SARTORIUS

User Manual

Octet® Analysis Studio

User Guide Version 13

Octet® Analysis Studio User Guide

3011603 A August 2022

Table of Contents

Chapter 1:

General Information

System Requirements

Table 1-1: System Requirements

Section	Description
Operating system	Windows® 10 32-bit or 64-bit, version 1607 "Anniversary Update" and newer Windows 11 version 21H2 or newer
Processor	1.5GHz or faster
RAM	2GB
Hard disk space	Approximately 100MB required for installation, additional for experiment data and results
Screen Resolution	1920x1080 recommended Display scale and layout of 100% recommended
Other	Microsoft® Excel® is required for creating Excel reports in the legacy (.xls) format. Not required for creating Excel reports in the modern (.xlsx) format.

About the Software

Octet® Analysis Studio software lets you analyze multiple data files at once. You can look at multiple experiments, biosensor trays or plates from the same or different experiments, and then append them together.

You can preview experiment data and create single datasets by:

- Appending or concatenating multiple experiments together
- Overlaying data from multiple biosensor trays in one experiment
- Overlaying data from multiple plates in different data folders

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 does not work if the guidelines are not followed.*

WARNING: *Informs the user that specific actions could cause irreversible consequences or damage.*

Contacting Technical Support

You can contact Sartorius Technical Support at:

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E-mail: octetsupport@sartorius.com

Chapter 2:

Getting Started

Launching the Software

Double-click the desktop icon to launch the software.

The Home screen appears (Figure 2-1).

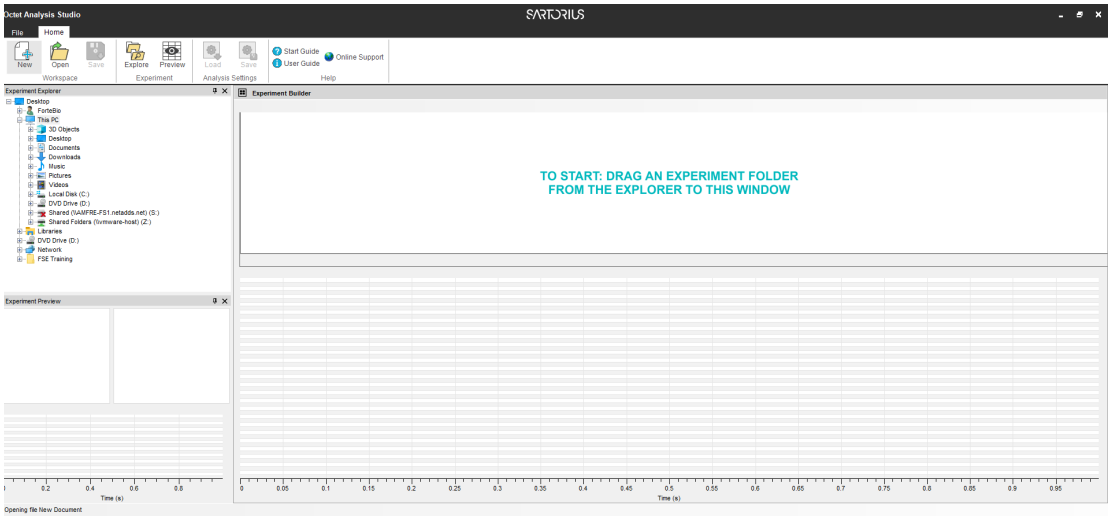


Figure 2-1: Home Screen

Previewing Experiment files

Use the Home screen to preview experiment files before doing editing or analysis, and to append or overlay multiple data files at the same time.

To preview experiments, use the folder tree in the **Experiment Explorer** window (Figure 2-2) to navigate to the location of your experiment files. Selectable Kinetic experiment folders are pink, and Quantitation experiment folders are teal.

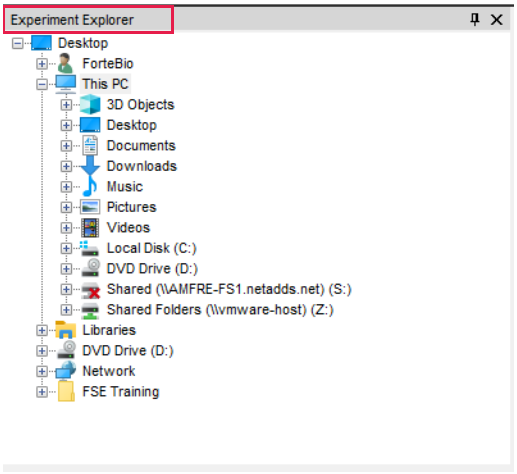


Figure 2-2: Experiment Explorer

Click a folder to display the experiment's Sensor Tray layout, Sample Plate layout(s), and sensorgrams in the **Preview window** (Figure 2-3). If more than one Sample Plate or Sensor Tray was used, each displays in its own tab. Click the tabs or use the arrow keys to view individual plates.

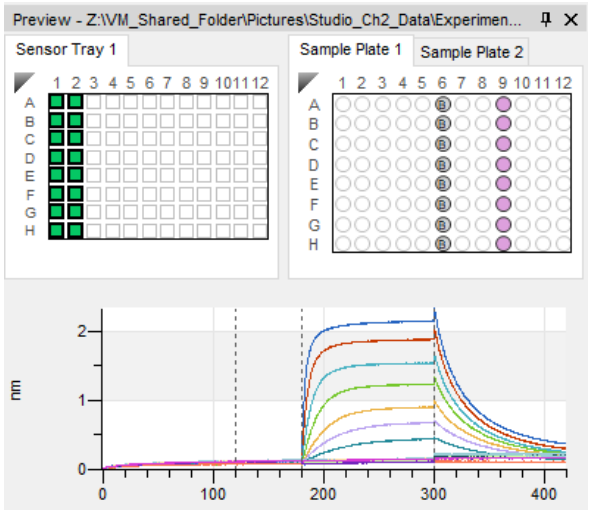


Figure 2-3: Preview Window

To view individual sensorgrams in the Preview window, click a location in the Sensor Tray (Figure 2-4):

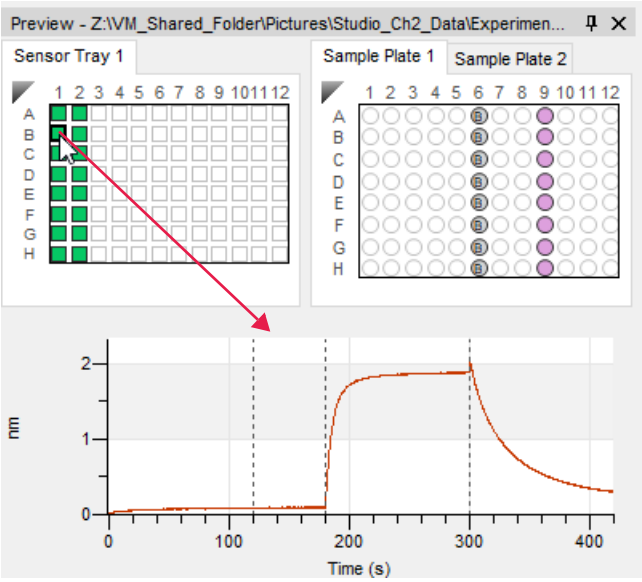


Figure 2-4: Viewing Individual Biosensor Data in the Preview Window

Opening Experiment files for Analysis

To view data in the Experiment Builder,

1. Open the data folder by doing one of the following:
   - double-click the data folder in Experiment Explorer
   - click the data folder and drag it to the Segment window (Figure 2-5).

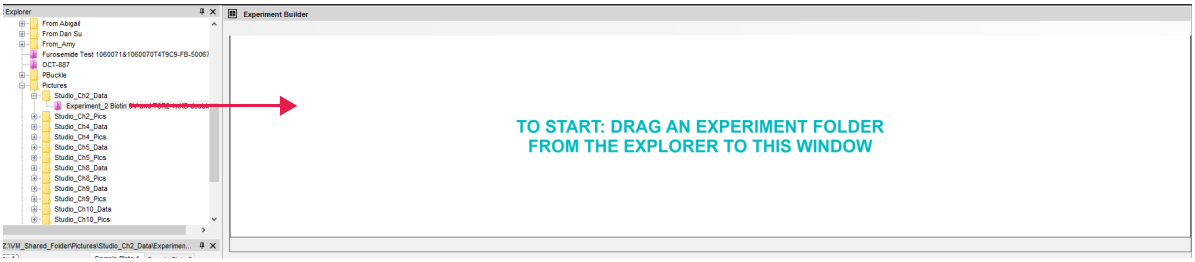


Figure 2-5: Drag Folder to the Segment Window to View

2. Release the mouse when the Segment window displays **Ok to drop this experiment here** (Figure 2-6).

Figure 2-6: OK to Drop Message

3. The sensorgrams are displayed in the Data Viewing area under the Segment window.

Figure 2-7: Data View

The file name, sensor trays, and the assay acquisition rate and steps used with each sensor tray also display.

To remove experiment file(s), right-click the **Experiment name**, **Sensor Tray or Assay** box for the file you want to remove and select **Delete** (Figure 2-8).

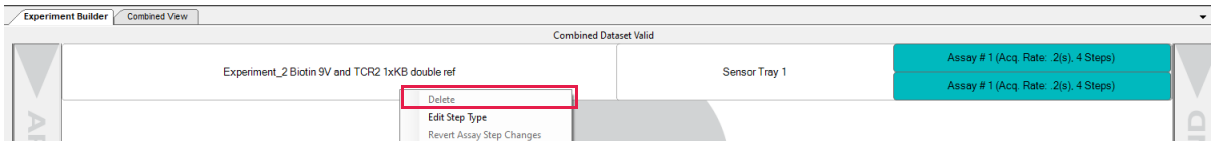


Figure 2-8: Deleting Files

Click  (**Open-Workspace**) to open a previously analyzed experiment (.efrd) file.

Resizing, Hiding and Closing Windows

To resize the windows in the Home screen, roll the cursor over the right edge of the Experiment Explorer or Preview windows until the resizing cursor displays (Figure 2-9).

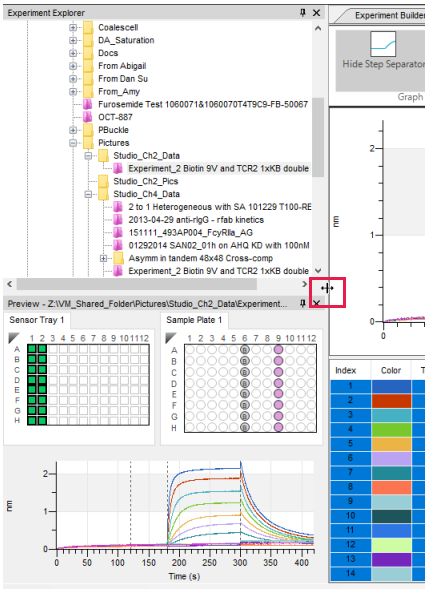


Figure 2-9: Window Resize Cursor

Click and drag the window to resize (Figure 2-10):

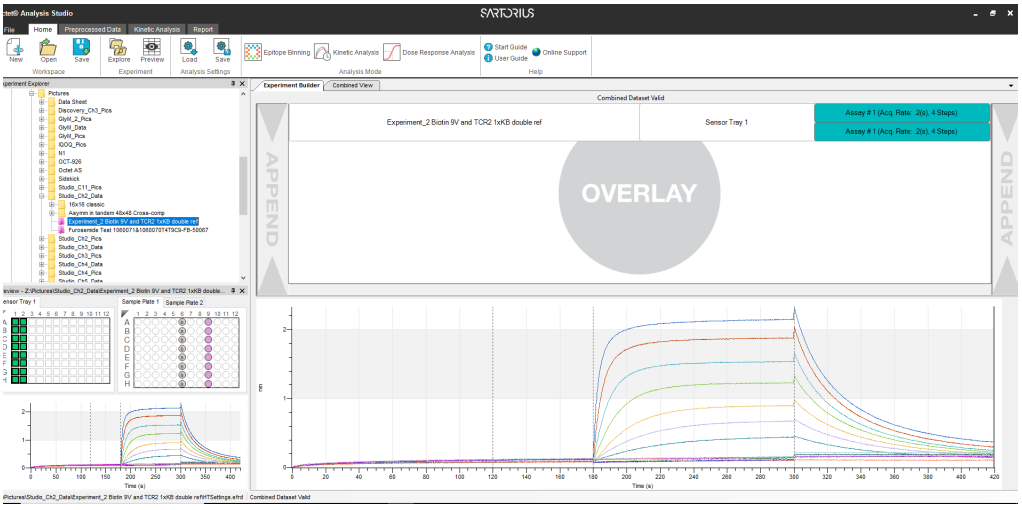


Figure 2-10: Resized Windows

Hide windows and minimize them to the sidebar by clicking the **Pin** icon (Auto Hide) in the upper right corner. (Figure 2-11).

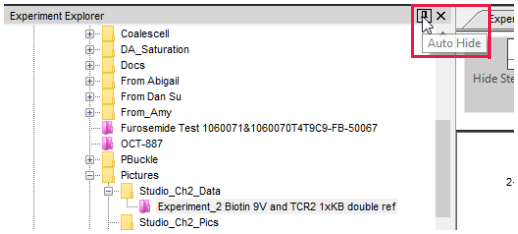


Figure 2-11: Hiding Windows

To see a hidden window, roll your mouse over the sidebar. This displays the window as long as the mouse stays on the sidebar (Figure 2-12).

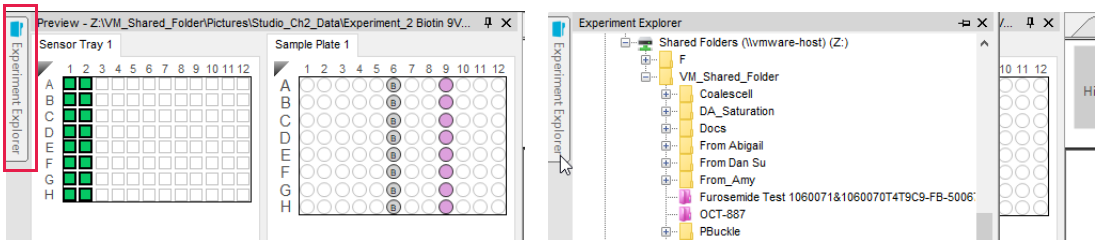


Figure 2-12: Viewing Hidden Windows

To restore a window on the Home screen, click the **Pin** icon (Auto Hide) (Figure 2-13).

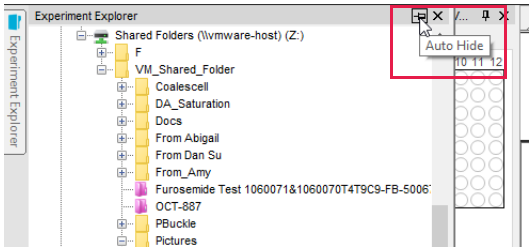


Figure 2-13: Unhiding Windows

To close the Experiment Explorer and Preview windows, click X (Close) in the upper right corner (Figure 2-14).

