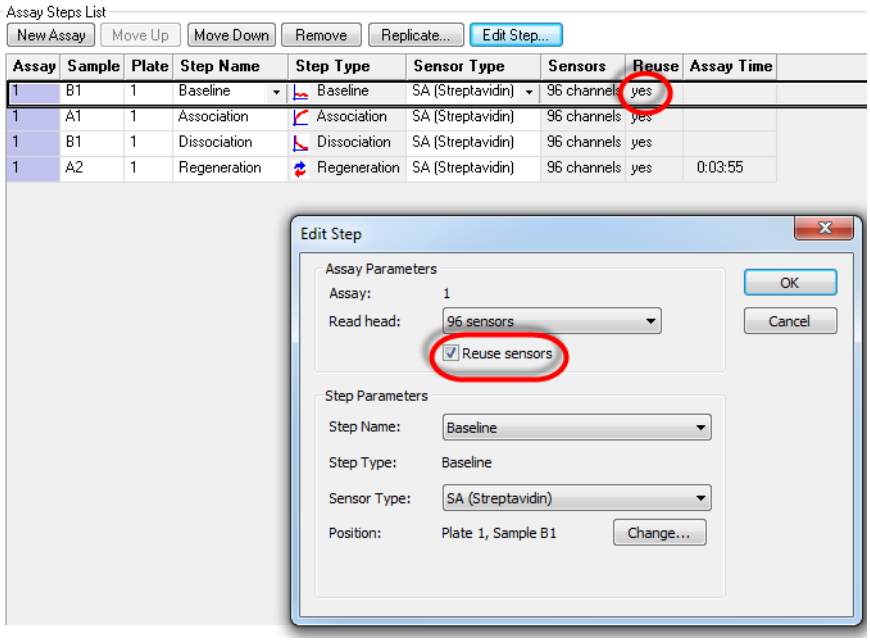


Figure 10-83: Changing the Biosensor Reuse Selection

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:

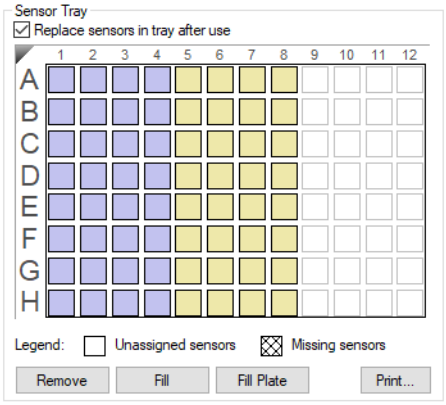


Figure 10-84: Replace Sensors in Tray After Use Check Box

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 ← → arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

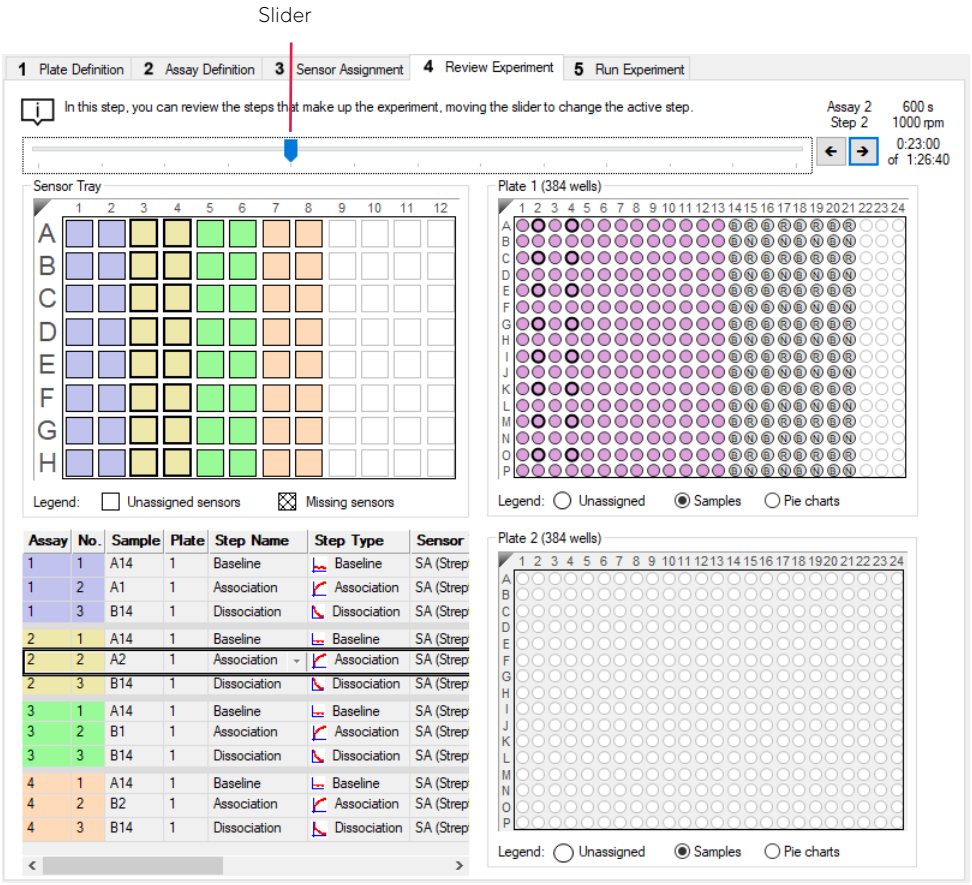


Figure 10-85: Review Experiment Window

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 factory-set location, the software may not function properly.*

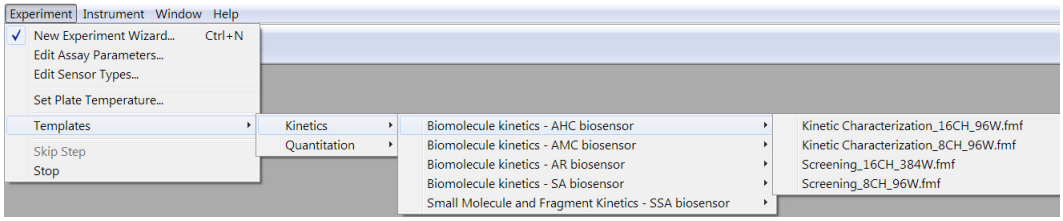


Figure 10-86: Saved Experiments in the Template Folder

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® 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).

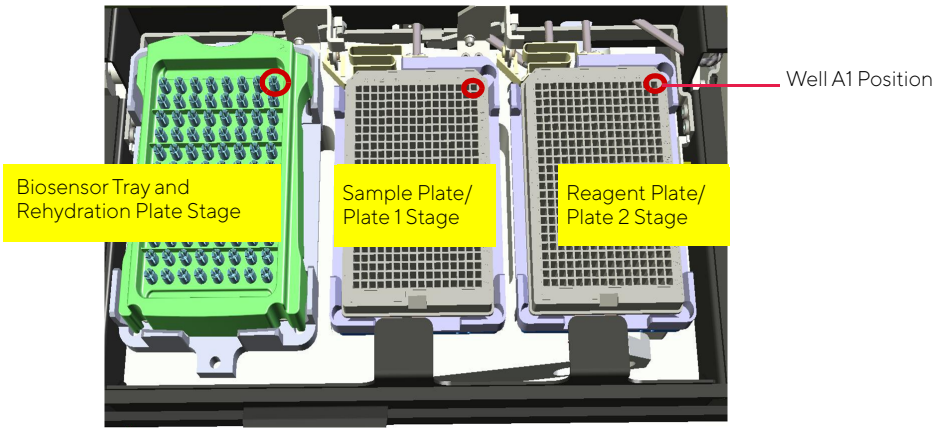


Figure 10-87: Octet® Instrument Stage Platform

IMPORTANT: *Ensure that the bottom of the sample plate, reagent plate and biosensor tray are flat on the stages.*

3. Click ▲ to close the Octet® 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 (→) to access the Run Experiment window (see Figure 10-88).

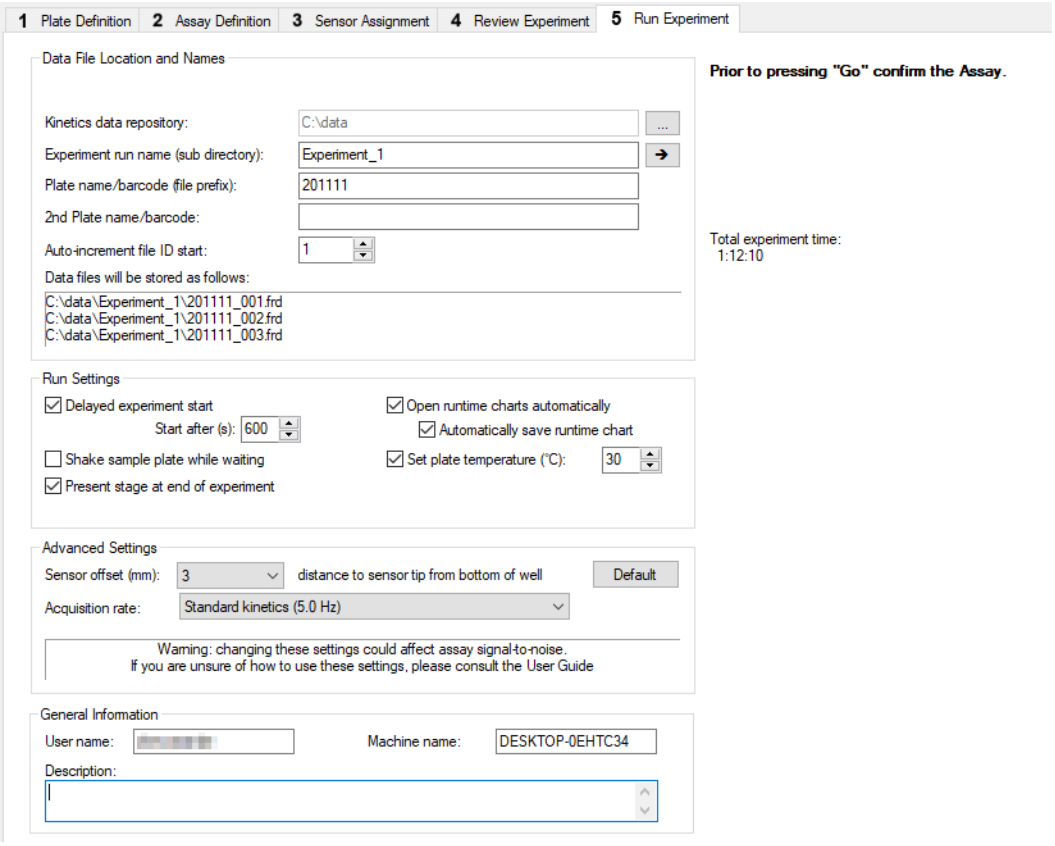


Figure 10-88: Run Experiment Tab—Octet®16

2. Confirm the default settings or enter new settings. See "Run Experiment Window Settings" on page 477 for more information on experimental settings.

NOTICE: *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 10-89).

NOTICE: *For more details about the Runtime Binding Chart, see "Managing the Runtime Binding Chart" on page 479.*

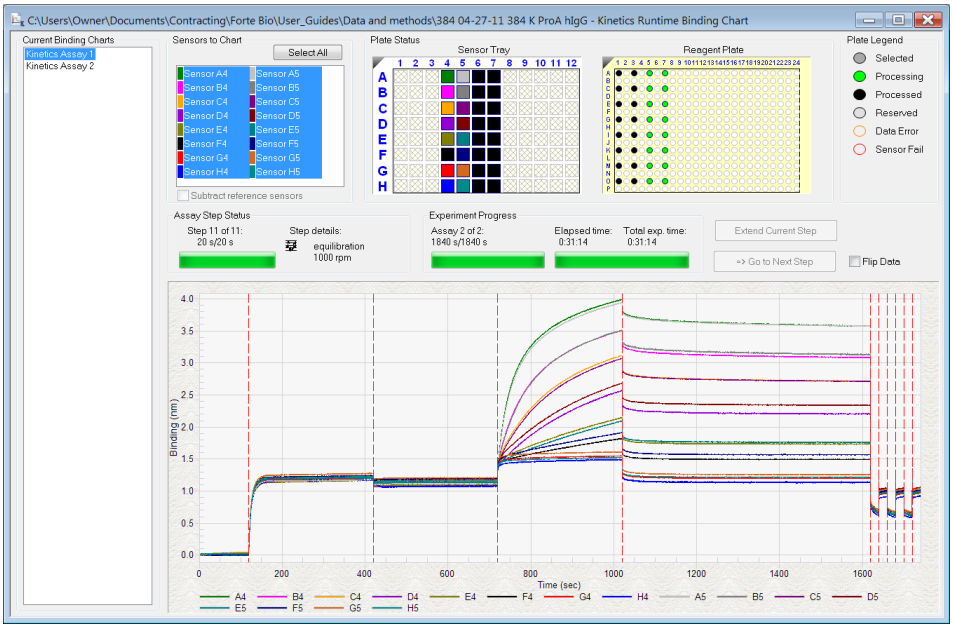


Figure 10-89: Runtime Binding Chart

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.

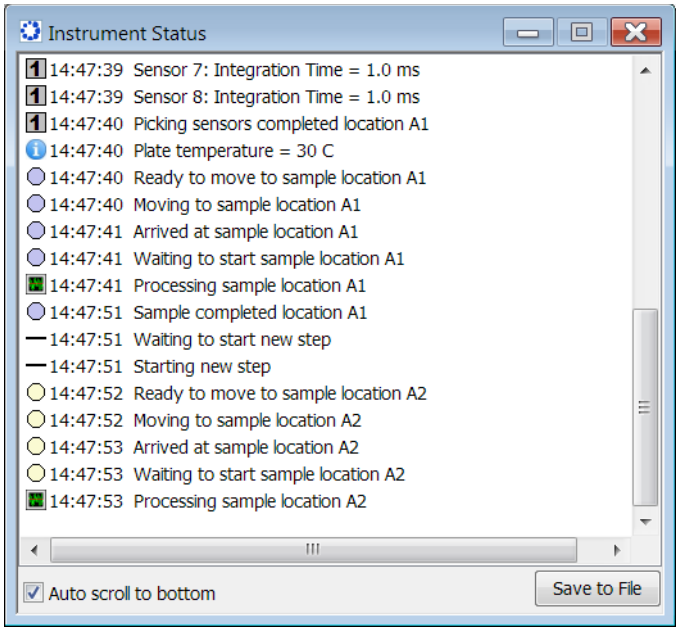


Figure 10-90: Instrument Status Log

WARNING: *Do not open the Octet® 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® 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 **Browse** to select another data location. ***NOTICE:*** *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 **GO**.
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 **Runtime Binding Chart** for the current biosensor during data acquisition.
Automatically save run-time chart	Saves an image (.jpg) of the **Runtime Binding Chart**. 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 **File > Options**. The factory set default temperature is 30 °C. ***NOTICE:*** *If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet® 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® 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® RH16 system:

Table 10-10: Advanced Settings Octet® RH16

Item	Description
Acquisition rate	• 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. • 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. • 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.
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® QK384 system:

Table 10-11: Advanced Settings Octet QK384

Item	Description
Acquisition rate	• 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. • 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.
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® 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 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 color-coded (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® 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.*

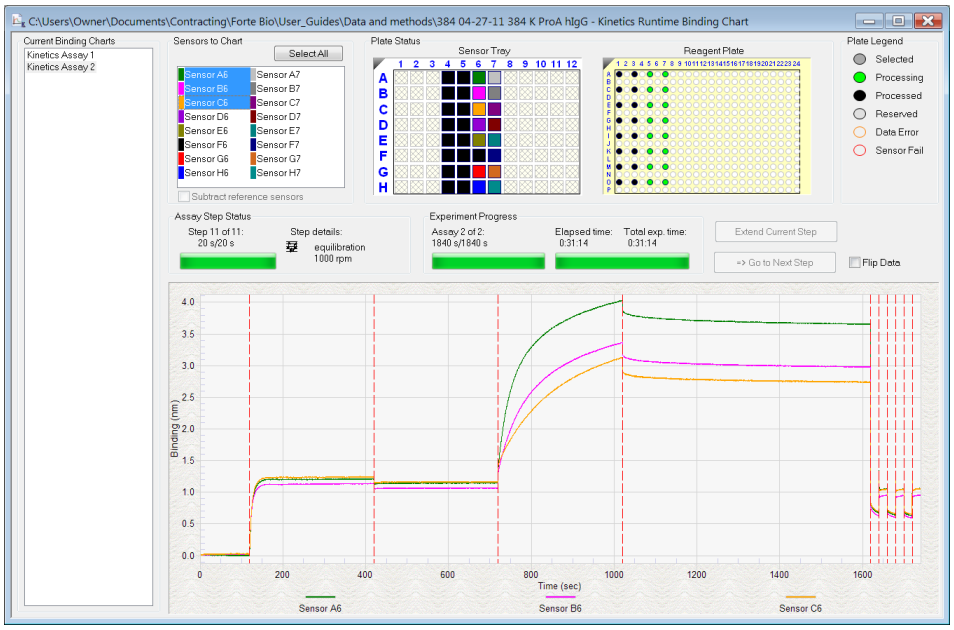


Figure 10-91: Runtime Binding Chart Window

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).

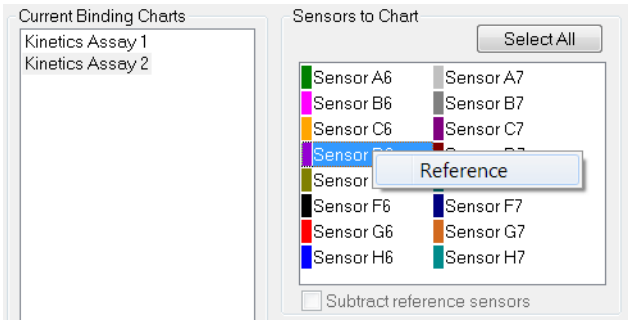


Figure 10-92: Designating a Reference Biosensor in the Runtime Binding Chart

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).

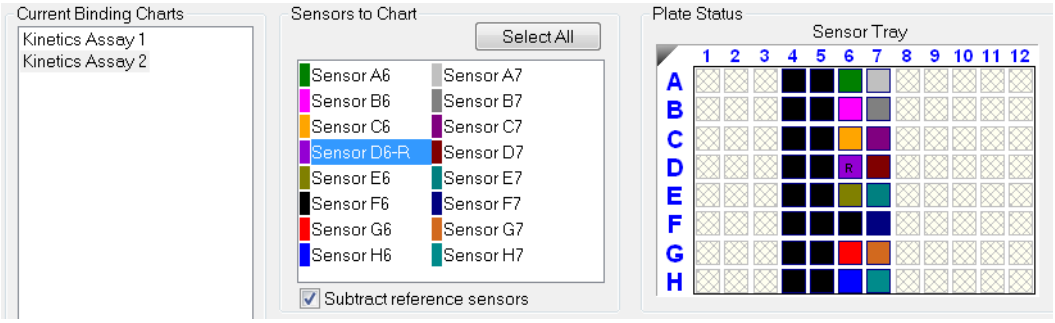


Figure 10-93: Subtract Reference Sensors check box in the Runtime Binding Chart

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.

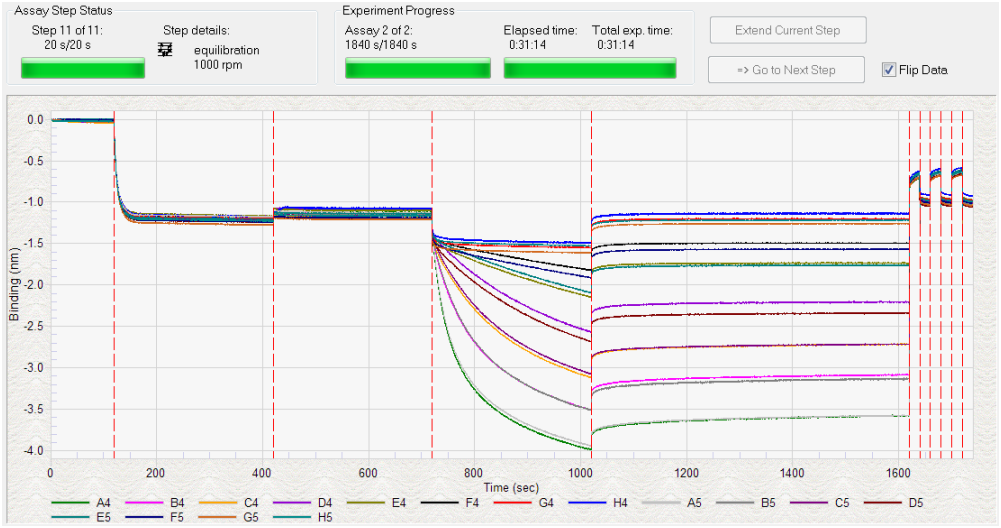


Figure 10-94: Data Inverted Using Flip Data Function

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**.

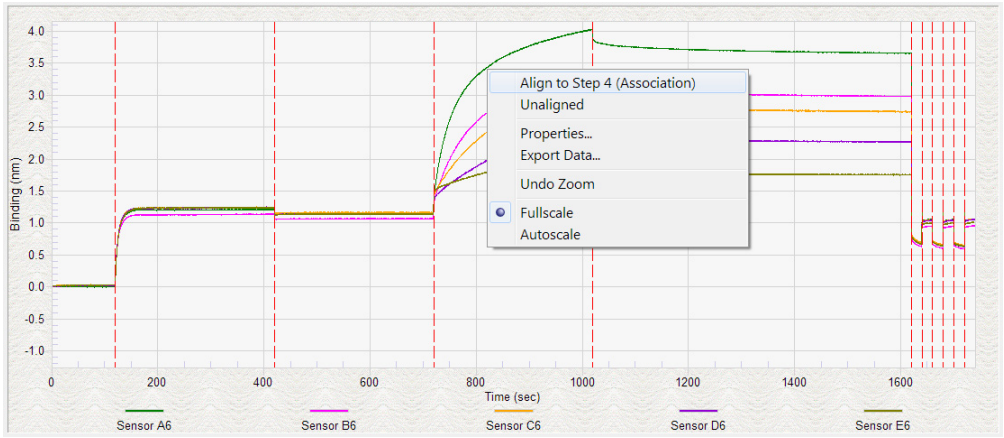


Figure 10-95: Runtime Binding Chart—Aligning the Data to a User-Selected Step

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**.

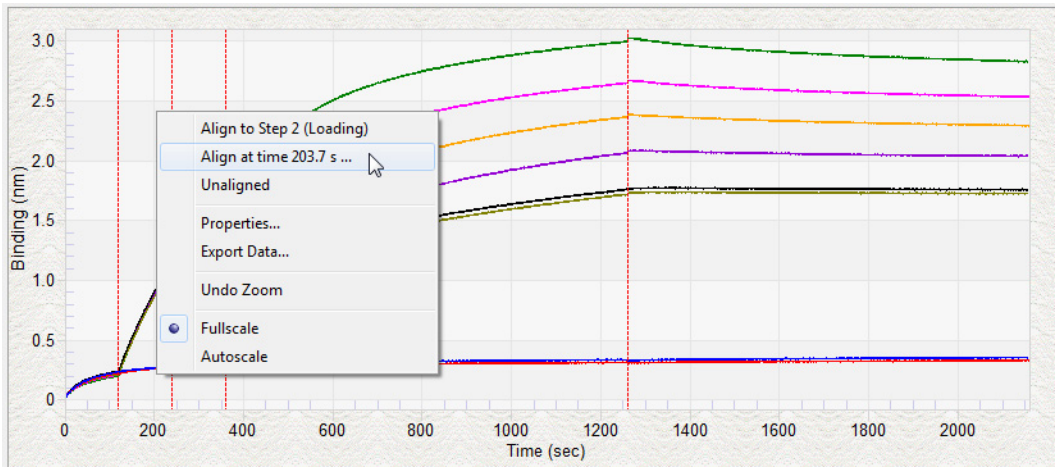


Figure 10-96: Runtime Binding Chart—Aligning the Data to a User-Specified Time

The Align at Time dialog box displays (Figure 10-97).

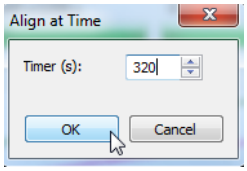


Figure 10-97: Align at Time Dialog Box

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**.

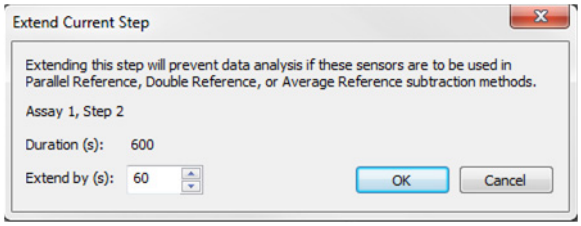


Figure 10-98: Extend Current Step Dialog Box

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

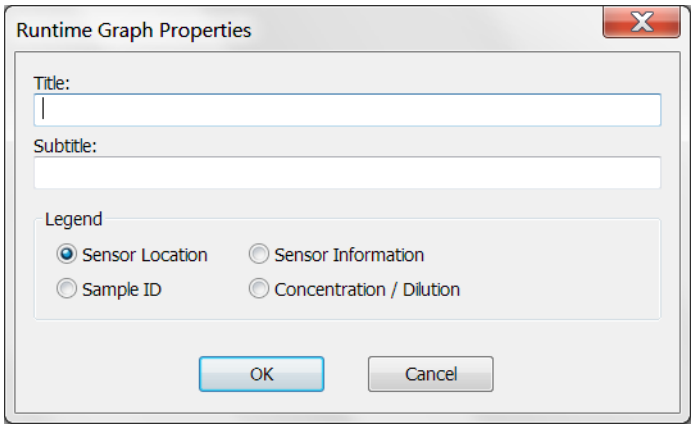


Figure 10-99: Selecting a Runtime Binding Chart Legend

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**.

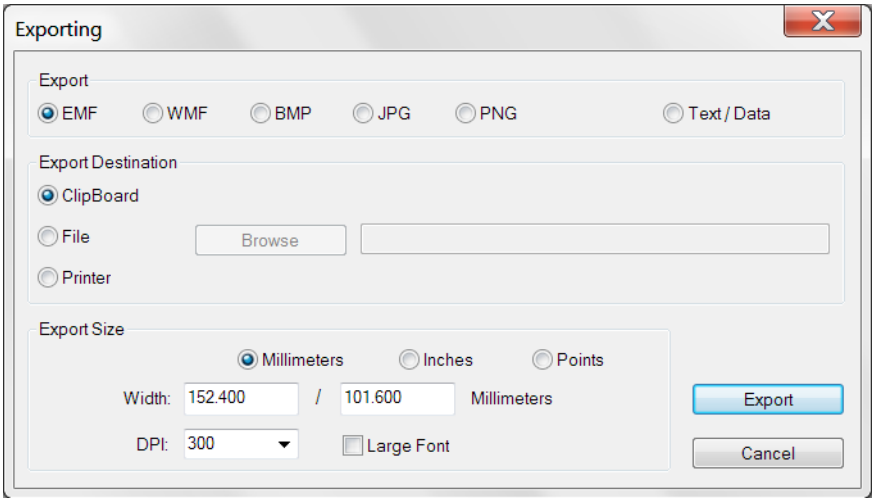


Figure 10-100: Exporting Dialog Box

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	✓		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 biosensor. 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		✓	Clipboard	Copies the chart to the system clip-board

Table 10-13: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Print the Runtime Binding Chart	✓	Printer		Opens the Print dialog box.

Managing Experiment Method Files

After you run an experiment, the Octet® 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 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® 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® system and the Octet® BLI Discovery software, follow the steps in Table 10-15 to set up and run an epitope binning experiment.

Table 10-15: Octet® BLI Discovery Steps for Epitope Binning Assays

Octet® Software	Functions
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). ***NOTICE:*** *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: Octet® RH16 or Octet® 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.

Tab 1 (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® QK384 and Octet® RH16, choose a plate format for both plate positions by clicking **Modify Plates**. Select either the 96- or 384 well format for each plate:

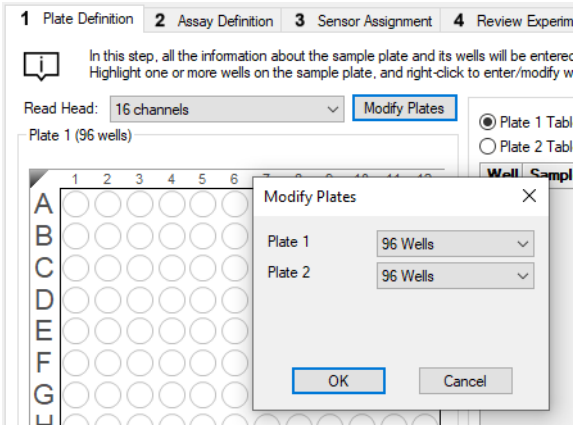


Figure 10-101: Select Plate Formats

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:\

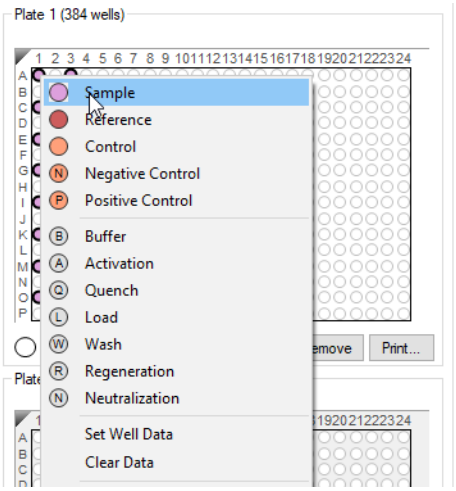


Figure 10-102: Designating Well Types

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:

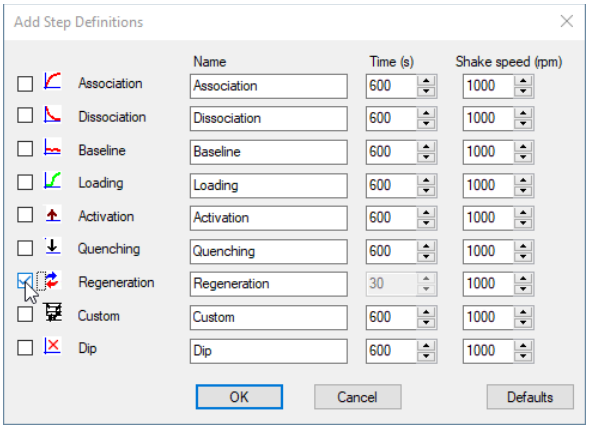


Figure 10-103: Add Step Definition Box

   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:

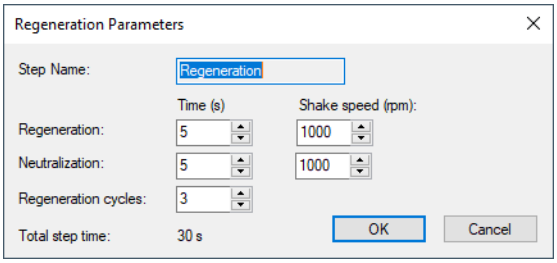


Figure 10-104: Regeneration Parameters Box

   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:

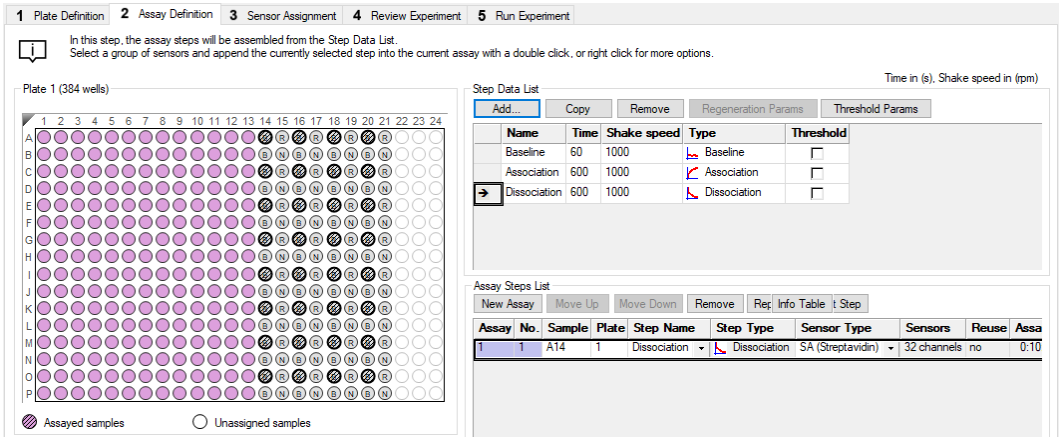


Figure 10-105: Assay Steps List

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:

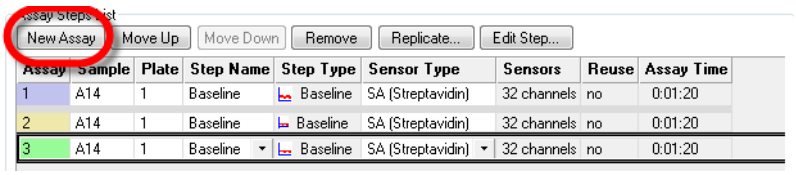


Figure 10-106: New Assay Button

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:

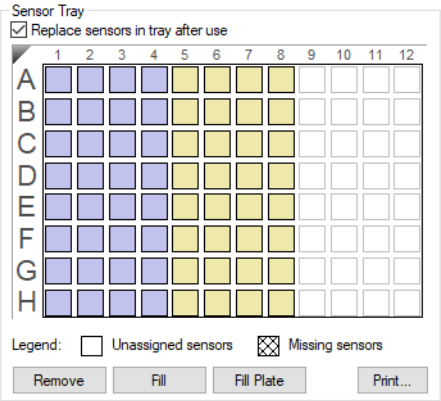


Figure 10-107: Replace Sensors in Tray After Use Check Box

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:

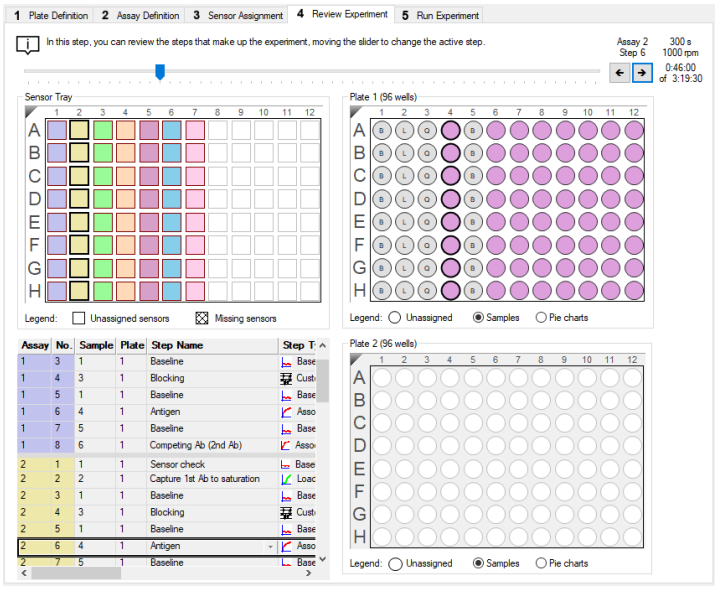


Figure 10-108: Navigating the Review Experiment Tab

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:

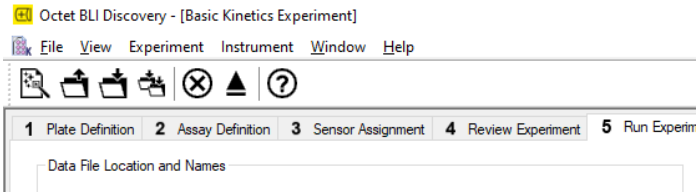


Figure 10-109: Present Stage Button

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:

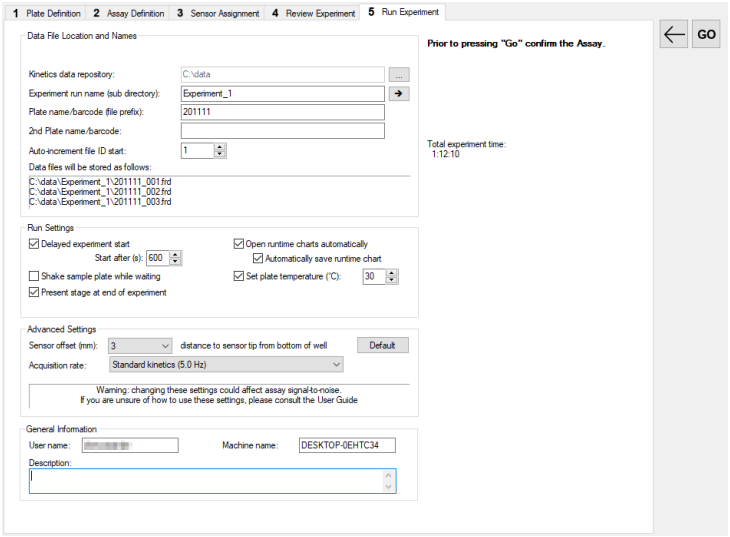


Figure 10-110: GO Button

Starting an Experiment: Octet® 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.