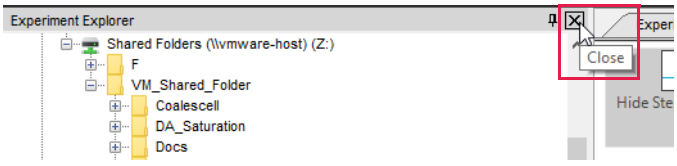


Figure 2-14: Closing Windows

To redisplay either window, click  or  .

Working with Graphs

The Octet® Analysis Studio software offers many types of graphs. This section describes features common to most graphs. The chapters about specific types of analysis will have the details for that specific graph type.

Selecting Traces

Usually, graphs are displayed with their corresponding data table. In some cases, a corresponding sensor tray or sample plate is also shown. The active selection is synchronized between graphs, the data table and the plate view.

Single Trace

To make a selection:

- click a graph trace,
- click a row in the data table,
- or click a sensor or sample well in the plate view.

In Figure 2-15, sensor B1 was selected in the Full Traces table. The graph displays that trace in an isolated view and the corresponding sensor well is highlighted in the plate view.

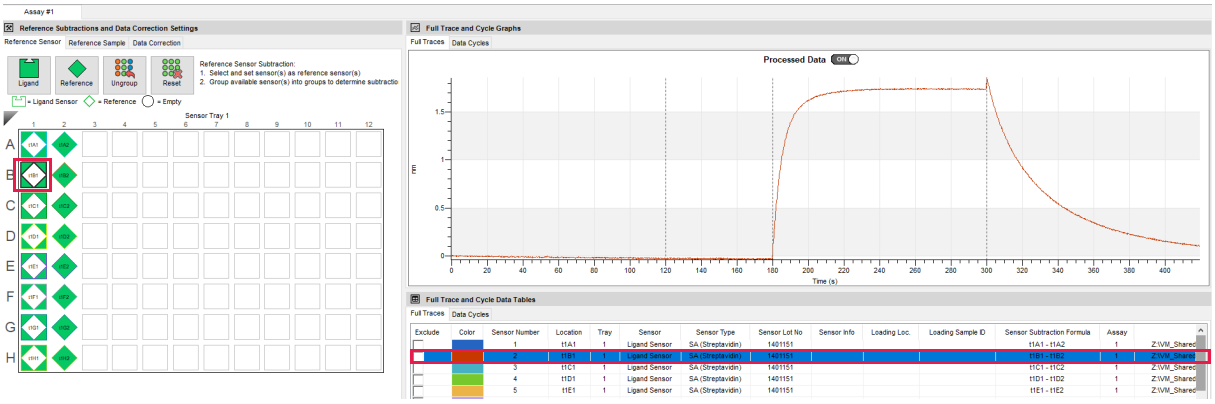


Figure 2-15: Viewing a Single Trace

Multiple Traces

You can view selected traces or all traces.

Selected Traces: press and hold Ctrl while making a selection on the data table or plate view.
In Figure 2-16 sensors A1, C1, and E1 were selected

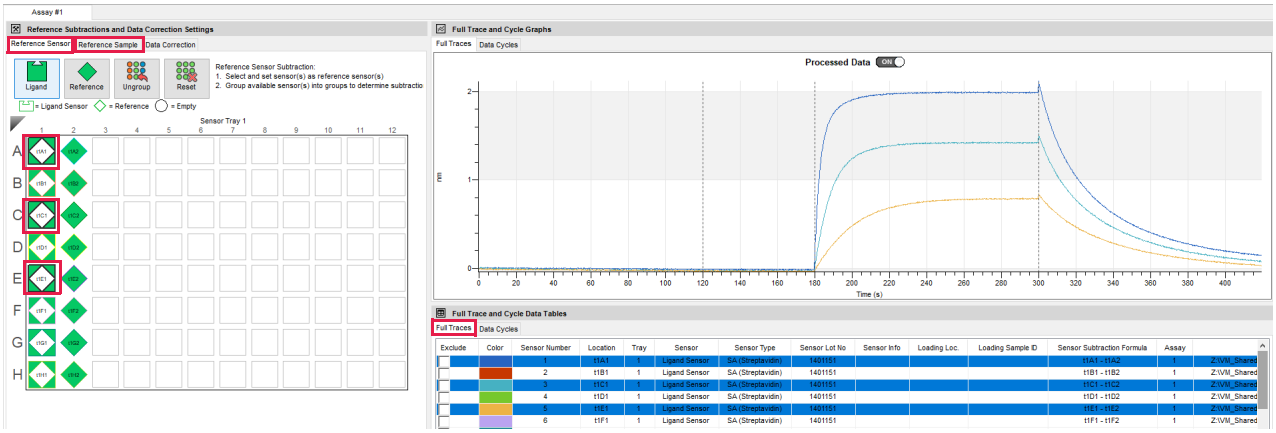


Figure 2-16: Viewing Multiple Sensorgrams

Changing the Graph View

-  (**Show All**) - After you view selected traces, click Show All.

-  (**Hide Step Dividers**) and  (**Show Steps Dividers**) - use these features to hide or show the vertical annotations that indicate a step transition. (Figure 2-17).

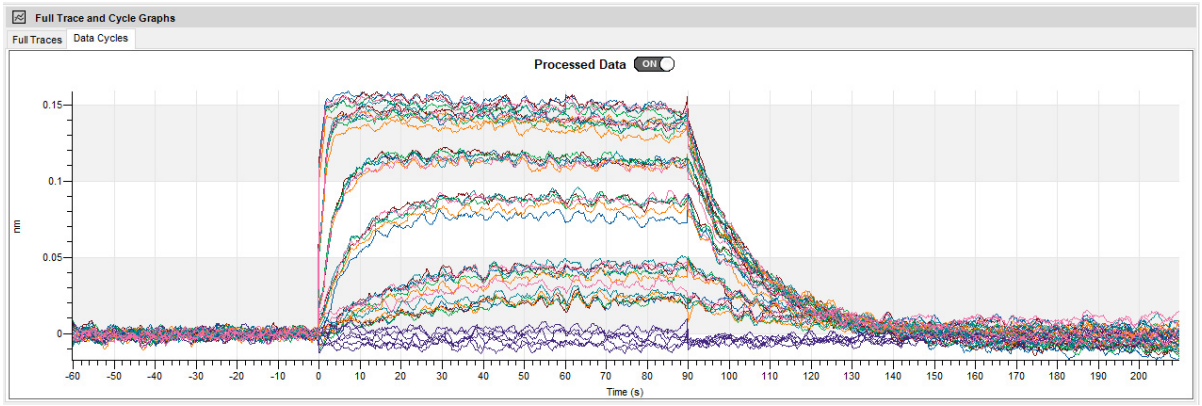


Figure 2-17: Step Dividers Hidden

- To make the sensorgram traces thicker, right click the graph and select **Thicken Line**:

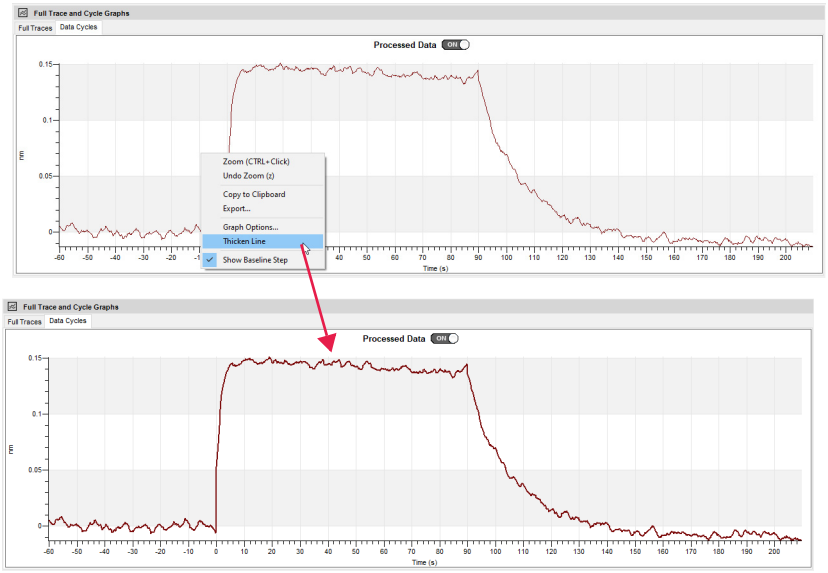


Figure 2-18: Selecting Thicken Line

- To see a zoomed-in version of the graph in a separate window, double click anywhere on the graph.
- To zoom in on a specific area, right-click in the graph area and select **Zoom** (Figure 2-3), or press **CTRL** and click.

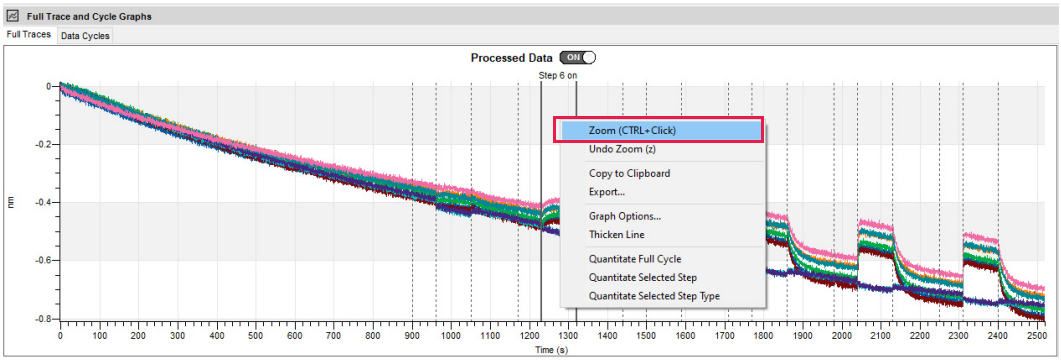


Figure 2-19: Selecting Zoom

Draw a box around the area you want to zoom in on with your mouse (Figure 2-4):

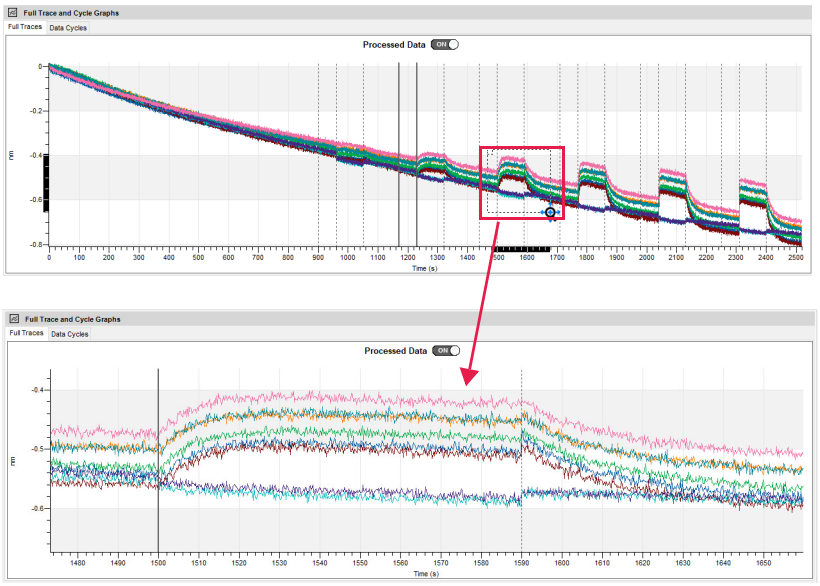


Figure 2-20: Selecting a Zoom Area

Right-click the graph area and select **Undo Zoom** or press **Z** on your keyboard to return to the full view.

Graph Options

To change the graph display options, right-click the graph and select **Graph Options**. The Graph Options window displays (Figure 2-21).Graph Options Window.

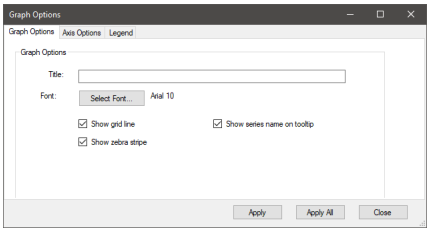


Figure 2-21: Changing Graph Options.

- **Title** - Adding text in the Title box displays that text above the graph (Figure 2-22).

Figure 2-22: Adding a Graph Title

- **Font Size** - Lets you select the font size of the Title in pixels.
- **Show Grid Line** - Checking or deselecting this box shows or hides the grid lines on the graph.
- **Show Zebra Stripe** - Checking or deselecting this box shows or hides (Figure 2-23) the alternating white and grey horizontal rows on the graph.

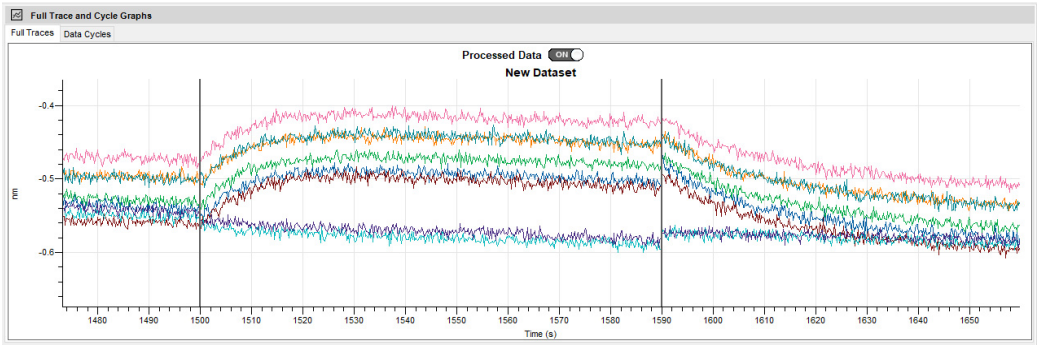


Figure 2-23: Show Zebra Stripe Deselected

- **Show Series Name on Tooltip** - Checking or deselecting this box shows (Figure 2-24) or hides the tooltip when you hover over a trace on the graph.

Figure 2-24: Show Series Name on Tooltip Selected

Axis Options

To change the graph's X and Y axis options, right-click the graph and select **Graph Options**, then select the **Axis Options** tab (Figure 2-25).

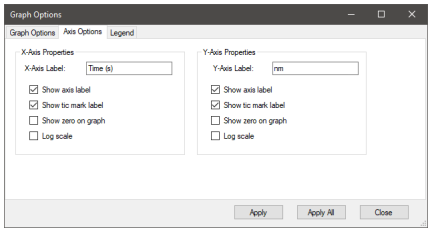


Figure 2-25: Axis Options Tab

- **Axis Label** - Changing text in the Axis Label boxes updates the X and Y axis labels on the graph.
- **Show Axis Label** - Checking or deselecting this box shows or hides the X and Y axis labels on the graph.
- **Show Tic Mark Label** - Checking or deselecting this box shows or hides the X and Y axes tic mark values on the graph.
- **Show Zero on Graph** - Shows the zero on the y-scale in the graph if the signals are too high and zero is not shown by default.
- **Log Scale** - Checking or deselecting this box changes the X and Y scale from linear to log scale (Figure 2-26).