Tab 1 (Plate Definition)

1. Choose the number of simultaneous wells to be read from the **Read Head** drop down list:

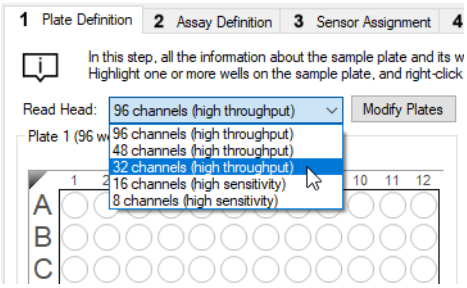


Figure 10-111: Select Wells to be Read

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:

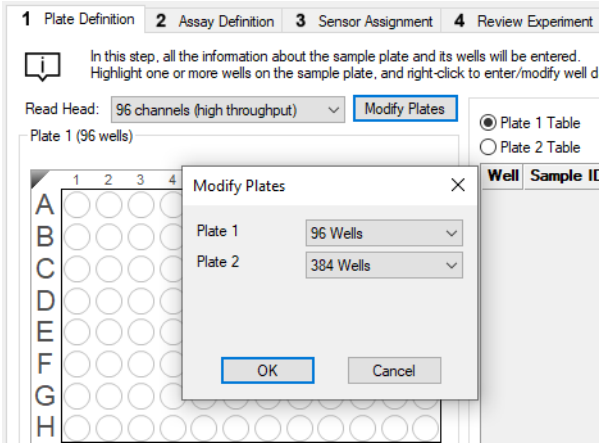


Figure 10-112: Select Plate 1 and Plate 2 Formats

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:

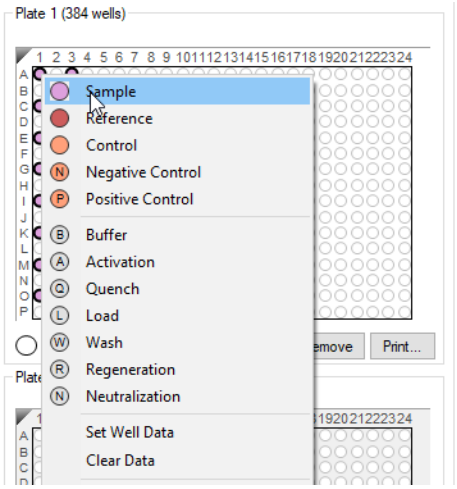


Figure 10-113: Designating Well Types

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:

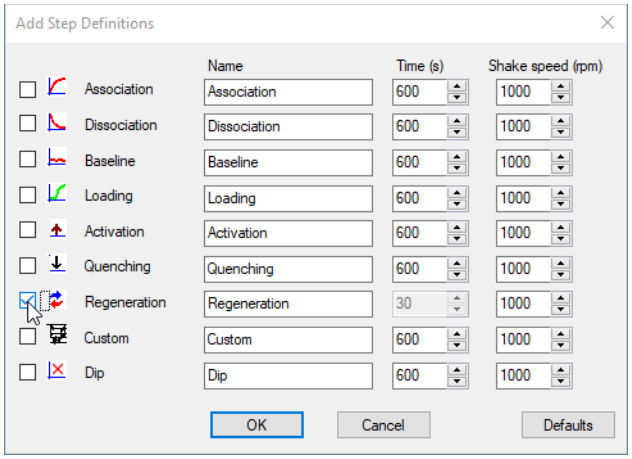


Figure 10-114: Add Step Definition Box

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:

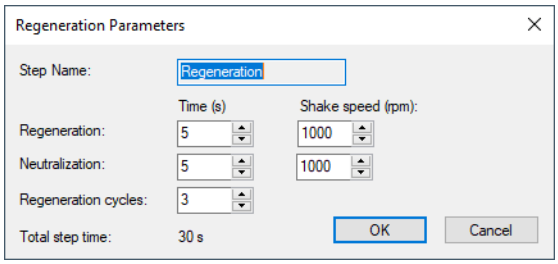


Figure 10-115: Regeneration Parameters Box

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:

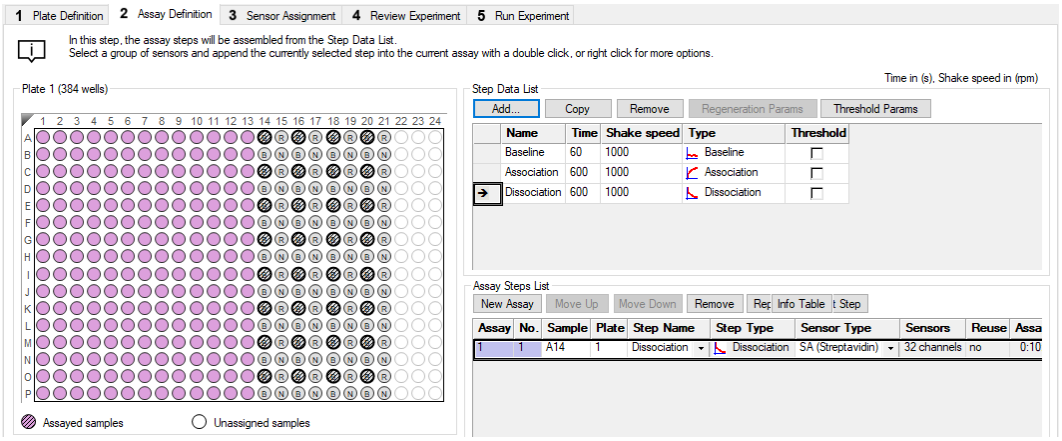


Figure 10-116: Assay Steps List

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:

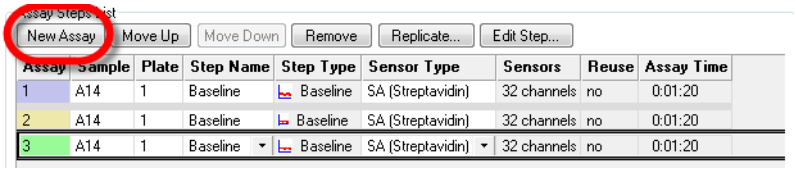


Figure 10-117: New Assay Button

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:

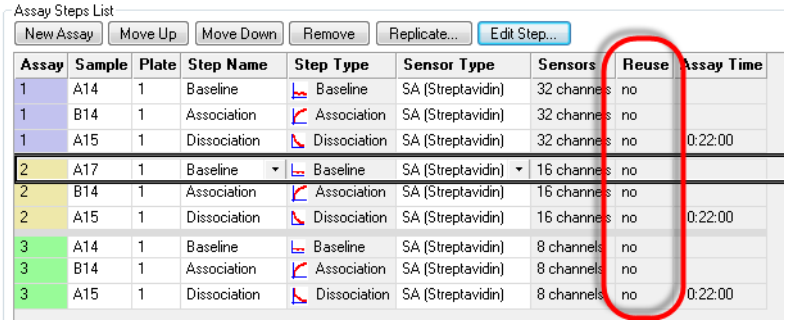


Figure 10-118: Default Biosensor Reuse Selection

NOTICE: *The Reuse option is available only for the Octet® 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.

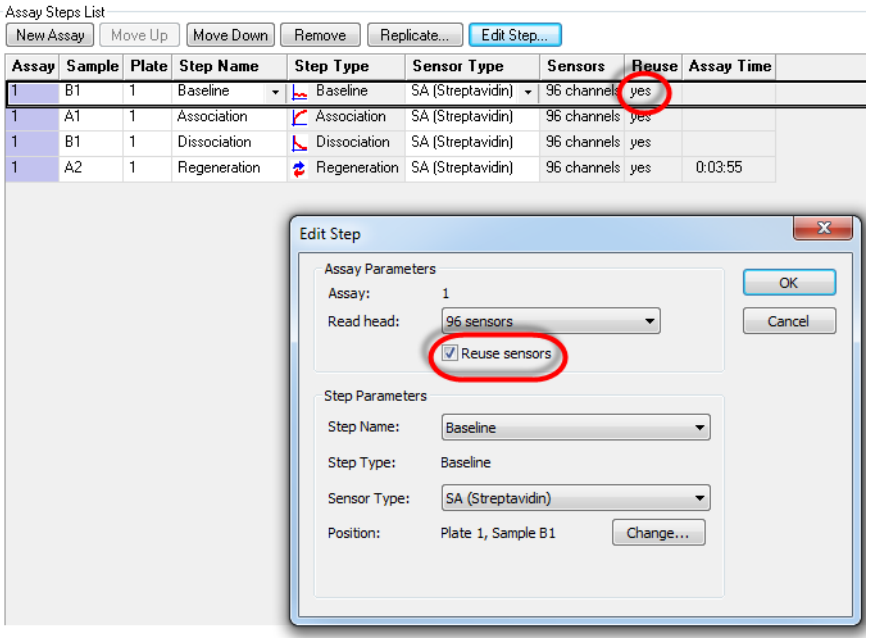


Figure 10-119: Changing the Biosensor Reuse Selection

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:

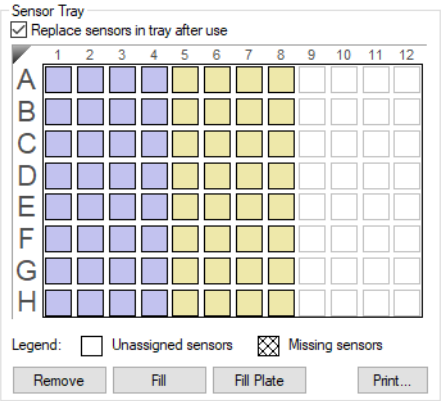


Figure 10-120: Replace Sensors in Tray After Use Check Box

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:

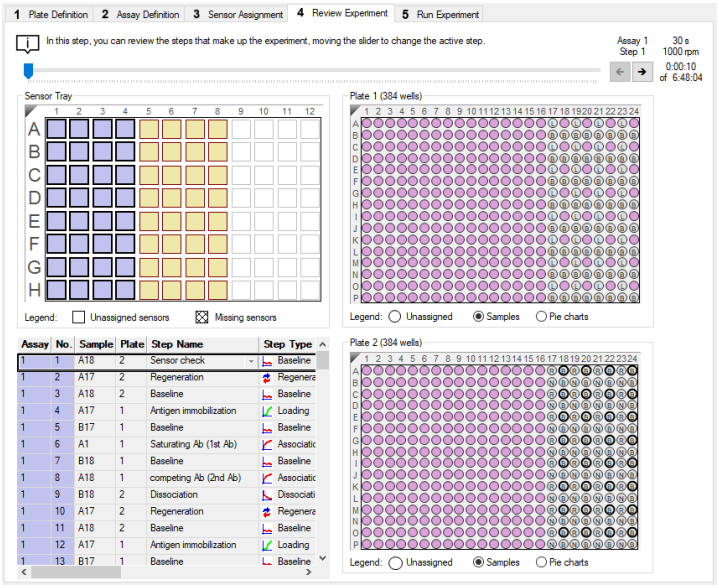


Figure 10-121: Navigating the Review Experiment Tab

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:

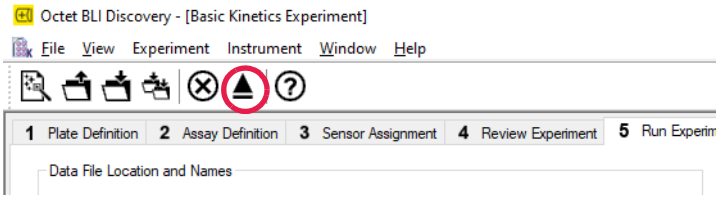


Figure 10-122: Present Stage Button

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:

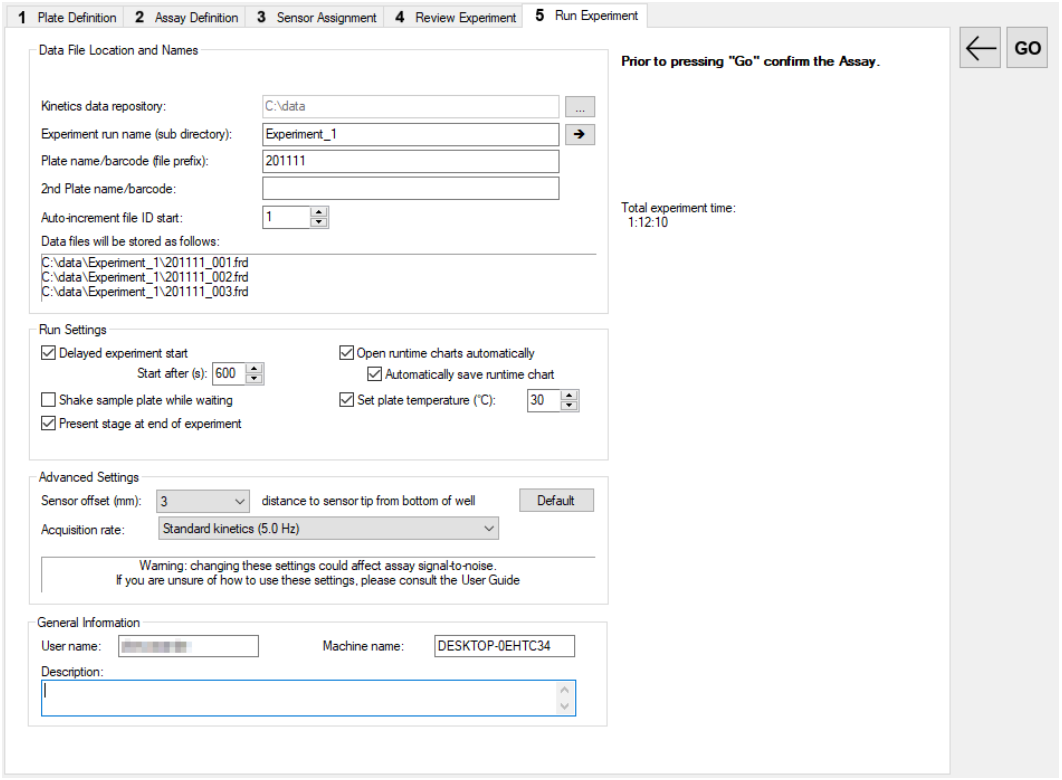


Figure 10-123: GO Button

Chapter 11:

Dose Response Experiments

Dose Response Analysis Overview

In an Octet® 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}).

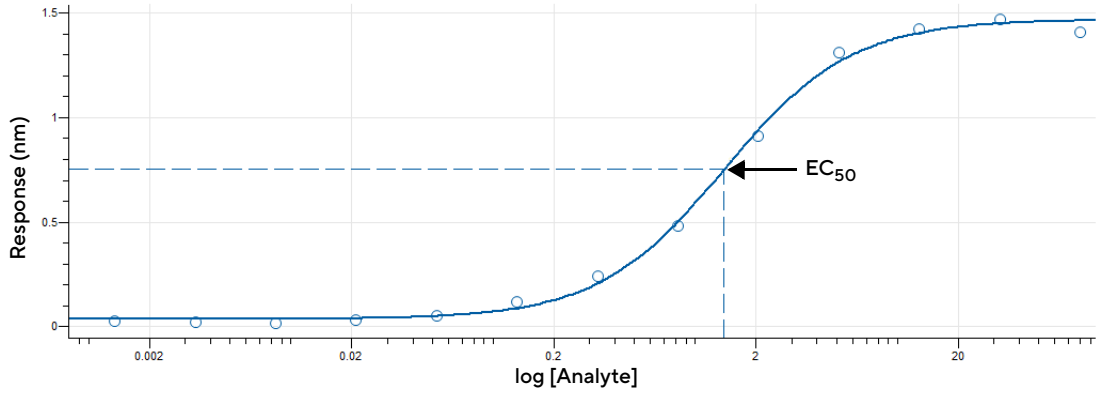


Figure 11-1: Determining EC_{50} from the Dose Response Curve

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® RH16 system, but the analysis remains largely the same regardless of the Octet® 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.

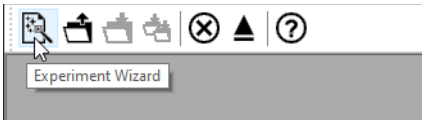


Figure 11-2: Starting the Experiment Wizard

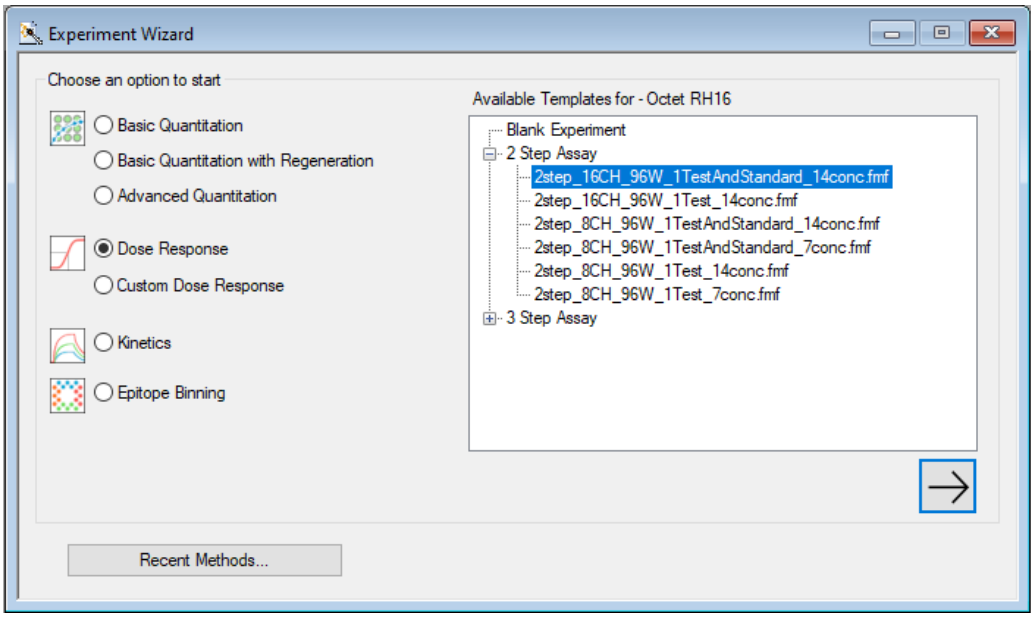


Figure 11-3: Experiment Wizard

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®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.

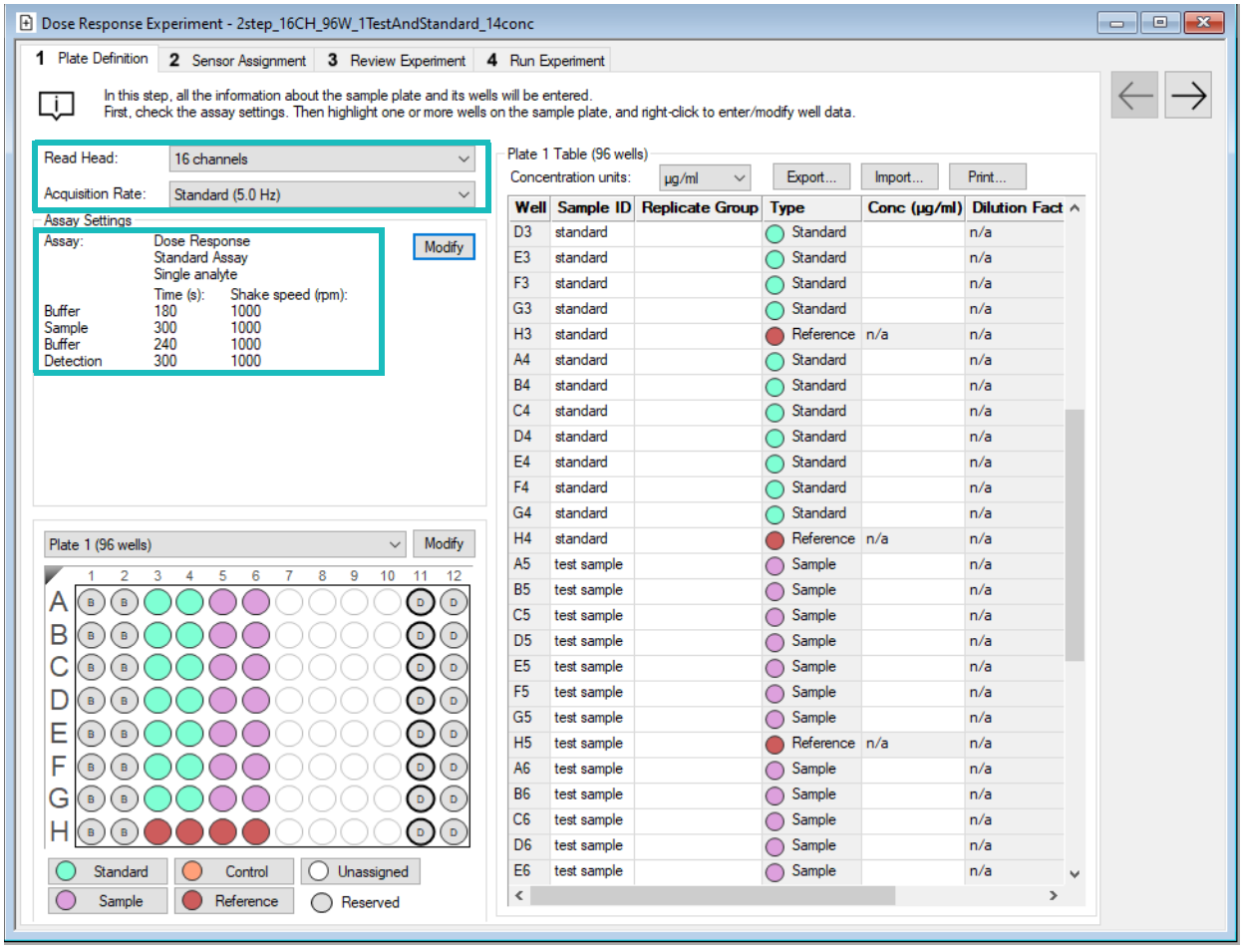


Figure 11-4: Plate Definition Tab Read Head and Assay Settings

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).

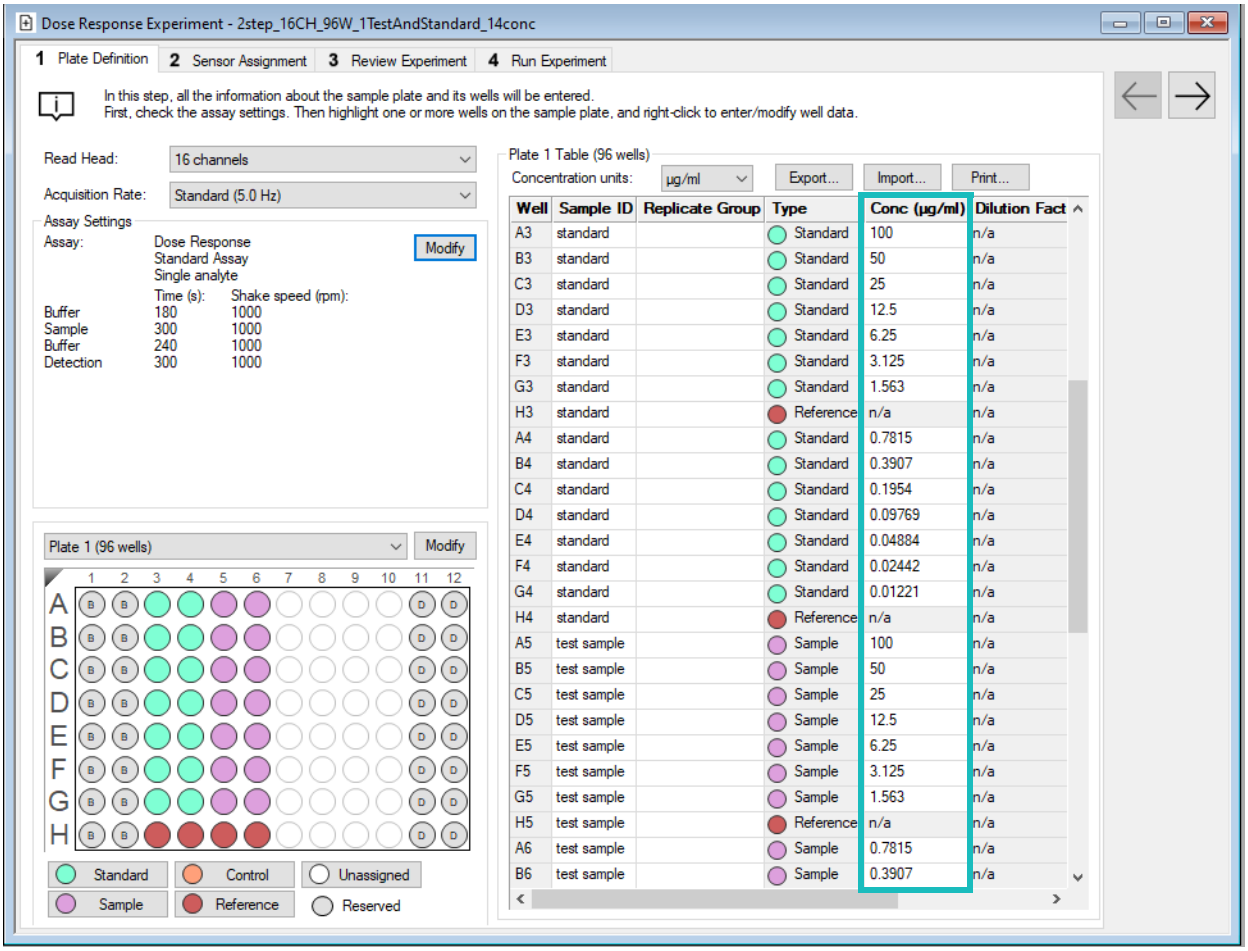


Figure 11-5: Plate Definition Tab Concentration

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 TNFα Lot 47. Using this, Octet® Analysis Studio Software can group all the related samples' data into one dose response curve fit. You should not put well-specific identifiers into the Sample ID or this will make it more difficult to group your related data for analysis. For example, IDs like TNFα 100 µg/mL, TNFα 50 µg/mL, TNFα 25 µg/mL, etc. add more work to the analysis because Octet®Analysis Studio Software will treat those samples as independent by default. If you wish to additional well-specific information, enter it into the table's **Information** column on the far right. Please see the Octet® 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.

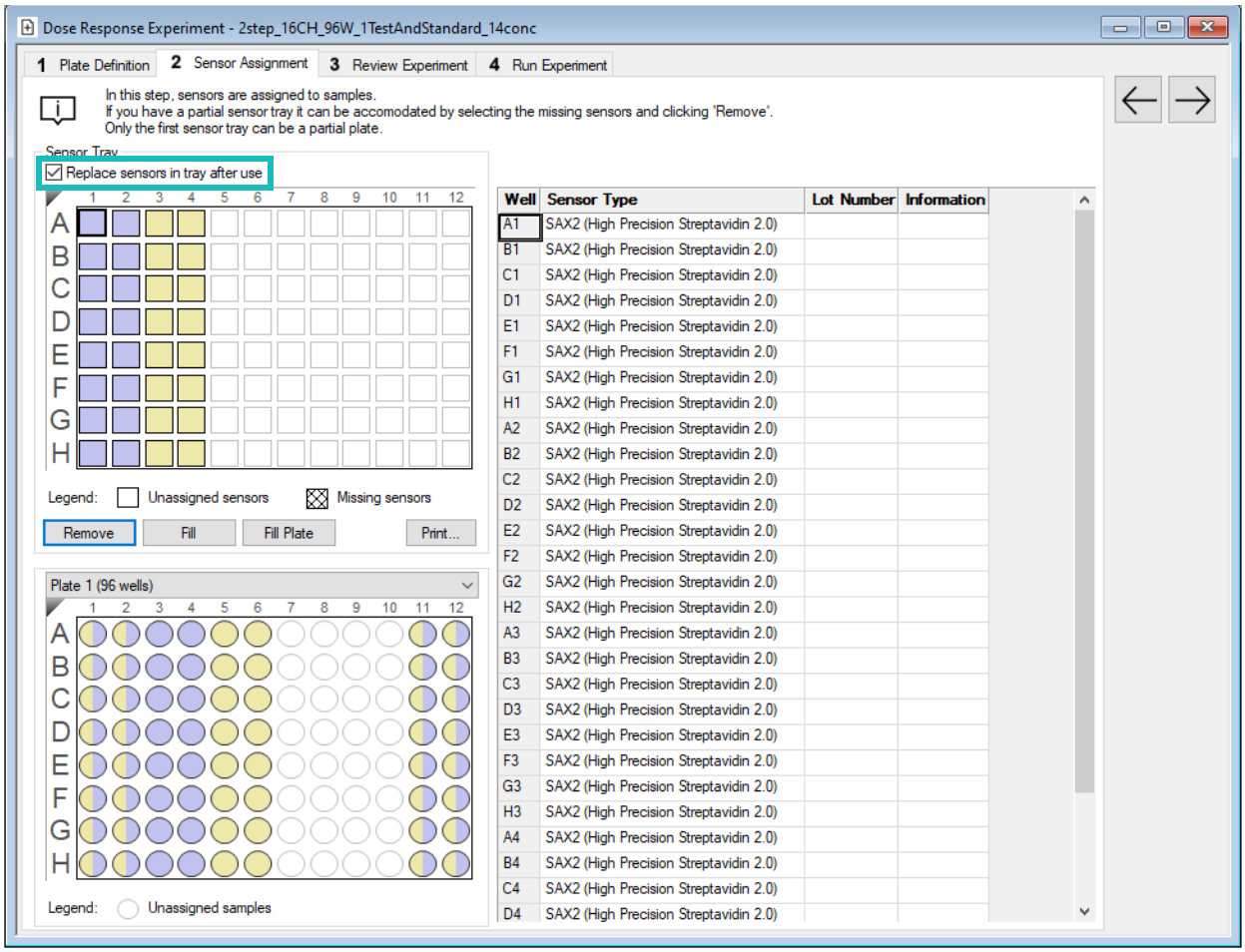


Figure 11-6: Selecting Biosensors for Reuse

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.

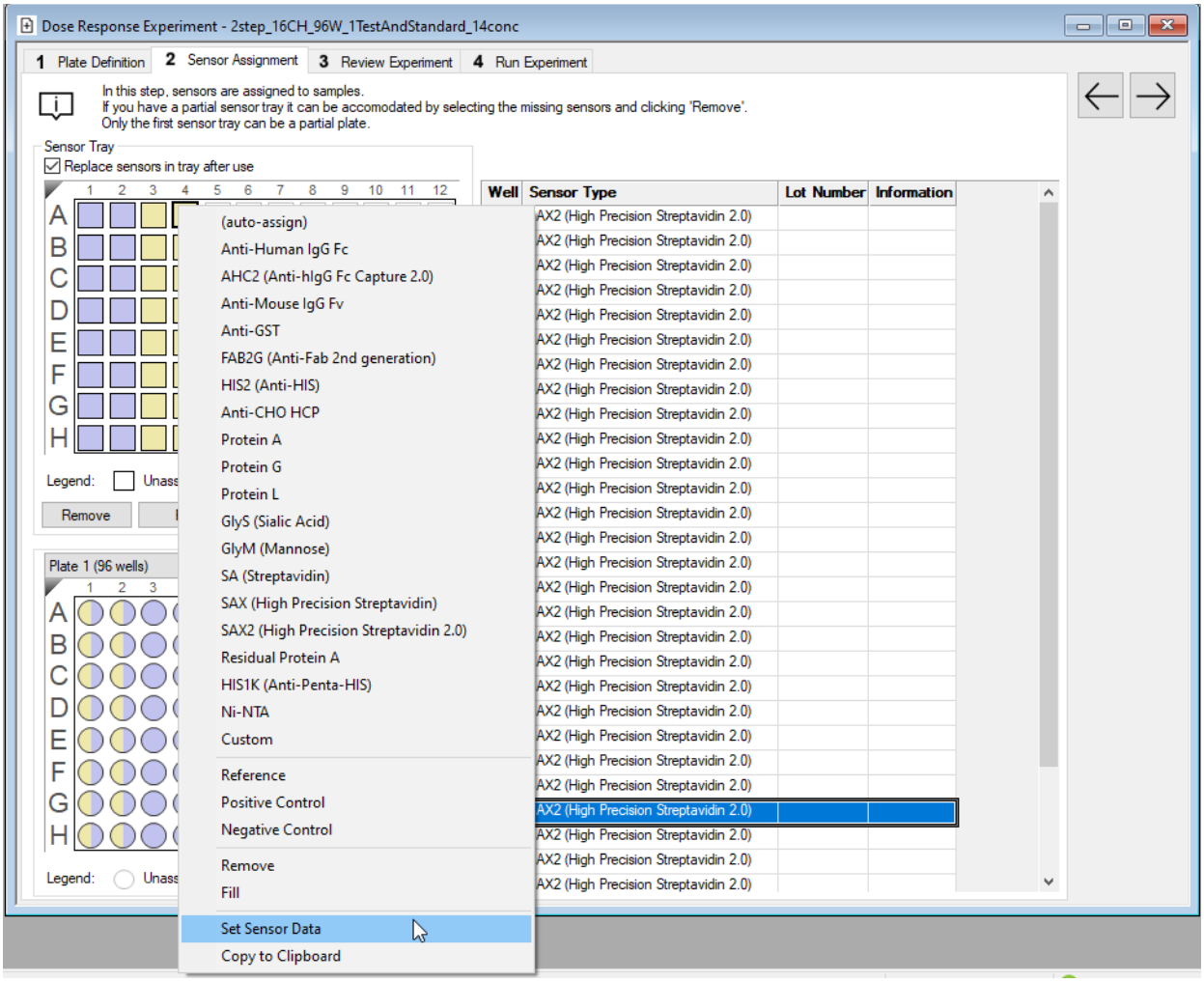


Figure 11-7: Setting Sensor Data

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 re-used 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**).

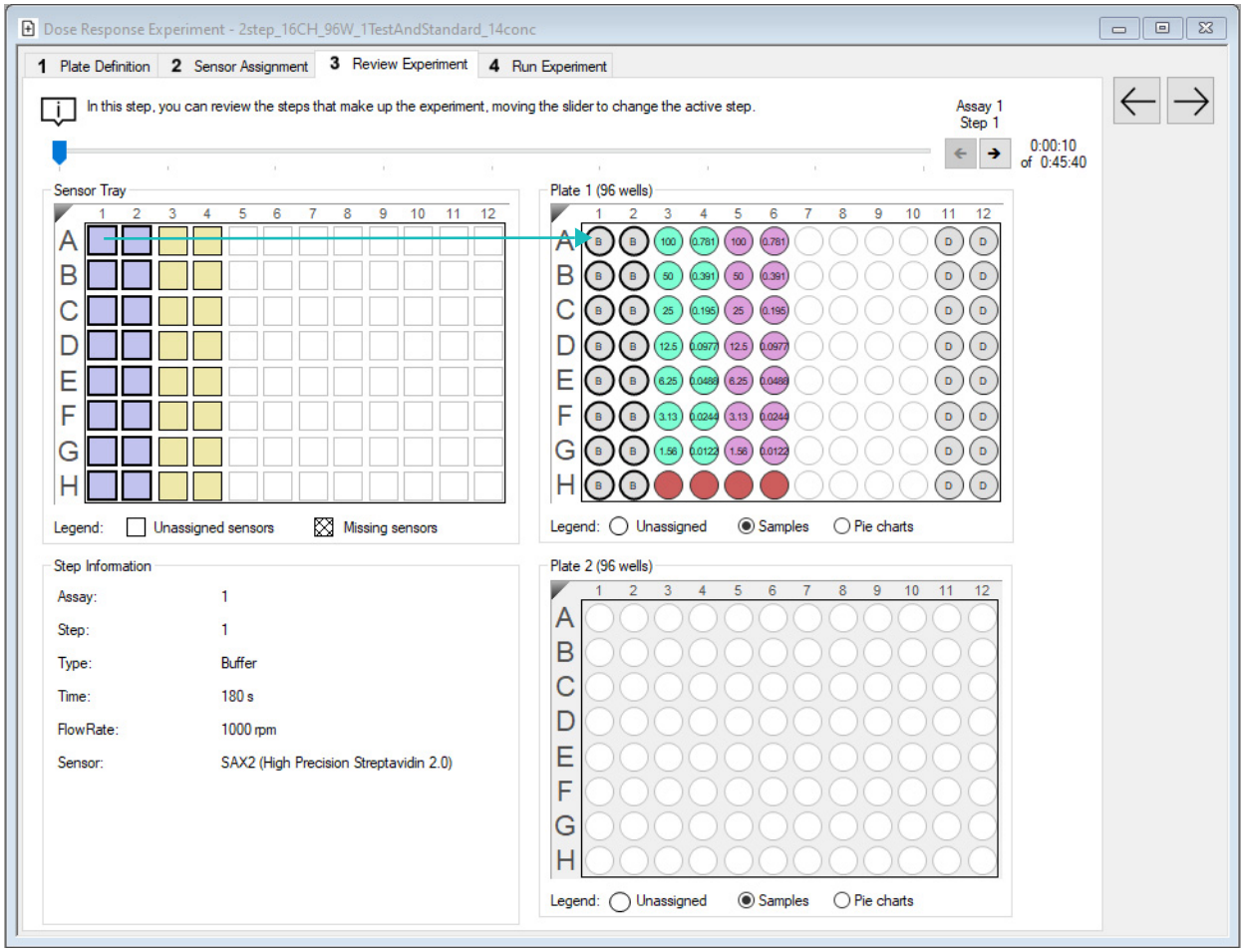


Figure 11-8: Review Experiment Tab

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.

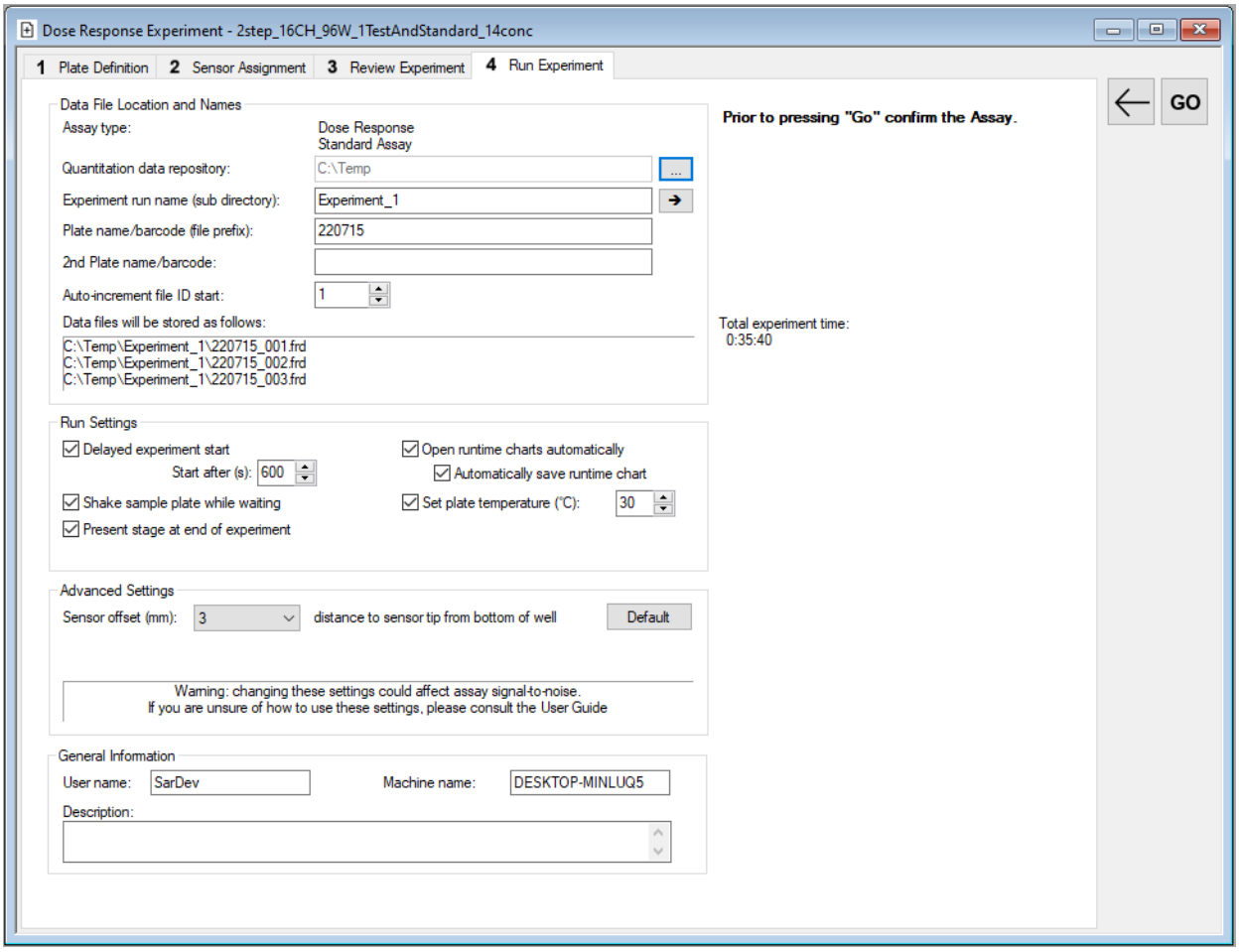


Figure 11-9: Run Experiment Tab

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.

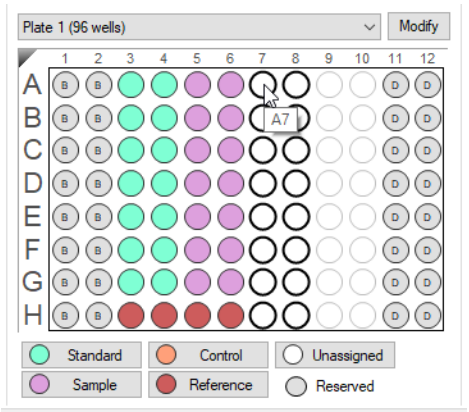


Figure 11-10: Adding Samples

While the cursor is over A7, right click to open the well pop-up menu and select **Sample**.

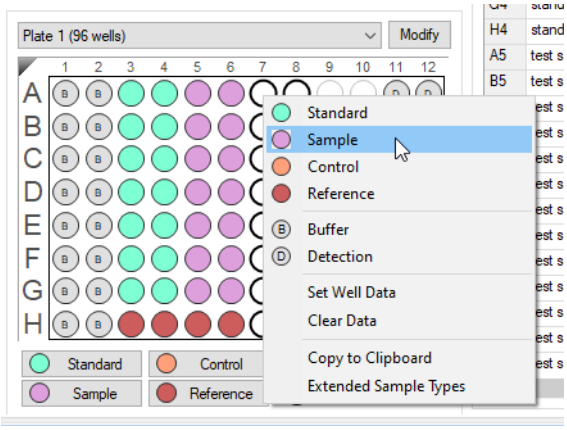


Figure 11-11: Setting Wells to Sample

All the unused wells in columns 7 and 8 are now samples (purple).

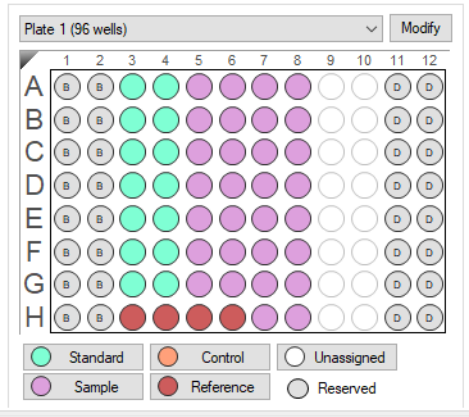


Figure 11-12: Sample Wells Set

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**.

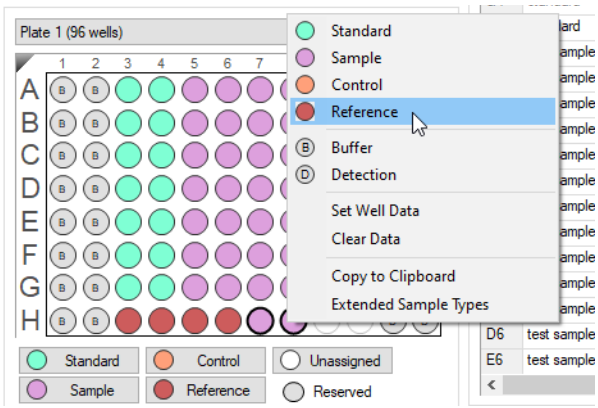


Figure 11-13: Setting Wells to Reference

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.

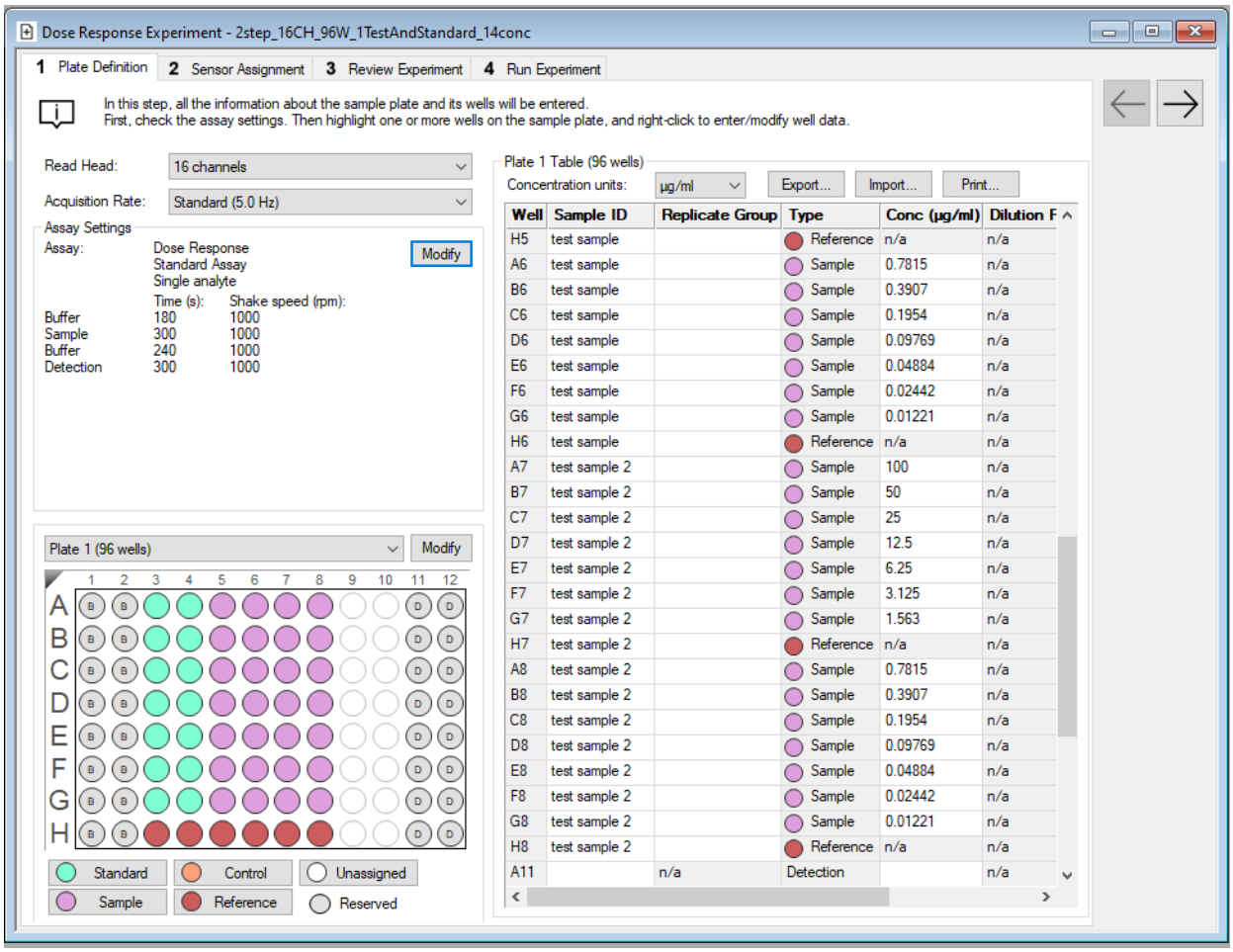


Figure 11-14: Updated Plate Definition Tab

Go to **Tab 2: Sensor Assignment** to see the additional biosensors (shown in green) used for the added assay.

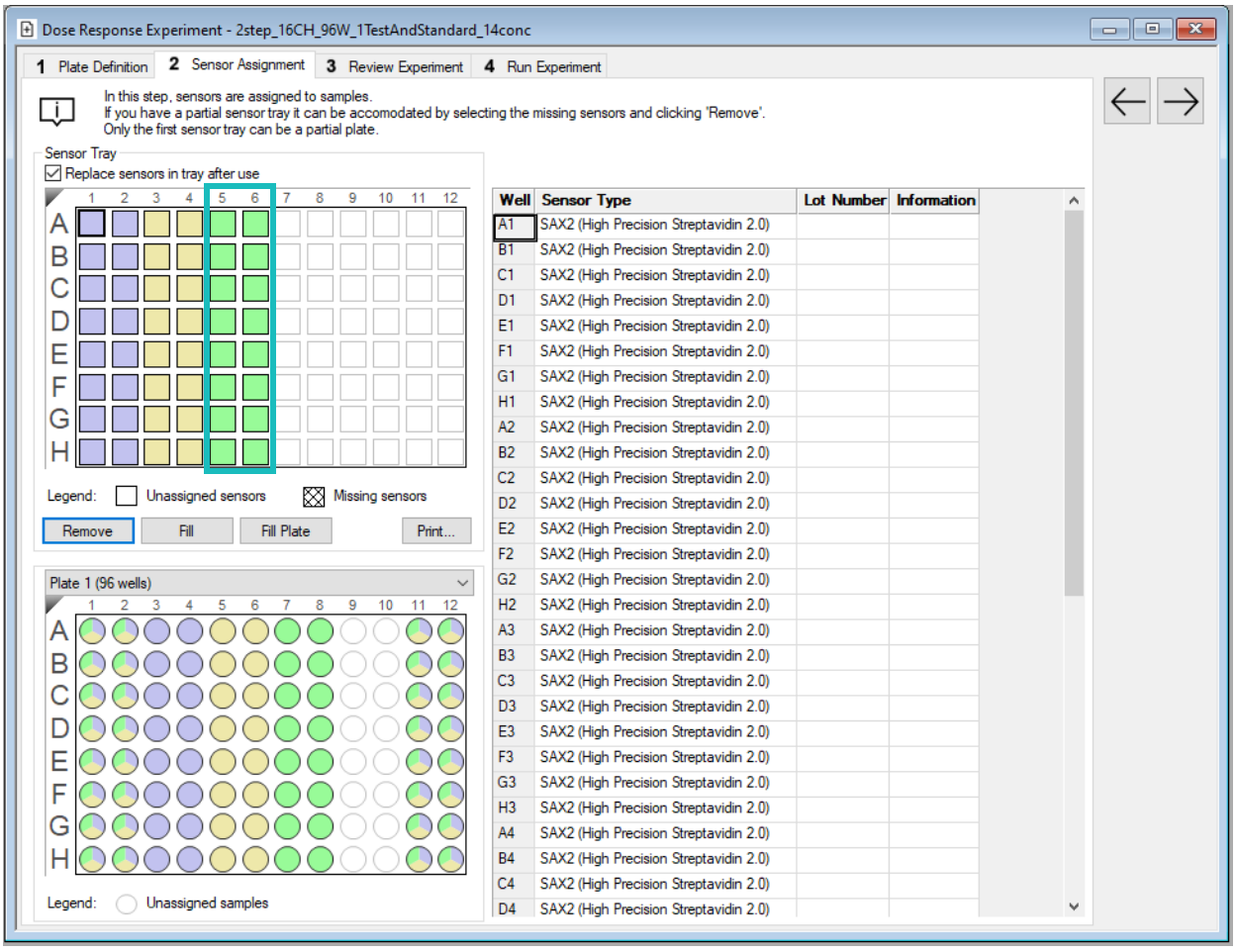


Figure 11-15: Updated Sensor Assignment Tab

Tab 3: Review Experiment also shows the additional biosensors (shown in green) used for the added assay.

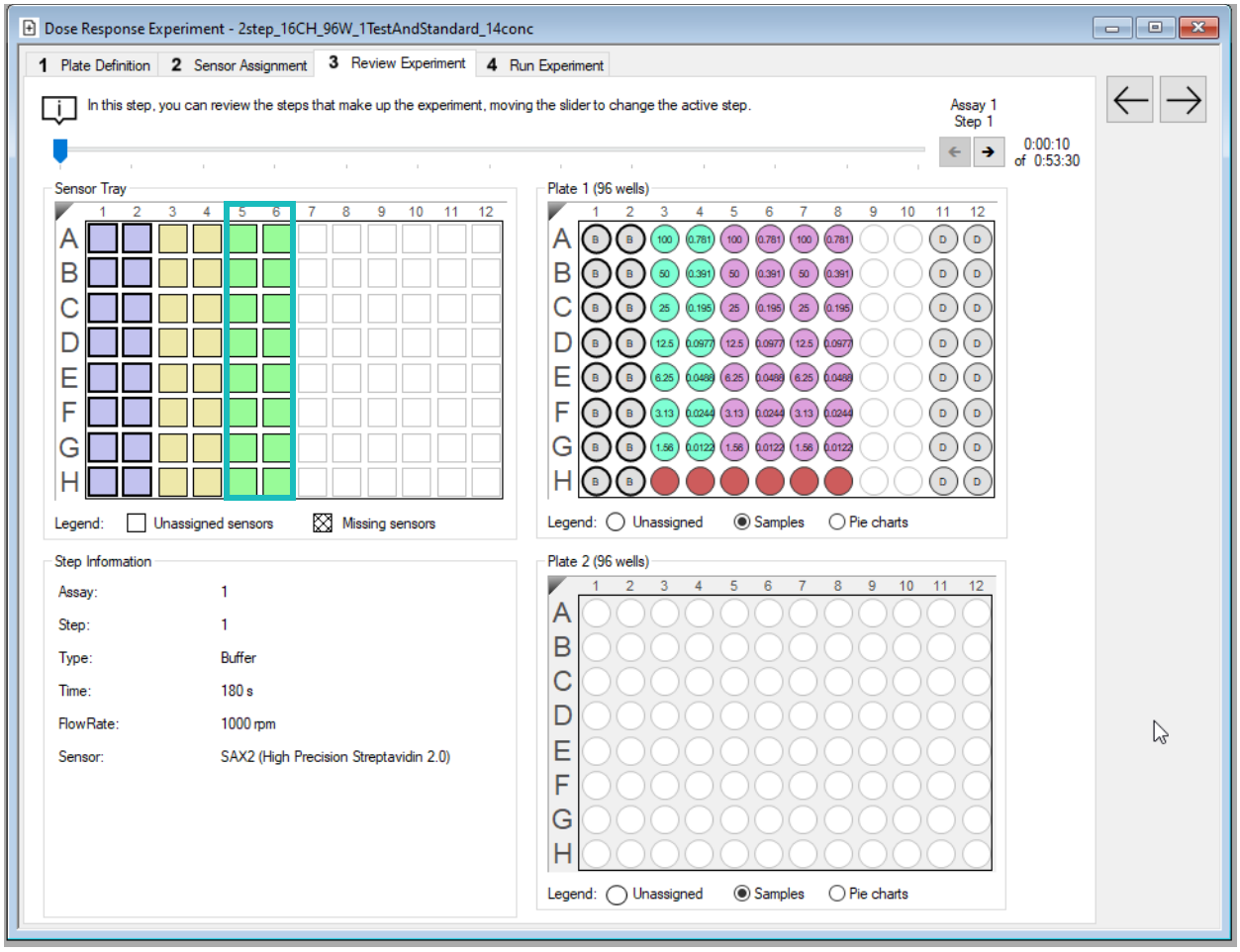


Figure 11-16: Updated Review Experiment Tab

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**.

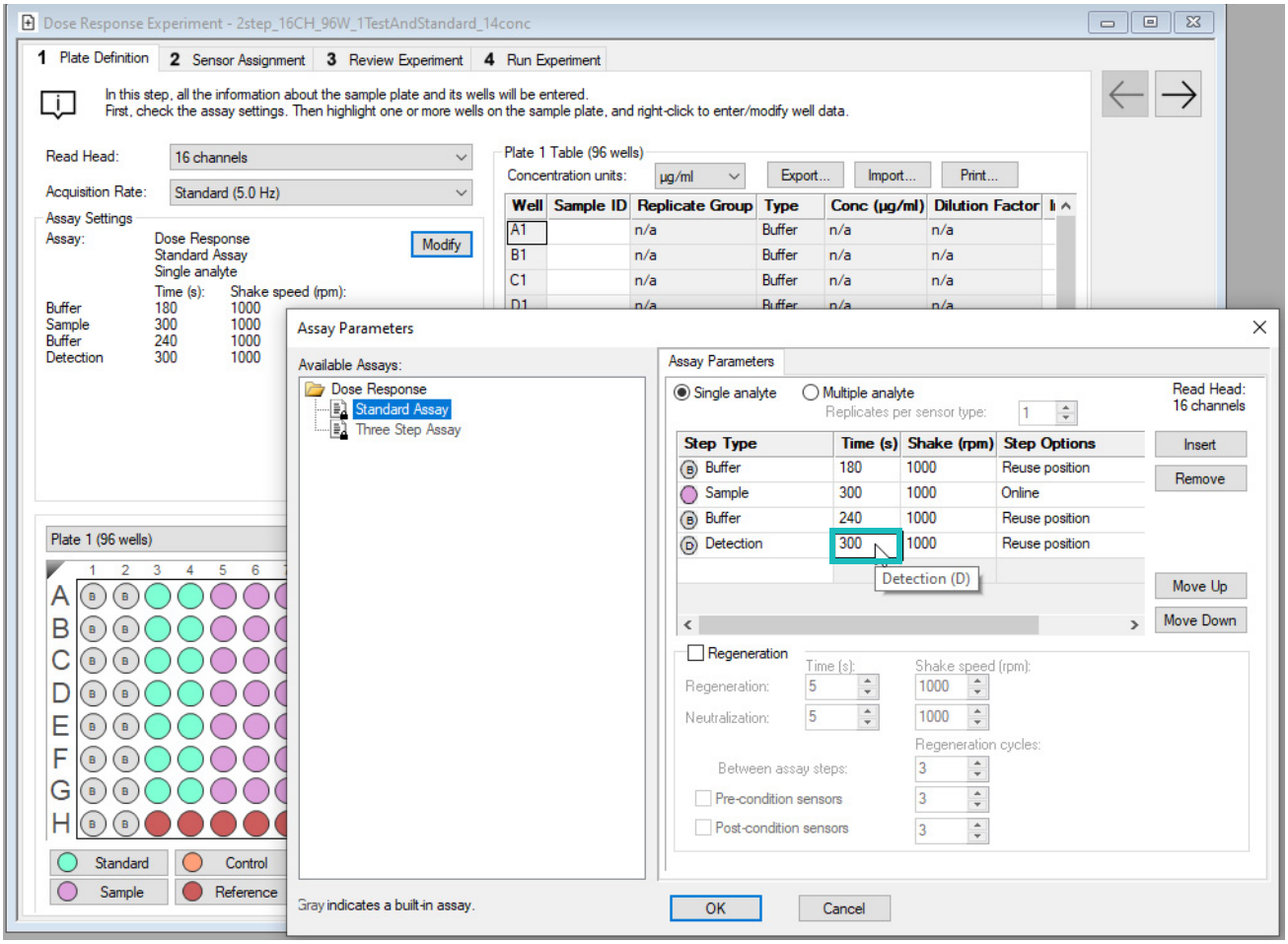


Figure 11-17: Changing the Detection Time

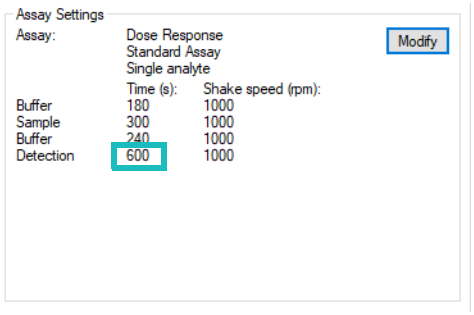


Figure 11-18: Assay Settings Showing Updated Detection Time

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.

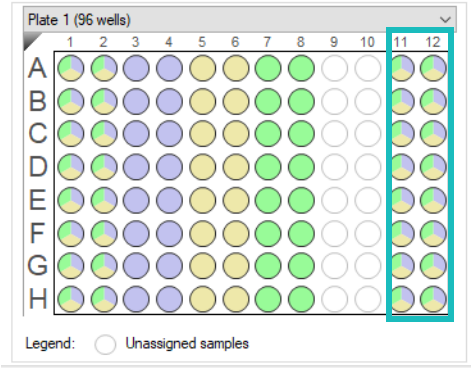


Figure 11-19: All Assays Use Detection Columns 11 and 12

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**.

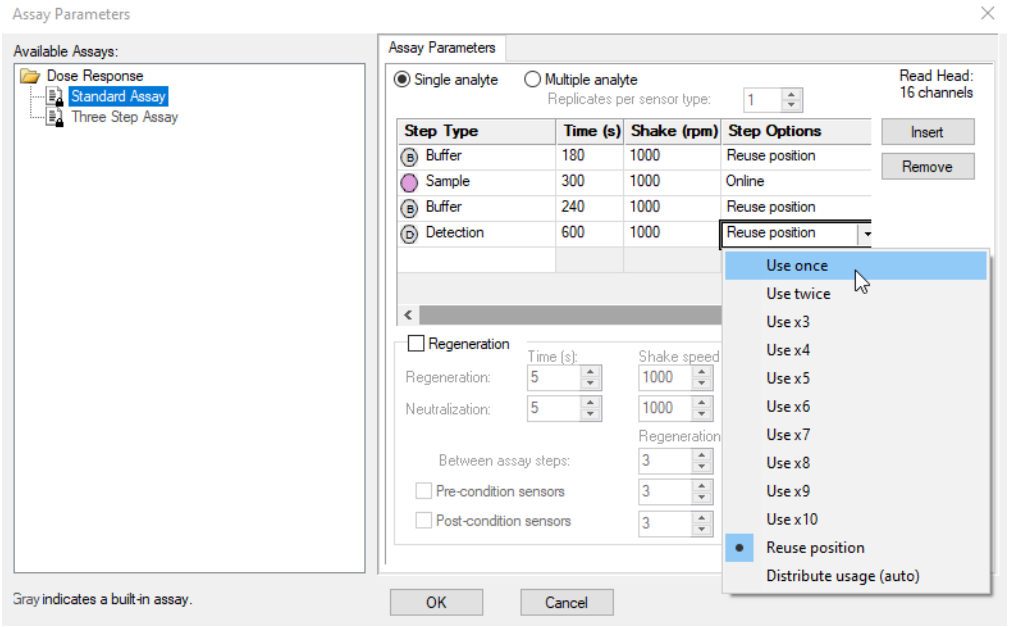


Figure 11-20: Selecting Use Once

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.

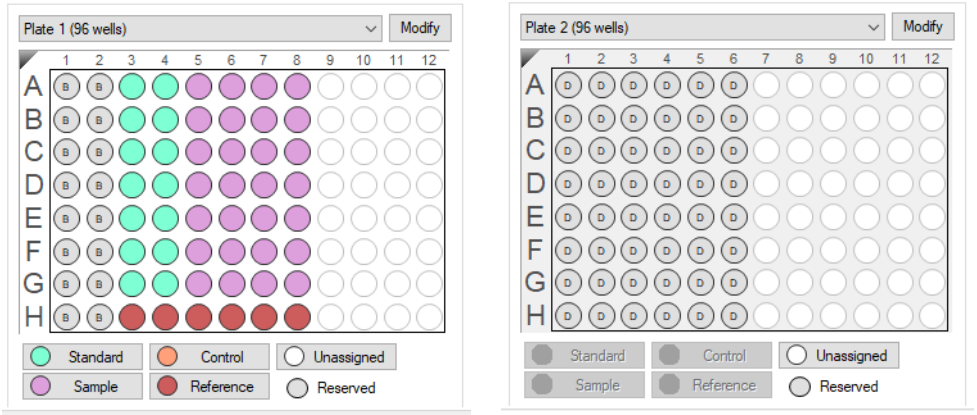


Figure 11-21: Final Layout of Plate 1 and 2 Showing 48 New Detection Wells

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.

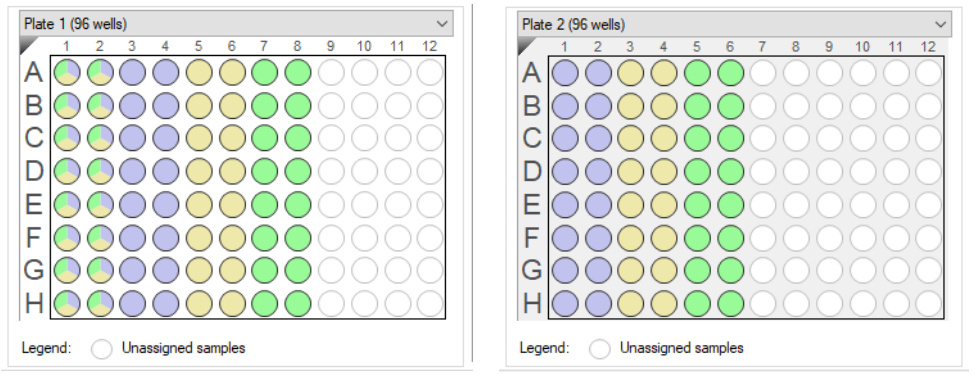


Figure 11-22: Detection Wells Showing Single Use in Plate 1, and All Wells in Plate 2 are Detection

Modifying a Template: Adding Replicates

To get a better, more statistically meaningful, determination of dose response parameters like EC_{50}, 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:

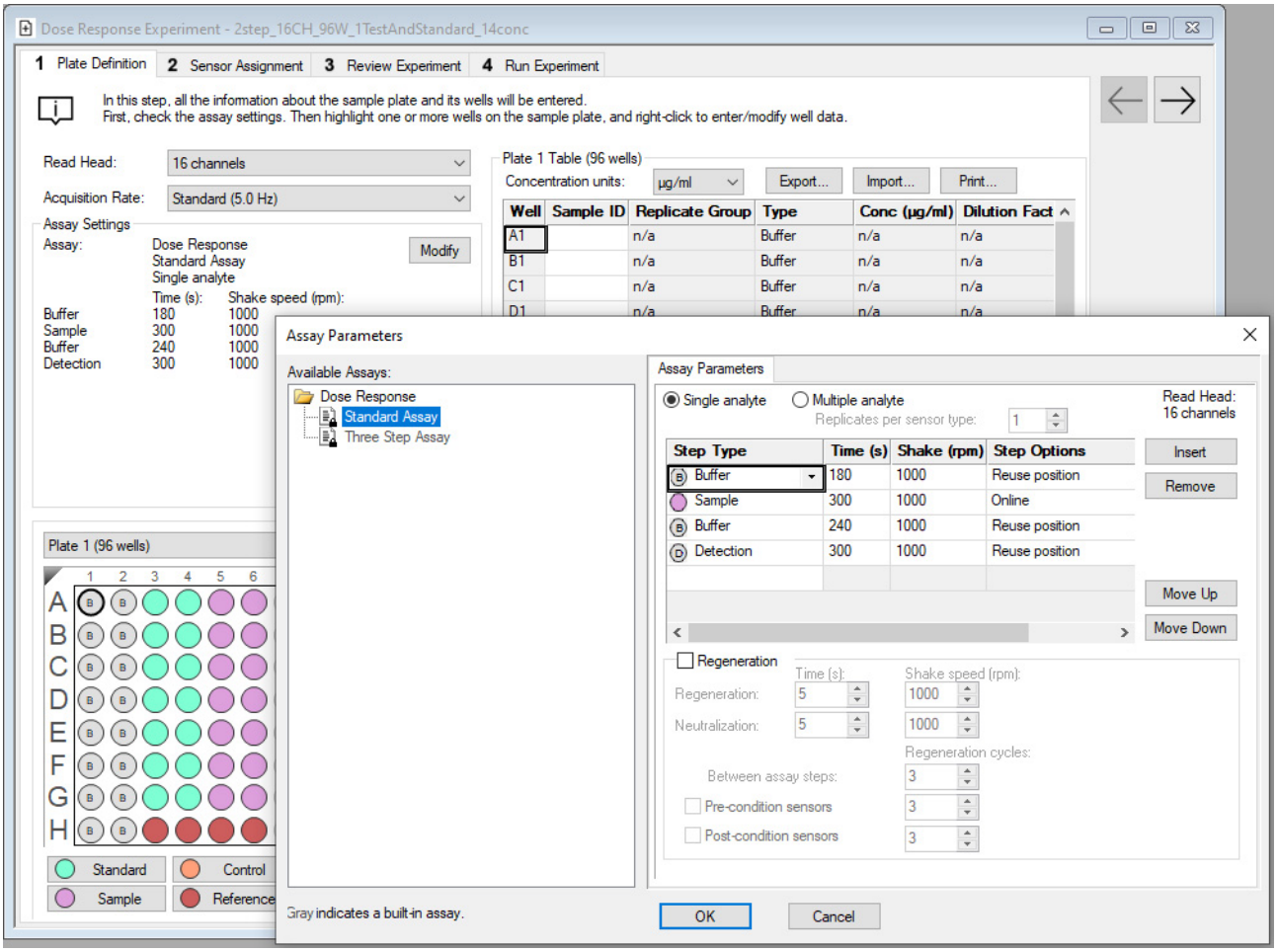


Figure 11-23: Assay Parameters Window

Select the **Multiple analyte** radio button, set the **Replicates per sensor type** to **3** and click **OK**.

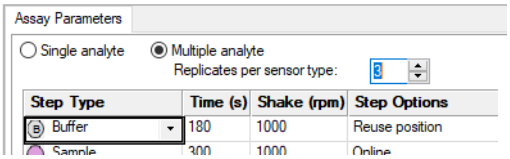


Figure 11-24: Setting Replicates Per Sensor Type

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.

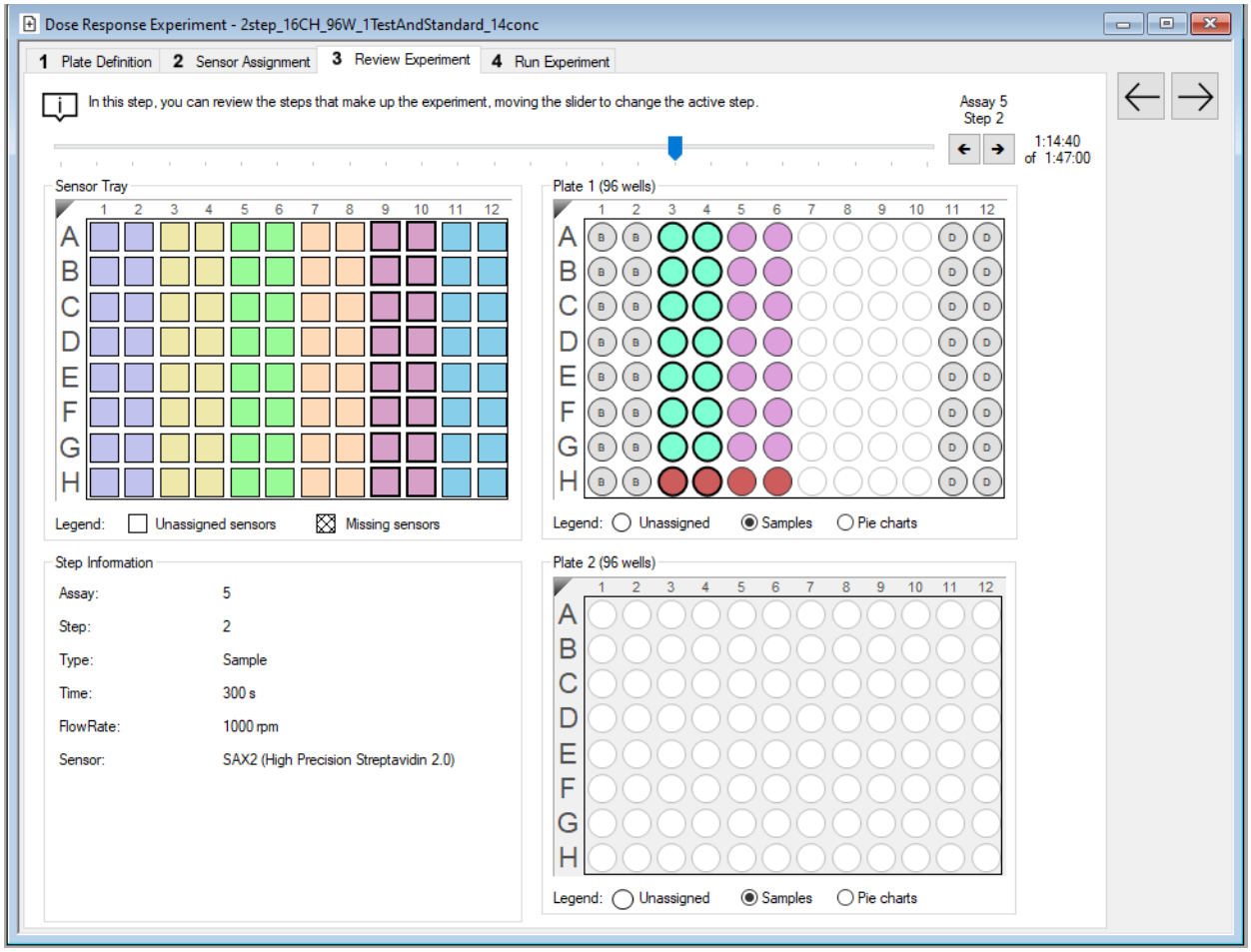


Figure 11-25: Experiment Using Replicates

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**.

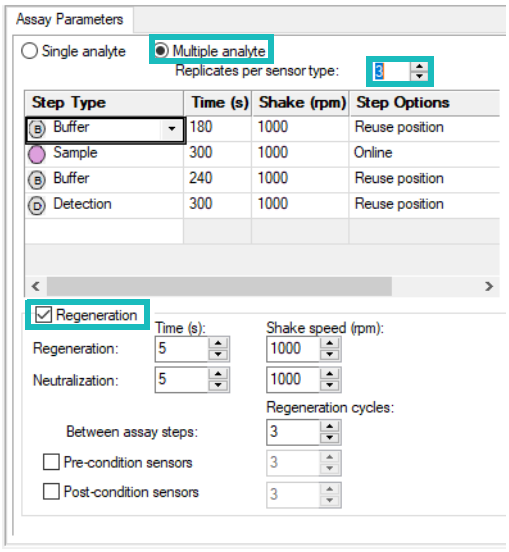


Figure 11-26: Assay Parameters with Multiple Analytes and Regeneration Selected

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.

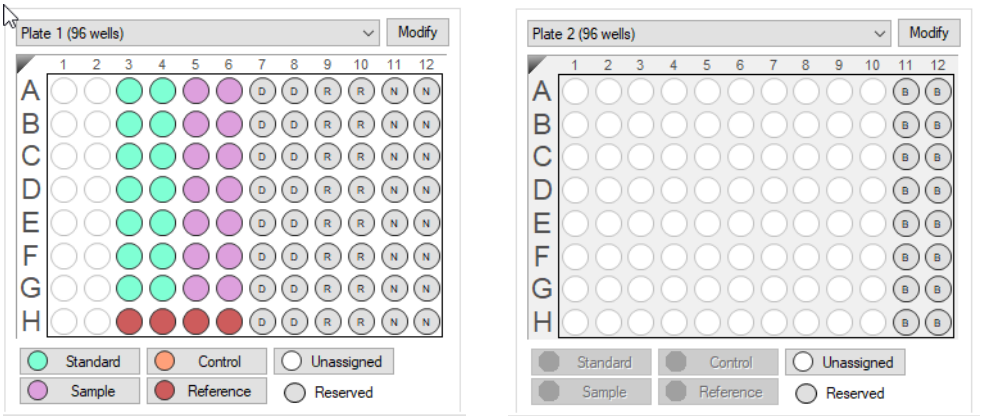


Figure 11-27: Updated Plate Layouts

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.

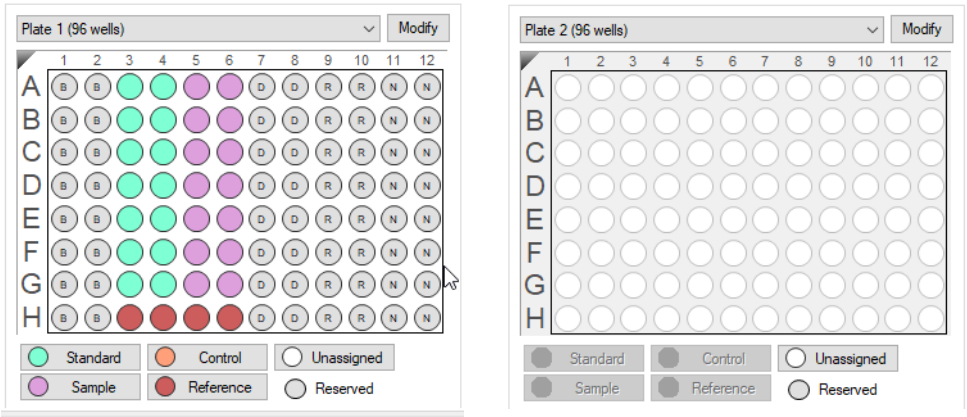


Figure 11-28: Buffer Wells Moved to Plate 1

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**.

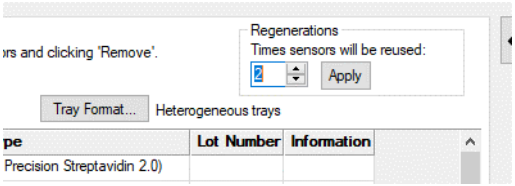


Figure 11-29: Setting Number of Times Sensors Will be Reused

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.

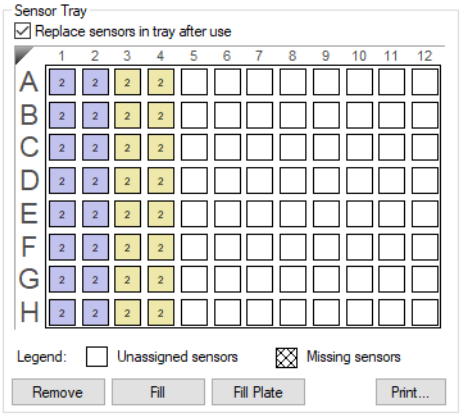


Figure 11-30: Sensor Tray Showing Biosensors are Regenerated Twice

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.

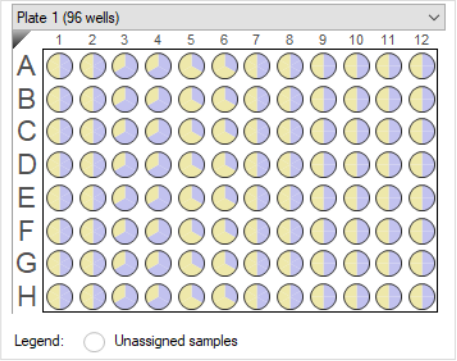


Figure 11-31: Plate Showing Biosensor Use

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® 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.

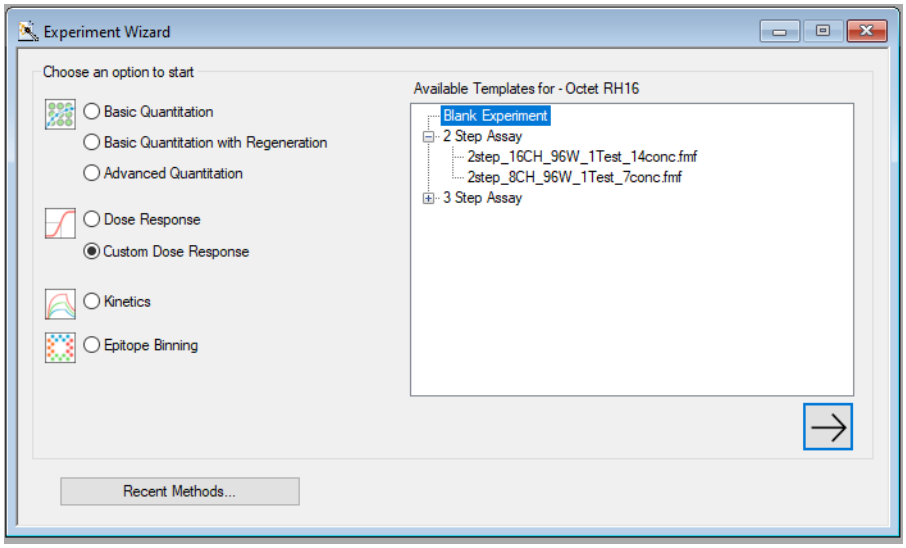


Figure 11-32: Selecting the Custom Dose Response Wizard

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.