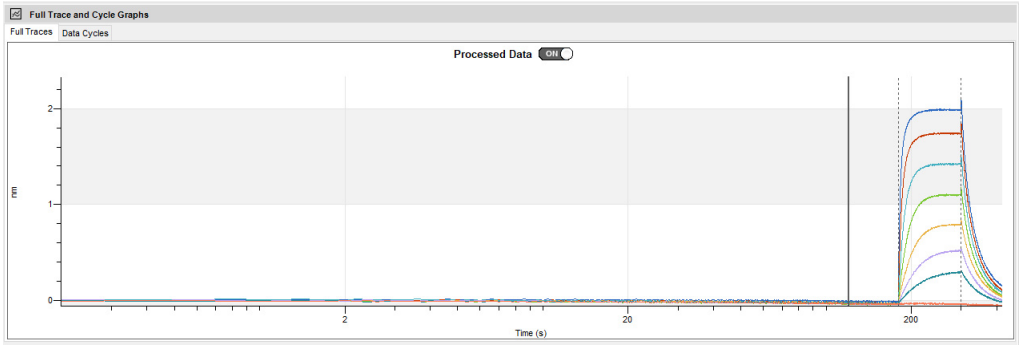


Figure 2-26: X-axis Log Scale Selected

Changing the Graph Axis Range

To change the graph's X and Y axis range, right-click the graph and select **Graph Options**, then select the **Custom Range** tab (Figure 2-27).

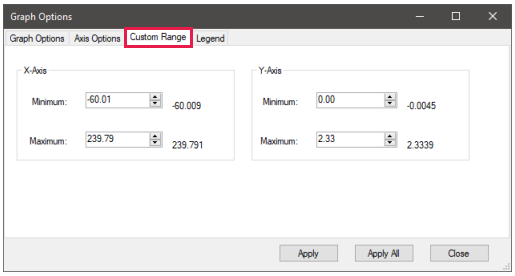


Figure 2-27: Custom Range Tab

Enter your own Minimum and Maximum values for the X and Y axes. After making your selections, click **Apply** to apply the change just to the graph you selected, or if you are viewing individual graphs, select **Apply All** to apply the change to all graphs.

Adding Fit Graph Legends

To show data legends on the graphs, right-click the graph and select **Graph Options**, then select the **Legend** tab (Figure 2-28).

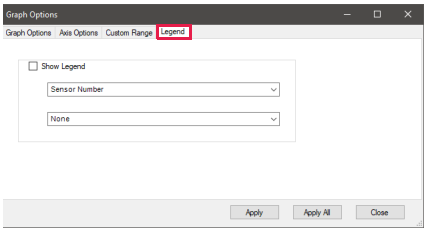


Figure 2-28: Legend Tab

Select up to two legend options from the drop down menus, then select Show Legends (Figure 2-29).

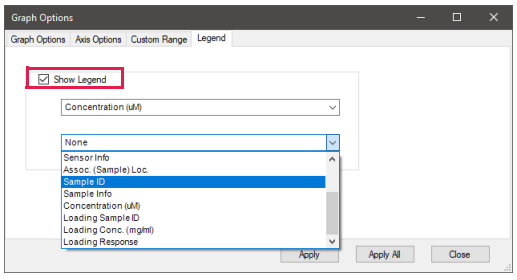


Figure 2-29: Selecting Legend Options

After making your selections, click **Apply** to apply the change. The legend option(s) selected displays at the bottom of the graph (Figure 2-30).

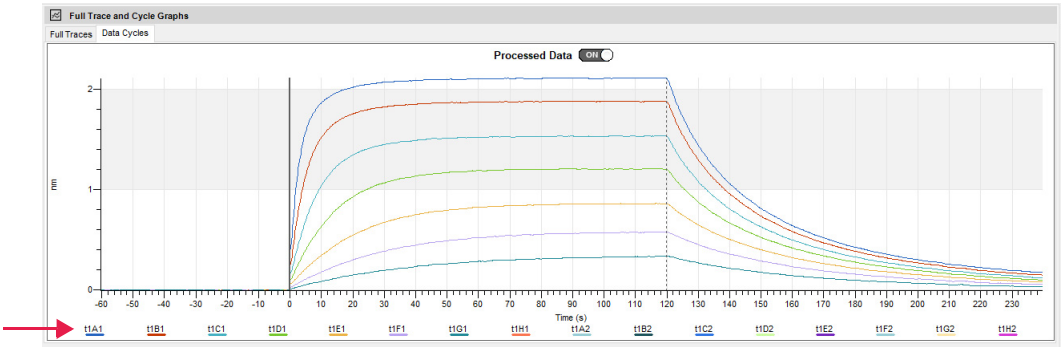


Figure 2-30: Legend Displayed

Saving and Importing Analysis Settings

If routine assays are being run where analysis settings are similar between runs, save time by loading the previously saved analysis settings. After the analysis settings are tailored for the current experiment, click SAVE in Analysis Settings. You will be prompted to name your settings file.

Once a new unanalyzed experiment is opened click **(Load)** in Analysis Settings to load previously saved analysis settings (Figure 2-31).

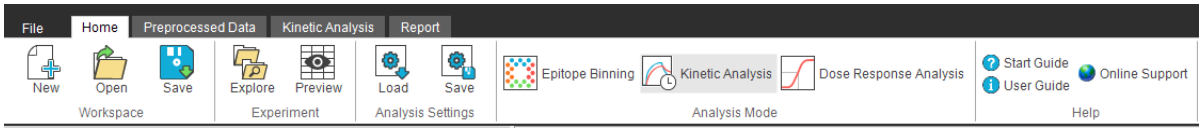


Figure 2-31: Analysis Settings

Appending Multiple Experiment Files

Often sequential steps need to be run in multiple experiments due to limited plate real estate. When this happens, the software lets you append (or concatenate) those multiple files together to create a complete experiment. For example, when 50x50 classical sandwich binning needs to be performed using Amine-Reactive 2nd Generation (AR2G) biosensors, immobilizing 50 mAbs onto the biosensors requires 50 wells of MES buffer, activation buffer, mAbs, quenching buffer and running buffer each. This doesn't leave enough space in the 384-well plate for antigen wells and 50 sandwiching mAbs. So, the loading of mAbs onto AR2G biosensors has to be done in one experiment, and the binding to the antigen and the second set of mAbs needs to be done in a second, separate experiment. You can now analyze the complete 50x50 dataset by appending the two experiments together.

Multiple experiments can be appended, but the final dataset is valid only if the number of sensor trays are the same for each file (or appended segment). The software assumes the sensor trays are full for each experiment. There are no step number, type or duration constraints when appending data, but the software displays an error to let you know when files cannot be appended.

To append experiment files and create a new dataset:

1. Drag the first experiment file to the Segment window (Figure 2-32).

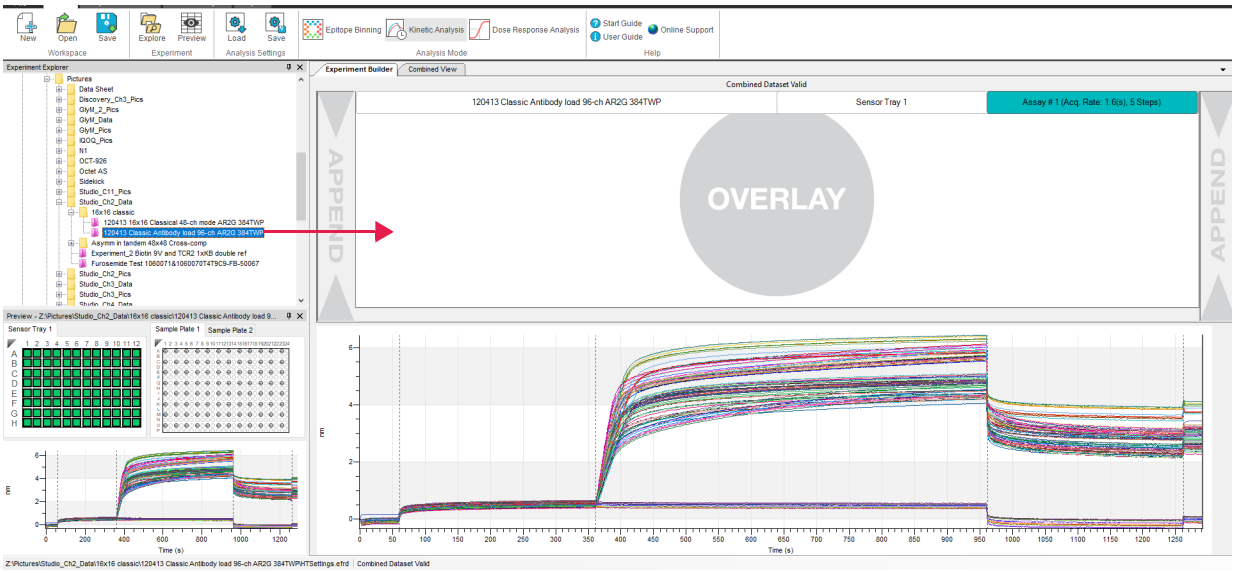


Figure 2-32: First File in Segment Window

2. Select the file you want to append to the existing experiment file and drag it to either the left or right **APPEND** areas of the Segment window. Dragging to the left adds the file to the beginning of the dataset, and dragging it to the right adds it to the end of the dataset. Release the mouse when the APPEND area turns green (Figure 2-33).

NOTICE: *The software displays CANNOT APPEND a file if it does not contain the same number of sensor trays as the one currently open.*

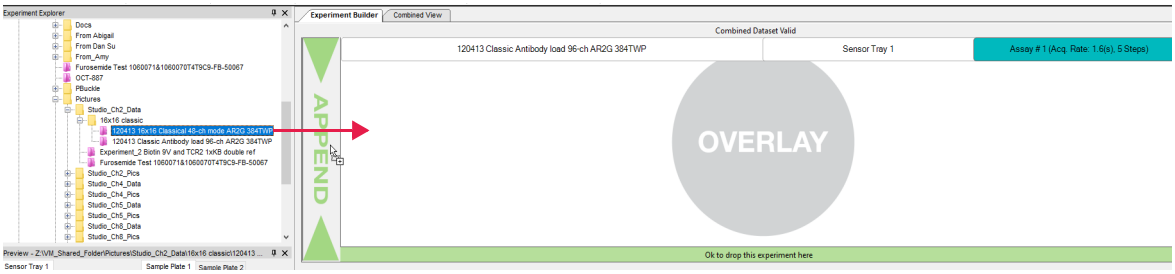


Figure 2-33: Drag Second File to Segment Window

The Data Viewing area displays the combined, appended dataset.

NOTICE: *Verify that combined dataset matches your assay setup before moving on to the next steps.*

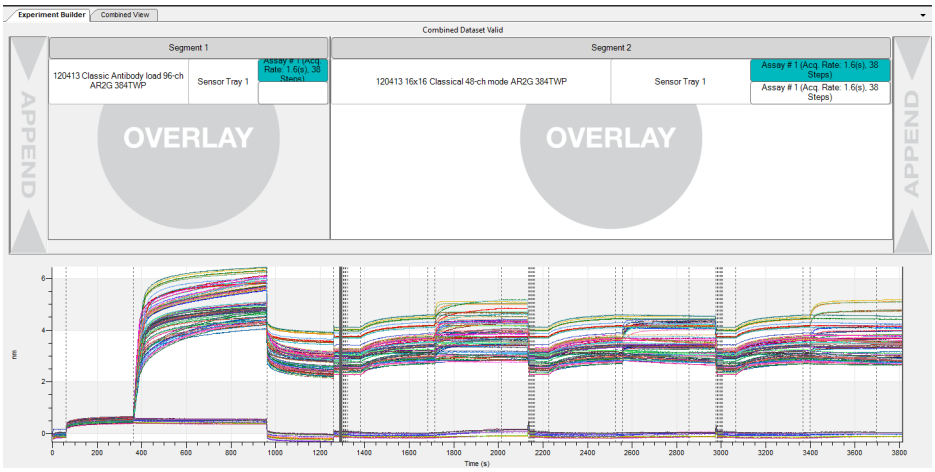


Figure 2-34: Appended Files

3. Drag the other files to the APPEND areas that you want to append to the existing experiments.
4. If you want to remove files, right click the **Experiment Name, Sensor Tray or Assay** box for the file you want to remove and select **Delete** (Figure 2-35).

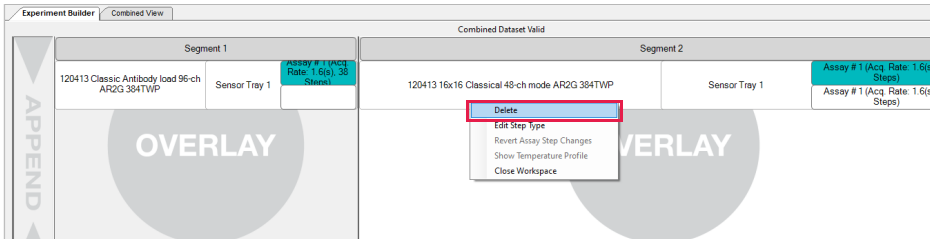


Figure 2-35: Deleting File.

5. Click  to save the appended dataset or click **File** > **Save As**. To edit the dataset before analysis see "Viewing Full Datasets 30" on page 29.

Editing Step Types

1. To edit the assay file step types, right click the **Experiment Name, Sensor Tray or Assay** box for the file you want to edit and select **Edit Step Type** (Figure 2-36).

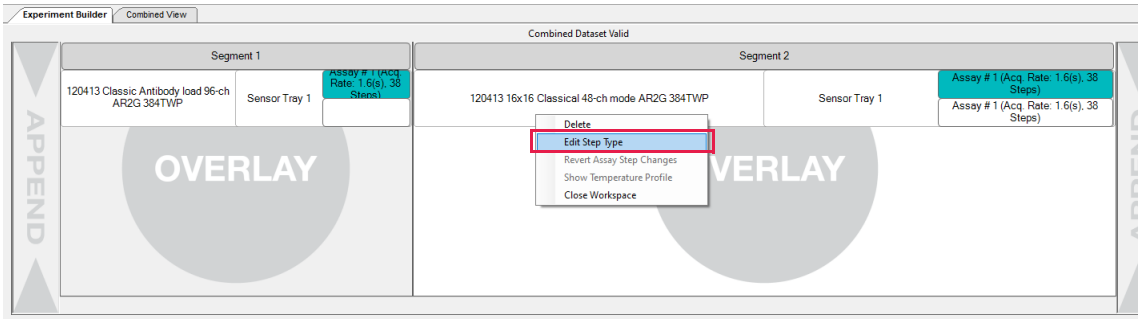
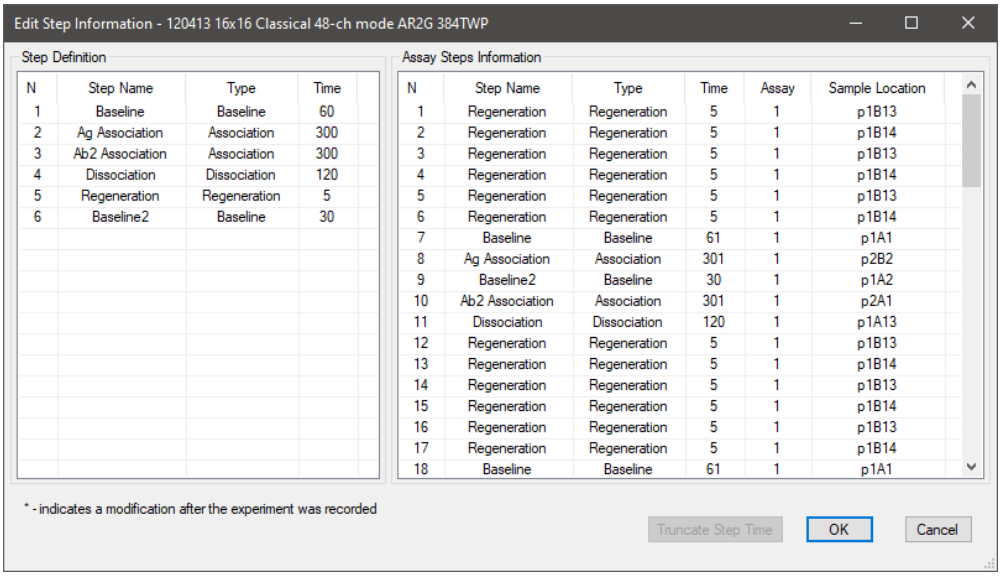


Figure 2-36: Editing Step Type.