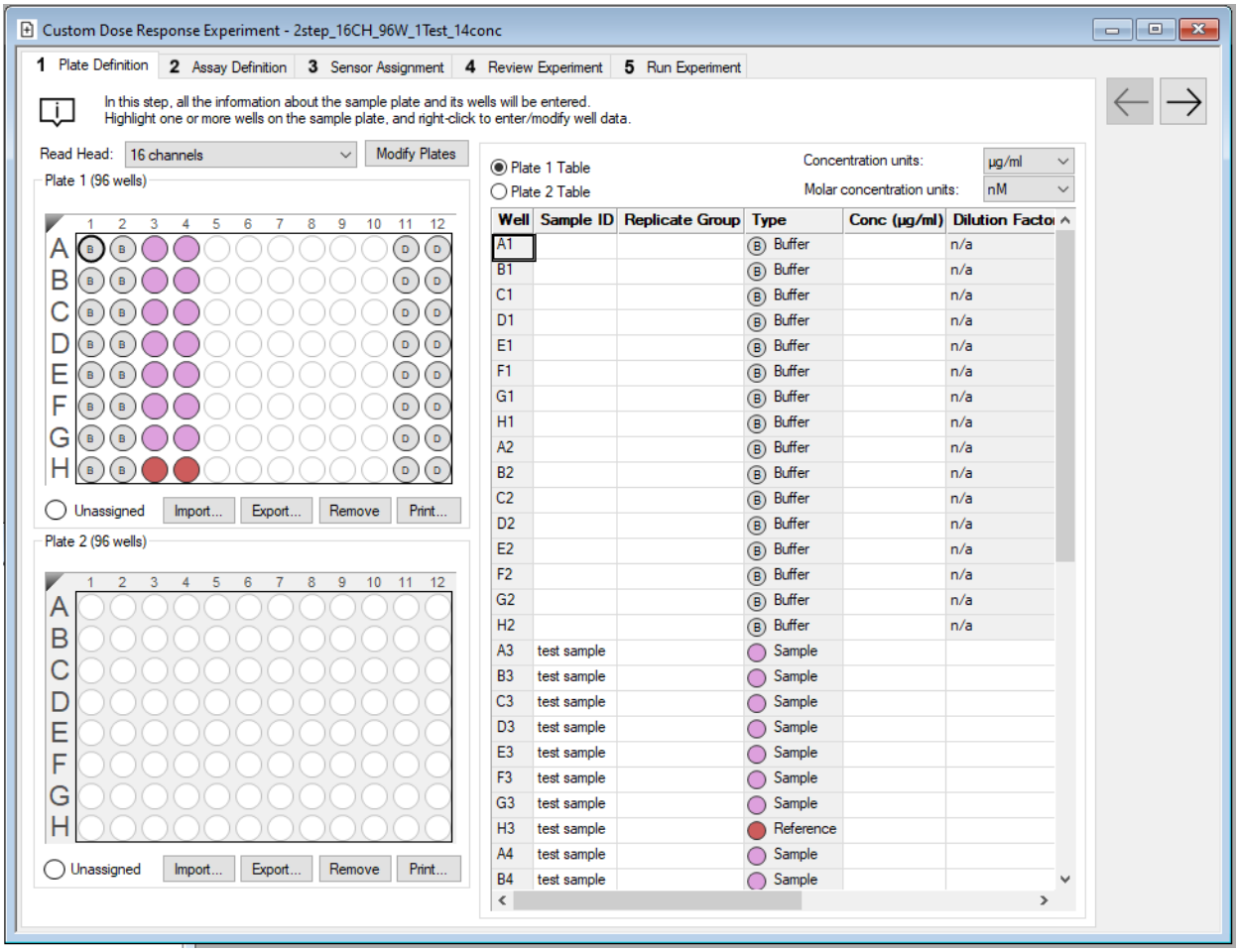


Figure 11-33: Basic Custom Dose Response Template

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.

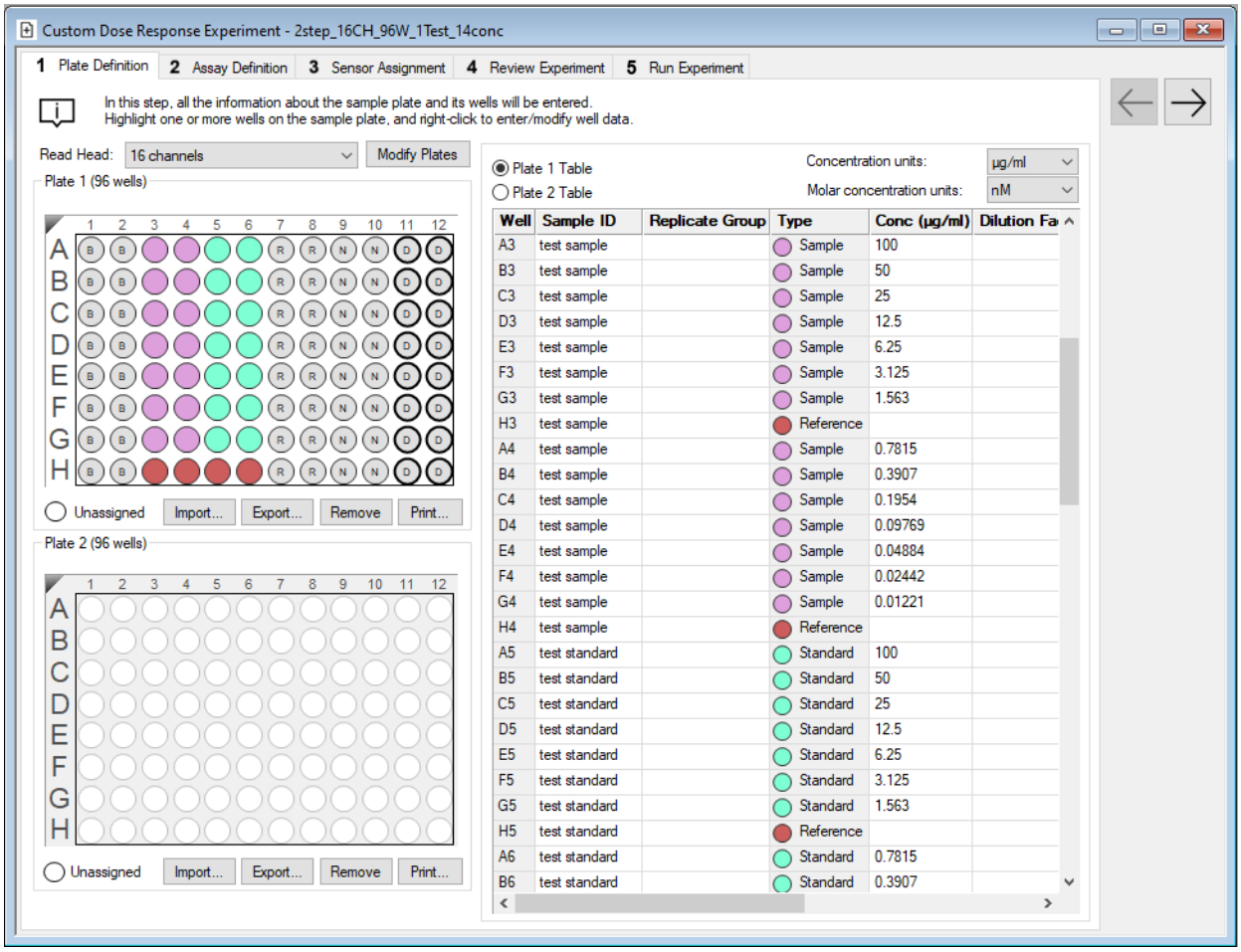


Figure 11-34: Plate Definition After Adding Standards with References, Regeneration and Neutralization Wells

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.

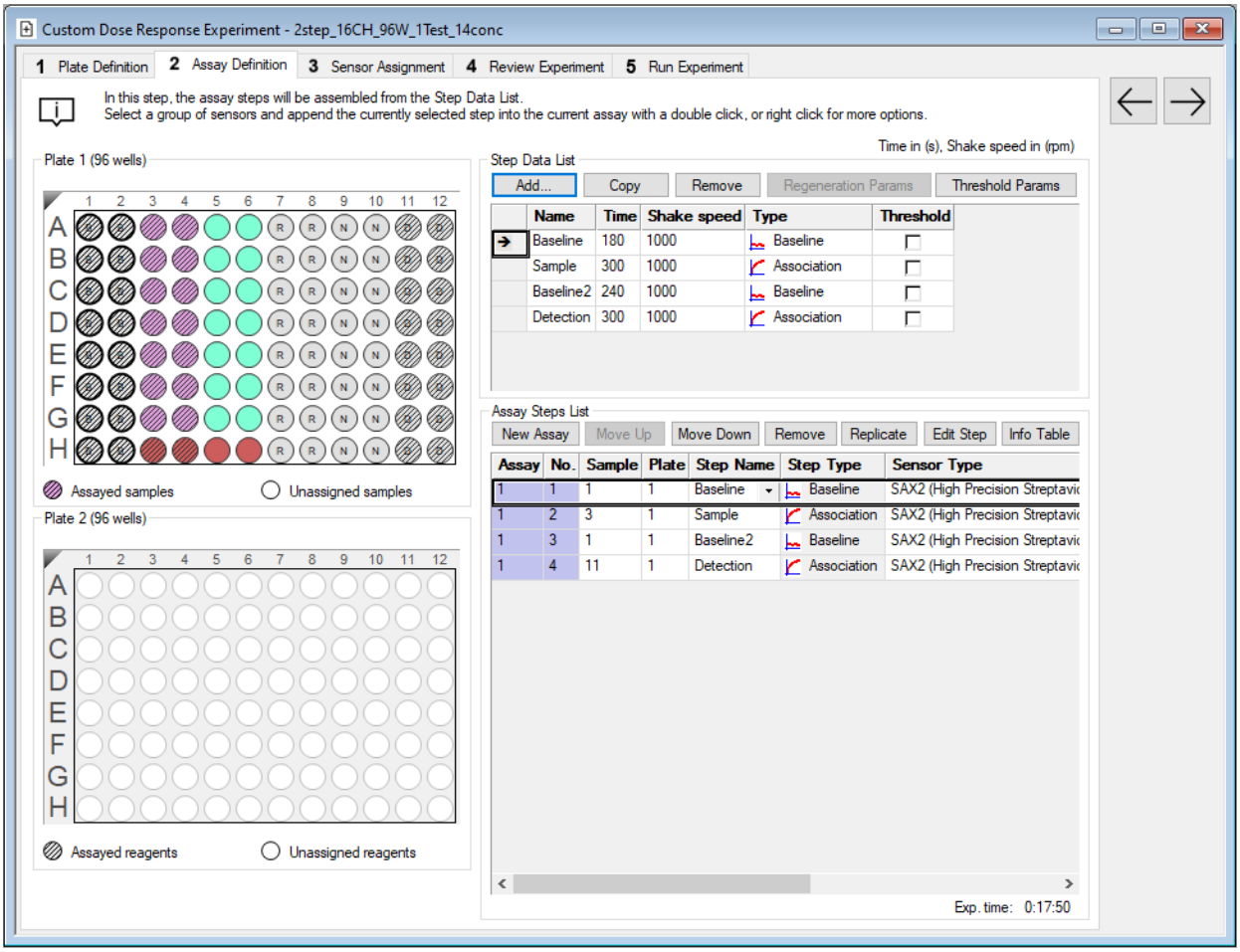


Figure 11-35: Assay Definition Tab Showing Striped Wells Used by One or More Assays

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.

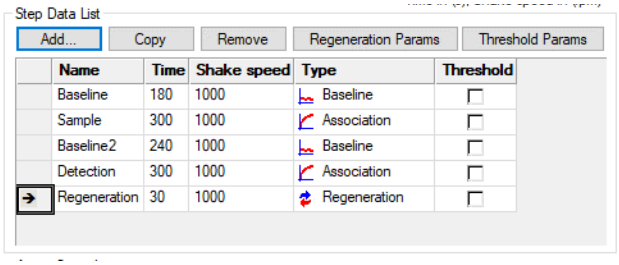


Figure 11-36: Adding a Regeneration Step

Check the regeneration settings by clicking the **Regeneration Params** button.

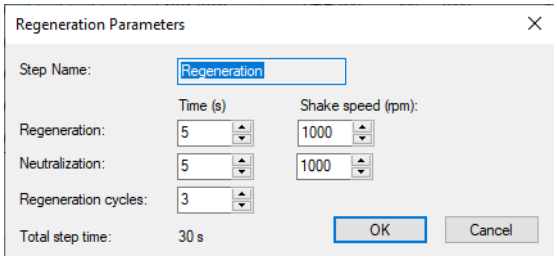


Figure 11-37: Regeneration Parameters

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.

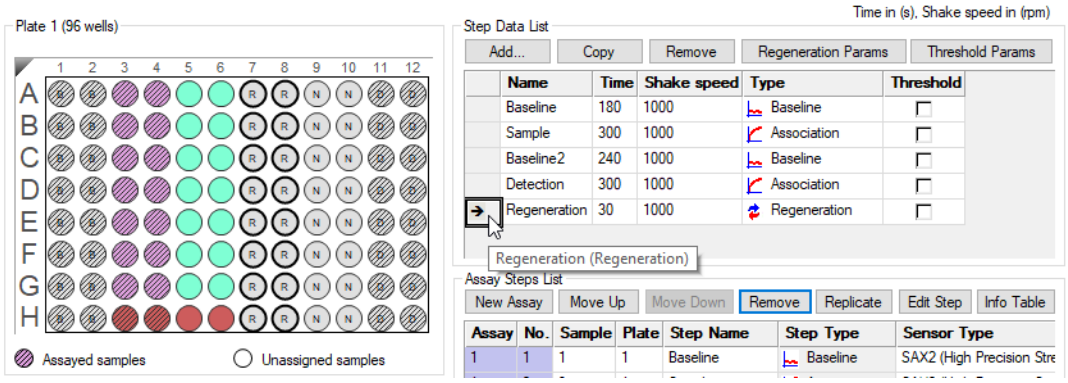


Figure 11-38: Associating a Step to a Well

You will now see a regeneration step in the assay and all the regeneration and neutralization wells will be striped.

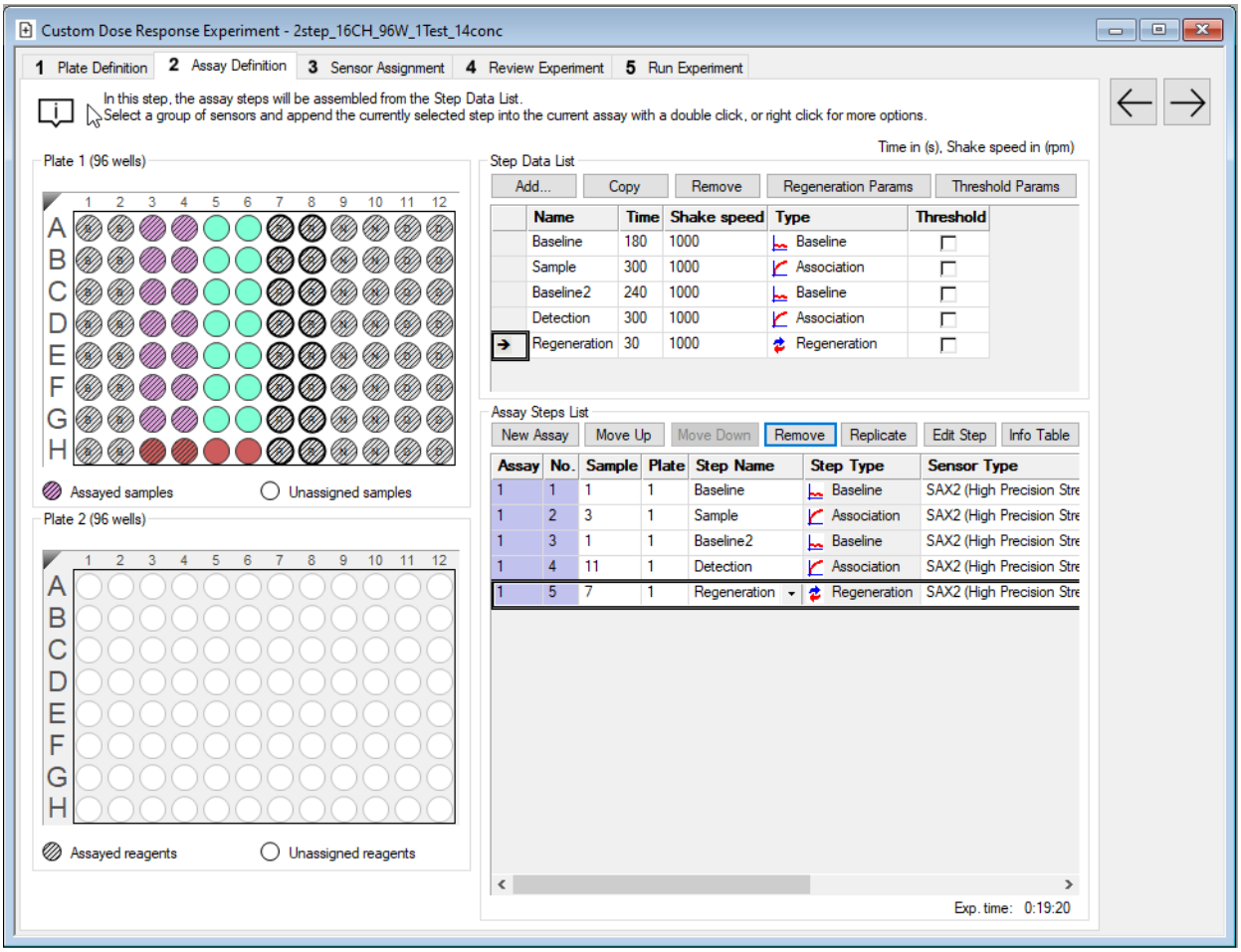


Figure 11-39: Sensor Regeneration Added

This represents one measurement of the set of samples. To add replicates, select all the steps of this assay and click the **Replicate** button.

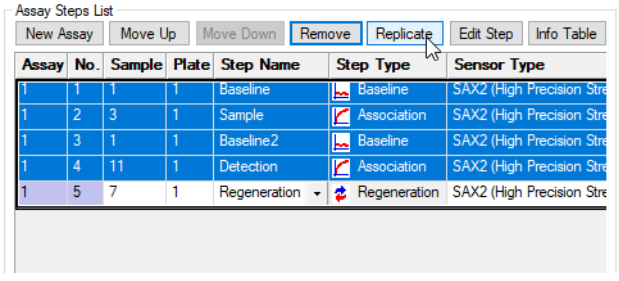


Figure 11-40: Adding Replicates

In the Replicate Step dialog, select the **Append to current assay** radio button and click **OK**.

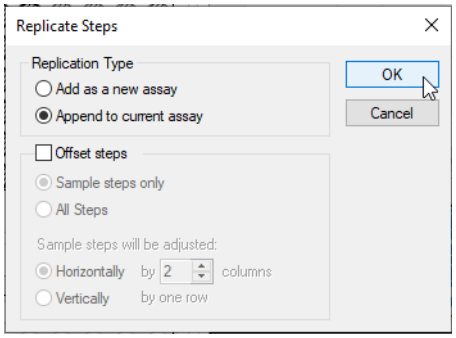
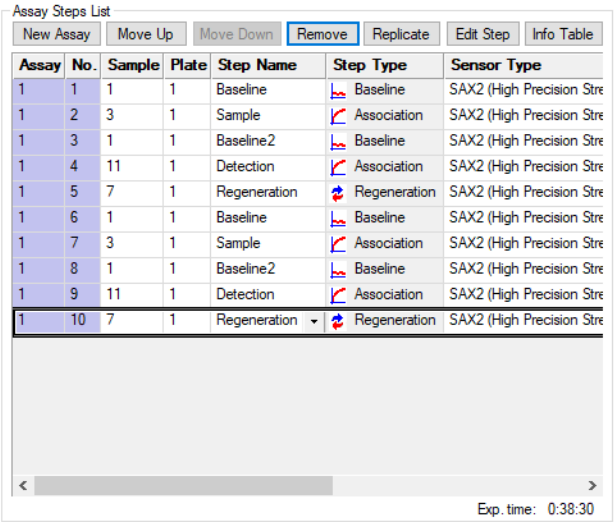


Figure 11-41: Replicate Steps Dialog

Now you see that the samples are measured twice.



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
1	6	1	1	Baseline	Baseline	SAX2 (High Precision Stre
1	7	3	1	Sample	Association	SAX2 (High Precision Stre
1	8	1	1	Baseline2	Baseline	SAX2 (High Precision Stre
1	9	11	1	Detection	Association	SAX2 (High Precision Stre
1	10	7	1	Regeneration	Regeneration	SAX2 (High Precision Stre

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.

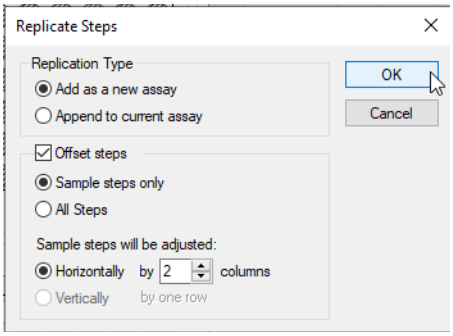


Figure 11-43: Adding Replicated Steps as a New Assay

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.

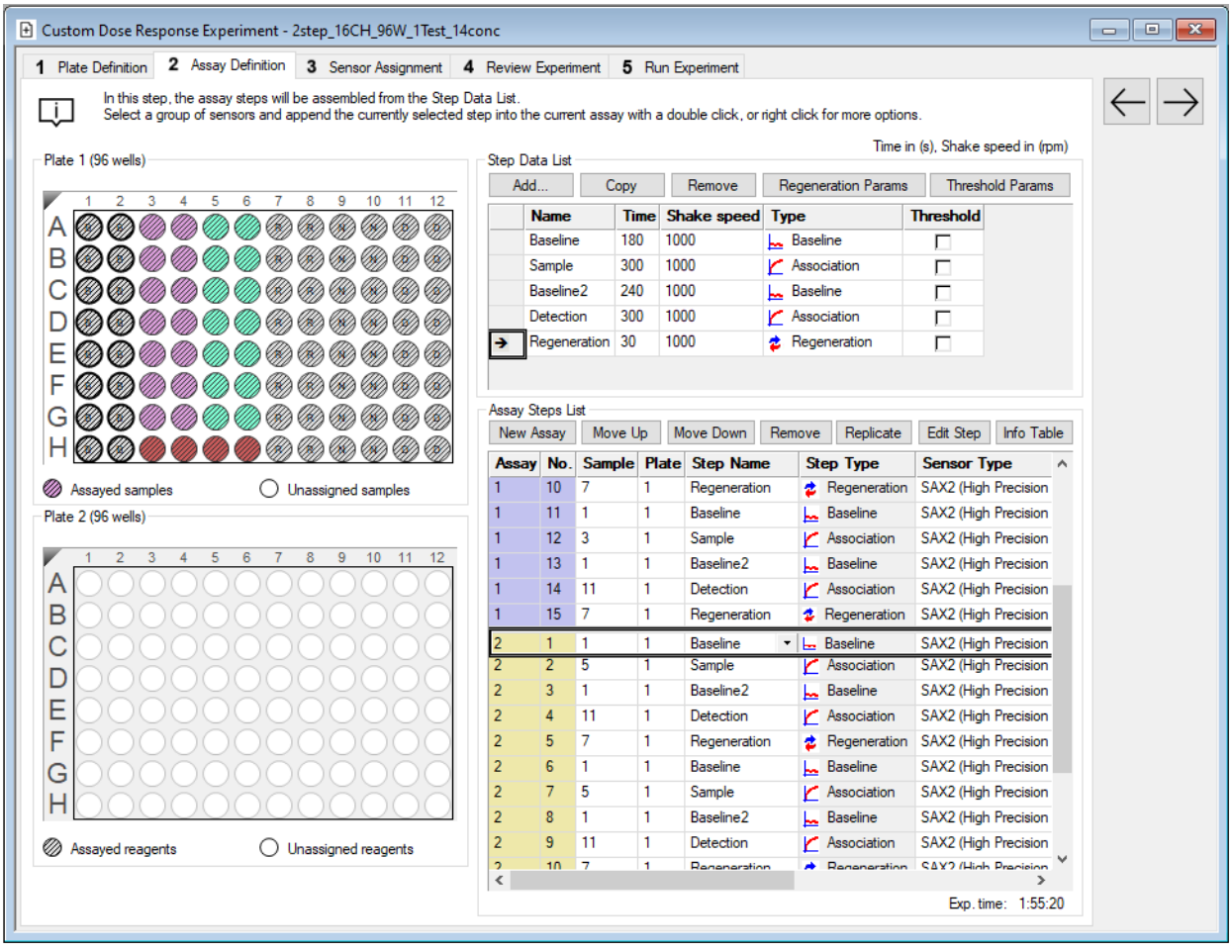


Figure 11-44: Biosensor Regeneration Added to the First Samples Assay

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).

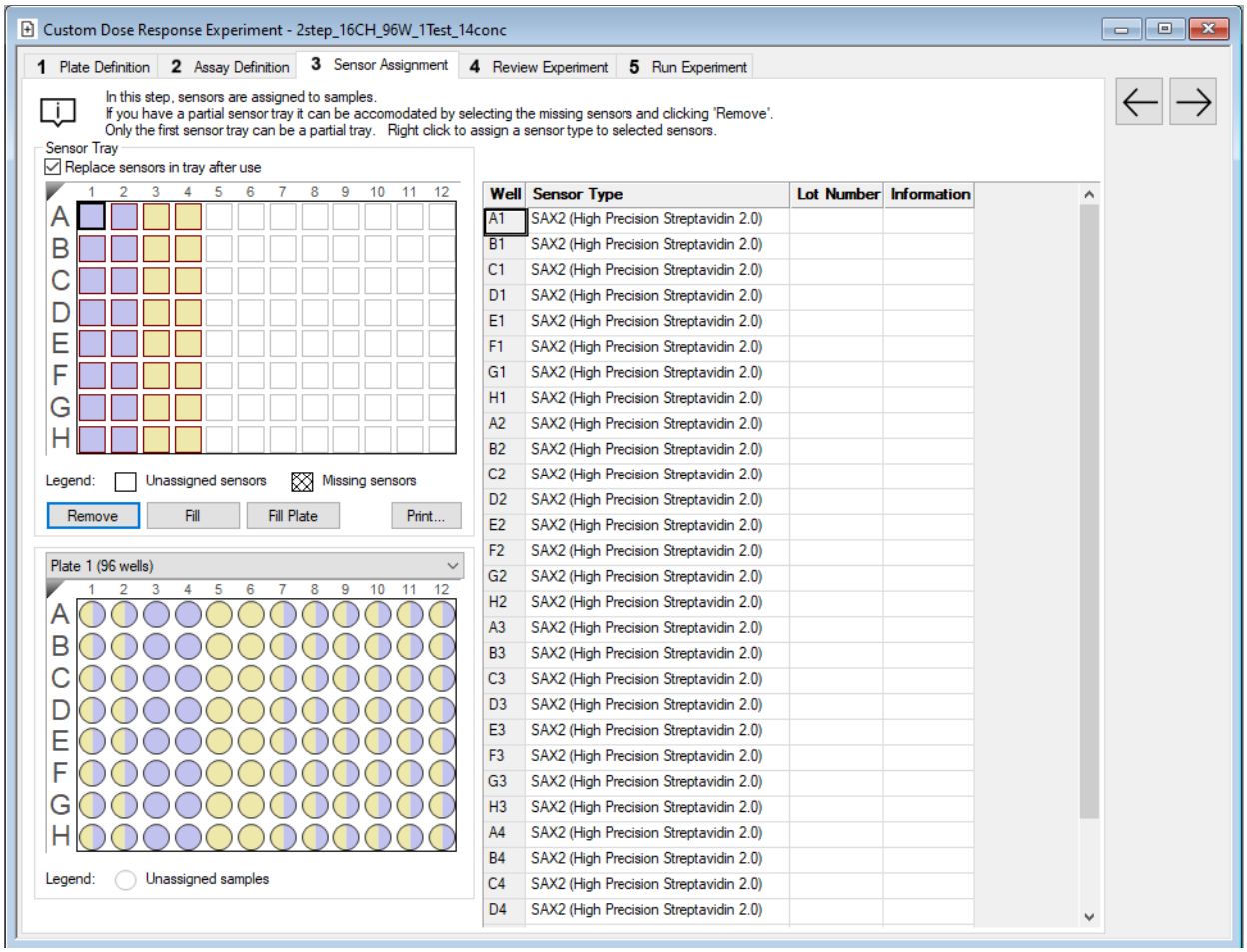


Figure 11-45: Solid Colors Indicate Samples and Standards Have Dedicated Biosensors

Experimental Design Tips

EC_{50}

- 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® 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 dose-response 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® 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® Analysis Studio Software User Guide.
- If possible, make the sample and detection steps long enough to reach equilibrium.

Chapter 12:

Maintenance

NOTICE: *For Octet® RH96 system maintenance-related questions, please contact your local Sartorius representative or Technical Support at octetsupport@sartorius.com or +1-650-322-1360.*

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® K2, Octet® R2, Octet® R4, Octet® R8, Octet® RED96e, and Octet® QKe Systems

Cleaning the Octet® 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: I*l 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® instrument regularly, clean the interior horizontal surfaces daily with a Kimwipe® tissue moistened with a 30–60% isopropyl alcohol solution. Otherwise, clean once a week or as needed.*

Routine cleaning of the Octet® 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.

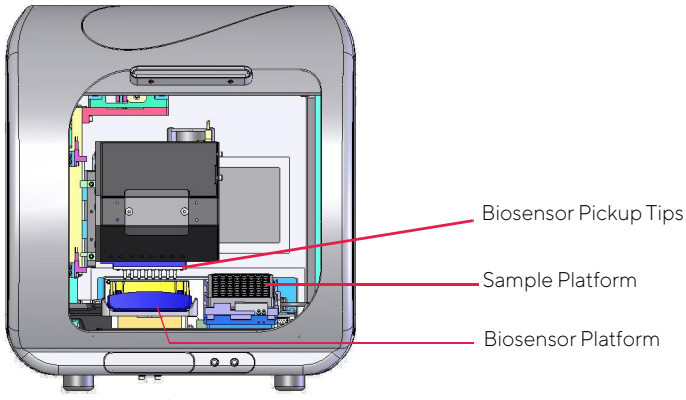


Figure 12-1: Octet® Instrument

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® 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®, 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.

NOTICE: *Sartorius recommends that the waste container be emptied after every run of a 96-biosensor tray.*

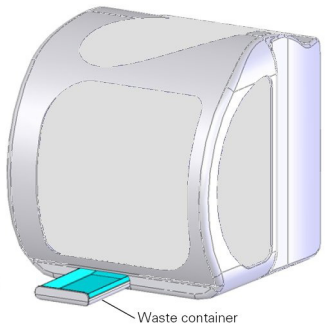


Figure 12-2: Waste Container for the Octet® Instrument

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® RH16, and Octet® QK384 Systems

Cleaning the Octet® Instrument

NOTICE: *If you use the Octet® 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® RH16 or Octet® 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.

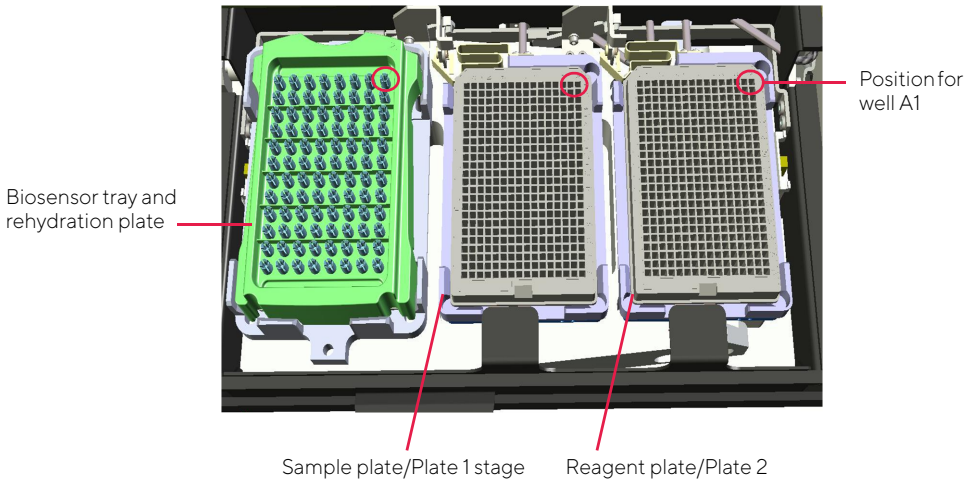


Figure 12-3: Octet® RH16 and Octet® QK384 Stage Platform

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® RH96 and Octet® 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® 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® 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® 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® RH16, Octet® RH96 and Octet® QK384 Systems with an Automation Interface

Overview

The Octet® 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® BLI Discovery software. The file is located in the C:\Program Files\Sartorius\OctetBLIDiscovery directory.

NOTICE:
The automation interface can only be used with the Octet® QK384, Octet ® RH16, and Octet® RH96 systems.
The examples that follow are illustrated using a TCP/IP connection, but the serial port connection behaves identically.

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® 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® 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® BLI Discovery software can be controlled via automation interface through a serial port (RS-232) or a TCP/IP socket.*

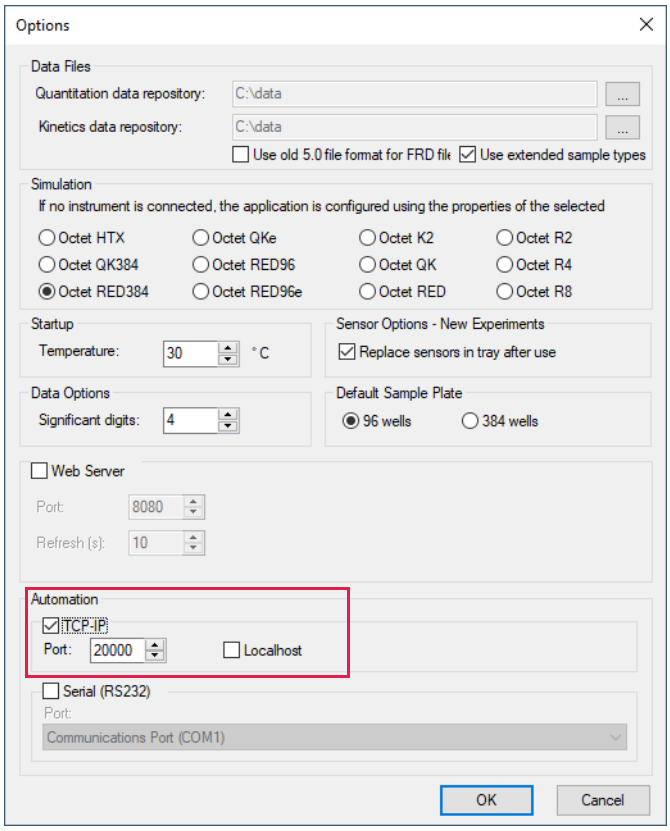


Figure A-1: Options Dialog Box—Automation Interface Selection

NOTICE: *The Localhost option can be useful in developing the automation client on the same computer that runs the Octet® 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® 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® BLI Discovery software via serial port (RS-232) port or TCP/IP socket.

To connect the Automation Client example application:

1. In the Octet® 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).

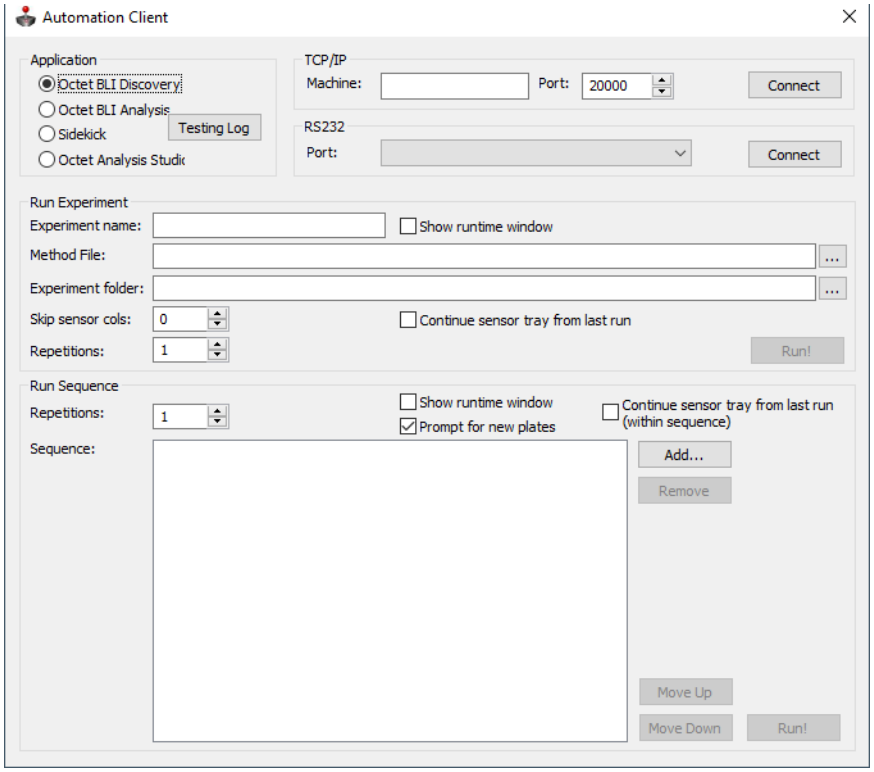


Figure A-2: Automation Client Window

4. Select the TCP/IP or RS-232 port selected previously in the Octet® 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:

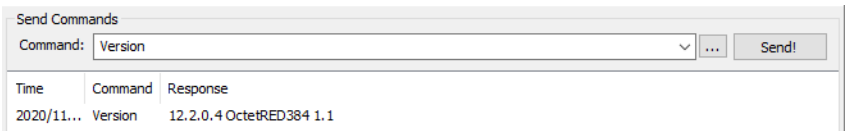


Figure A-3: Send Commands—Command Field

The response indicates that the **Automation Client** has connected to the Octet® BLI Discovery software. This example indicates that version 6.1.0.75 of the Octet® BLI Discovery software is controlling an Octet® instrument using version 1.0 of the automation interface.

Automation Commands

Table A-1 summarizes the commands supported by the Octet® BLI Discovery software automation interface.