All assay steps are displayed in the Edit Step Information window (Figure 2-37).



N	Step Name	Type	Time
1	Baseline	Baseline	60
2	Ag Association	Association	300
3	Ab2 Association	Association	300
4	Dissociation	Dissociation	120
5	Regeneration	Regeneration	5
6	Baseline2	Baseline	30

N	Step Name	Type	Time	Assay	Sample Location
1	Regeneration	Regeneration	5	1	p1B13
2	Regeneration	Regeneration	5	1	p1B14
3	Regeneration	Regeneration	5	1	p1B13
4	Regeneration	Regeneration	5	1	p1B14
5	Regeneration	Regeneration	5	1	p1B13
6	Regeneration	Regeneration	5	1	p1B14
7	Baseline	Baseline	61	1	p1A1
8	Ag Association	Association	301	1	p2B2
9	Baseline2	Baseline	30	1	p1A2
10	Ab2 Association	Association	301	1	p2A1
11	Dissociation	Dissociation	120	1	p1A13
12	Regeneration	Regeneration	5	1	p1B13
13	Regeneration	Regeneration	5	1	p1B14
14	Regeneration	Regeneration	5	1	p1B13
15	Regeneration	Regeneration	5	1	p1B14
16	Regeneration	Regeneration	5	1	p1B13
17	Regeneration	Regeneration	5	1	p1B14
18	Baseline	Baseline	61	1	p1A1

Figure 2-37: Edit Step Information Window

2. You can change a Step Definition and have that change applied anywhere the step type is used for all assays in the experiments you're previewing. Click the row you want to edit. Then right-click the row, roll your mouse over **Change Step Type** and select the new step type (Figure 2-38) then click **OK**.

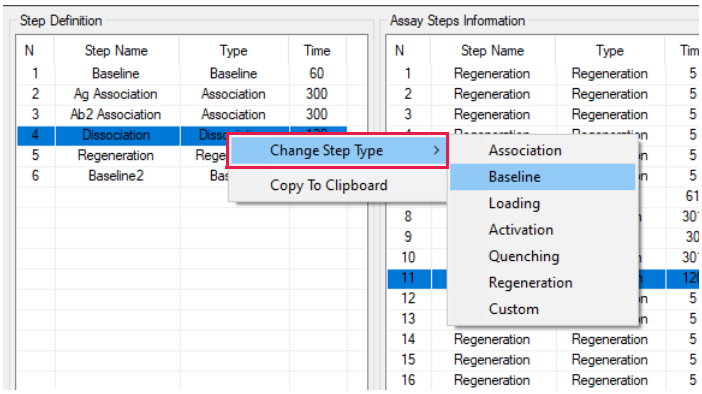


Figure 2-38: Changing a Step Definition

3. To change Assay Step Info for a specific step only, click the row you want to edit. Then right-click the row, roll your mouse over **Change Step Type** or **Change Step Name** and select the new type or name (Figure 2-39) then click **OK**. This only changes the step specified, even if the same sample location was used in another step.

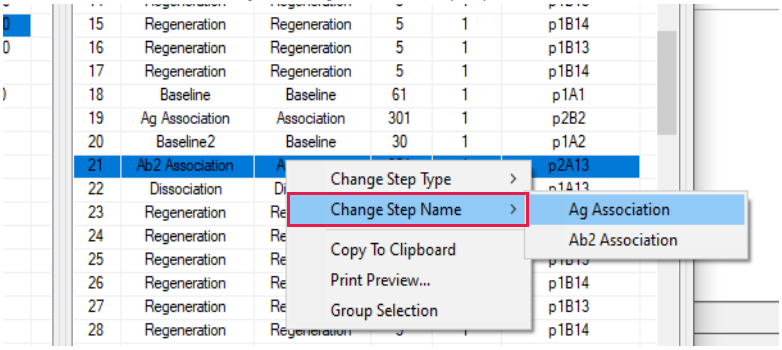


Figure 2-39: Changing Assay Steps Info

4. When there are two or more assays with matching step sequence but different step times, use the **Truncate Step Time** button to shorten the step times to create matching assays that can be analyzed together. This is feature is useful when the assay setup in Octet® BLI Discovery software uses a "threshold" criteria to terminate a step when it reaches a specified signal level.

5. The * next to the **Step Time** in the table in Figure 2-40 indicates the step time that has been truncated.

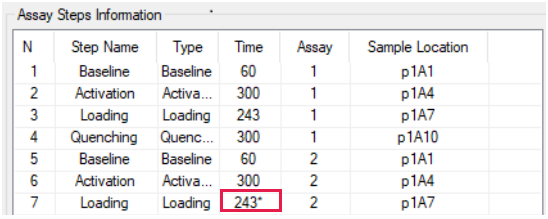


Figure 2-40: Truncated Assay Step Time

6. When you need to remove all assay step changes and revert to the original Assay Step Information, right-click the Experiment and choose **Revert Assay Step Changes**.

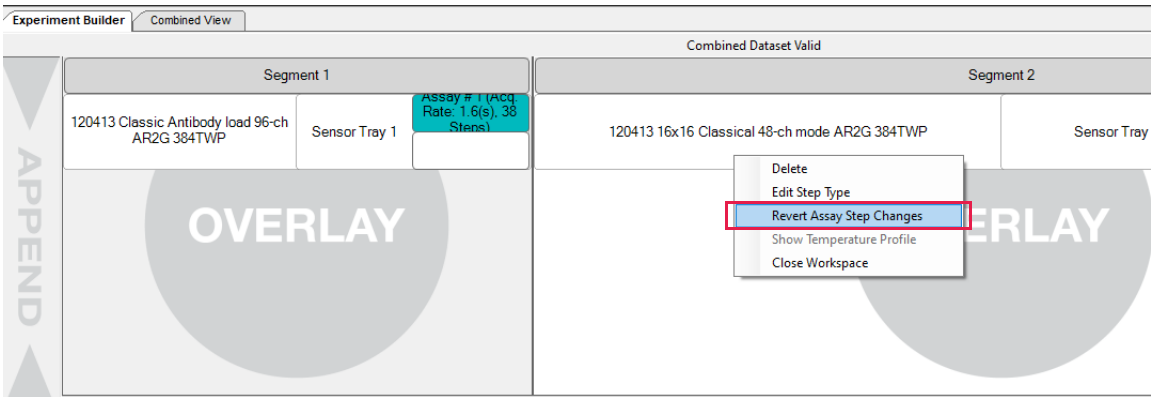


Figure 2-41: Reverting Assay Step Changes

Overlaying Multiple Experiment files

When you need to analyze the data from multiple plates and/or multiple sensor trays that use the same protocol or method file, you can overlay them in the software. Multiple experiments can be overlaid as long as they have the same number of assay steps, same step time and the assay steps are of the same length. The software displays an error to let you know if files can't be overlaid.

To overlay experiment files and create a new dataset:

1. Drag the first experiment file to the Segment box (Figure 2-42).

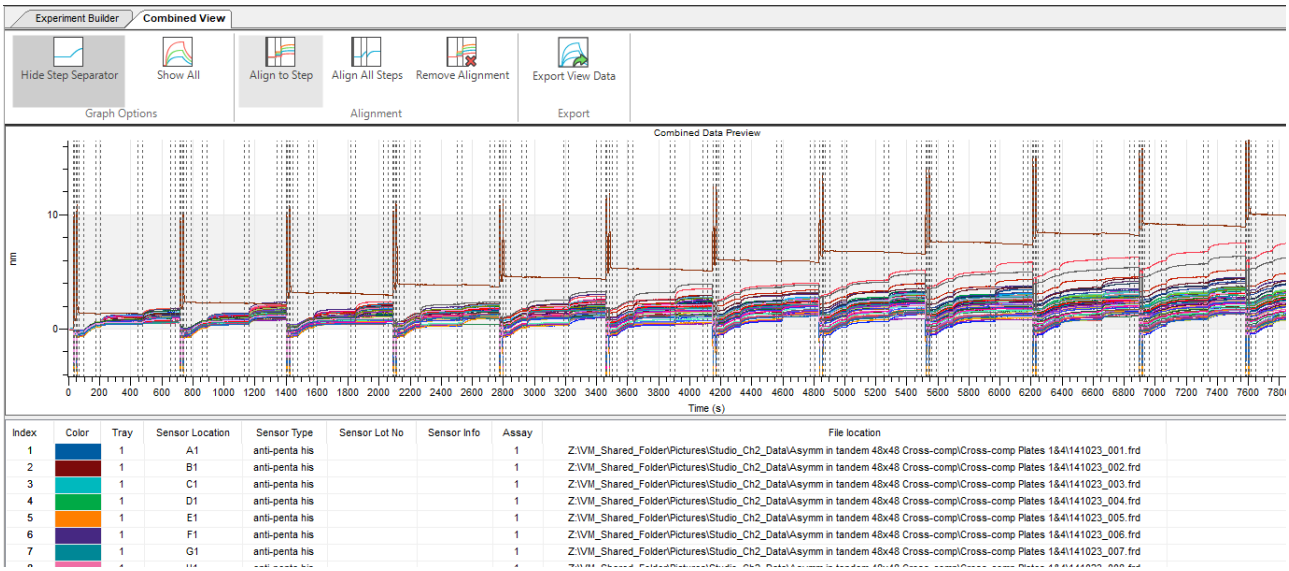


Figure 2-42: First File in Segment Window

2. Select the file you want to overlay onto the existing experiment file and drag it to the **OVERLAY** area of the Segment box. Release the mouse when the OVERLAY area turns green (Figure 2-43).

NOTICE: *The software displays CANNOT OVERLAY if the file you want to overlay does not contain the same number of assay steps or if the steps are different lengths than the one currently open.*

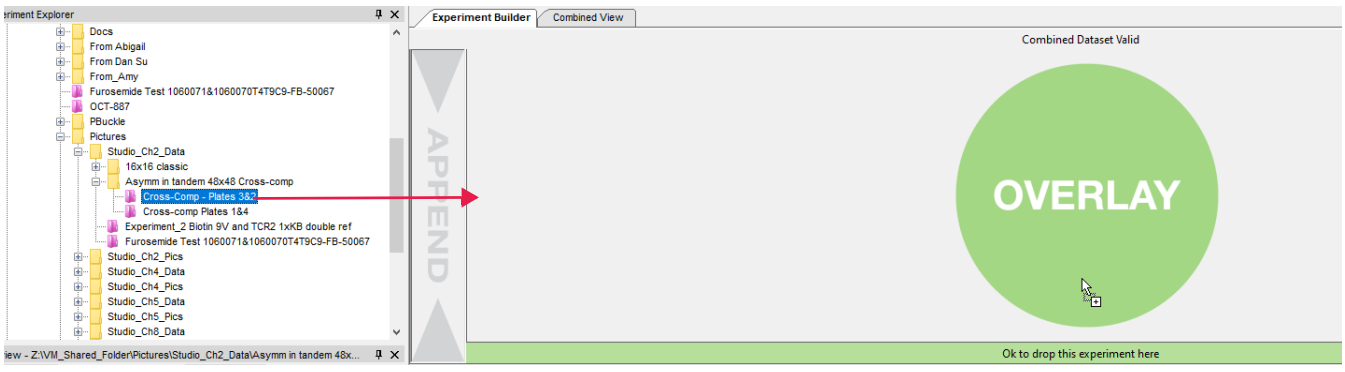


Figure 2-43: Dragging Second File to Segment Window

The Data Viewing area displays overlaid sensorgrams for all files.

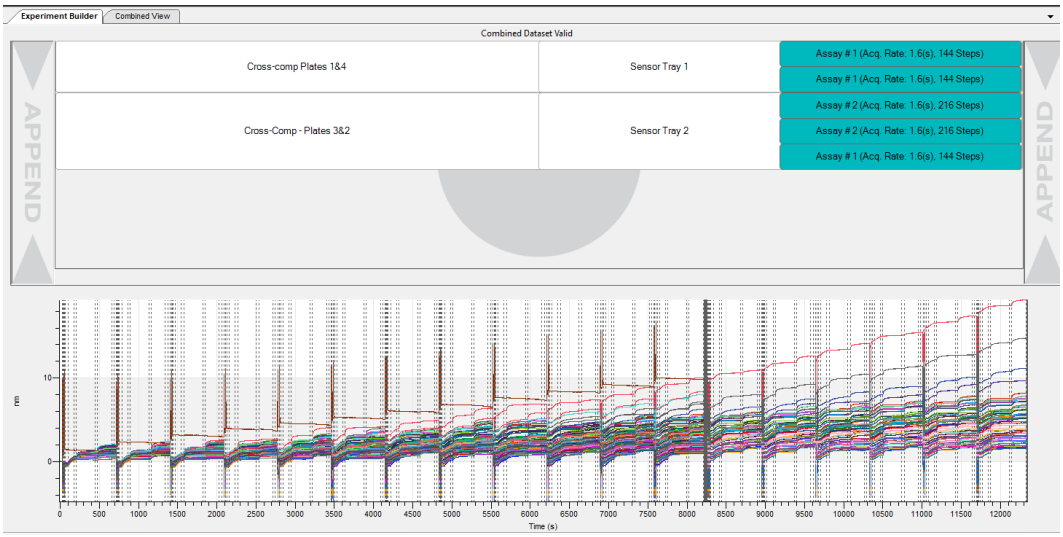


Figure 2-44: Overlaid Files

3. Drag any other files to the OVERLAY area that you want to add to the existing experiments.
4. If you want to remove files, right click the **Experiment Name, Sensor Tray or Assay** box for the file you want to remove and select **Delete** (Figure 2-45).

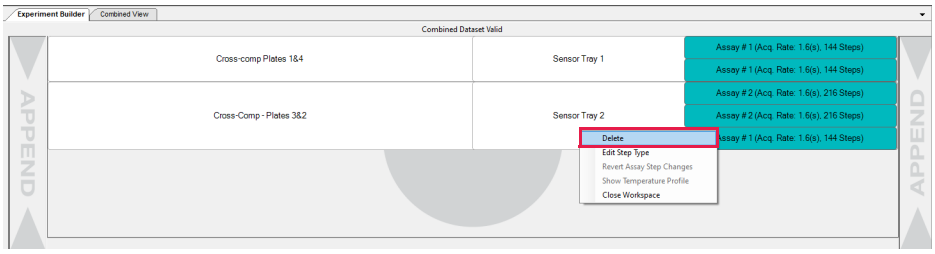


Figure 2-45: Deleting Files

5. Click  to save the overlay file or click **File** > **Save As**. To edit the dataset before analysis see "Viewing Full Datasets" on page 30.

Masking Data

The Mask Data feature lets you make a copy of your experiment with all proprietary info such as sample ID, sample info, sensor type and sensor info hidden, or 'masked'.

NOTICE: *The Mask Data feature is not available in Octet® Analysis Studio 21 CFR Part 11 software.*

To do this:

1. In the Data Selection tab, right-click an experiment in the folder tree.
2. Select **Mask Data** (Figure 2-46)

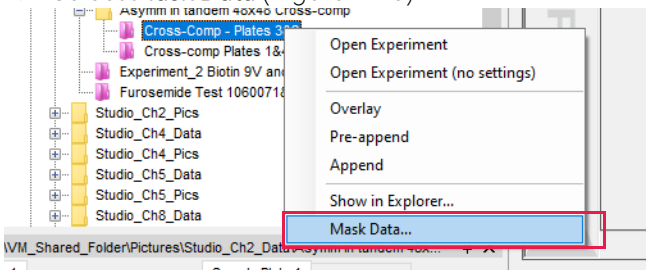


Figure 2-46: Selecting Mask Data Option

Save a masked copy of your experiment in the desired location.

Microsoft Excel Reports

Octet® Analysis Studio can generate reports in Excel format. By default, reports will use the modern .xlsx Excel file format. When using the .xlsx format, Microsoft Excel does not need to be installed on the same computer as Octet® Analysis Studio.

For compatibility purposes, the default Excel report format can be changed to the legacy Excel 97-2003 (.xls) format. To change the default format, click the **File** menu and choose **Preferences**. Check the option to **Use legacy Excel 97-2003 Format (.xls)**. When using the legacy Excel format, Microsoft Excel must be installed on the same computer as Octet® Analysis Studio.

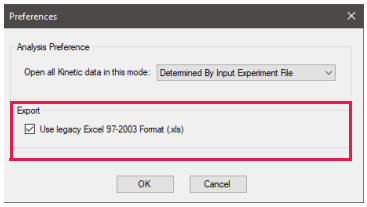


Figure 2-47: Legacy Excel Option

Help

Click  to view the software user guide.

Chapter 3:

View Data

Viewing Full Datasets

After you appended or overlaid a set of experiments in the Home screen, click **Combined View** to see experimental details for the full dataset in the View screen (Figure 3-1). The View screen lets you confirm your dataset was correctly overlaid and/or appended. If it isn't, just go back to the Experiment Builder tab to make changes.

NOTICE:

See "Resizing, Hiding and Closing Windows" on page 11. for information on working with the software windows.
See "Graph Options" on page 16. for information on graph display options.

Figure 3-1: View Screen

Changing the Sensorgram View

You can view individual sensorgrams, overlay multiple sensorgrams and zoom in on specific assay steps in the Combined Data Preview area.

To view individual sensorgrams, click a biosensor row in the table (Figure 3-2).

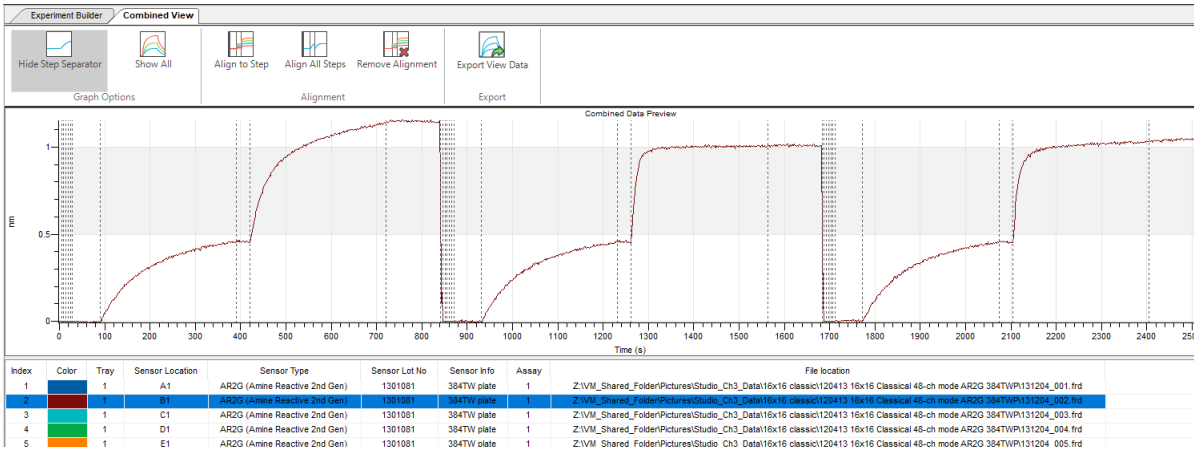


Figure 3-2: Viewing a Single Sensorgram

To view multiple sensorgrams, press and hold **Ctrl**, then select the biosensor rows in the table you want to view.

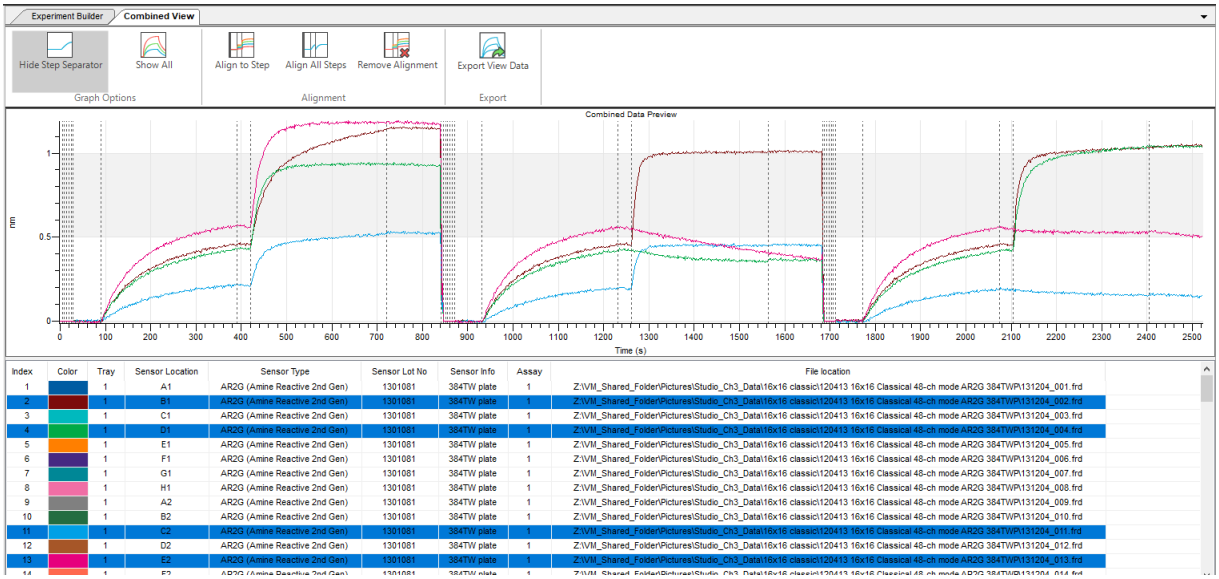


Figure 3-3: Viewing Multiple Sensorgrams

- Click  (**Show All**) to view all sensorgrams in the dataset again, or right-click the Combined Data Preview area and click **Select All Traces**.

- Click  (**Hide Step Separator**) to hide step dividers (Figure 3-4) or  (**Show Step Separator**) to show them.

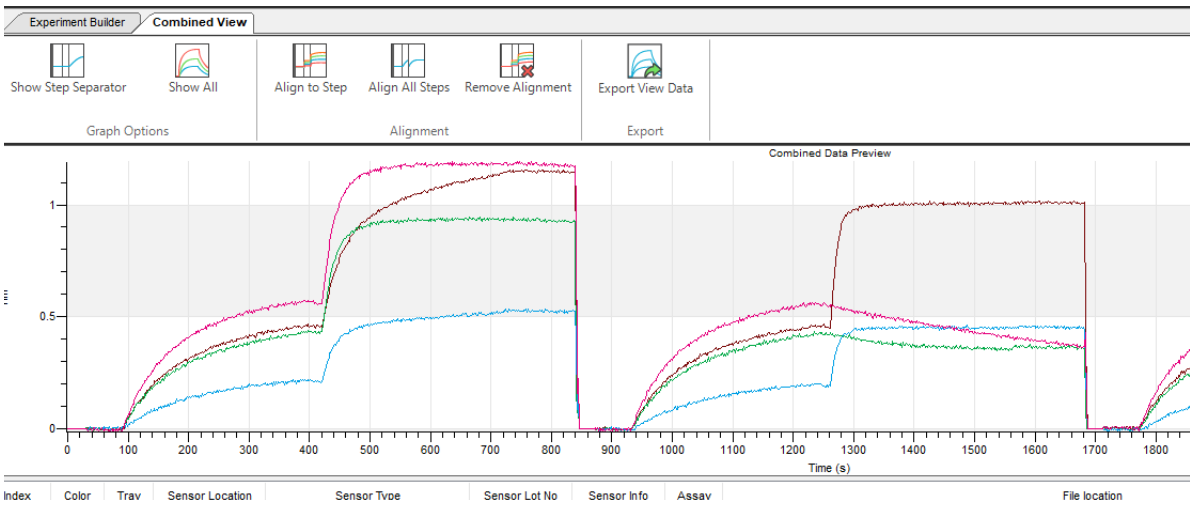


Figure 3-4: Step Dividers Hidden

To make the sensorgram traces thicker, right-click the graph and select **Thicken Line**:

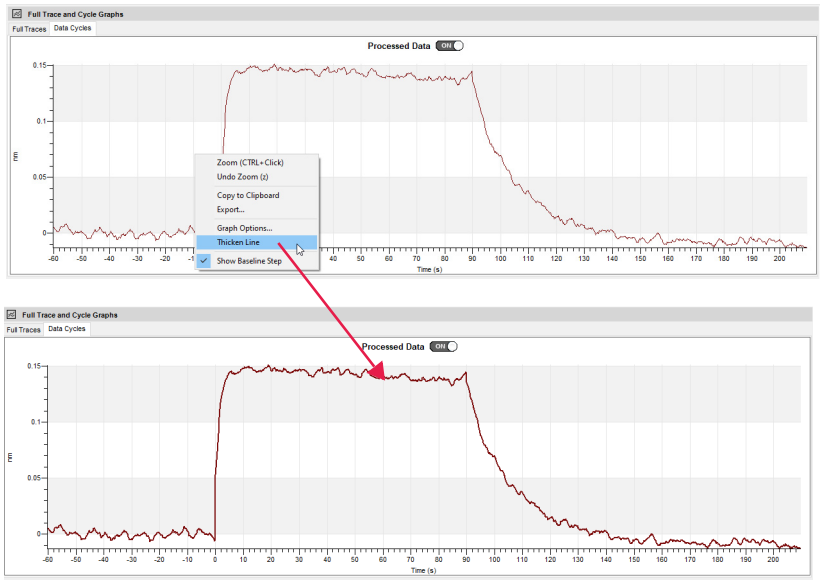


Figure 3-5: Selecting Thicken Line

To zoom in on a specific area, right-click the Combined Data Preview area and select **Zoom** (Figure 3-6), or press **CTRL** and click.

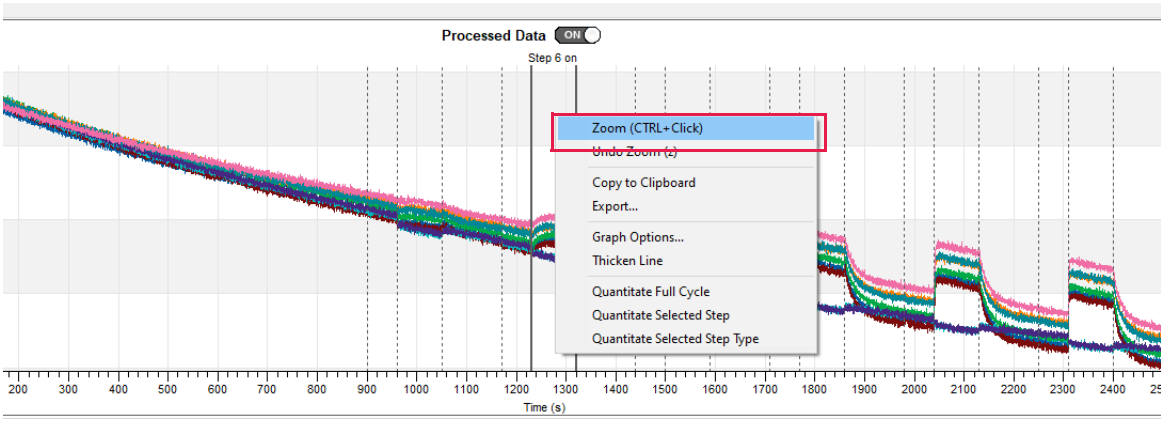


Figure 3-6: Selecting Zoom

Draw a box around the area you want to zoom in on with your mouse (Figure 3-7):

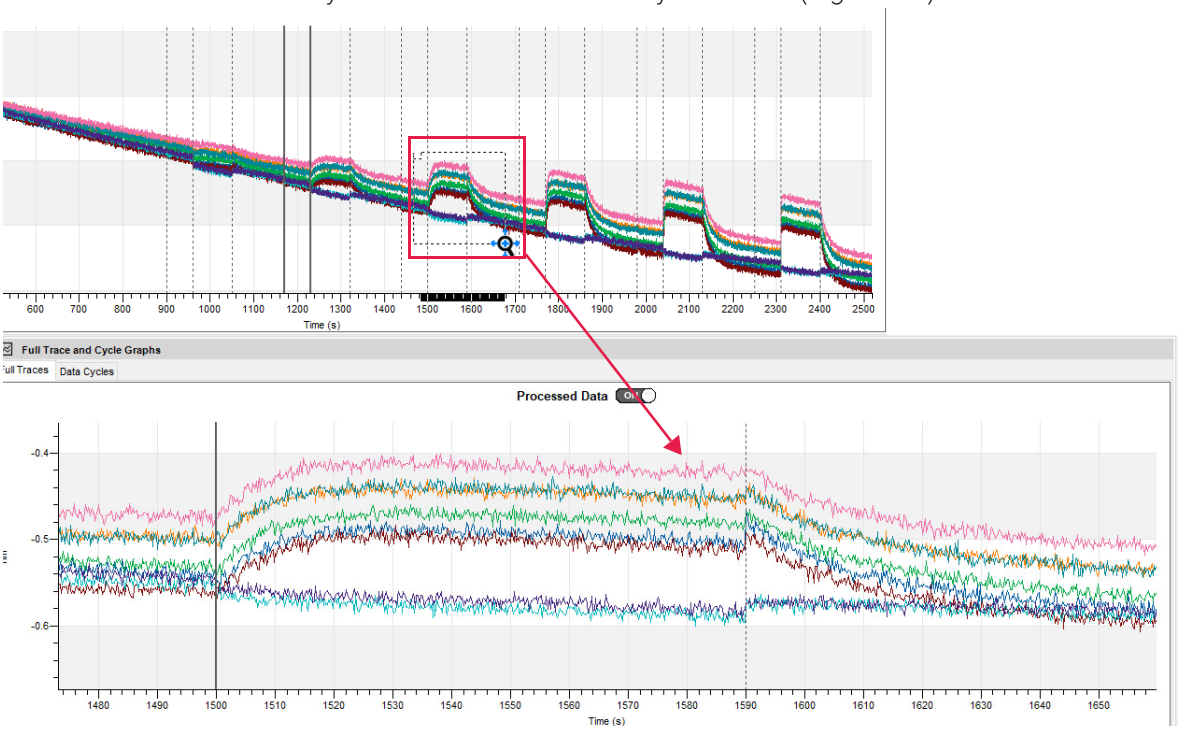
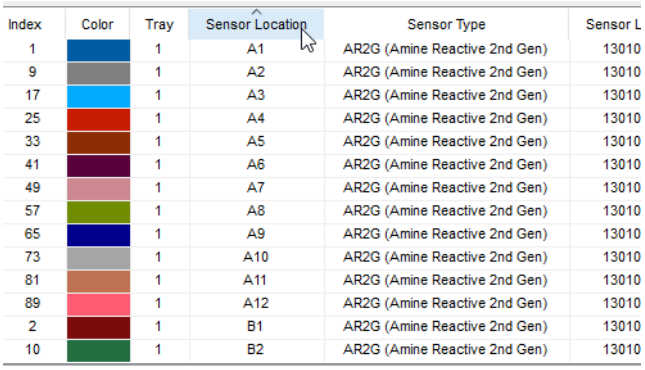


Figure 3-7: Selecting a Zoom Area

Right-click the Combined Data Preview area and select **Undo Zoom** or press **Z** on your keyboard to return to the full view.