NOTICE: *The symbolic names are provided for C++ clients who connect using the interface as defined in the AutomationAPI.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 automation 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 running.
Stop	AUT_CMD_STOP	Stops a running experiment, ejecting the sensors if necessary.
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 progress 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 **Run** command without the **-s** 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® 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)

{
CCountdownTimer Timer(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 = 'l'; // 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)
// Response:
// "OK\r\n"
// "Busy\r\n"
// "Running (nn%)\r\n"
// "Error: <reason>\r\n"

#endif // INC_ANALYSIS_AUTOMATIONAPI_H
```

Appendix B:

Octet® GxP Server Module

Overview

The Octet® 21 CFR Part 11 software portfolio is a client-server architecture for managing digital records created with Octet® BLI systems and analyzed using Octet® analysis applications. A CFR administrator is responsible for configuring user accounts for users of the Octet® CFR system. The 21 CFR Part 11 editions of the Octet® applications, including Octet® BLI Discovery, and Octet® Analysis Studio, enforce user logins prior to performing any operations with the software. During the user session, the Octet® 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® and Windows software.

Octet® GxP Server Components

The Octet® GxP Server software has three modules.

- Octet® 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® GxP Configuration Tool is an application for configuring basic details of the Octet® GxP Server such as the network port.
- Octet® 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® 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® GxP Server System Requirements

Table B-1: Octet® 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® CFR applications. This configuration does not require internet access.

The Octet® 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® GxP Server in a production environment.

Network Configuration

Recommended Configuration

Install the Octet® GxP Server onto a dedicated administrator computer. This can be a dedicated physical server workstation, or a virtual machine. Typically, the Octet® GxP Server computer will be under local IT control. Ensure that the server is always on and available for client connections. The Octet® hardware controller PC must have network connectivity to the Octet® GxP Server. Other optional workstations with the Octet® Analysis Studio software, will also need network connectivity to the Octet® GxP Server.

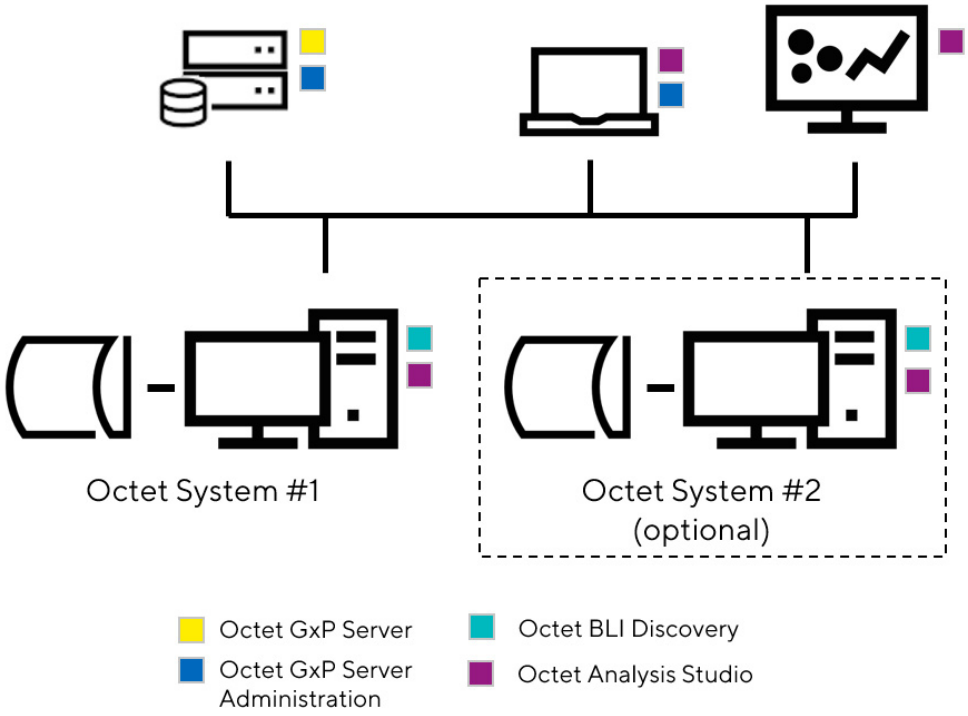


Figure B-1: Network layout

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® 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® GxP Server software on a single computer along with the Octet® BLI Discovery software. This is a last resort.

See the following section for compliance considerations if using this option.

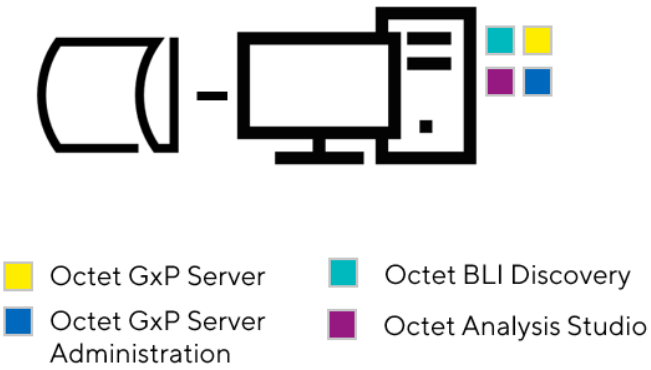


Figure B-2: Layout of a single computer with the Octet® system

Ensuring Compliance

The Octet® GxP Server is a key part of keeping records compliance. Strictly control the access to the computer hosting the Octet® 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® system, for example local IT, or the Quality department.

Control the Octet® 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® system users as Windows standard accounts. Any Windows user with Administrative privileges on the computer will have access to the Octet® GxP Server database files.*

Installation of the Octet® GxP Server Module

NOTICE: I*f you are upgrading from an earlier version of Octet® 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® GxP Server software. You can also install the Octet® GxP Administration component on other client computers such as the Octet® controller computer. In general, only install the Octet® GxP Server component on one computer in your network.

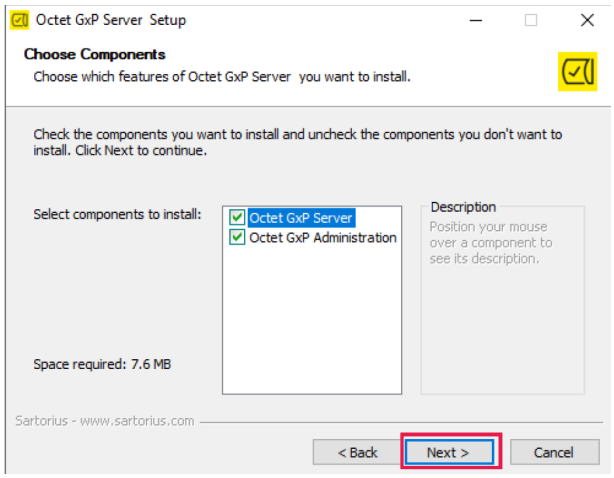


Figure B-4: Choose Components dialog box

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.

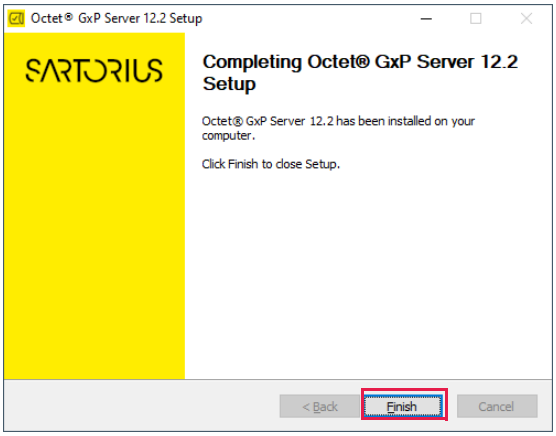


Figure B-5: Completing the GxP Server Setup

Initial Configuration

The default configuration for Octet® 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® GxP Server software uses, see "Additional Server configuration options" on page 577. The default port for the Octet® GxP Server is port 20002

Initial Administrator Login

A new installation of Octet® 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® GxP Server software.

1. Double-click the desktop short cut (Figure B-6) to launch the Octet® GxP Server Administration tool.

Figure B-6: Octet® 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**.

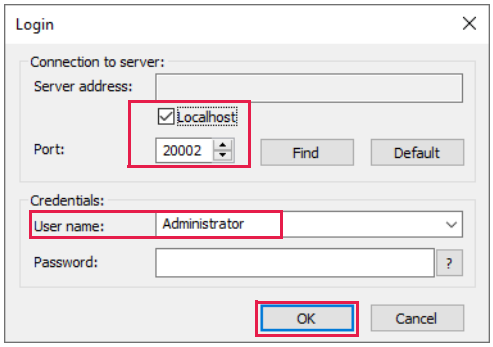


Figure B-7: Login Dialog Box

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.

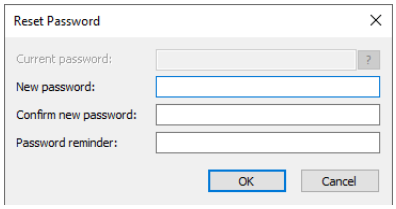


Figure B-8: Reset password dialog

5. After changing the password, the Octet® 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® 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® GxP Server. This can be the Octet® hardware controller PC or a personal workstation. You must connect the client computer to the same network as the Octet® GxP Server.

You can use any of the Octet® client applications to test connectivity to the server. These include the 21 CFR Part 11 editions of Octet® BLI Discovery, Octet® Analysis Studio, or the Octet® GxP Server Administration client. The connectivity test is the same.

1. Launch the client software of your choice. This example uses the Octet® BLI Discovery software

   The Login dialog box appears.

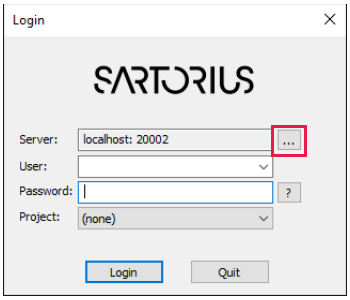


Figure B-9: Login dialog box

2. Click the... (Browse) to display the Octet® GxP Server dialog box.

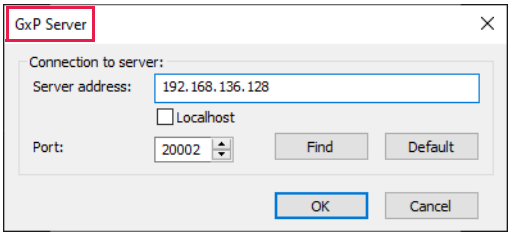


Figure B-10: GxP Server dialog box

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® GxP Server. Click **OK**.

NOTICE: *If the Octet® 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.

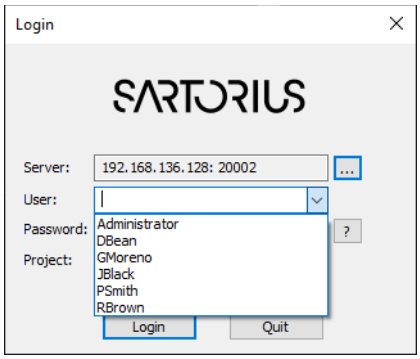


Figure B-11: Login screen with server address configured

5. After a successful login, you have access to the client application. This confirms connectivity to the Octet® GxP Server. If you are not able to log in, see the Troubleshooting section.
6. After you select the Octet® 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® GxP Server.

Backup the Database

The Octet® 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® GxP Server Module.
2. Open Windows® 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® 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® 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® GxP Server (if upgrading from version 12.0 or older, locate the GxP Server in the list of apps).

2. Click on Octet® GxP Server, and then click **Uninstall** (Figure B-12).

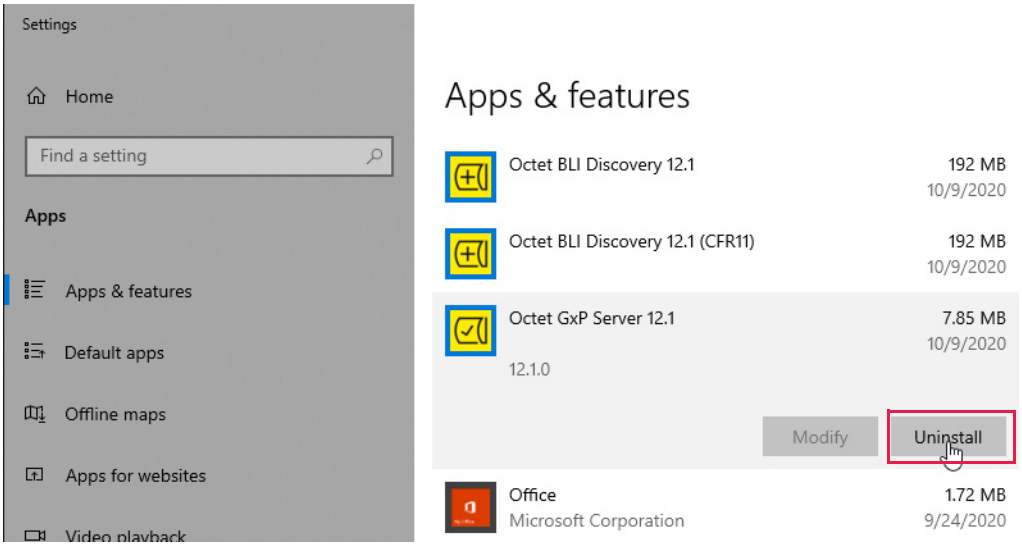


Figure B-12: Uninstall Octet® GxP Server

3. To install the Octet® GxP Server module: See "Installation of the Octet® GxP Server Module" on page 569.

After the Octet® 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® GxP Server Windows Service: Launch the Windows Services snap-in.
2. Locate Octet® 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.

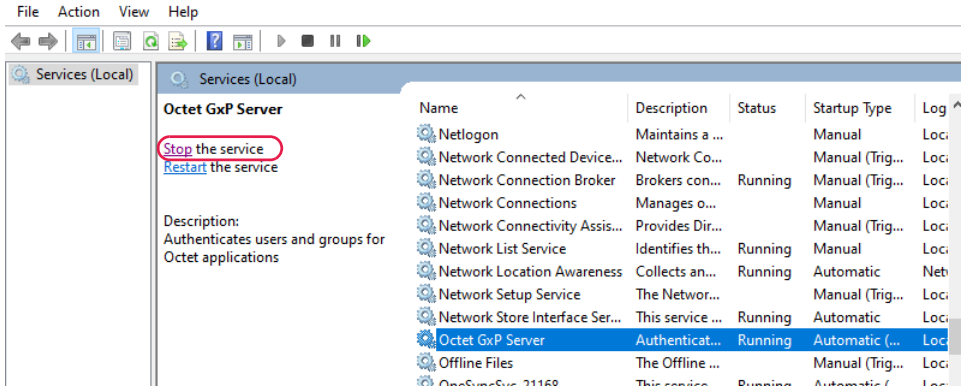


Figure B-13: Stop the service

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® GxP Server Windows Service: Return to the Services window. Select Octet® GxP Server in the list of services and choose **Start** the service (Figure B-14). Confirm that the service status changes to Running.

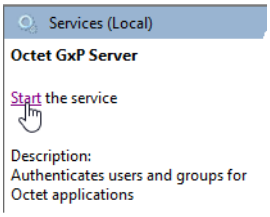


Figure B-14: Start the service

Moving the Octet® GxP Server to a New Host

Follow these instructions to change host computer for the Octet® GxP Server software.

1. Create a backup of the existing database files.
2. Install the Octet® 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® GxP Server Administration client on the new host using Localhost for the server connection.
5. To complete the move, uninstall the Octet® GxP Server software from the old host.
6. Update the server address for all client computers.

Restarting the Octet® GxP Server Module

If you cannot find the host location of the Octet® GxP Server module during user login or if users with valid credentials are unable to login, the Octet® 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® GxP Server module.*

Double-click on the OctetGxPServer.exe file (Figure B-15) in the Octet® GxP Server folder from the installed location:

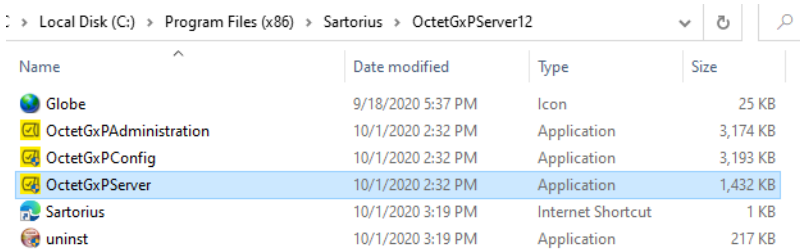


Figure B-15: Restart the Octet® GxP Server

Additional Server configuration options

The Octet® 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® GxP Server software, navigate to the installation folder and double-click on the OctetGxPConfig.exe program (Figure B-16).

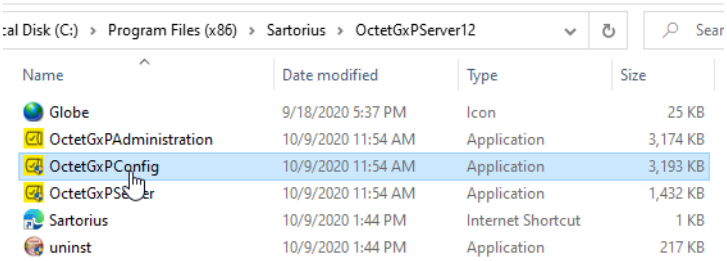


Figure B-16: Octet® GxP Server Configuration Tool

2. Log in to the Octet® GxP software configuration software using the Administrator account or an account that has the administrator privileges.

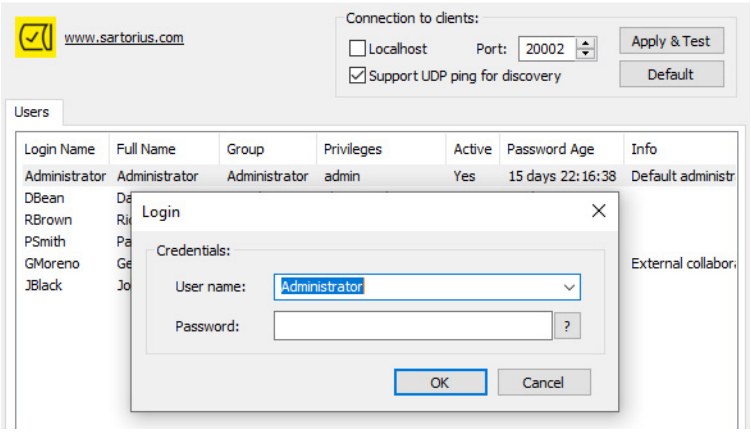


Figure B-17: Administrator login

3. In the Connections to Clients box (Figure B-18), make changes to the server settings as needed.

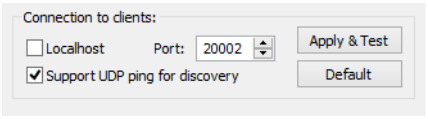


Figure B-18: Connection to clients box

4. Click **Apply & Test.** If the Octet® GxP Server module is functioning properly, this message appears (Figure B-19).

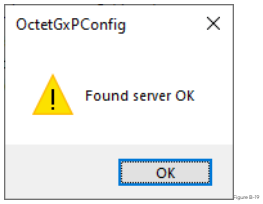


Figure B-19: Found server

To return to the originally configured Octet® GxP Server module settings, go to the Connections to clients box (Figure B-18) and click Default.

Appendix C:

21 CFR Administrator Guide

Description

The Octet® 21 CFR Part 11 compliant ready software consists of three software products:

- Octet® 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® Analysis Studio 21 CFR Part 11 software
  - Analyze binding data and view analysis results.
- Octet® GxP Server module software
  - Manage the user database and store Audit Trail data.

NOTICE: *Octet® BLI Analysis CFR is available if needed for compatibility.*

Data integrity

The CFR Administrator uses the Octet® 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® 21 CFR Part 11 software. All data acquired using Octet® 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® 21 CFR Part 11 software environment invalidates the digital signature. The Octet® 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® 21 CFR Part 11 software environment. An Audit Trail of all activities performed in any Octet® 21 CFR Part 11 application is stored in the Octet® GxP Server database.

Data files created using Octet® 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® 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® BLI Discovery 21 CFR Part 11 software is acquiring data from an Octet instrument. After data acquisition begins, Octet® 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® GxP Server Administration tool.

- Double-click the Octet® GxP Server Administration Desktop Shortcut (Figure C-1):

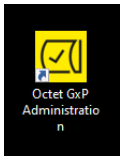


Figure C-1: Octet® GXP Server Administration Desktop Shortcut

The Octet® GxP Server Administration tool can be installed on the primary Octet® 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® GxP Server module, and choose ONLY the Octet® GxP Administration component during setup (Figure C-1).

The Octet® GxP Server Administration window displays (Figure C-2):

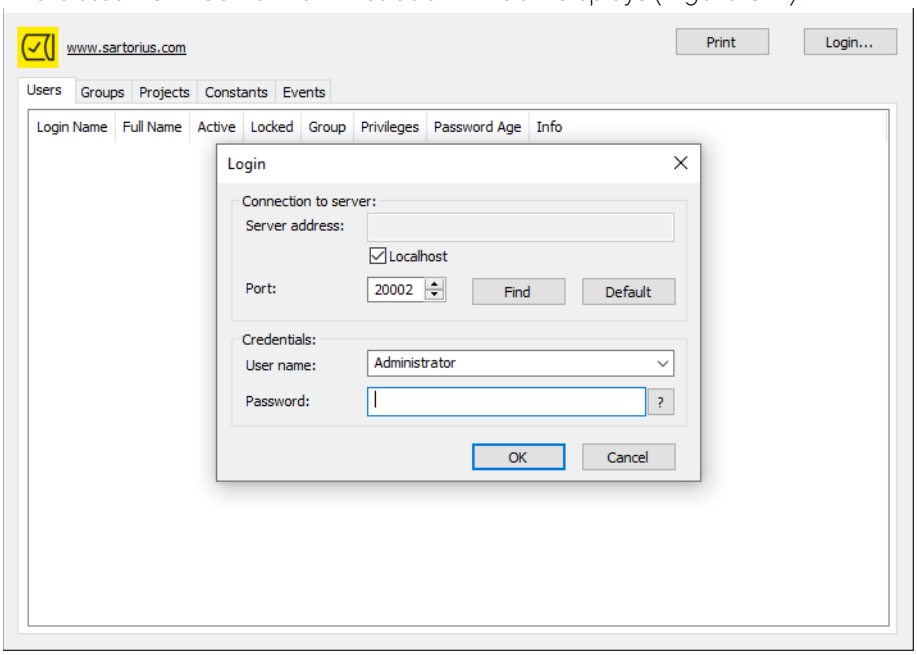


Figure C-2: Octet® GxP Server Administration with Login Dialog Box

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® GxP Server Administration Users Tab (Figure C-3).

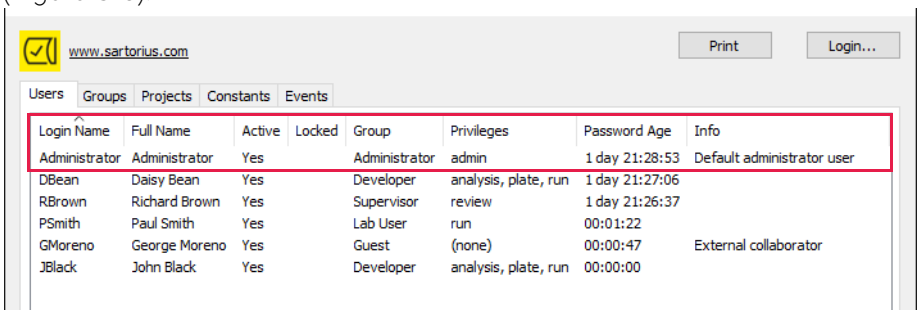


Figure C-3: Octet® GxP Server Administration Users Tab

Five tabs are available in the Octet® 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

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.

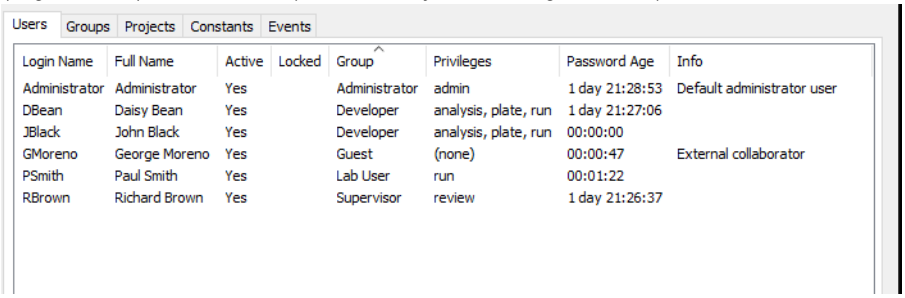


Figure C-4: User Tab Information Sorted by Group

Creating a New User Account

1. Right-click in a blank area in the **Users Tab**. The Tab menu appears,.

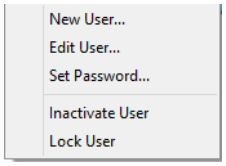


Figure C-5: Users Tab Menu New User Option

2. Select **New User** to display the New User dialog box.

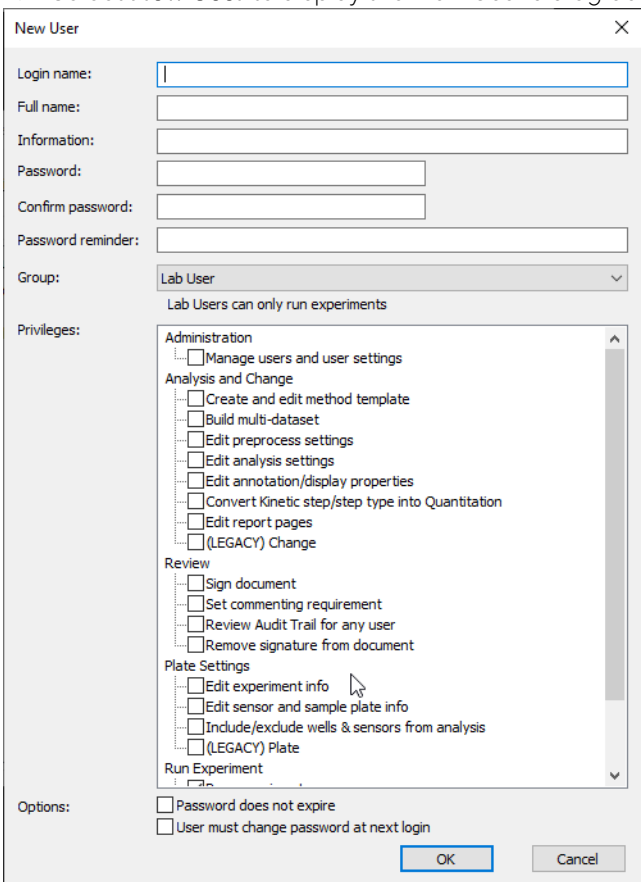


Figure C-6: *New User Dialog Box*

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	✓				
Supervisor			✓		
Developer		✓		✓	✓
Lab User			✓		
Guest					

NOTICE: *Analysis and Change, Review and Plate applies only to the Octet® BLI Analysis software, not the Octet® Analysis Studio software. See the Octet® 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	✓				
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			✓		

Table C-2: Default User Privileges (Continued) (Sheet 2 of 2)

	Default Group Privileges				
	Administrator	Supervisor	Developer	Lab User	Guest
Edit report pages			✓		
(LEGACY) Change			✓		
Review					
Sign Document		✓			
Set commenting requirement		✓			
Review Audit Trail for any user		✓			
Remove signature from document					
Plate Settings					
Edit experiment info			✓		
Edit sensor and sample plate info			✓		
Include/exclude wells and sensors from analysis			✓		
(LEGACY) Plate			✓		
Run Experiment					
Run Experiment				✓	
Import analysis settings template to new dataset				✓	
Export data and Excel report				✓	
Choose repository directory 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.

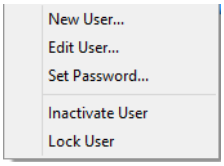


Figure C-7: User Tab Menu

2. Select **Edit User**: Make changes to privileges by selecting/deselecting check boxes.

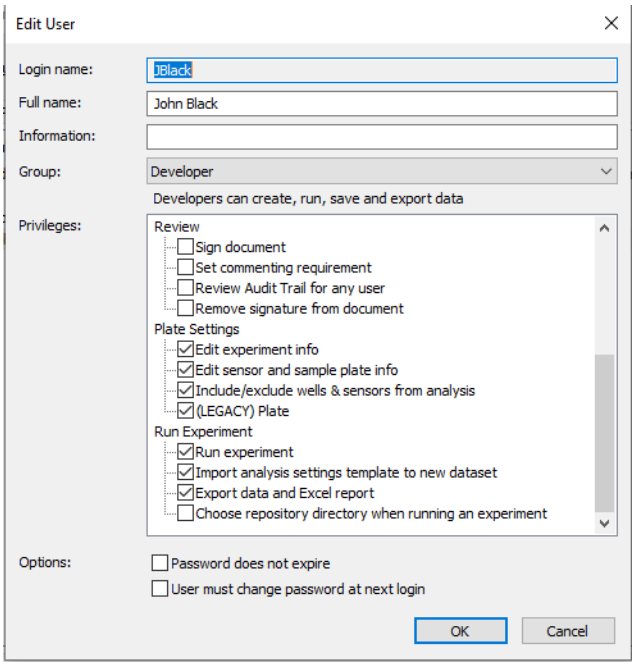


Figure C-8: Edit User Dialog Box

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):

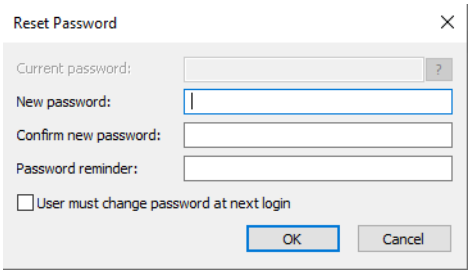


Figure C-9: Change Password Dialog Box

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 ® 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):

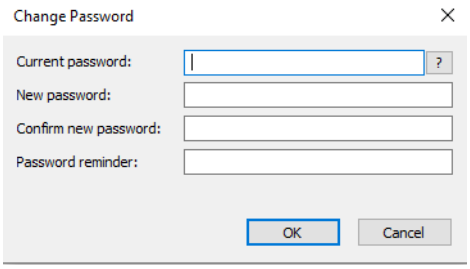


Figure C-10: Change Password Dialog Box

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® 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.

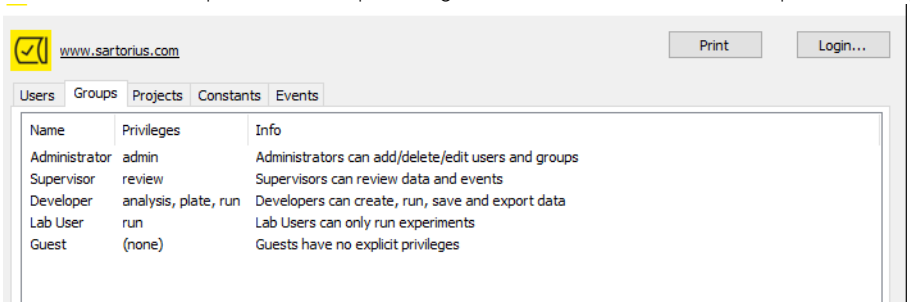


Figure C-11: Groups Tab

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).

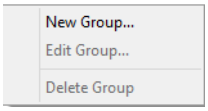


Figure C-12: Group Tab Menu

2. Click **New Group** to display the New Group dialog box (Figure C-13).

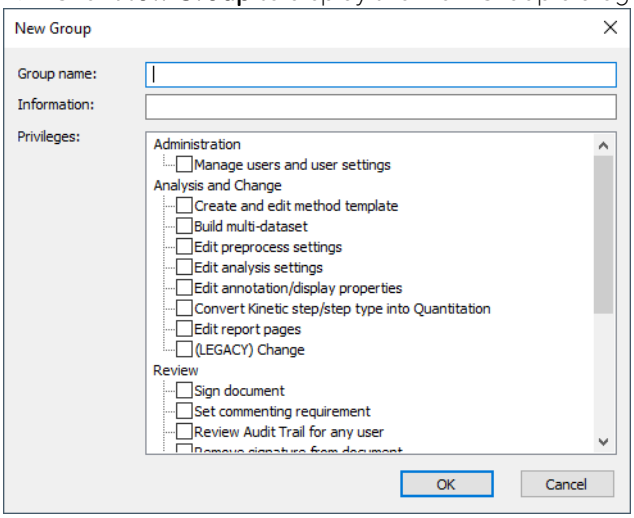


Figure C-13: New Group Dialog Box

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).

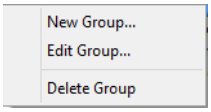


Figure C-14: Group Tab Menu with All Options Active

2. Select **Edit Group** to display the Edit Group dialog box (Figure C-15).

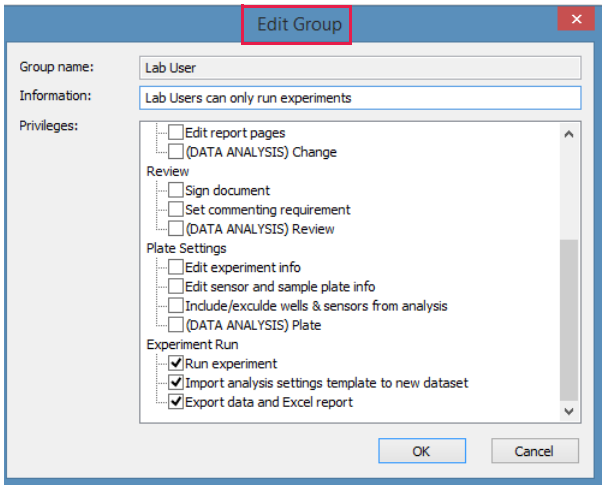


Figure C-15: Edit Group Dialog Box

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® BLI Discovery, or Octet® 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.

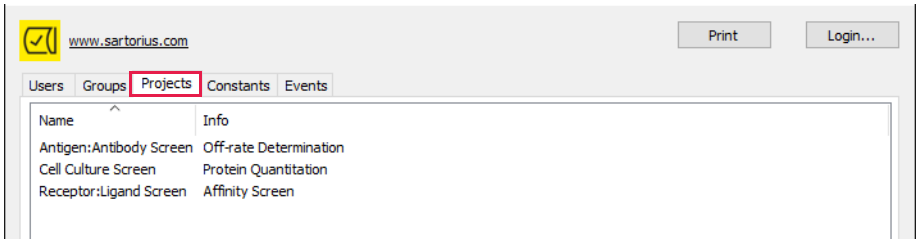


Figure C-16: Projects Tab

Creating a New Project

1. Right-click in a blank area in the **Projects** Tab to display the Tab menu (Figure C-17).

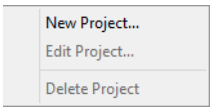


Figure C-17: Projects Tab Menu.

2. Select **New Project** to display the New Project dialog box (Figure C-18).

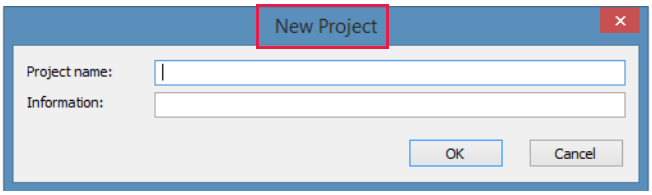


Figure C-18: New Project Dialog Box

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).

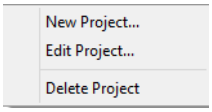


Figure C-19: Projects Tab Menu with All Options Active

2. Select **Edit Project** to display the Edit Project dialog box (Figure C-20).

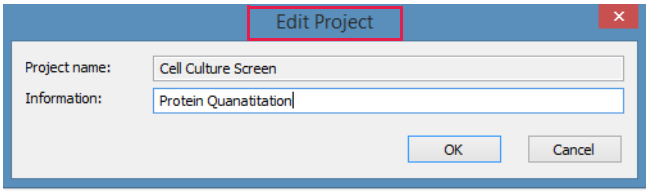


Figure C-20: Edit Project Dialog Box

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® GxP Server constant settings.

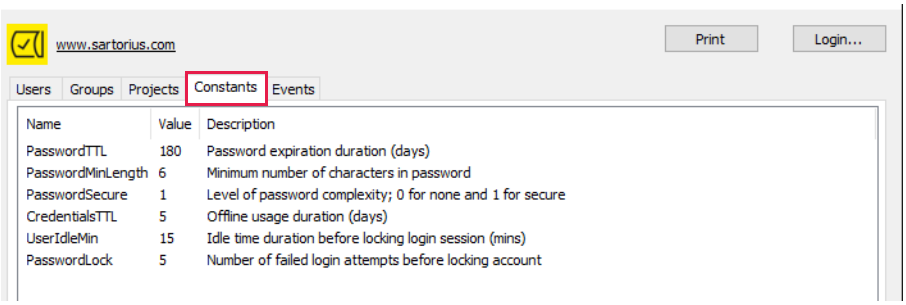


Figure C-21: Constants Tab

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 operate in case the server is temporarily down. Data is saved on the local computer and, upon the next connection to the database, the cached events automatically 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 constant 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
UserIdleMin	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).