Changing the Table View

Click a column header to sort the data. When a column header is blue, it indicates the table data is currently sorted by that parameter (Figure 3-8).



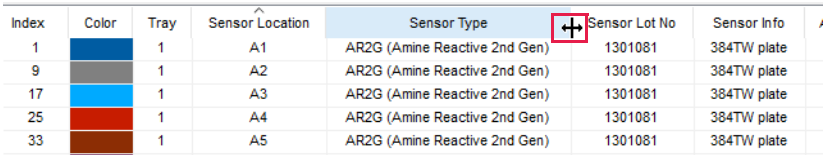
Index	Color	Tray	Sensor Location	Sensor Type	Sensor L
1		1	A1	AR2G (Amine Reactive 2nd Gen)	13010
9		1	A2	AR2G (Amine Reactive 2nd Gen)	13010
17		1	A3	AR2G (Amine Reactive 2nd Gen)	13010
25		1	A4	AR2G (Amine Reactive 2nd Gen)	13010
33		1	A5	AR2G (Amine Reactive 2nd Gen)	13010
41		1	A6	AR2G (Amine Reactive 2nd Gen)	13010
49		1	A7	AR2G (Amine Reactive 2nd Gen)	13010
57		1	A8	AR2G (Amine Reactive 2nd Gen)	13010
65		1	A9	AR2G (Amine Reactive 2nd Gen)	13010
73		1	A10	AR2G (Amine Reactive 2nd Gen)	13010
81		1	A11	AR2G (Amine Reactive 2nd Gen)	13010
89		1	A12	AR2G (Amine Reactive 2nd Gen)	13010
2		1	B1	AR2G (Amine Reactive 2nd Gen)	13010
10		1	B2	AR2G (Amine Reactive 2nd Gen)	13010

Figure 3-8: Sorting Data Table Columns by Sensor Location.

Table 3-1: Description of Data Table Columns

Column	Description
Index	Sort by Index Number: • Click once to sort the data by index number first to last starting at 1. • Click again to sort the data by index number last to first.
Color	Sort by Color.
Tray	Sort by Tray: • Click once to sort the data by tray by first to last starting at 1. • Click again to sort the data by tray last to first.
Sensor Location	Sort by Sensor Location: • Click once to sort the data by sensor location first to last starting at 1. • Click again to sort the data by sensor location last to first.
Sensor Type	Sort by Sensor Type: • Click once to sort the data by sensor name alphabetically A-Z. • Click again to sort the data by sensor name alphabetically Z-A.

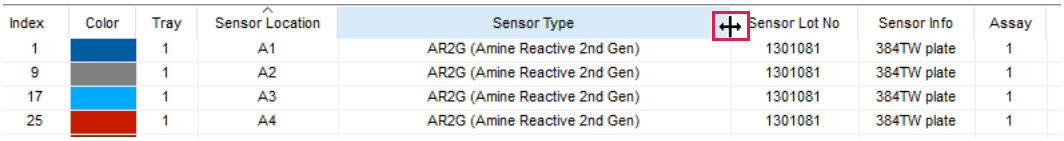
To resize the table columns, roll the cursor over the border between the column headers until the resizing cursor displays (Figure 3-9).



Index	Color	Tray	Sensor Location	Sensor Type	Sensor Lot No	Sensor Info
1		1	A1	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate
9		1	A2	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate
17		1	A3	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate
25		1	A4	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate
33		1	A5	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate

Figure 3-9: Column Resize Cursor

Then click and drag the column to resize (Figure 3-10).



Index	Color	Tray	Sensor Location	Sensor Type	Sensor Lot No	Sensor Info	Assay
1		1	A1	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate	1
9		1	A2	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate	1
17		1	A3	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate	1
25		1	A4	AR2G (Amine Reactive 2nd Gen)	1301081	384TW plate	1

Figure 3-10: Resized Column

Table Display Options

Right-click a populated table row or column to see the menu (Figure 3-11).

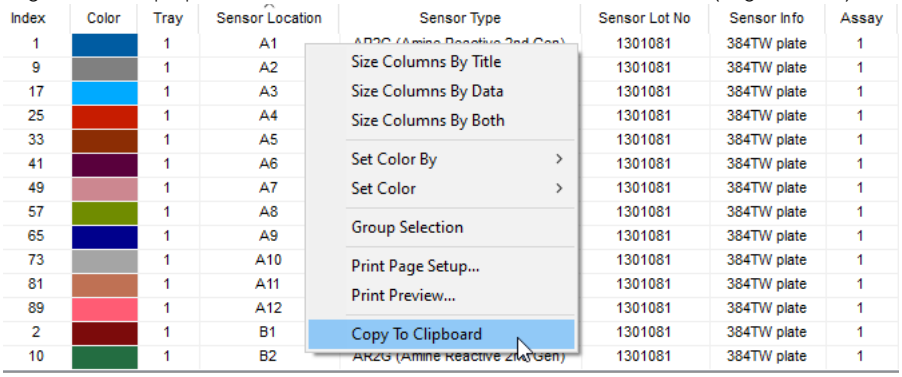


Figure 3-11: Table Display Menu

- **Size Columns by Title**—Sets all column widths to fit the column titles.
- **Size Columns by Data**—Sets all column widths to fit the data.
- **Size Columns by Both**—Sets all column widths to best fit both the column titles and the data.

- **Set Color By**—Lets you color-code sensorgrams and table rows by Index, Sensor, Sensor Tray, Sample Sensor Plate, Sensor Type, Associated (Sample) Location, Sample ID or Assay (Figure 3-12). If you'd like the sensorgrams and table rows for each biosensor to be a different color, we recommend using **Set Color By Index**.

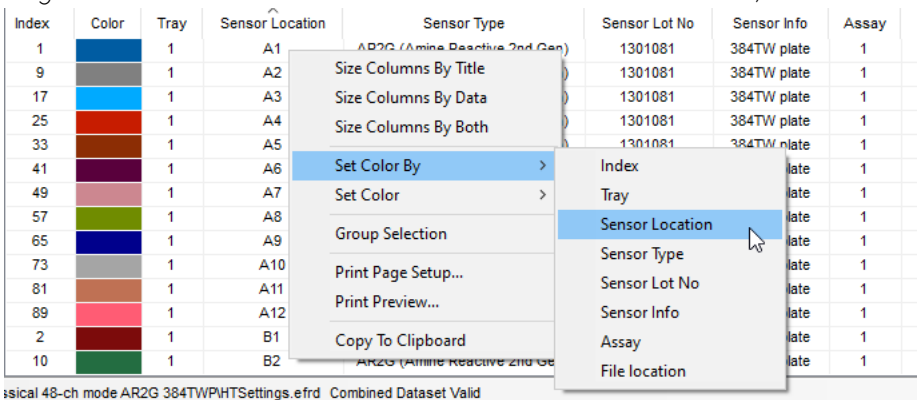


Figure 3-12: Set Color By

- **Set Color**—Rapidly set the color for the table row(s) that are currently selected. Choose **Custom...** to open the color palette and choose a custom color (Figure 3-13):

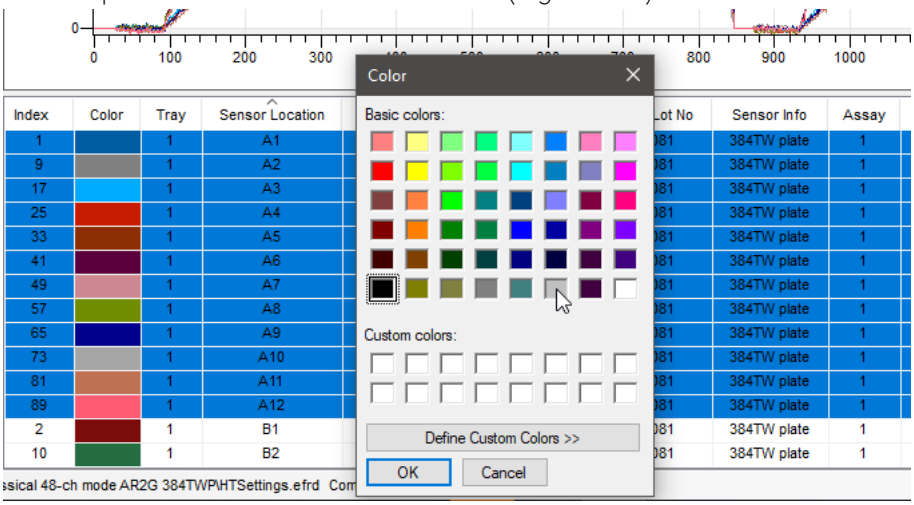


Figure 3-13: Set Color

- **Group Selection**—Lets you manually group selected rows together and move them to the top of the table.
- **Print Page Setup**—Lets you adjust the print settings (before printing the table).

- **Print Preview**—Shows a print preview of the table. You can select the number of pages to print, and then print the table (Figure 3-14):

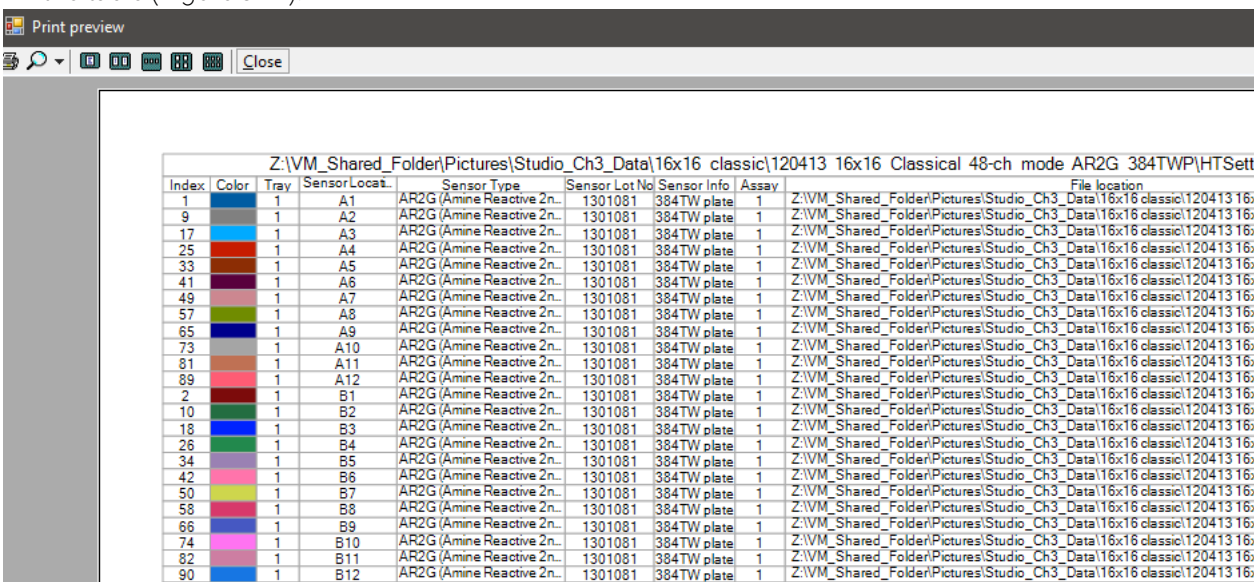


Figure 3-14: Print Preview

- **Copy to Clipboard**—Copies the table to the clipboard so you can paste it into other programs (Microsoft® Excel®, Microsoft Word®, electronic notebooks, etc.).

Click **File** > **Save** or **Save As** to save changes to the dataset before analysis.

Exporting Raw Data

- Click  (**Export View Data**) to export the raw, uncorrected data of the experiment or a combined data set in .csv format.

- To export the sensorgram view shown, right-click the Combined Data Preview and select Export (Figure 3-15).

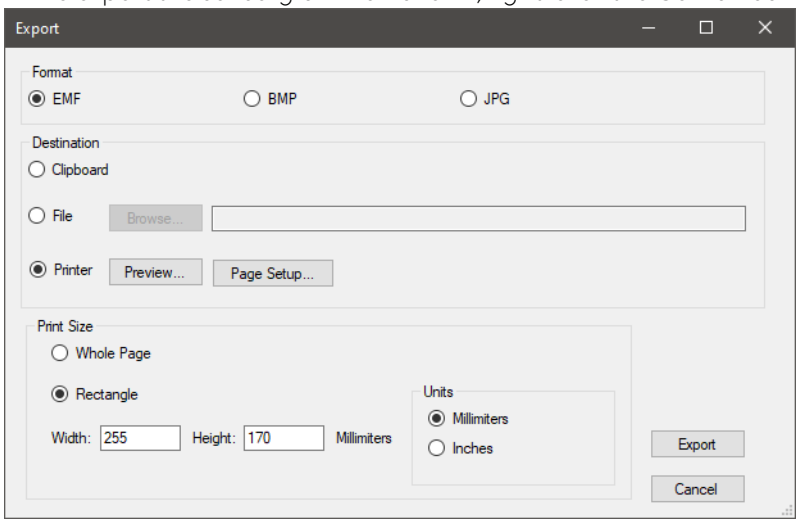


Figure 3-15: Export Dialog Box

Chose a format (EMF, BMP or JPG), an export destination (Clipboard, save to File, or Printer), set print size parameters if needed then click **Export**.