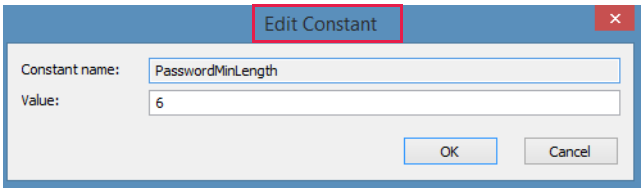


Figure C-22: Edit Constant Dialog Box

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® GxP Server module. Audit trails are stored on the Octet® GxP Server, not in individual files.

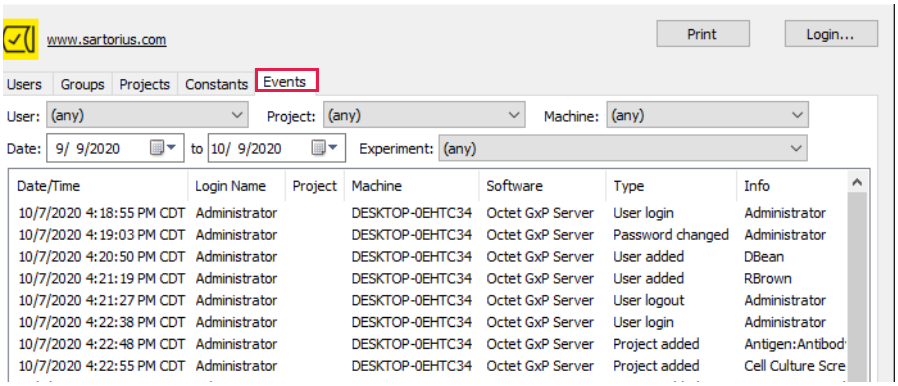


Figure C-23: Events Tab

NOTICE: *Octet® 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® 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® and BLItz instruments for Octet® GxP Server module versions 8.2 and higher)
- **Software** - Which software the event was logged in (available in Octet® GxP Server module versions 8.2 and higher only, includes Octet® 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.

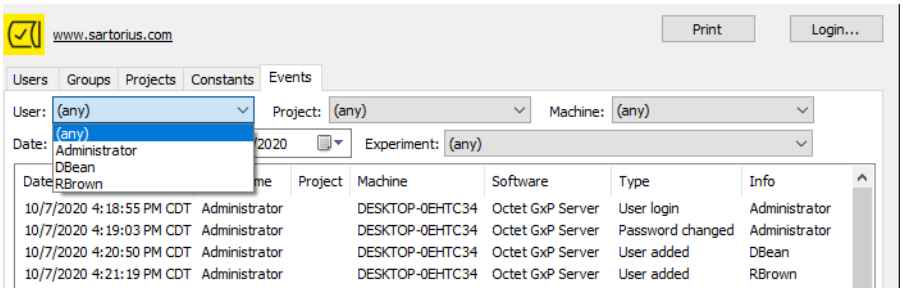


Figure C-24: Selecting Events by User Name

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).

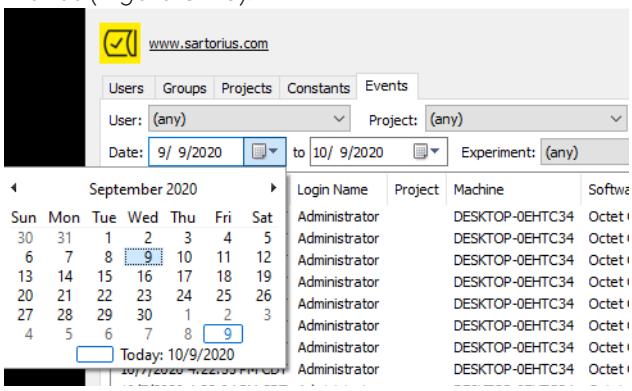


Figure C-25: Events Displayed for User Name

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).

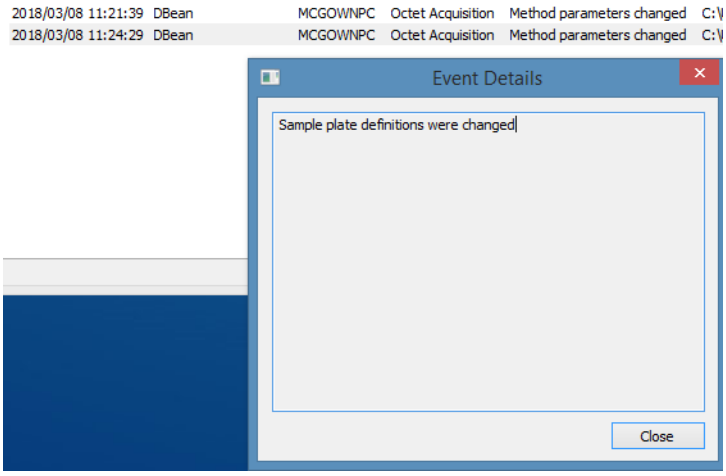


Figure C-26: Event Details Describing Method Parameter Changes

Appendix D:

Ambr® 15 and Octet® Data Exchange

Overview

Octet® Software version 13 has been designed to work with the Ambr® 15 Software in a way that allows easy transfer of sample information. Plate maps can be quickly and easily configured to run on Octet®systems. After analyzing the Octet® data, results can be exported back to Ambr® 15 Software for review in aggregate with other key metrics from the cell culture assay.

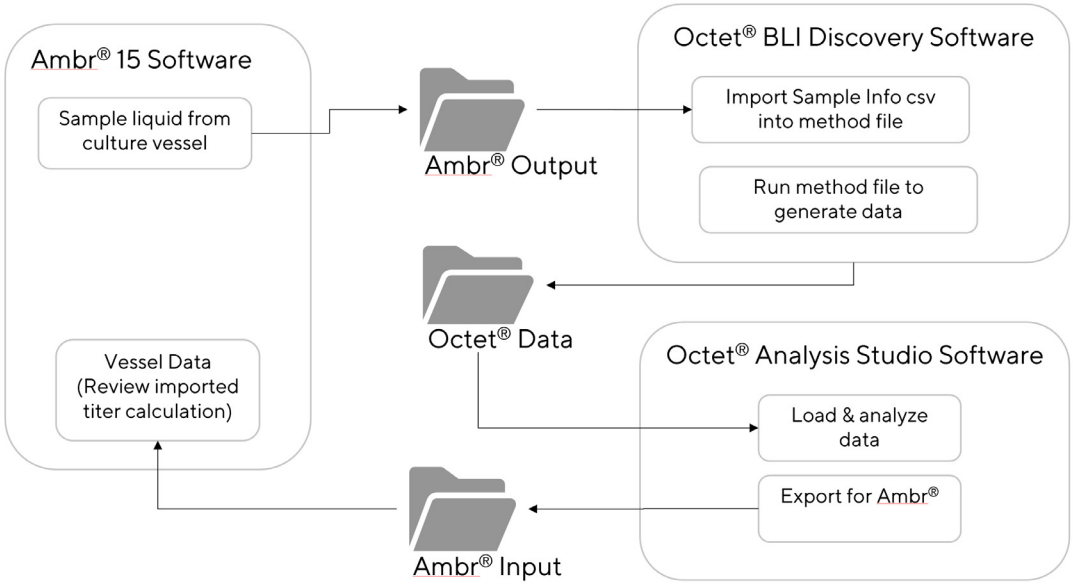


Figure D-1: Octet® and Ambr ®Software Workflow

Ambr® 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® 15 Software also monitors a designated import folder. When results from Octet® Software are placed in the import folder, Ambr® 15 Software automatically reads and imports the Octet® data.

Initial Setup

Step 1: Plan Data Exchange Locations

For the Ambr®-Octet® data exchange to work correctly, Ambr® and Octet® Software should both have access to a shared folder location. If the Ambr® and Octet® Software are running on the same computer, this can be any local folder. If the Ambr® and Octet® Software are on two different computers, we recommend you set up a shared network folder and map the drive on both the Ambr® and the Octet® 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® export folder is the same as the Octet® import folder. This is the folder where the Ambr® Software will create plate maps when sampling from the bioreactors. This folder is used with the Octet® BLI Discovery Software to plan and run the method. A recommended folder name is AmbrOutOctetIn.
2. The Octet® export folder is the same as the Ambr® import folder. Results from the Octet® analysis will be exported to this folder and automatically loaded into Ambr® Software. A recommended folder name is AmbrInOctetOut.

In the following steps, we will use a network folder mapped as the S: drive on the Octet® and Ambr® computers. The drive has the following folders defined:

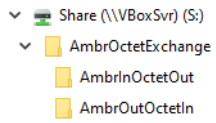


Figure D-2: S: Drive Folders

Step 2: Install Ambr® 15 Software

The Ambr® 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® 15 Software. If you do not see Data Import and Data Export listed, contact Ambr® support to resolve the license issue.

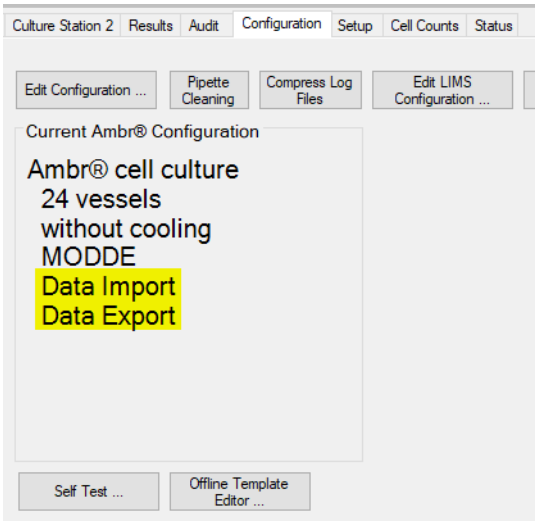


Figure D-3: Ambr® Software Configuration Tab

Step 3: Install Octet® Software

Install the version 13 Octet® Software by running the installers for Octet® BLI Discovery and Analysis Studio Software. Version 13 can be installed side-by-side with previous versions.

Step 4: Configure Ambr® 15 Import and Export Locations

On the Ambr®15 computer, look under the C:\Ambr®\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:

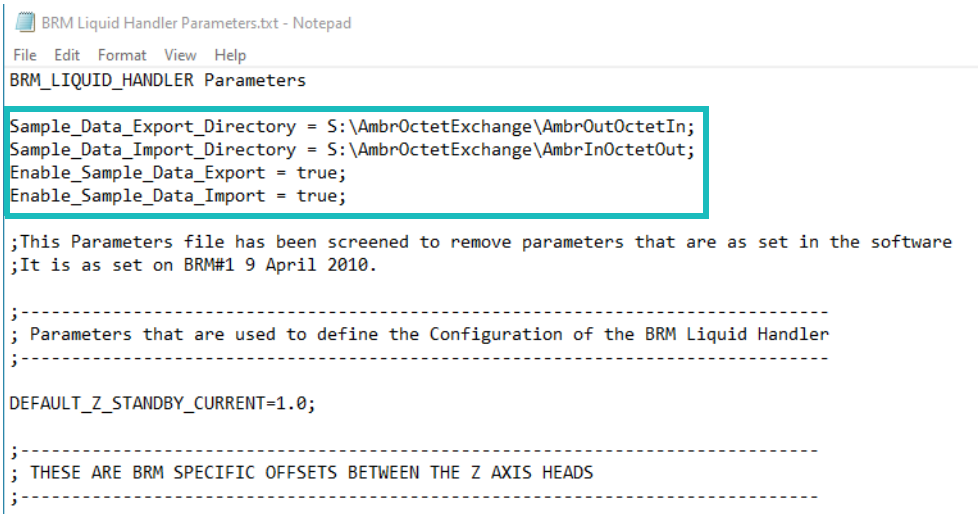


Figure D-4: BRM Liquid Handler Parameters File

Step 5: Configure Octet® Import and Export Locations

Open Octet® 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.

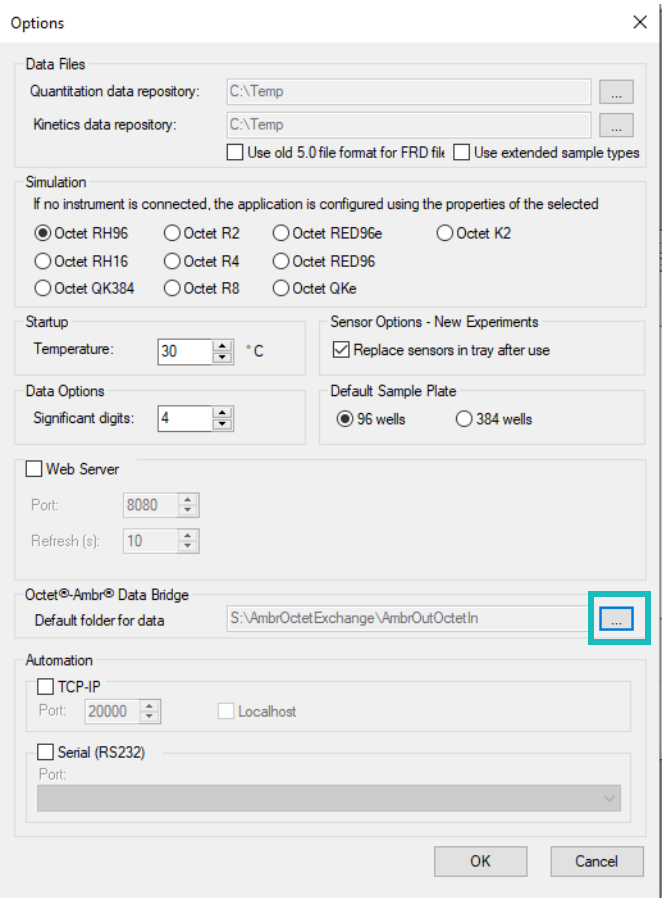


Figure D-5: Selecting the AmbrOutOctetIn Folder Location

Open Octet® 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.

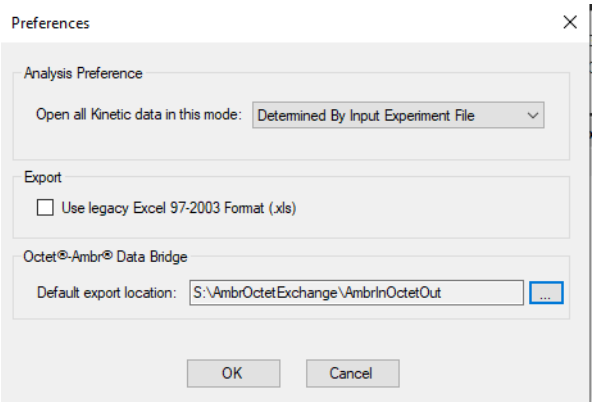


Figure D-6: Selecting the AmbrInOctetOut Folder Location

Step 6: Test the Ambr® Data Export

Prior to running a full experiment on the Ambr® 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® experiment to test the data export function.

Configure a process step to Sample Liquid from Culture Vessel.

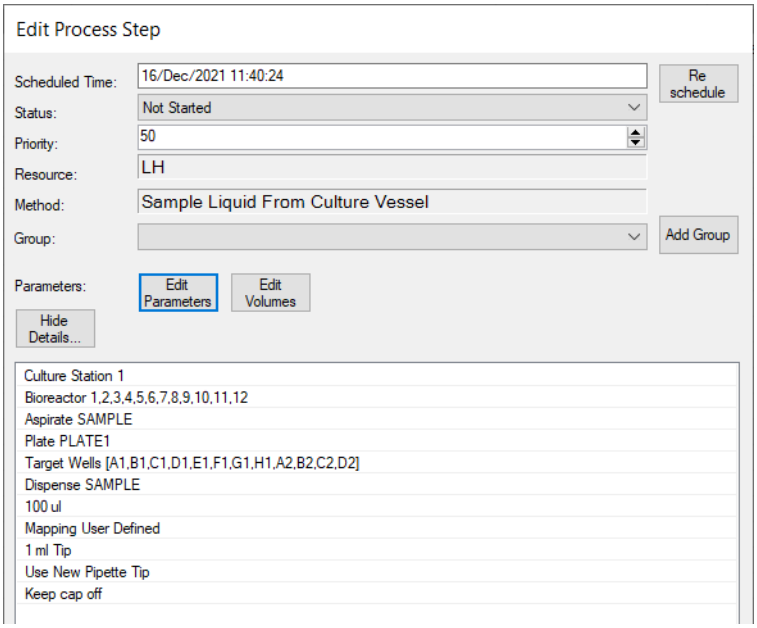


Figure D-7: Configuring a Process Step

After the Ambr® 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® Experiment

See Appendix E on page 611 for instructions on how to create a simple Ambr® experiment.

Import Samples to Octet® 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® 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.

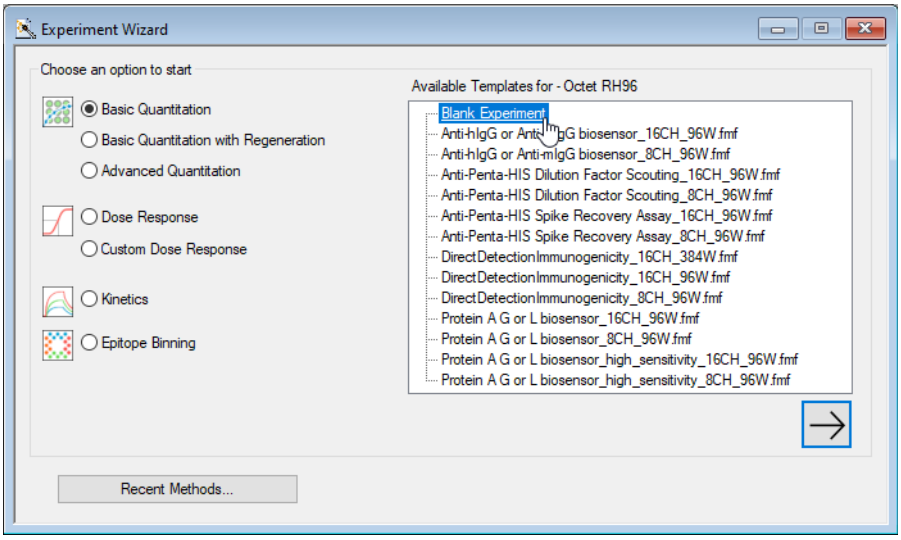


Figure D-8: Experiment Wizard

In **Tab 1 Plate Definition**, click the **Import** button above the sample table. Choose **Import Ambr data file** from the menu.

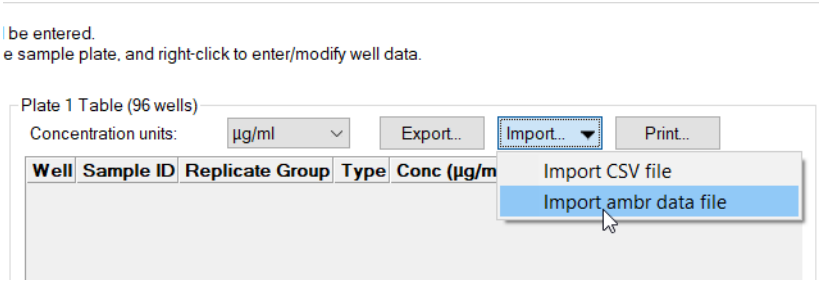


Figure D-9: Importing Ambr® Data File

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.

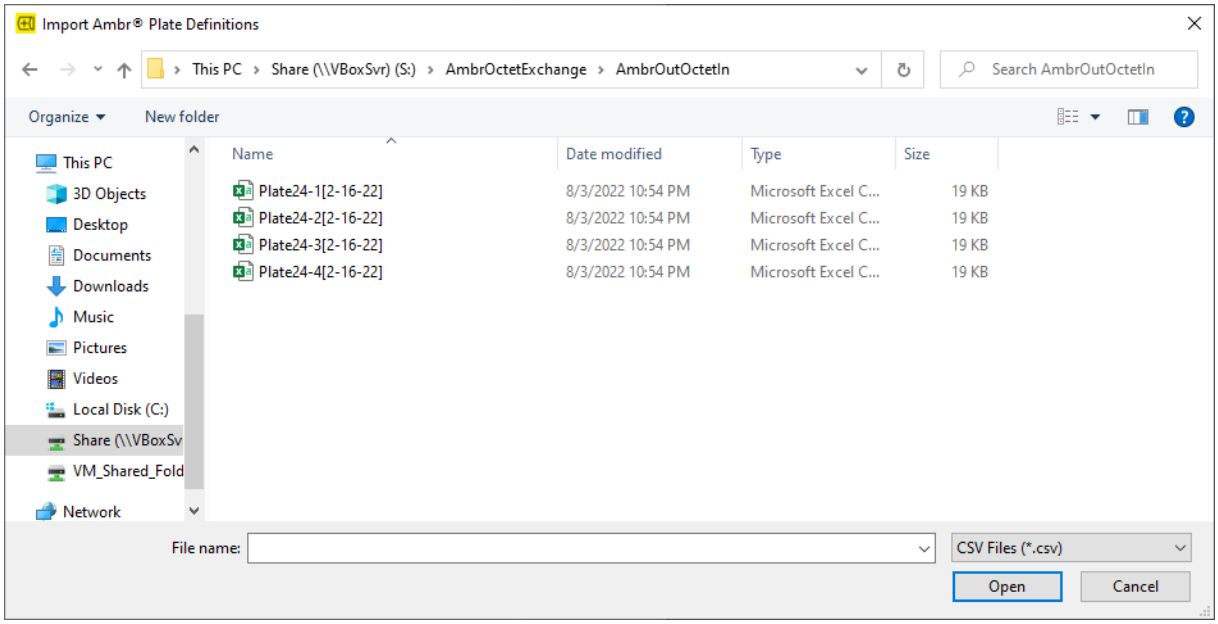


Figure D-10: Selecting Plate Maps

The Ambr® Data Import wizard allows you to review the incoming data before loading into the current method.

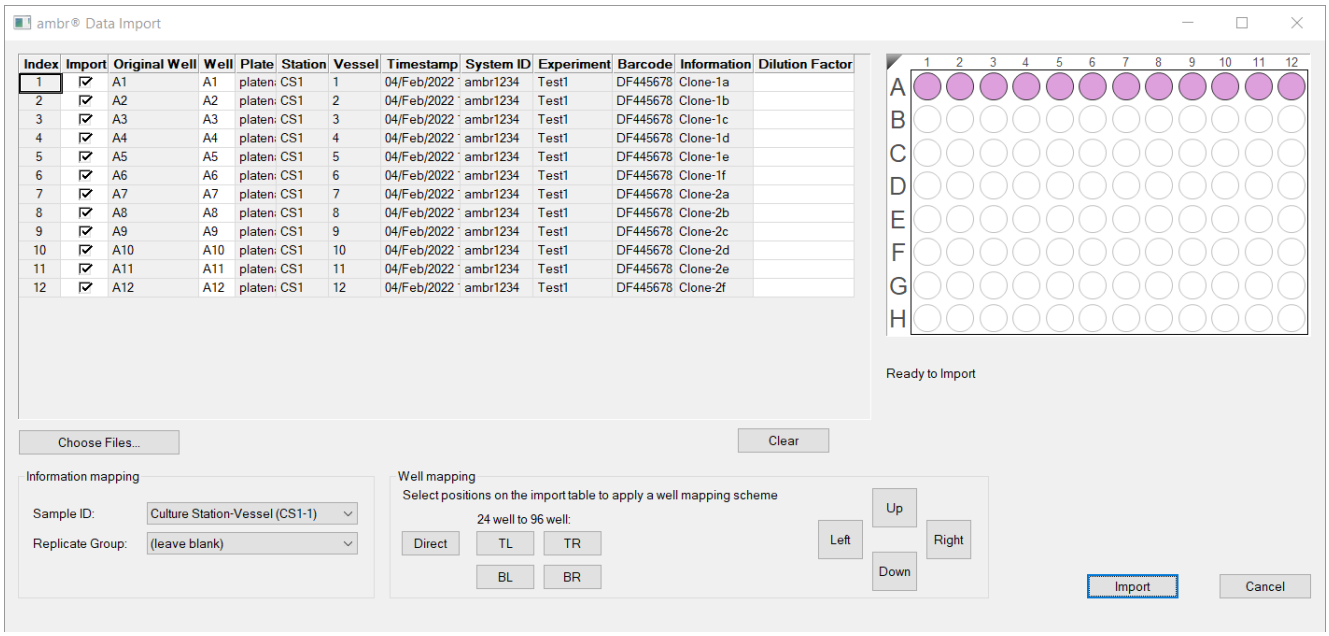


Figure D-11: Reviewing Incoming Data

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® 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® 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® liquid handler placed the sample. The Well column indicates the position where the sample will be when analyzed by the Octet®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® 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® sample data fields. Ambr® data fields can be mapped to the Octet® Sample ID and/or Replicate Group fields using the dropdown options. The required Ambr® data is automatically mapped into the Octet® 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® 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® Software is used directly as the Octet® 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® data (only for 384-well capable Octet® 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® plate. You can also manually type in the Octet® 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® well locations, or multiple rows that map to the same Octet® well.

When you are satisfied with the plate definition, click **Import** to load the definition into the Octet® method editor.

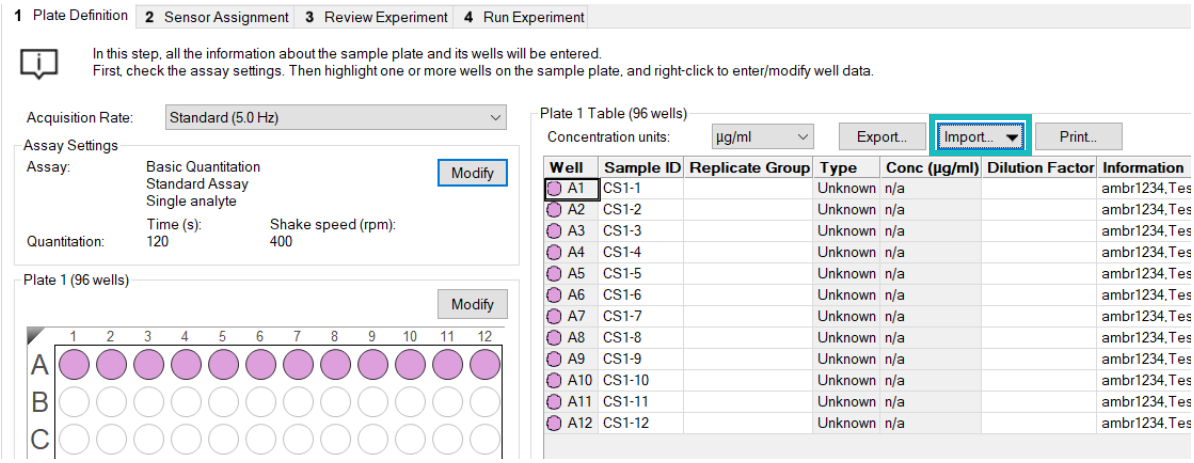


Figure D-12: Import Button in Tab 1 Plate Definition

Note in Figure D-12 that the Octet® Information column has been populated with the Ambr® sample data. Do not edit this information manually. The Sample ID, Replicate Group, and Dilution Factor can be edited without affecting Ambr® data exchange.

Run the Octet® Experiment

Configure other aspects of the method as needed. Prepare the sample plate(s) and run the Octet® experiment.

Analyze Octet® Results

When the experiment is complete, use the Octet® Analysis Studio Software to analyze the data. Quantitation analysis is outside the scope of this document. Refer to the Octet® Analysis Studio User Guide for details.

Export Octet® Results

When you are satisfied with the results of the analysis, export back to the Ambr® Software by clicking the **Export** button and choosing **Export to Ambr format**.

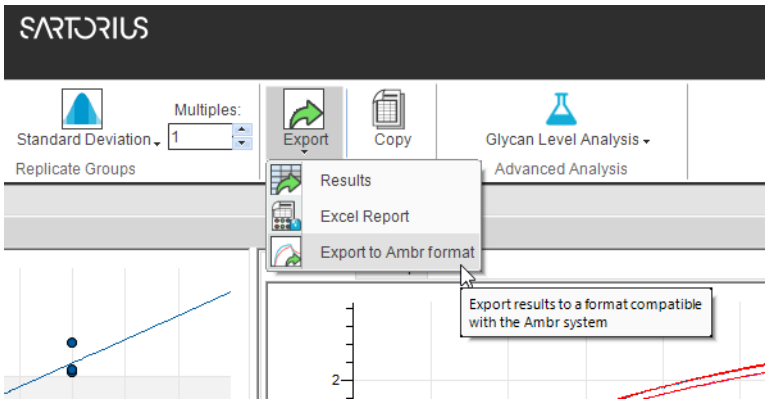


Figure D-13: Selecting Export to Ambr Format

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® result to export or leave as (none), and type the variable name that should be used in the Ambr® Software.

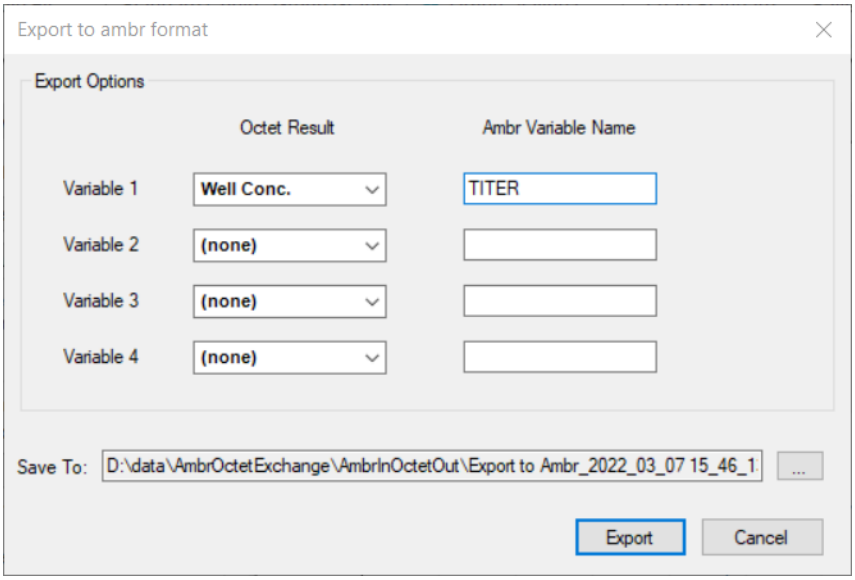


Figure D-14: Adding Variable Names For Ambr® Software

By default, the results will be exported to the AmbrInOctetOut folder you selected in the initial setup.

NOTICE: *When the Ambr® Software successfully imports the results, it will automatically delete the file.*

Appendix E:

Ambr® 15 and Octet® Data Exchange Simulation

Experiment Simulation

Presented here is a simple, simulated Ambr® experiment where you can confirm that the Ambr® output data exchange function is working correctly.

Start up the application and click the **Create New Experiment** button.

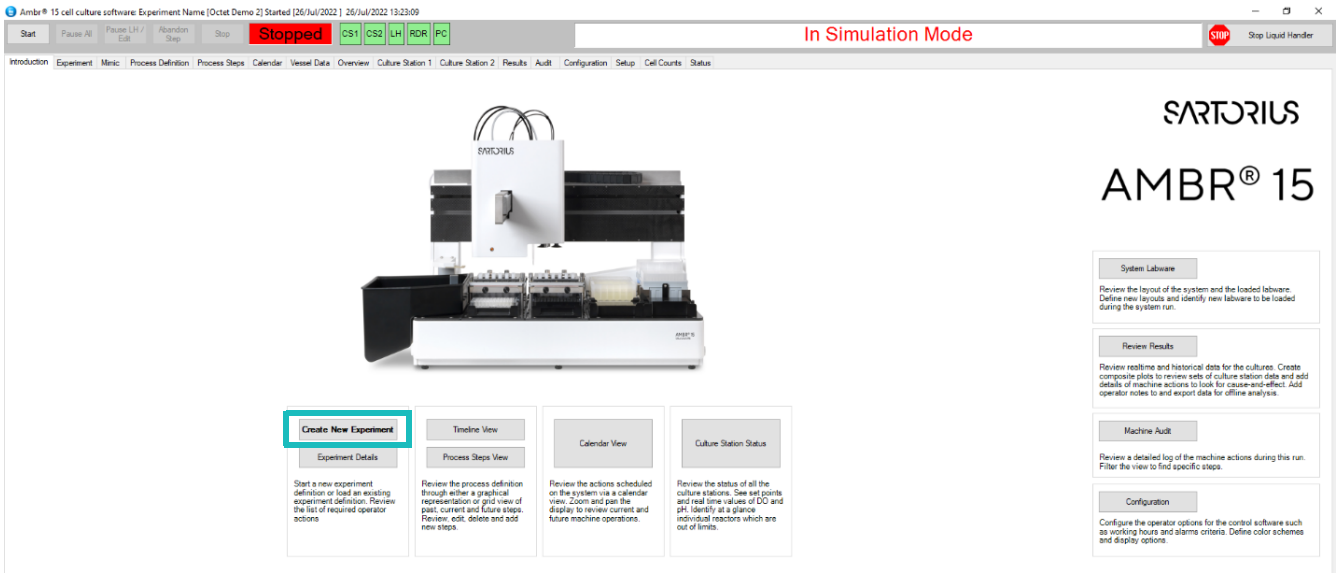


Figure E-1: Creating a New Experiment

When asked if you would like to base the new experiment on a previous one, click **No**.

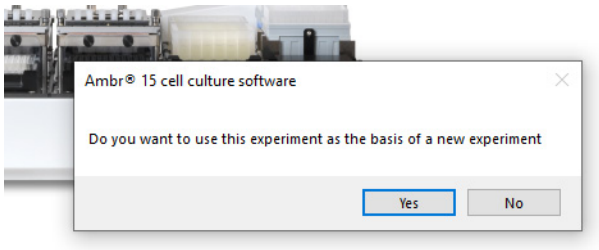


Figure E-2: Experiment Dialog

Click **Yes** to start the New Experiment Wizard.

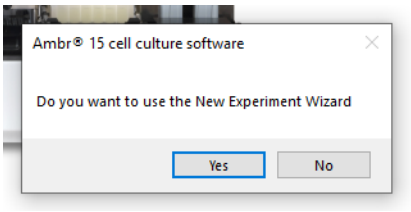


Figure E-3: Experiment Wizard Dialog

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**.

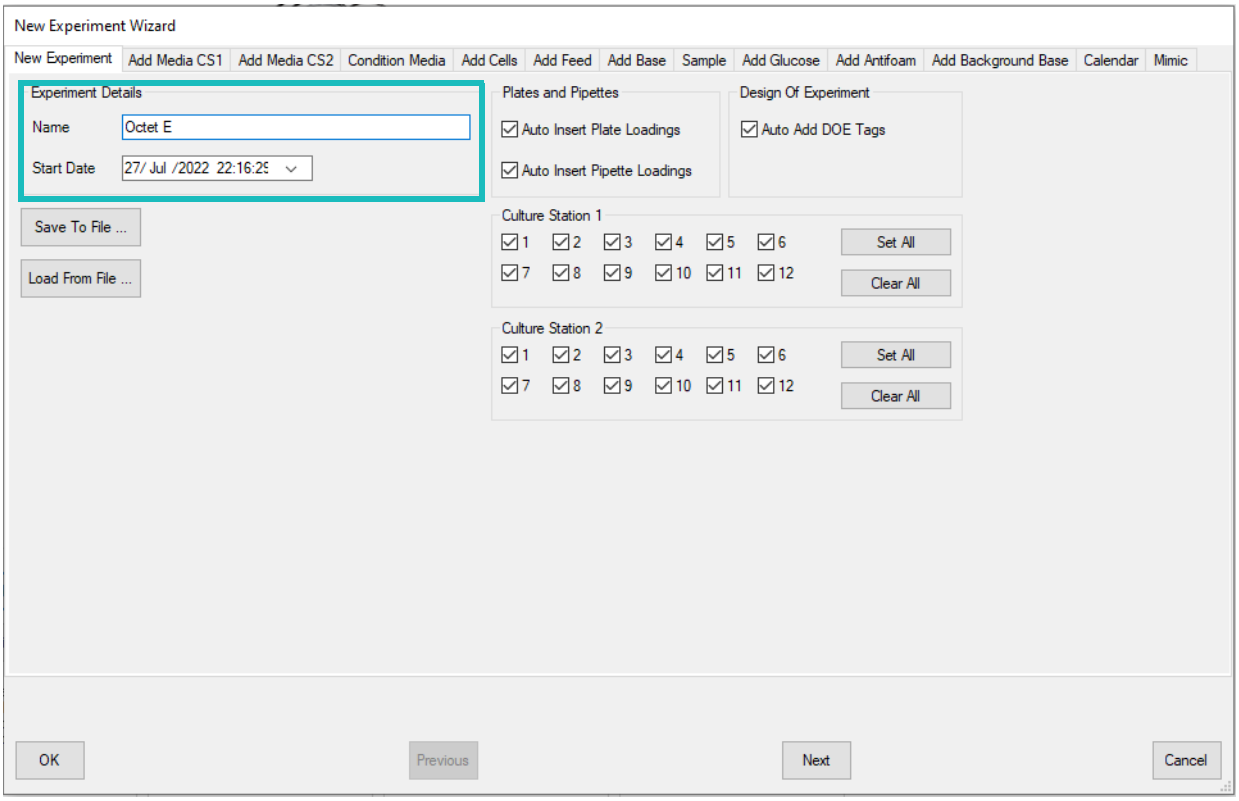


Figure E-4: New Experiment Tab

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.

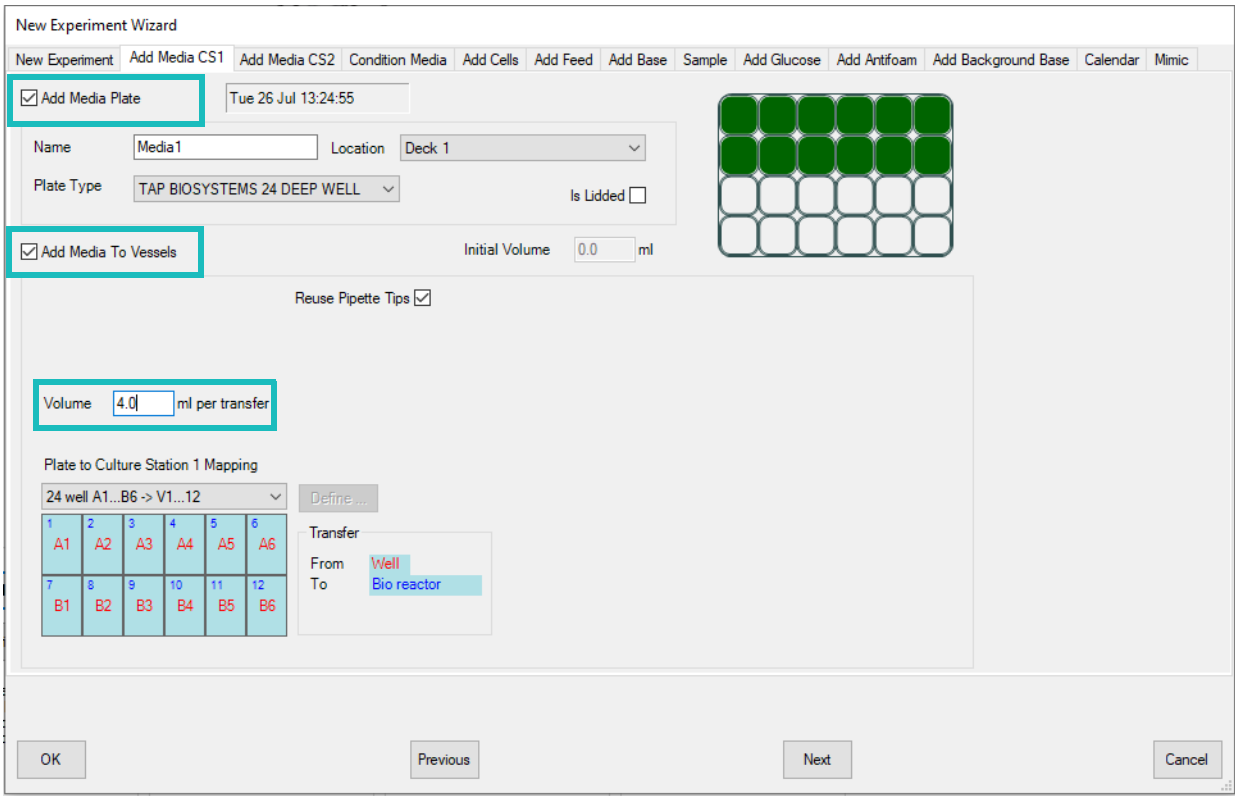


Figure E-5: Add Media CS1 Tab

The second media plate will be pipetted into Culture Station 2. It will be placed onto Deck 2 in the Add Media CS2 Tab.

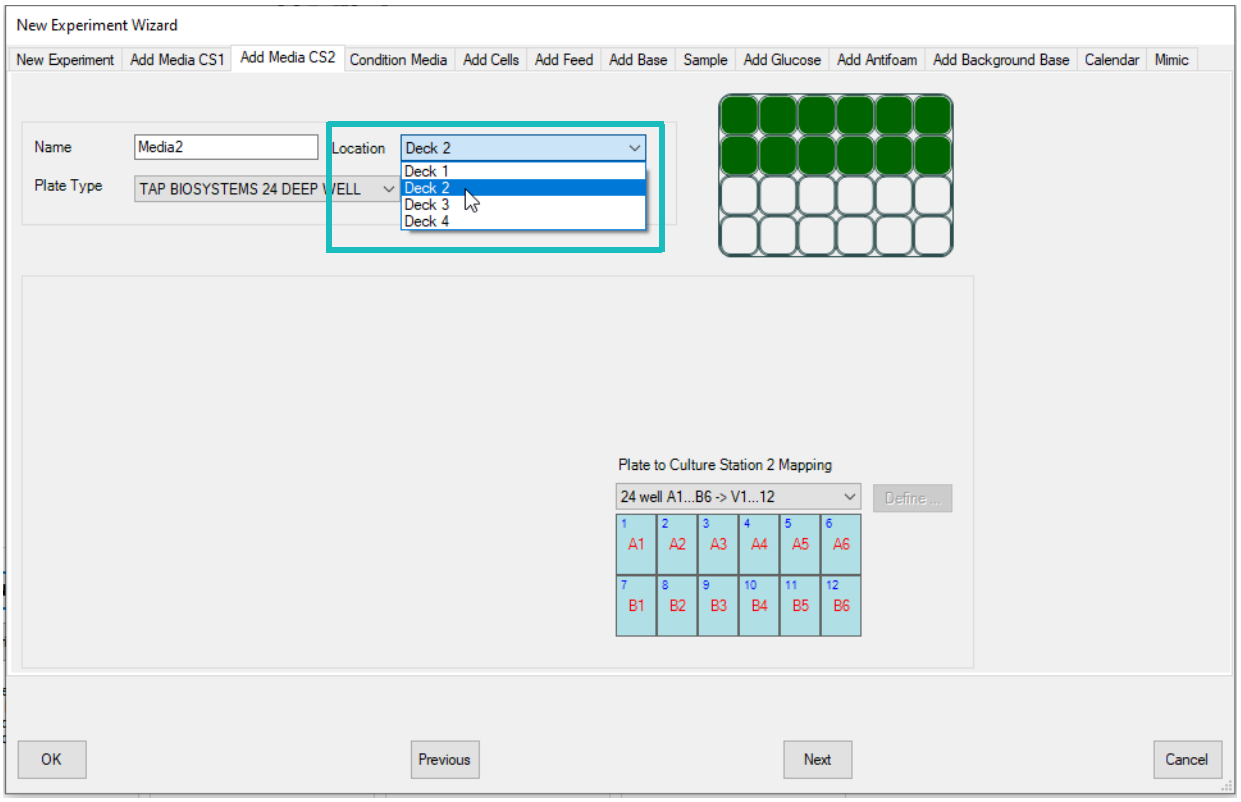


Figure E-6: Add Media CS2 Tab

Check the **Condition Media** checkbox in the Condition Media Tab.

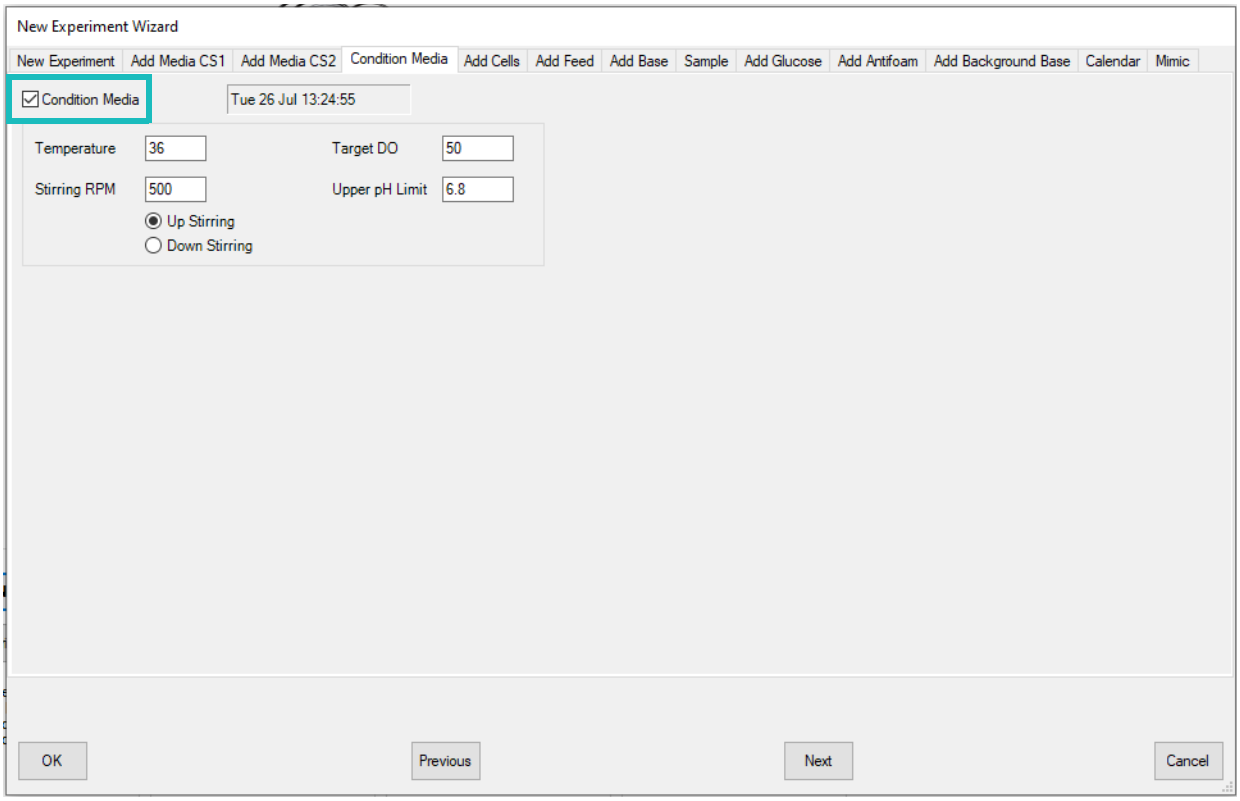


Figure E-7: Condition Media Tab

Skip the remaining tabs and go to the last one, **Mimic**. Click **OK**.

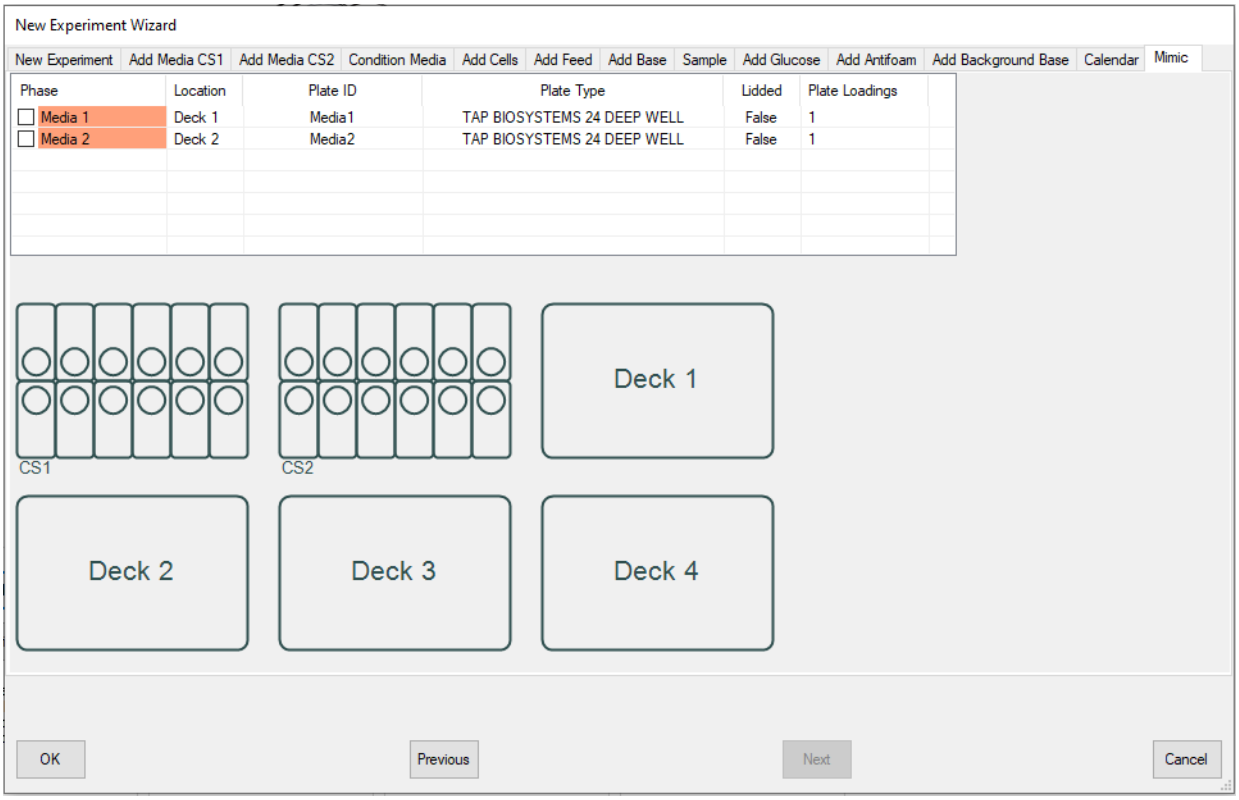


Figure E-8: Mimic Tab

When asked if you want to create the experiment, click **Yes**.

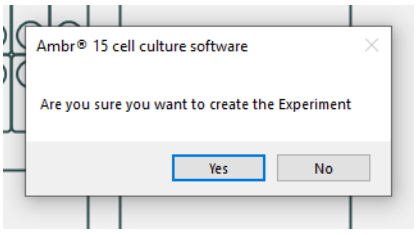


Figure E-9: Create Experiment Dialog

In the main screen, click the **Mimic** Tab.

This shows the placement of plates and pipettes on the Ambr® 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.

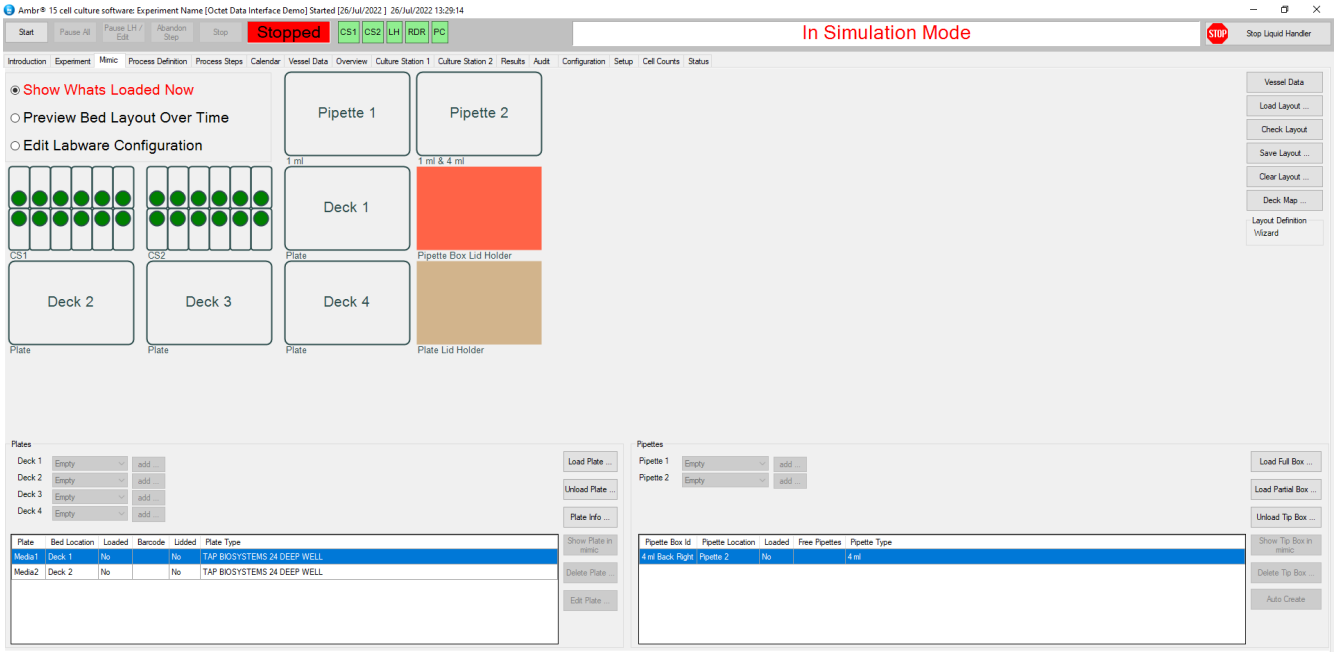


Figure E-10: Mimic Tab in Main Screen

We need to add a 96-well plate that will contain the culture station samples to be analyzed in the Octet® 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.

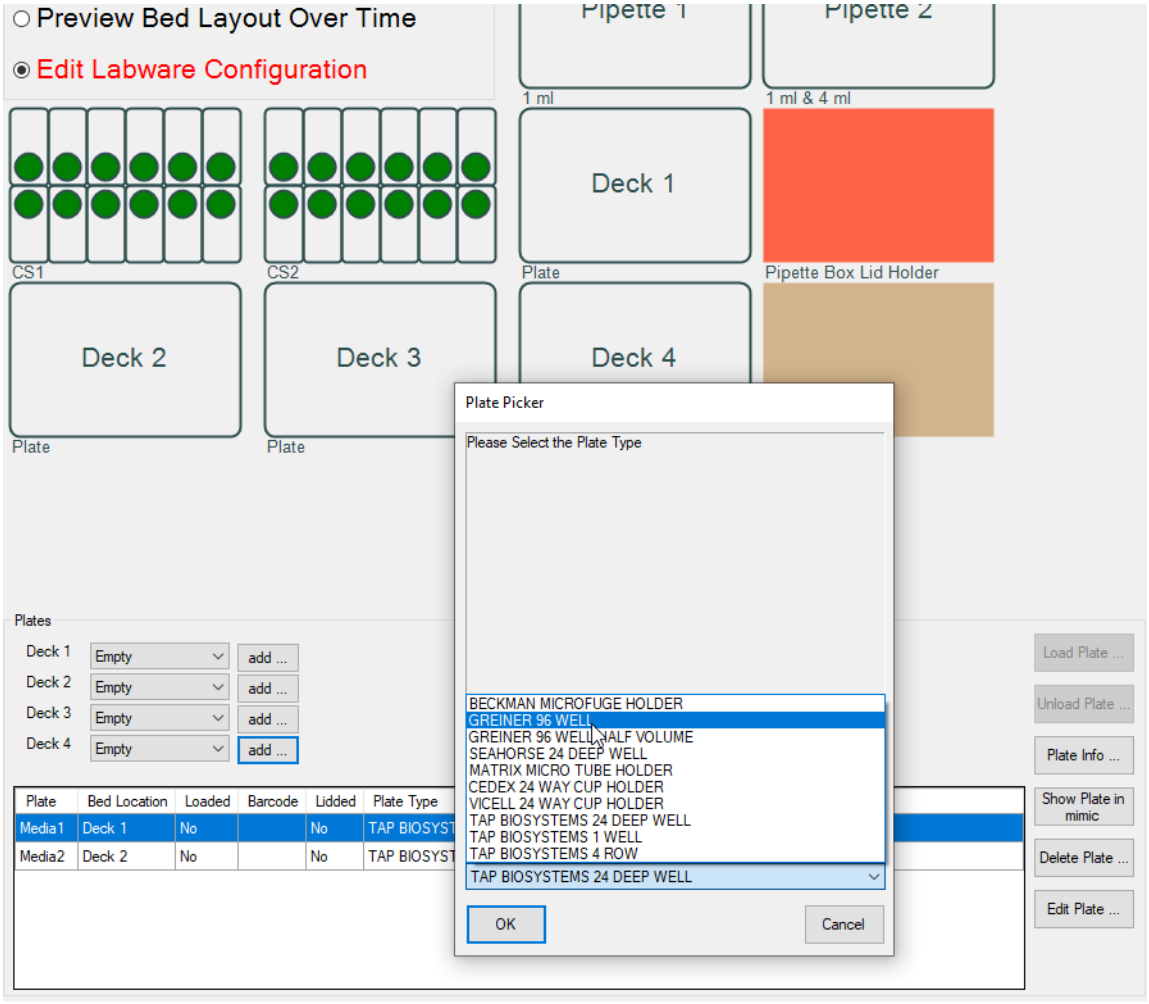


Figure E-11: Selecting a Plate Type

On the right next to Pipette 1, click **add...** and follow the prompts to add 1 ml pipettes.

When you are finished adding the necessary labware, the Mimic tab should look like this:

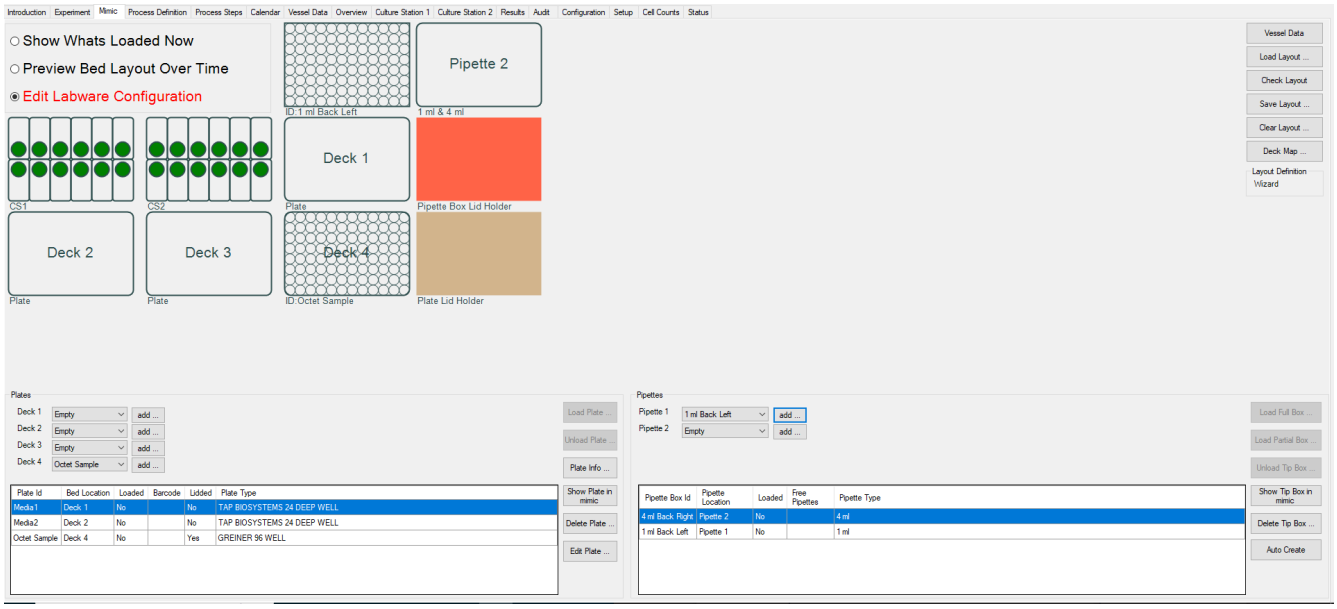


Figure E-12: Mimic Tab

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® system. What is missing is the 96-well Octet® sample plate and the 1 ml pipettes to transfer culture station samples to this plate.

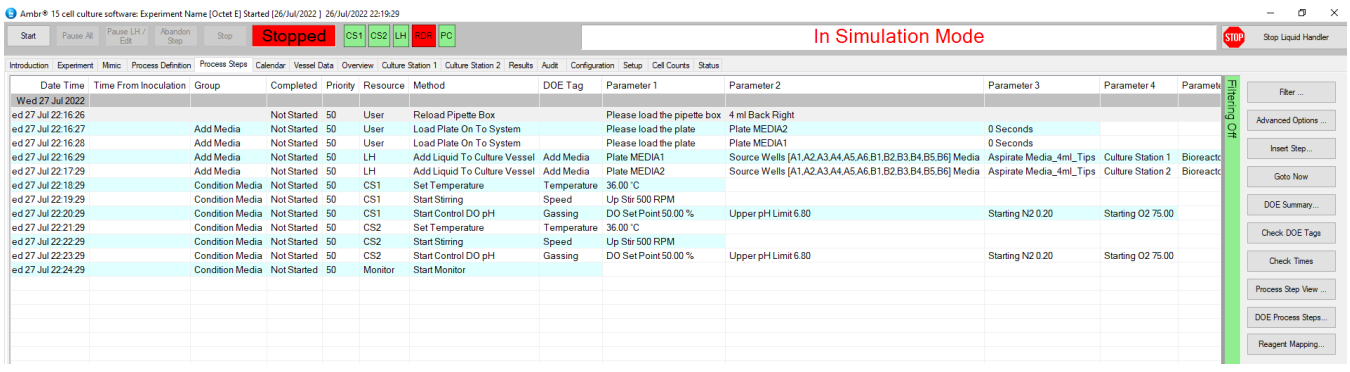


Figure E-13: Process Steps Tab

In the next few screens, we will add a step to place the 1 ml pipettes on the Ambr® system.

Right click any step and select **Process Steps | Insert New**.

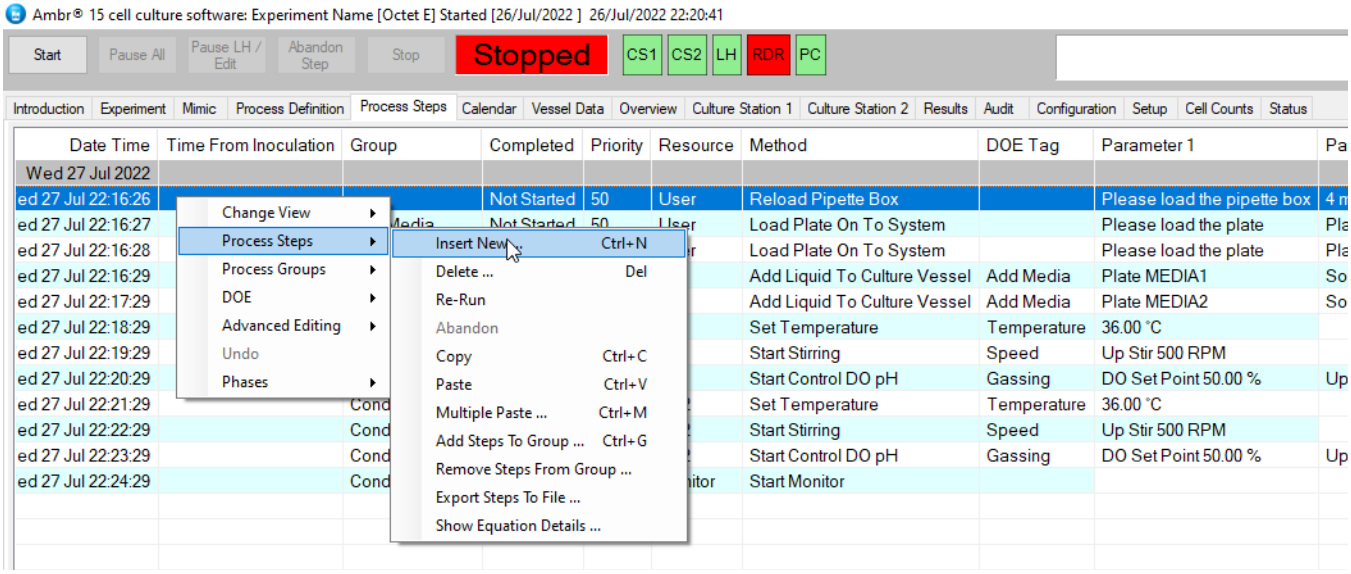


Figure E-14: Inserting a New Process Step

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.

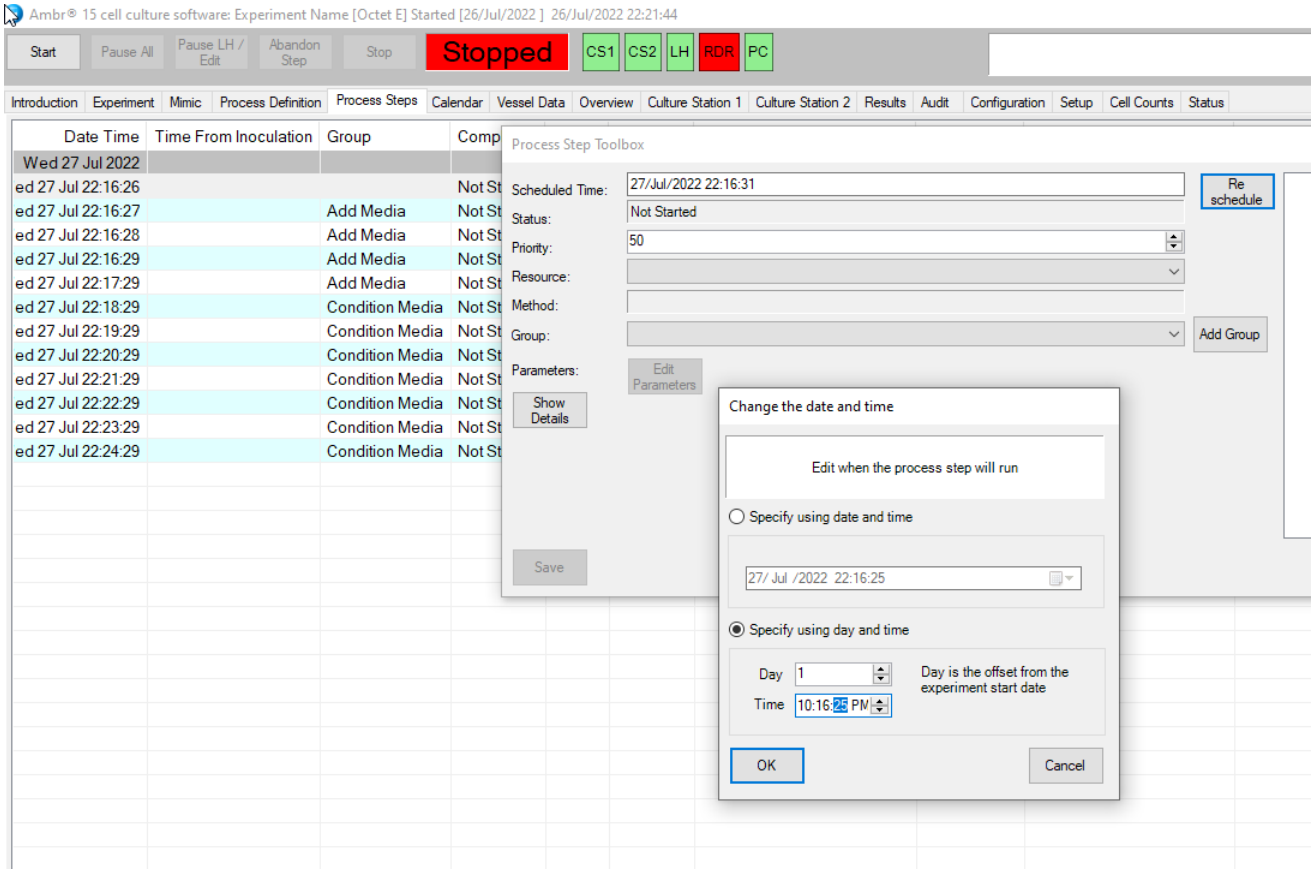


Figure E-15: Rescheduling the Process Step

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.

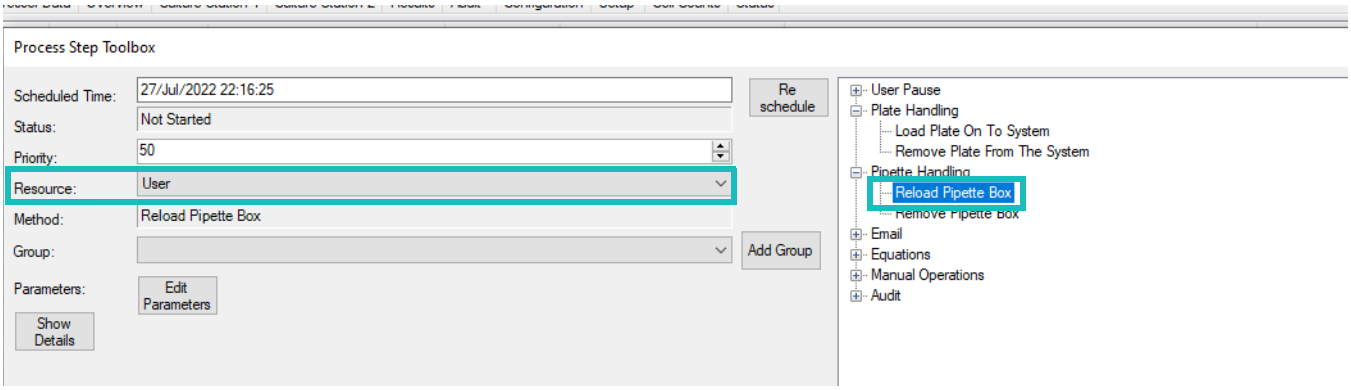


Figure E-16: Selecting Reload Pipette Box in the Process Step Toolbox Dialog

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**.

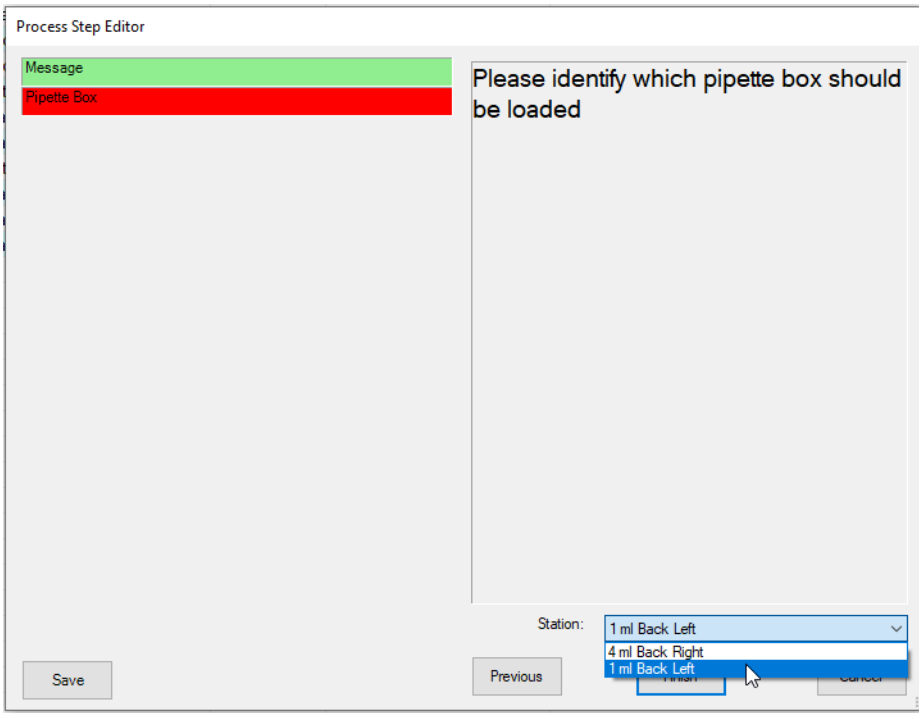


Figure E-17: Process Step Editor

To add the 96-well plate to the Ambr®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.

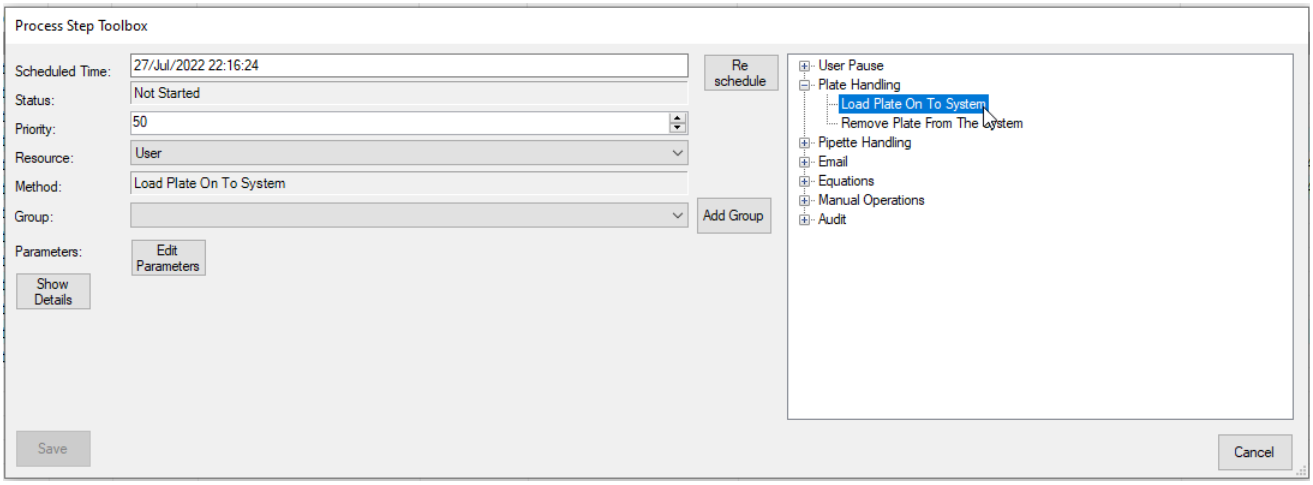


Figure E-18: Selecting Load Plate On To System

In the **Plate** selector, choose **OCTET SAMPLE** (or the name you gave the 96-well plate previously defined in the Mimic tab). Click **Save**.

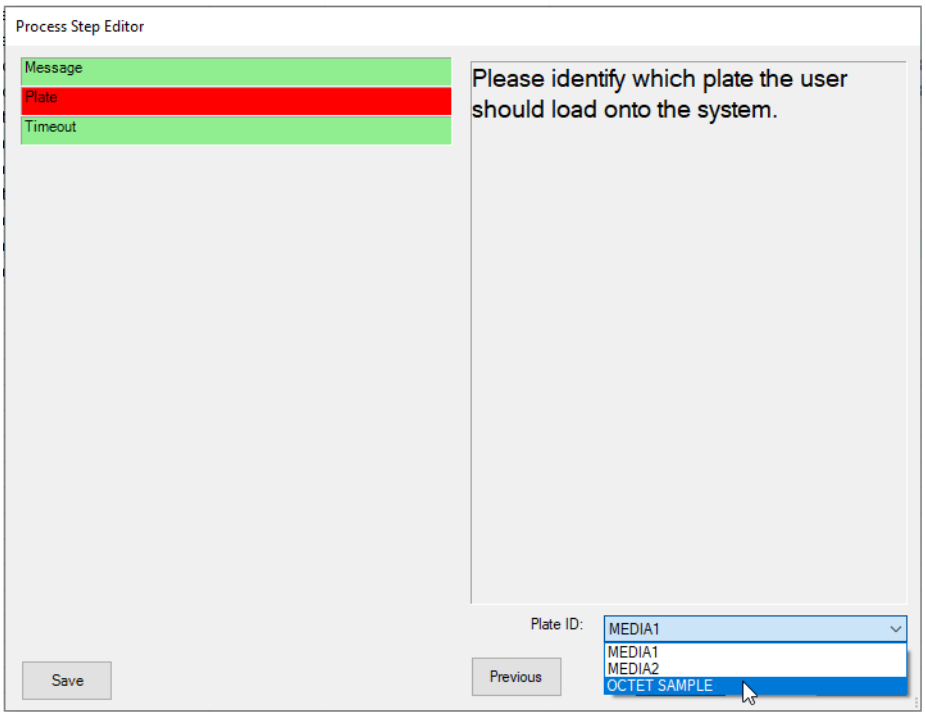


Figure E-19: Selecting the Plate

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
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
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
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.

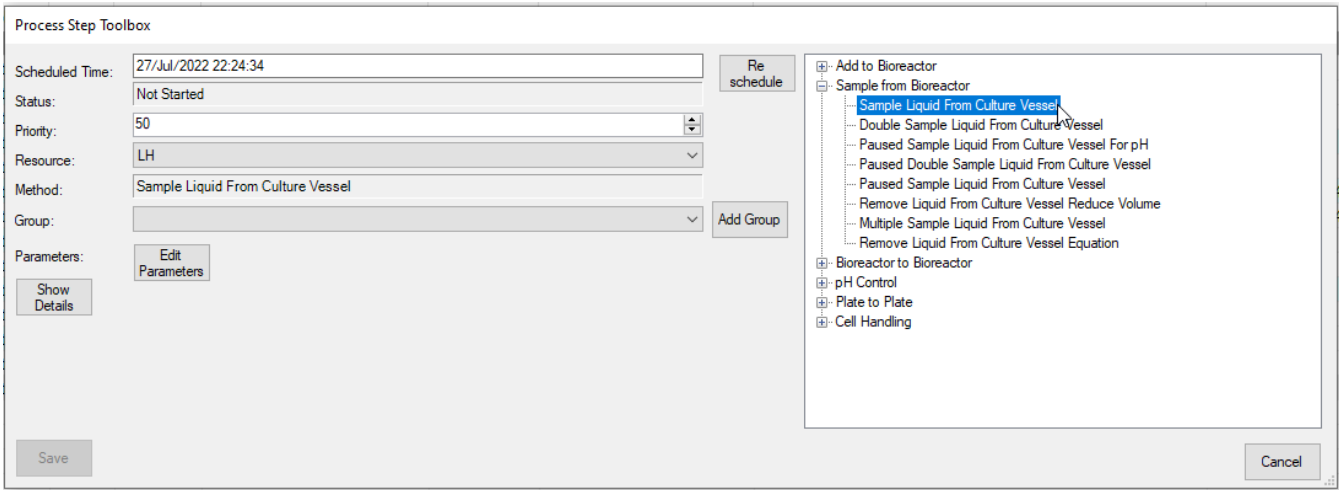


Figure E-21: Selecting Sample Liquid From Culture Vessel

To make this step a little easier to spot, we'll create an Octet® group and assign this step to it. Click the **Add Group** button and type **Octet** into the **Add Group Name** dialog.

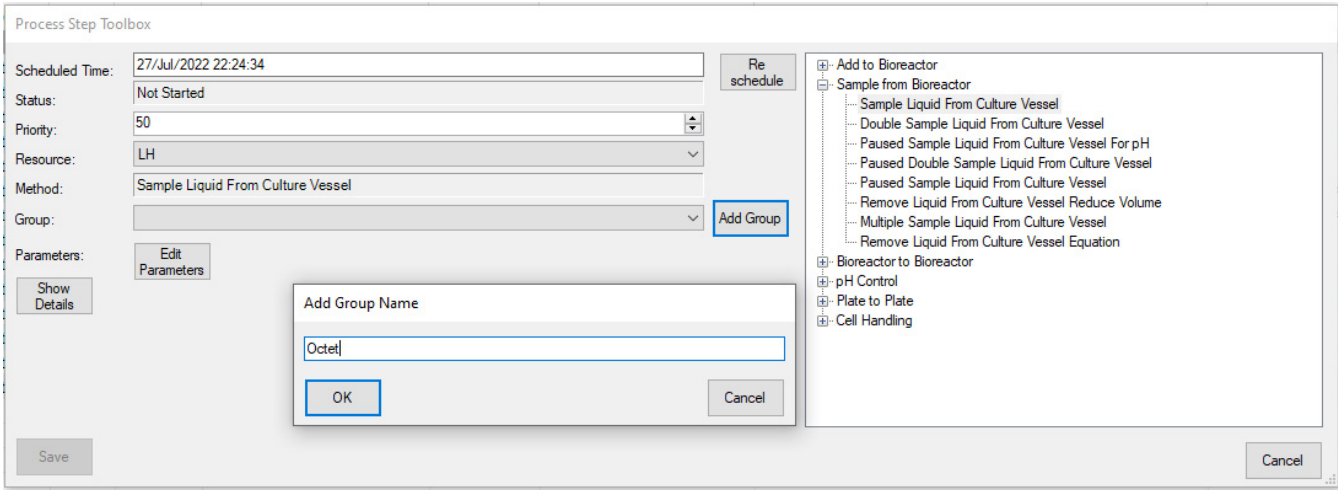


Figure E-22: Adding the Group Name

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).

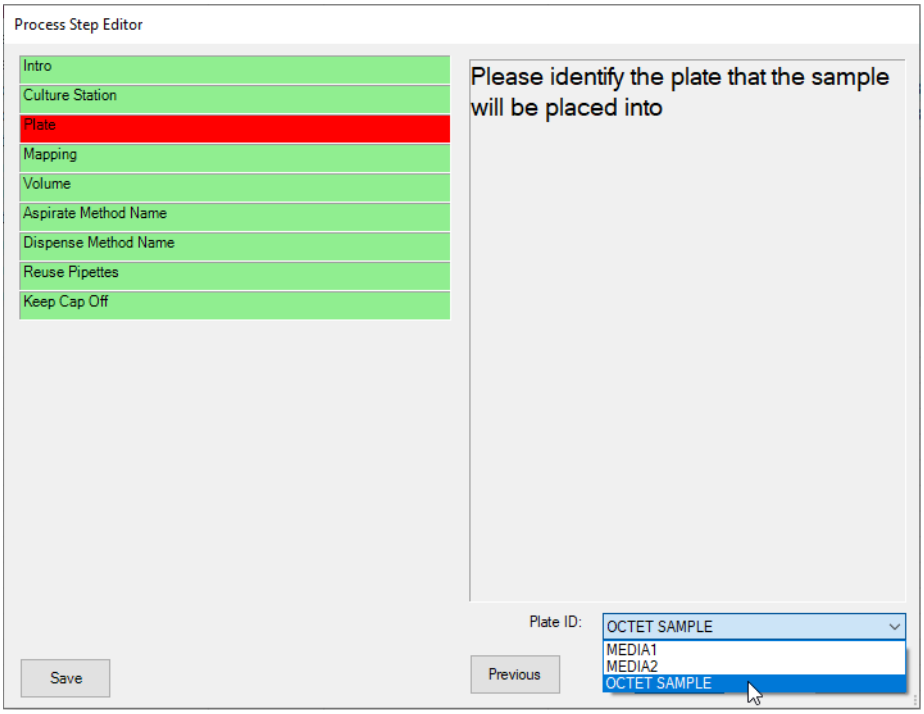


Figure E-23: Selecting the Sample Destination

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**.

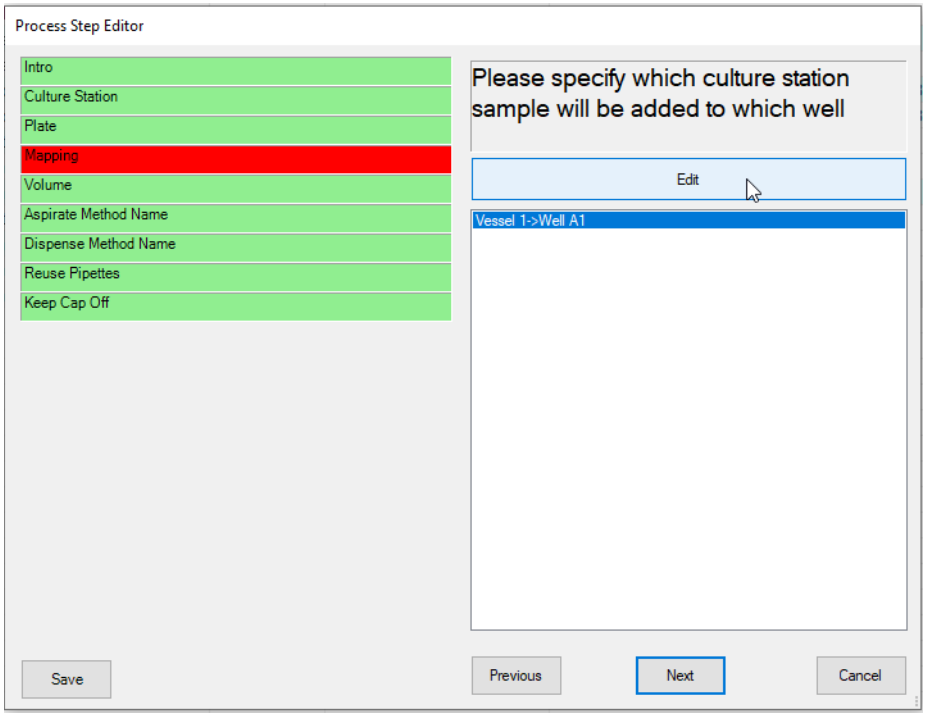


Figure E-24: Sample Mapping

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.

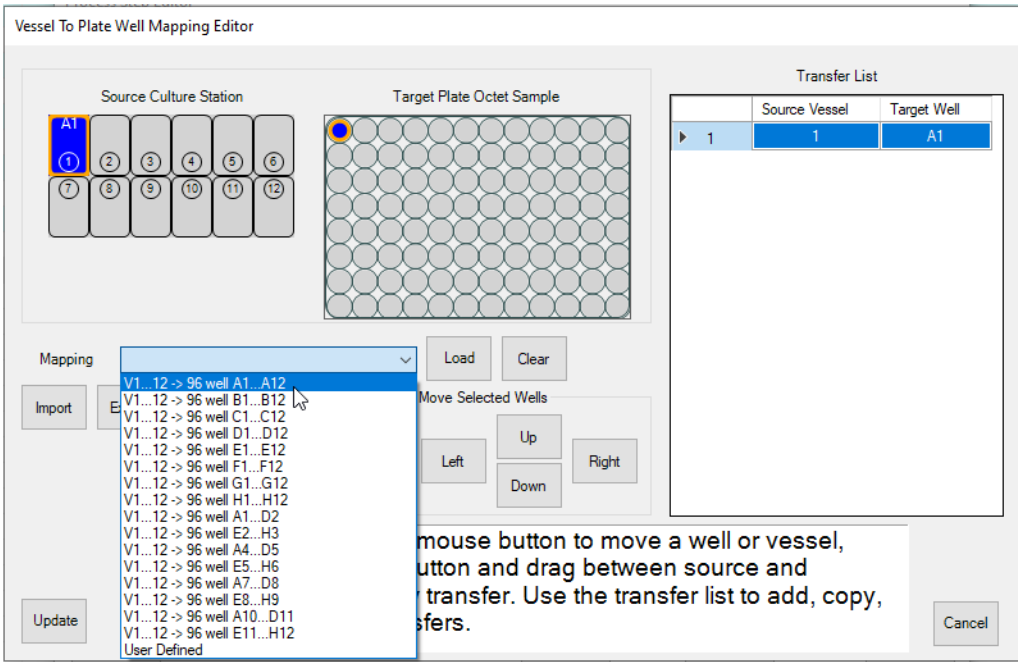


Figure E-25: Mapping Editor

This view now shows all 12 vessels mapped to the first row of the sample plate. Click **Update** to accept this mapping.

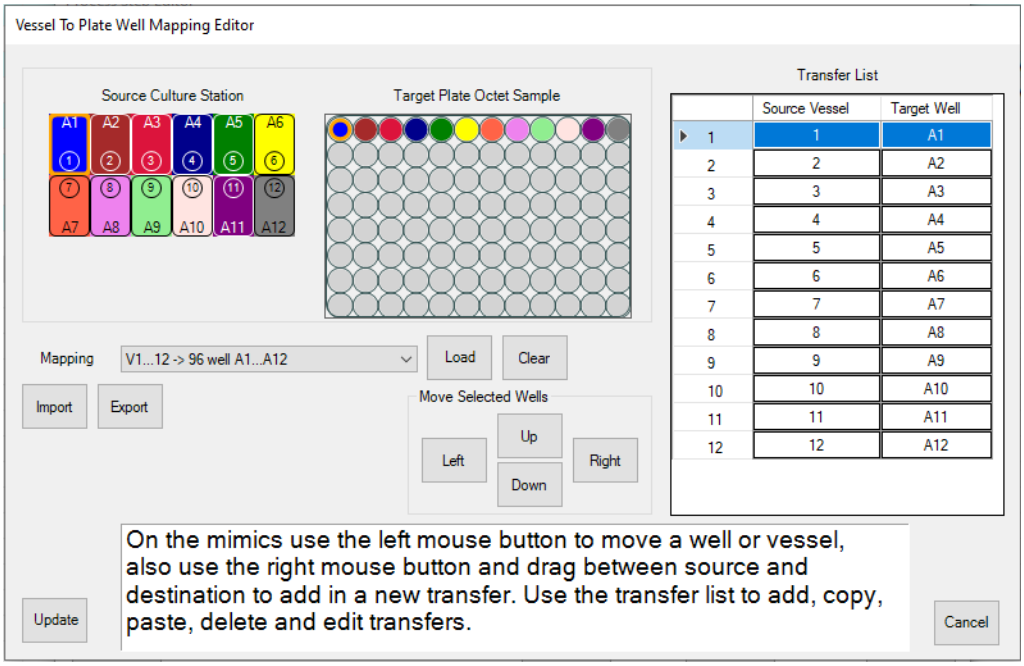


Figure E-26: Updated Mapping

In the **Volume** settings, set the amount of sample to pipette. All other options can be left at their default settings. Click **Save**.

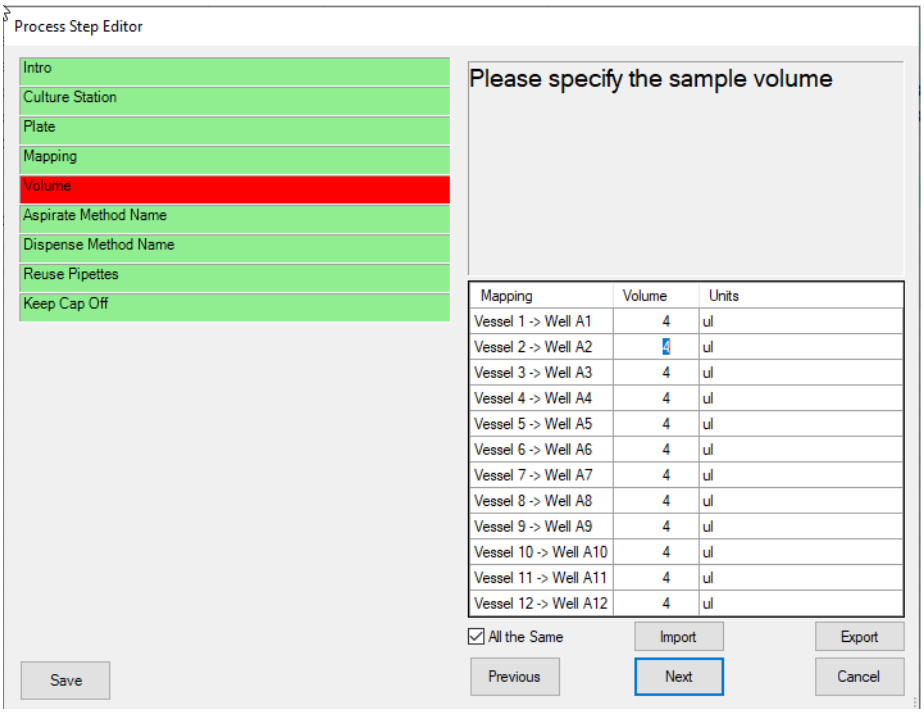


Figure E-27: Setting the Amount of Sample to Pipette

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 box
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 0 field, make sure the date is set for **today**. Next, click the **Reschedule** button.

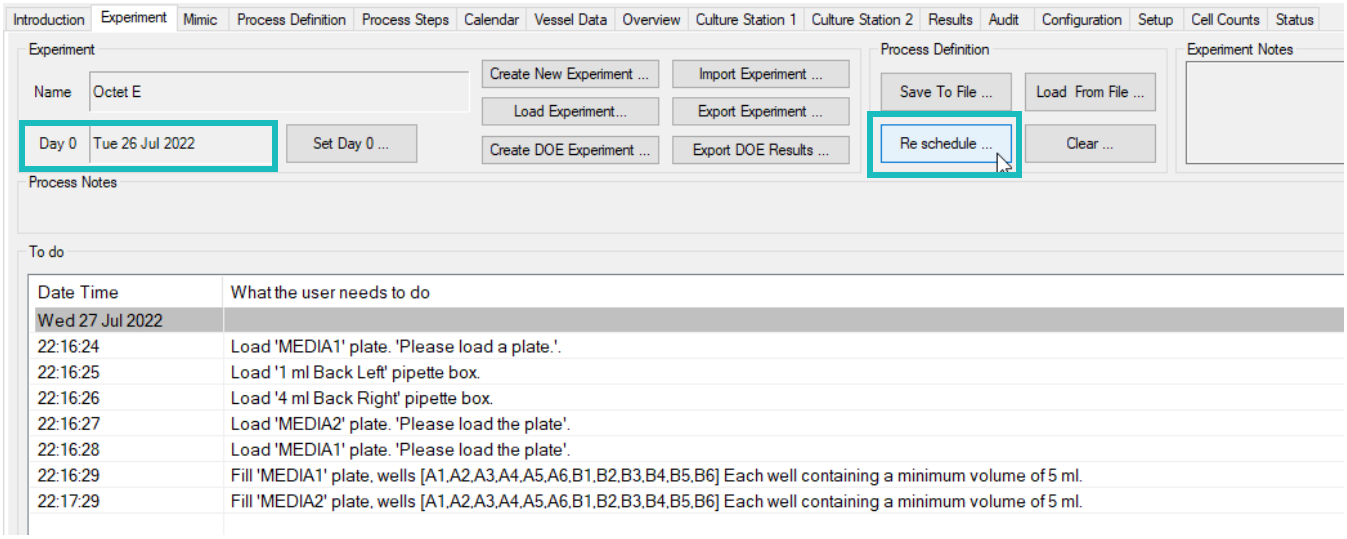


Figure E-29: Rescheduling the Experiment for Today

Set the **Day** to **0** and **Time** to 3-4 minutes from the current time.

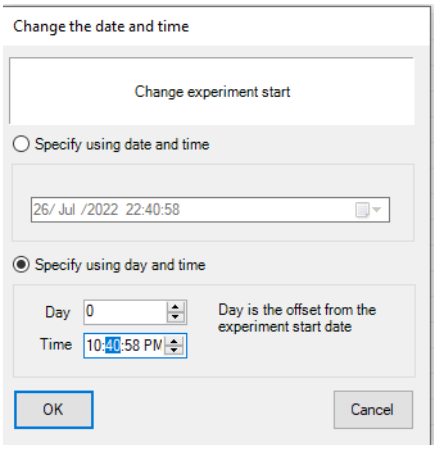


Figure E-30: Setting the Experiment Date and Time

Click the **Start** button to start the experiment.

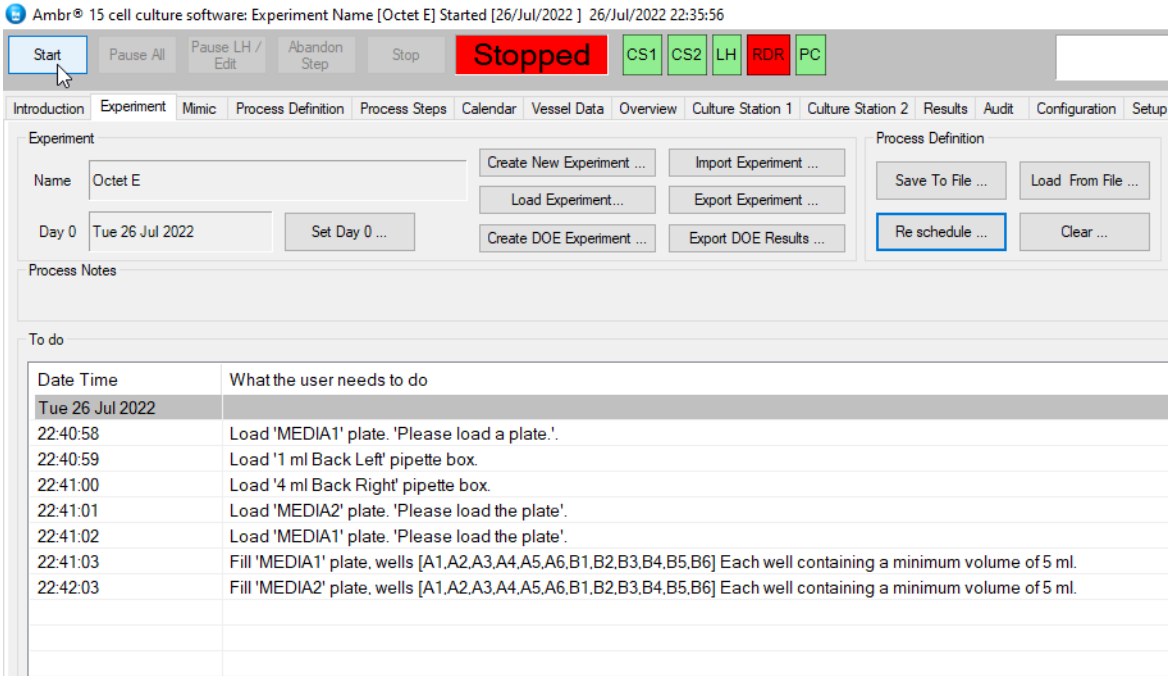


Figure E-31: Starting the Experiment

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**.

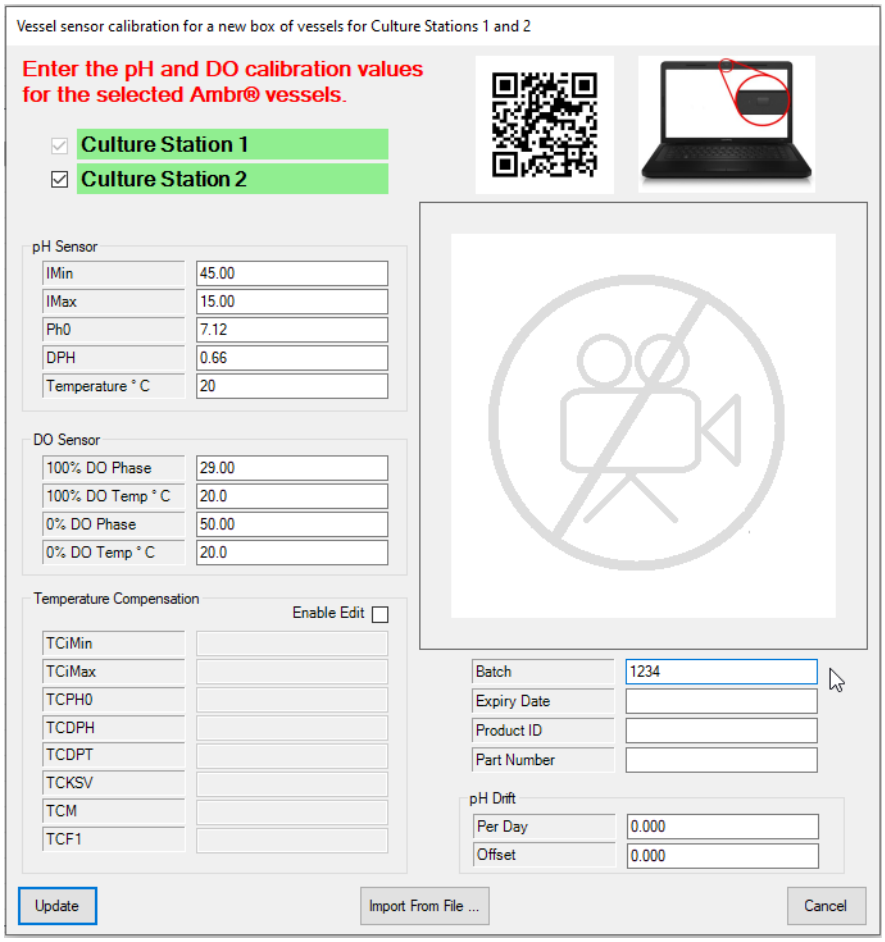


Figure E-32: Setting the Batch Number

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.

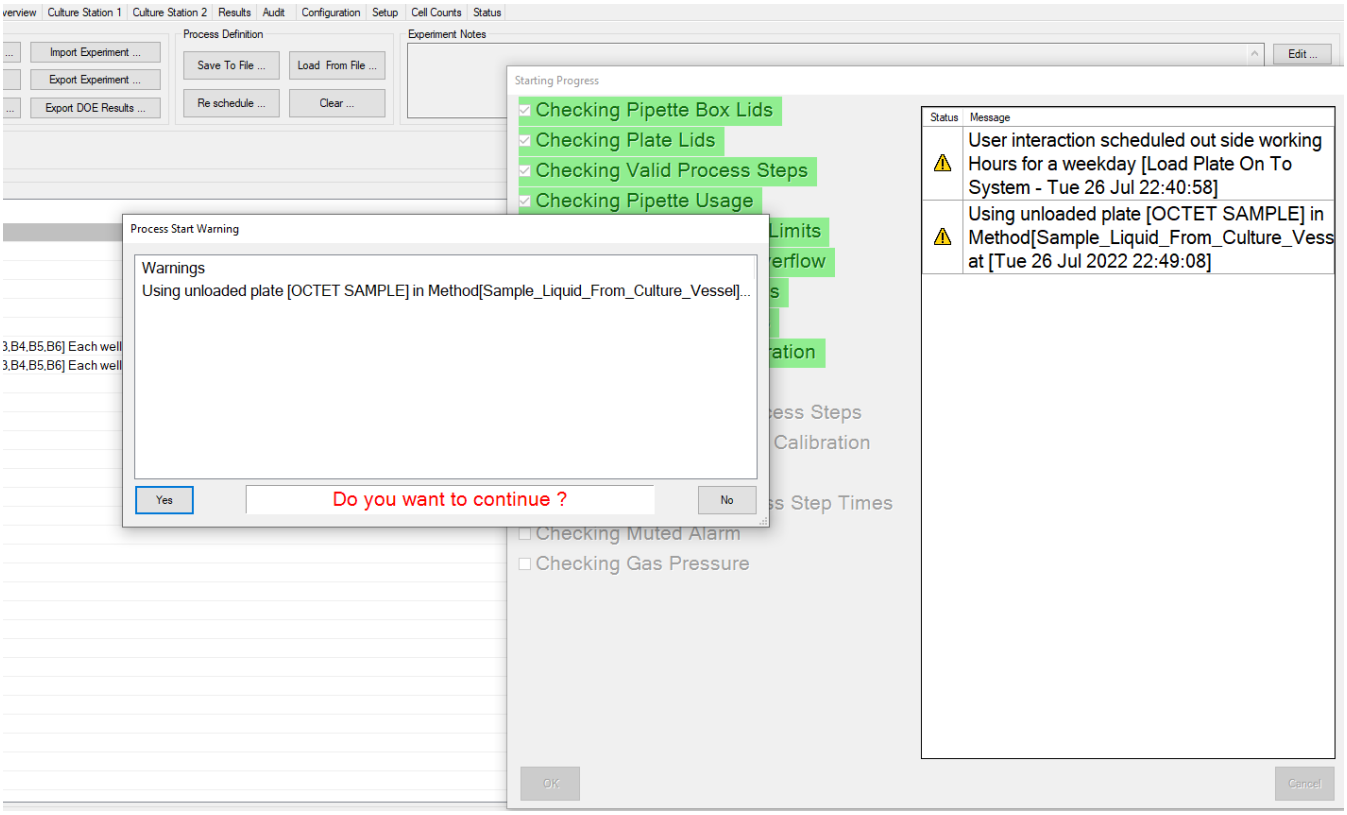


Figure E-33: Using Unloaded Plate Warning

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.

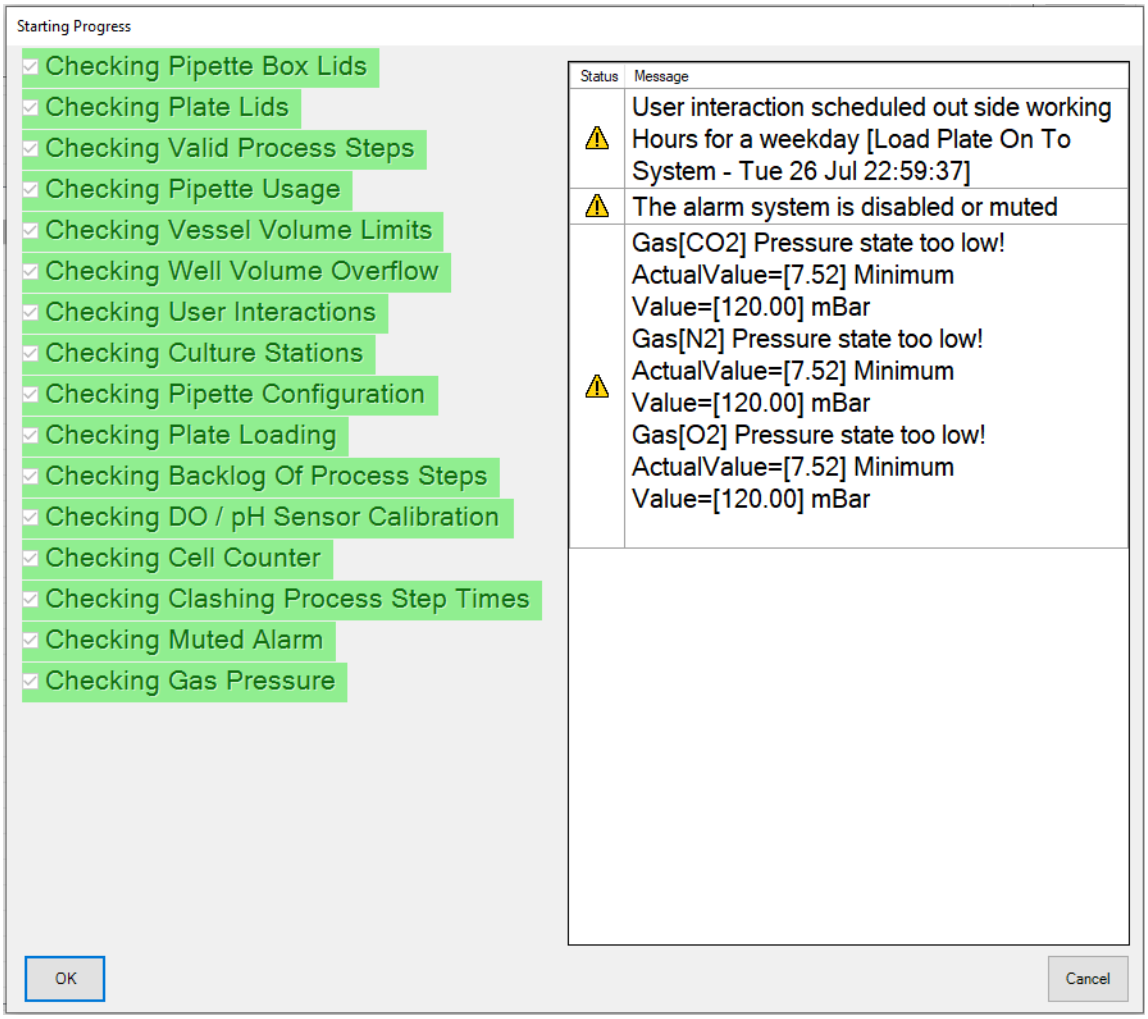


Figure E-34: Messages in Starting Progress Dialog Showing Green Status

The first few steps will inform you to place plates and pipettes onto the Ambr® system. Click **OK** for each prompt.

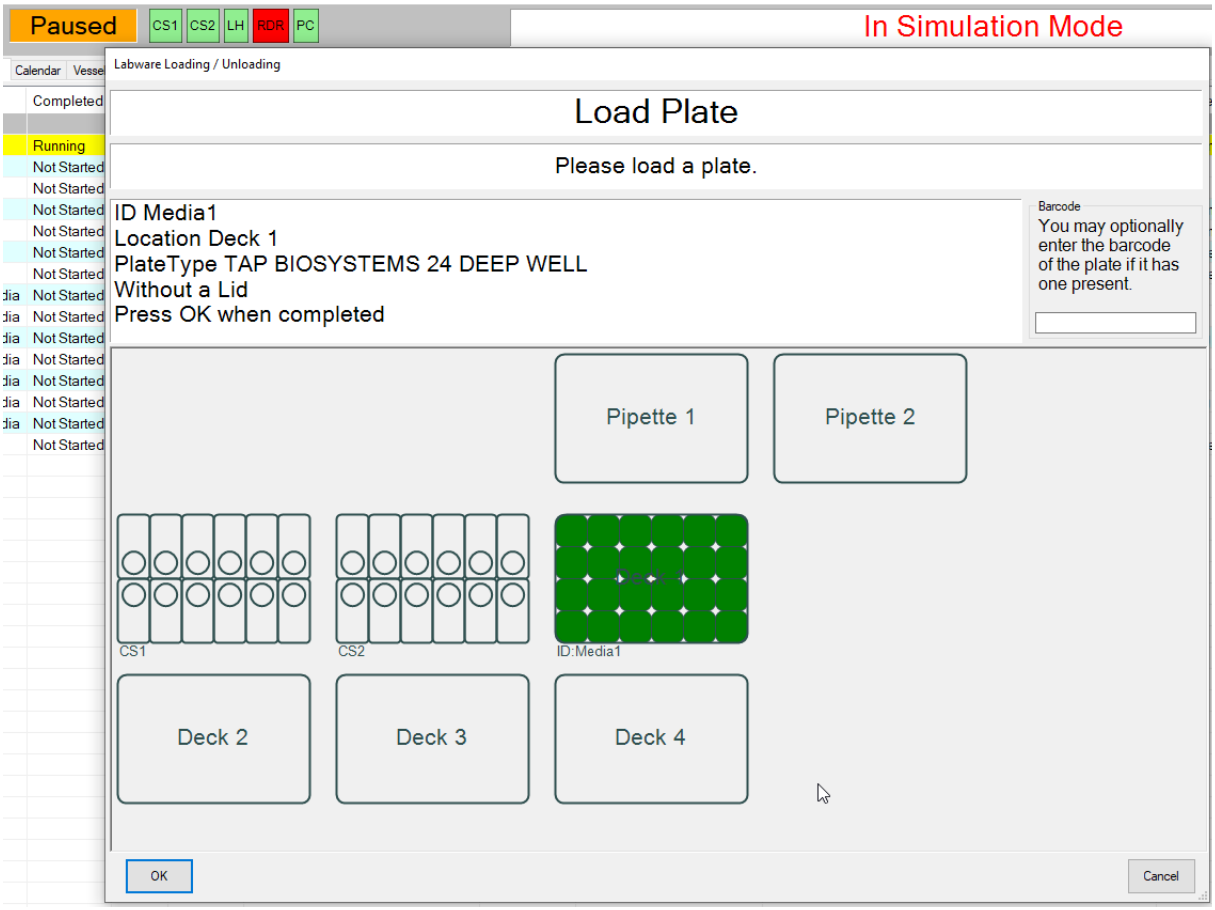
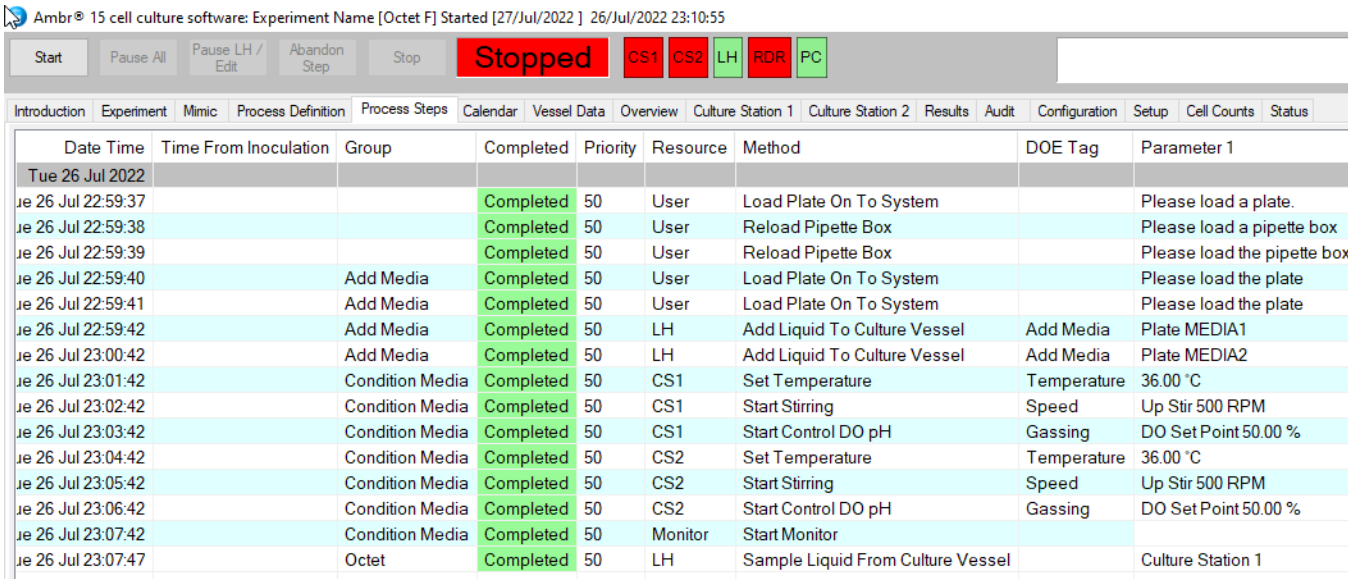


Figure E-35: Load Plate Prompt

The Process Steps table shows the status of each step in the experiment. When completed, all steps should be green.



Date Time	Time From Inoculation	Group	Completed	Priority	Resource	Method	DOE Tag	Parameter 1
Tue 26 Jul 2022								
ue 26 Jul 22:59:37			Completed	50	User	Load Plate On To System		Please load a plate.
ue 26 Jul 22:59:38			Completed	50	User	Reload Pipette Box		Please load a pipette box
ue 26 Jul 22:59:39			Completed	50	User	Reload Pipette Box		Please load the pipette box
ue 26 Jul 22:59:40		Add Media	Completed	50	User	Load Plate On To System		Please load the plate
ue 26 Jul 22:59:41		Add Media	Completed	50	User	Load Plate On To System		Please load the plate
ue 26 Jul 22:59:42		Add Media	Completed	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA1
ue 26 Jul 23:00:42		Add Media	Completed	50	LH	Add Liquid To Culture Vessel	Add Media	Plate MEDIA2
ue 26 Jul 23:01:42		Condition Media	Completed	50	CS1	Set Temperature	Temperature	36.00 °C
ue 26 Jul 23:02:42		Condition Media	Completed	50	CS1	Start Stirring	Speed	Up Stir 500 RPM
ue 26 Jul 23:03:42		Condition Media	Completed	50	CS1	Start Control DO pH	Gassing	DO Set Point 50.00 %
ue 26 Jul 23:04:42		Condition Media	Completed	50	CS2	Set Temperature	Temperature	36.00 °C
ue 26 Jul 23:05:42		Condition Media	Completed	50	CS2	Start Stirring	Speed	Up Stir 500 RPM
ue 26 Jul 23:06:42		Condition Media	Completed	50	CS2	Start Control DO pH	Gassing	DO Set Point 50.00 %
ue 26 Jul 23:07:42		Condition Media	Completed	50	Monitor	Start Monitor		
ue 26 Jul 23:07:47		Octet	Completed	50	LH	Sample Liquid From Culture Vessel		Culture Station 1

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®system.

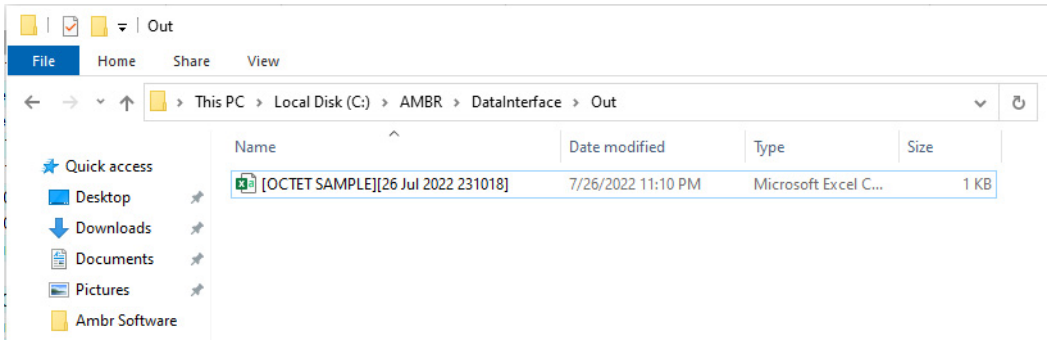
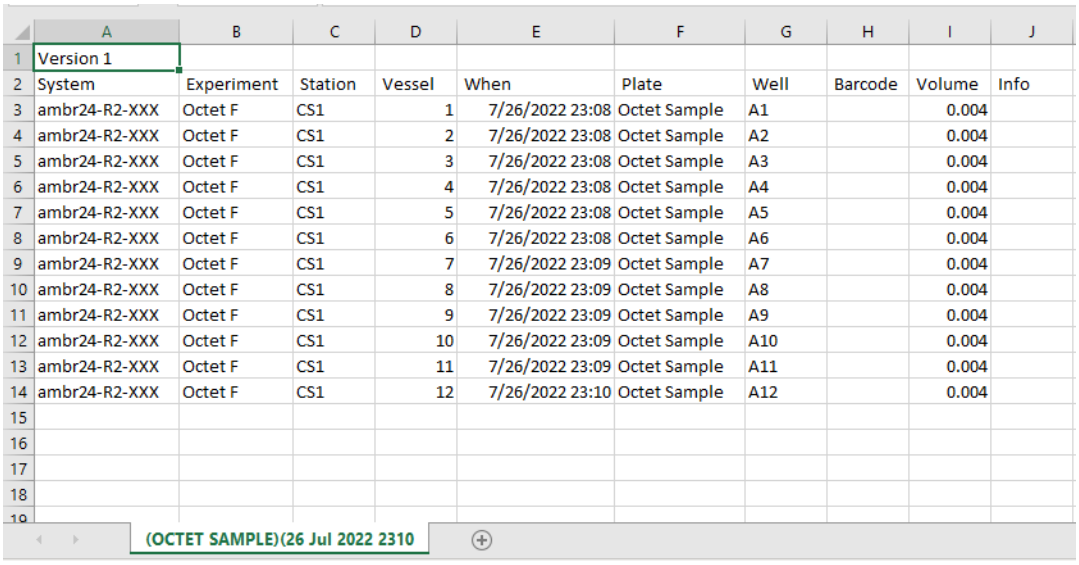


Figure E-37: Output Directory



	A	B	C	D	E	F	G	H	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										
19										

(OCTET SAMPLE)(26 Jul 2022 2310

Figure E-38: Plate Map CSV File

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