Chapter 4:

Preprocessing Kinetic Datasets

Pre-analysis Data Correction

Before analysis the Preprocess Data Screen lets you subtract any non-specific binding or baseline drift data and make other data corrections if necessary. Click the **Preprocess Data** tab to view the Preprocess Data screen (Figure 4-1).

NOTICE:

See "Resizing, Hiding and Closing Windows" on page 11 for information on working with the software windows.
See "Graph Options" on page 16 for information on graph display options.

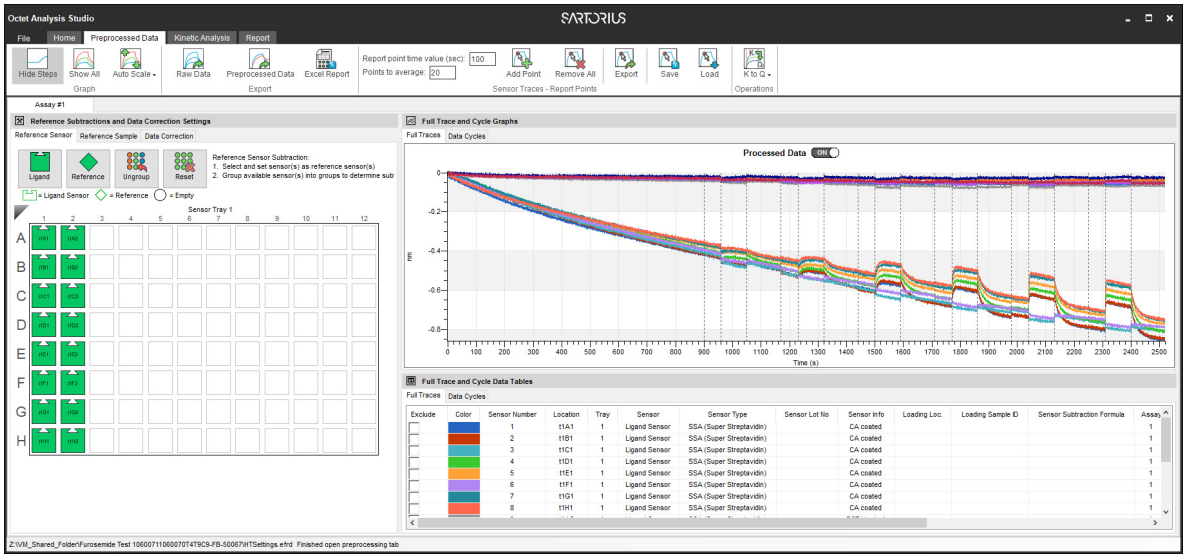


Figure 4-1: Preprocess Data Screen

Reference Sensor Subtraction

To subtract non-specific binding in experiments where reference biosensors were used for analyte binding with no ligand present, click the **Reference Sensor** tab in the Reference Subtractions and Data Corrections window (Figure 4-2). For more information about the tab refer to "Reference Sensor (Non-specific Binding) Subtraction" on page 47.

NOTICE: To subtract baseline drift for ligand-bound biosensors that are dipped into buffer or zero-concentration analyte, go directly to "Reference Sample Well Subtraction" on page 57.

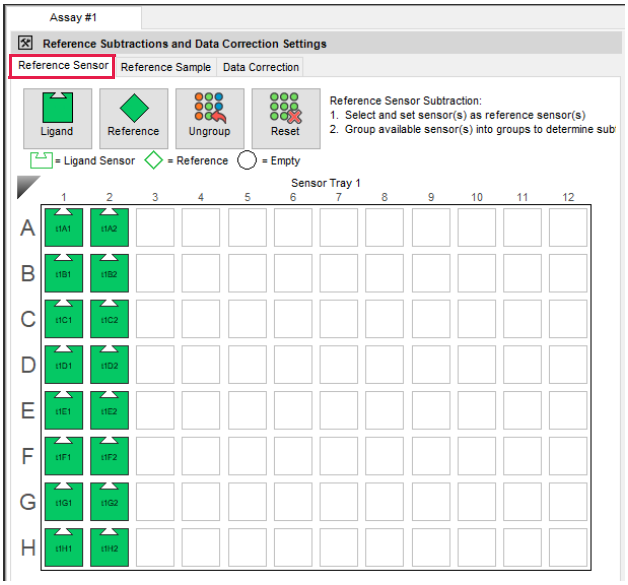


Figure 4-2: Reference Sensor Tab

Viewing Data

To view data for different assays in the experiment or combined data set, click the **Assay** tab above the Reference Subtractions and Data Corrections window (Figure 4-3).

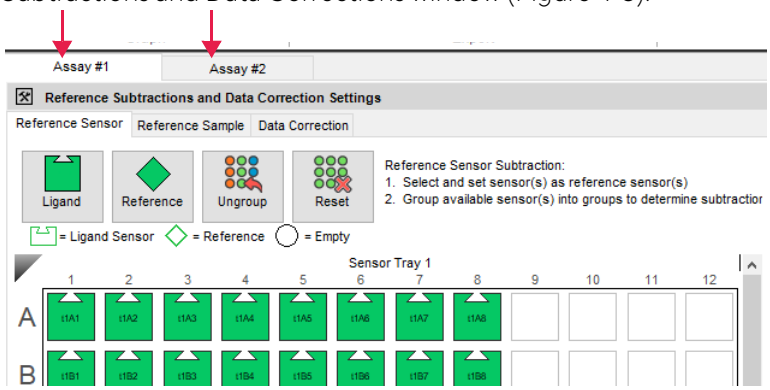


Figure 4-3: Assay Tabs

If you used more than one tray of biosensors in the experiment or combined dataset, use the scrollbar to see the other trays (Figure 4-4).

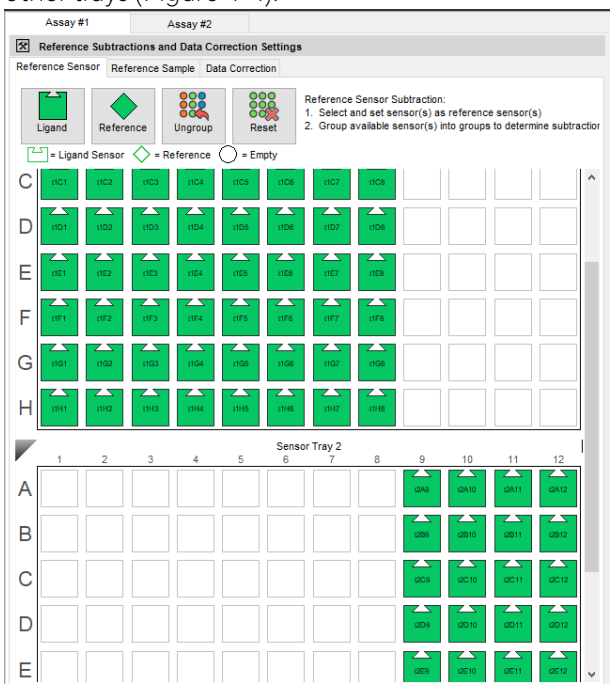


Figure 4-4: Scrolling Between Biosensor Trays

- Biosensors used in the experiment are green in the tray map and are set to ligand or reference sensors according to how they were originally assigned in the BLI Discovery software.
- Biosensors are identified by their tray and well number. For example t1A3 is the biosensor in well A3 on tray 1, t2H5 is the biosensor in well H5 on tray 2 (Figure 4-3 and Figure 4-4).

Clicking on rows, columns or specific wells in a tray displays the data for that biosensor in the Full Traces and Data Cycles window, and highlights the selected data in the Sensor Trace and Kinetic Data tables (Figure 4-5)

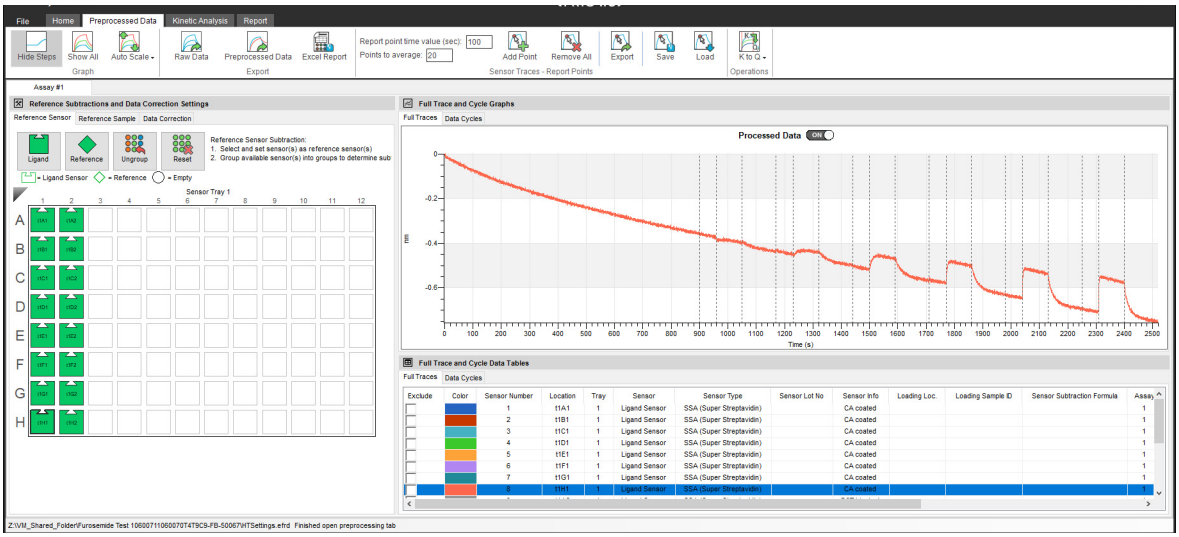


Figure 4-5: Biosensor Selection Data Display

The Full Traces tab shows data for all steps of the experiment including initial baseline, loading, baseline and association/dissociation (Figure 4-6).

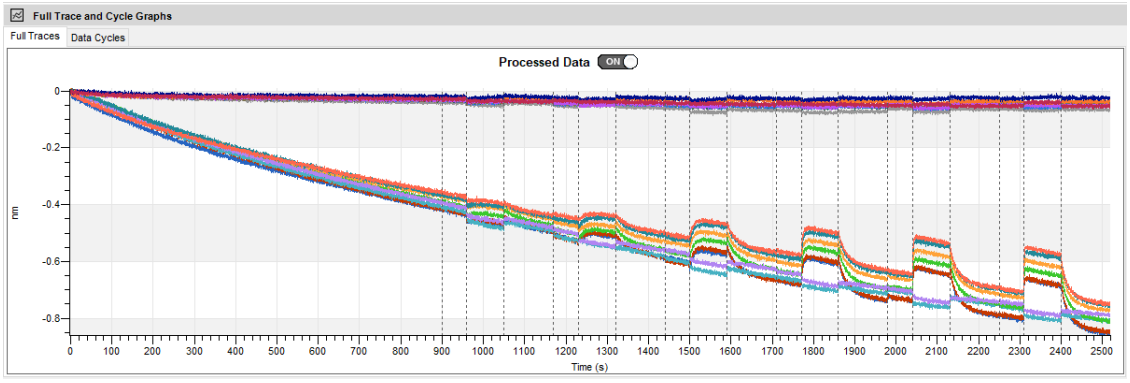


Figure 4-6: Full Traces Tab

To switch between viewing corrected and uncorrected data, click the **Processed Data** toggle at the top of the graph (Figure 4-7).

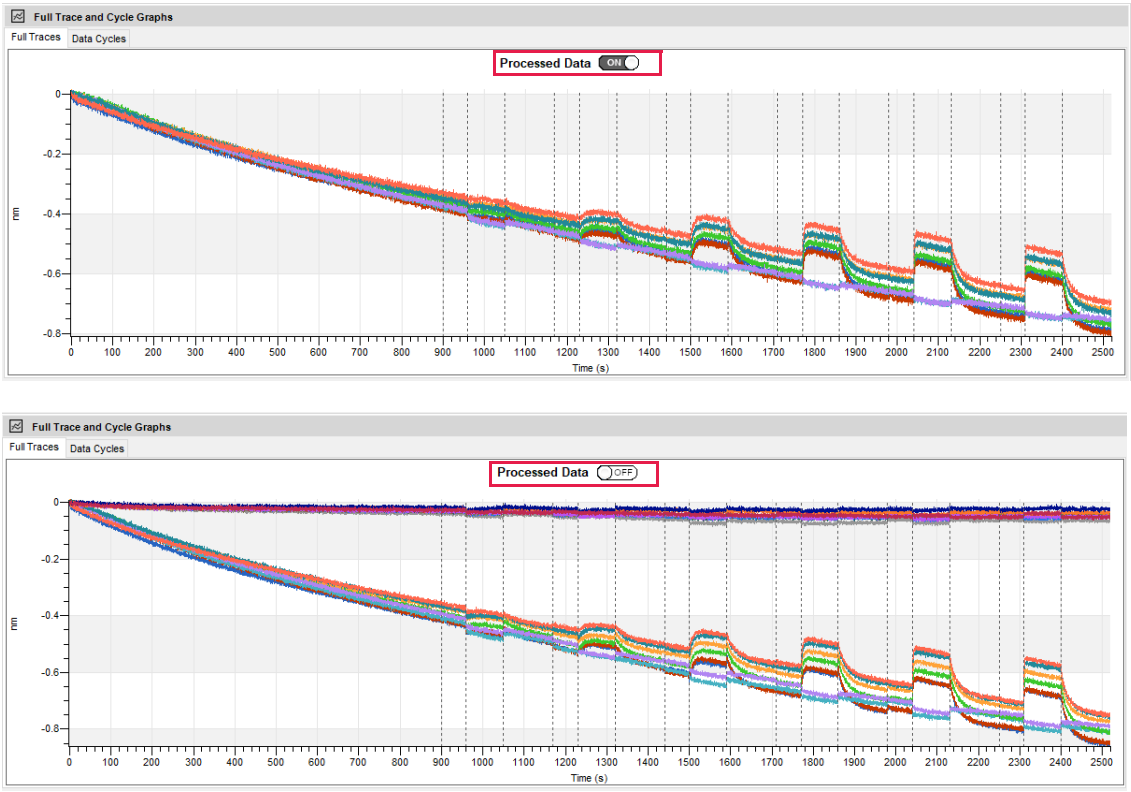


Figure 4-7: Processed Data ON (Top) and OFF (Bottom)

The Data Cycles tab shows only the baseline after ligand loading and the association/dissociation steps (Figure 4-8)

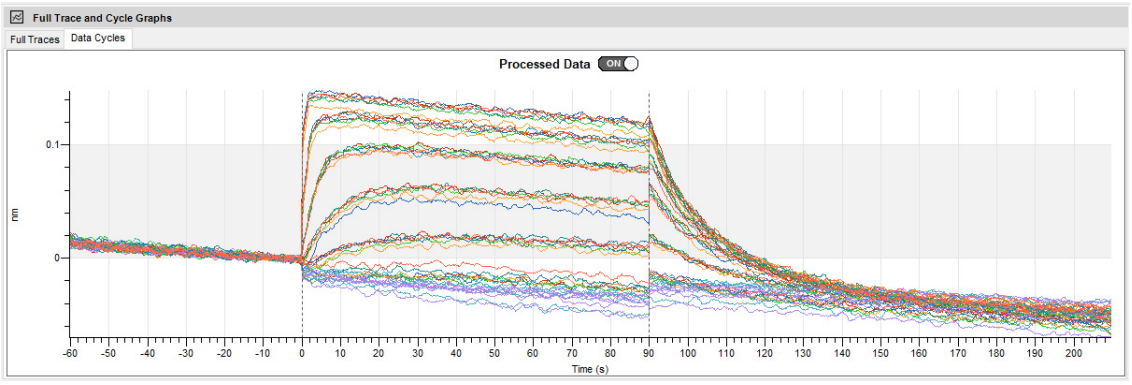
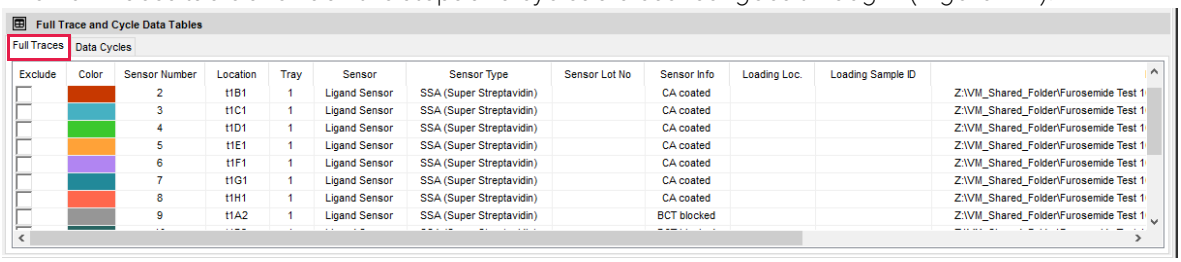


Figure 4-8: Data Cycles Tab

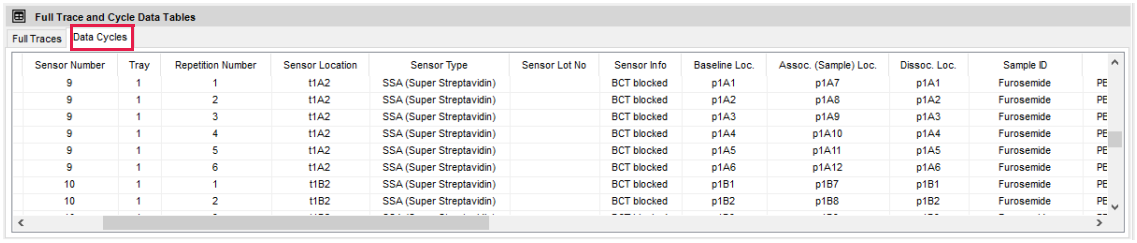
The Full Traces table shows all the steps and cycles a biosensor goes through (Figure 4-9).



Exclude	Color	Sensor Number	Location	Tray	Sensor	Sensor Type	Sensor Lot No	Sensor Info	Loading Loc.	Loading Sample ID	
		2	t1B1	1	Ligand Sensor	SSA (Super Streptavidin)		CA coated			Z:\VM_Shared_Folder\Furosemide Test 1
		3	t1C1	1	Ligand Sensor	SSA (Super Streptavidin)		CA coated			Z:\VM_Shared_Folder\Furosemide Test 1
		4	t1D1	1	Ligand Sensor	SSA (Super Streptavidin)		CA coated			Z:\VM_Shared_Folder\Furosemide Test 1
		5	t1E1	1	Ligand Sensor	SSA (Super Streptavidin)		CA coated			Z:\VM_Shared_Folder\Furosemide Test 1
		6	t1F1	1	Ligand Sensor	SSA (Super Streptavidin)		CA coated			Z:\VM_Shared_Folder\Furosemide Test 1
		7	t1G1	1	Ligand Sensor	SSA (Super Streptavidin)		CA coated			Z:\VM_Shared_Folder\Furosemide Test 1
		8	t1H1	1	Ligand Sensor	SSA (Super Streptavidin)		CA coated			Z:\VM_Shared_Folder\Furosemide Test 1
		9	t1A2	1	Ligand Sensor	SSA (Super Streptavidin)		BCT blocked			Z:\VM_Shared_Folder\Furosemide Test 1

Figure 4-9: Full Traces Table

The Data Cycles table shows kinetic-specific cycle information (Figure 4-10).



Sensor Number	Tray	Repetition Number	Sensor Location	Sensor Type	Sensor Lot No	Sensor Info	Baseline Loc.	Assoc. (Sample) Loc.	Dissoc. Loc.	Sample ID	
9	1	1	t1A2	SSA (Super Streptavidin)		BCT blocked	p1A1	p1A7	p1A1	Furosemide	PE
9	1	2	t1A2	SSA (Super Streptavidin)		BCT blocked	p1A2	p1A8	p1A2	Furosemide	PE
9	1	3	t1A2	SSA (Super Streptavidin)		BCT blocked	p1A3	p1A9	p1A3	Furosemide	PE
9	1	4	t1A2	SSA (Super Streptavidin)		BCT blocked	p1A4	p1A10	p1A4	Furosemide	PE
9	1	5	t1A2	SSA (Super Streptavidin)		BCT blocked	p1A5	p1A11	p1A5	Furosemide	PE
9	1	6	t1A2	SSA (Super Streptavidin)		BCT blocked	p1A6	p1A12	p1A6	Furosemide	PE
10	1	1	t1B2	SSA (Super Streptavidin)		BCT blocked	p1B1	p1B7	p1B1	Furosemide	PE
10	1	2	t1B2	SSA (Super Streptavidin)		BCT blocked	p1B2	p1B8	p1B2	Furosemide	PE

Figure 4-10: Data Cycles Table

- To view a single row or column of biosensor data, click the number above the column or the letter next to the row (Figure 4-11).

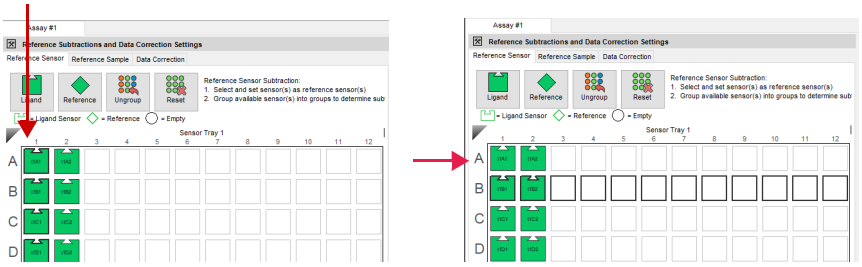


Figure 4-11: Selecting a Single Row or Column

- To view data for multiple sequential rows or columns, click the letter of the first row or the number of the first column you want to view, then hold the mouse and drag it across all the other row/columns you want to view (Figure 4-12).

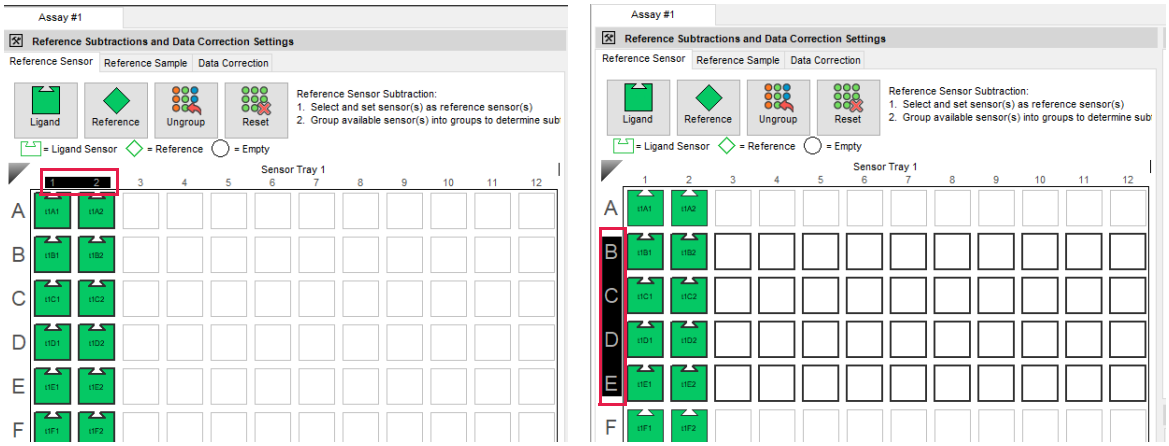


Figure 4-12: Selecting Multiple, Sequential Rows or Columns

- To view data for multiple, non-sequential rows or columns, click the number above the first column or letter next to the first row you want to view, hold **Ctrl** and select the other rows/columns you want to view.
- To view data for multiple sequential biosensors, use your mouse to draw a box around the biosensors (Figure 4-13).

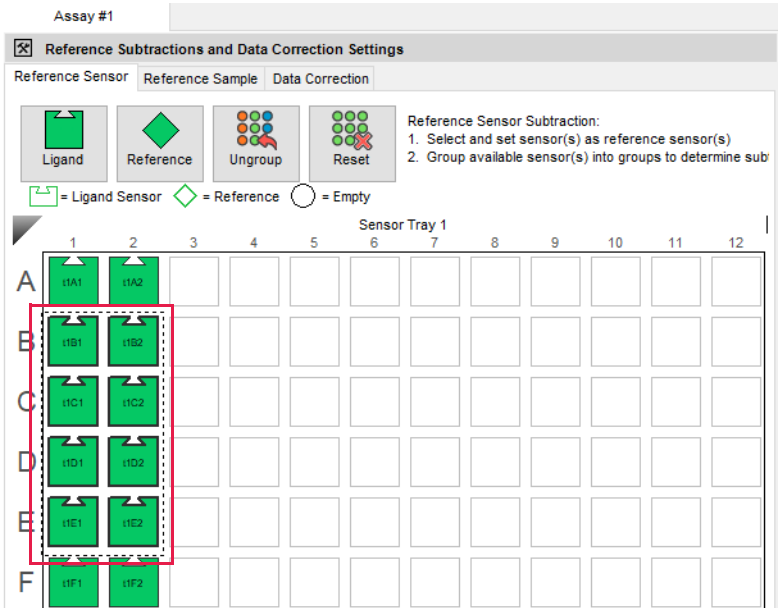


Figure 4-13: Selecting Multiple, Sequential Biosensors

- To view data for multiple, non-sequential biosensors, click the first biosensor you want to view, hold **Ctrl** and select the others you want to view.
- To view data for multiple biosensors according to the instrument read-head pattern, hold the **Shift** button while clicking on a sensor.

Reference Sensor (Non-specific Binding) Subtraction

The experimental data shown for this example uses two columns of biosensors. Samples run with the biosensors in column 1 used an immobilized ligand (Figure 4-14), and the samples run with biosensors in column 2 had an irrelevant ligand immobilized or used blank buffer (no ligand present) as shown in Figure 4-15.

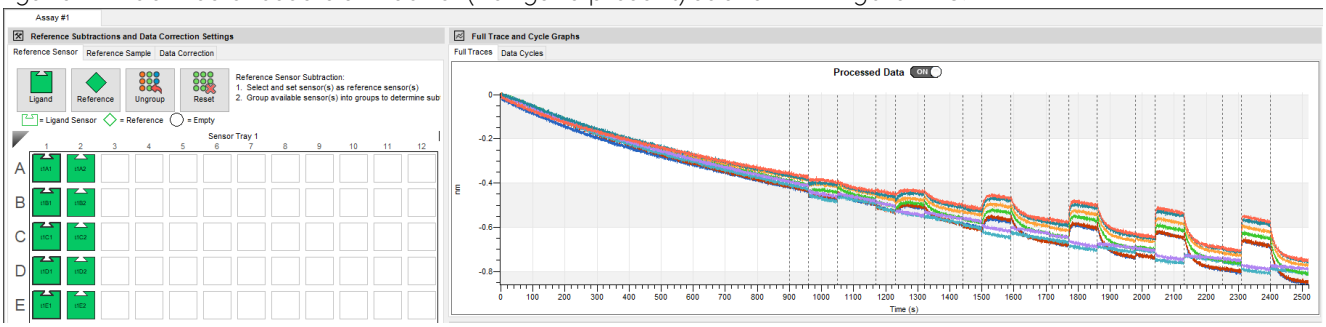


Figure 4-14: Biosensor Column with Ligand

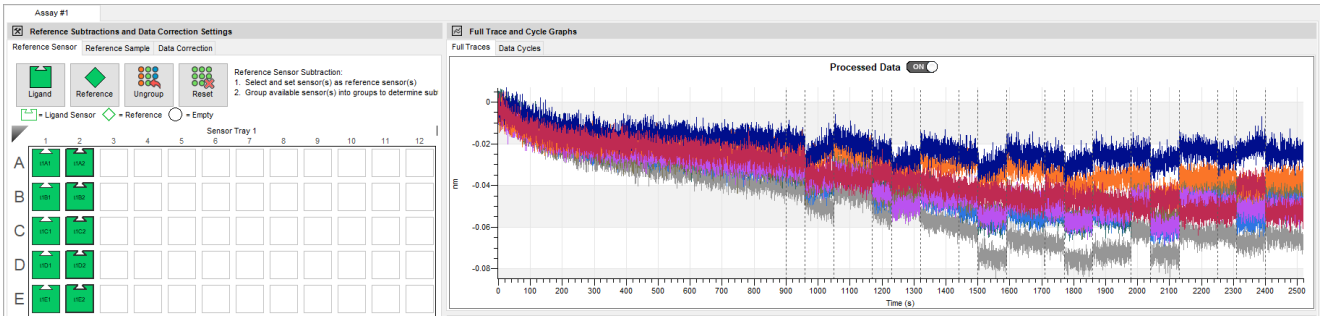


Figure 4-15: Biosensor Column with No Ligand

1. The software initially identifies ligand and reference sensors from the method file. Wells not used display as empty. To identify and set reference biosensors, select the biosensors you want to use to correct for non-specific binding. These should be the biosensors you ran with an irrelevant ligand or with buffer (no ligand). You can select one sensor, row or column (Figure 4-16) or multiple sensors, rows and columns.

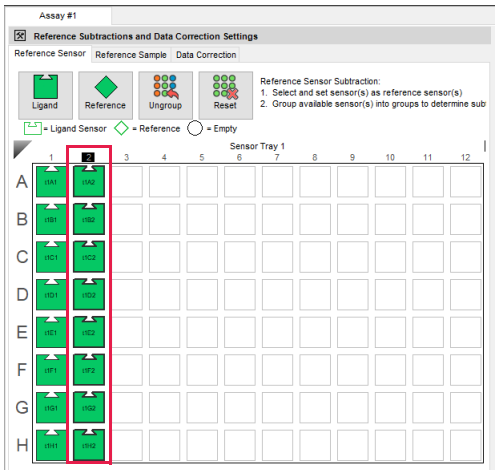


Figure 4-16: Selecting a Biosensor Column

2. Right-click a selected biosensors and select **Reference Sensor** (Figure 4-17). You can also click the Reference Sensor button to change the sensor type.

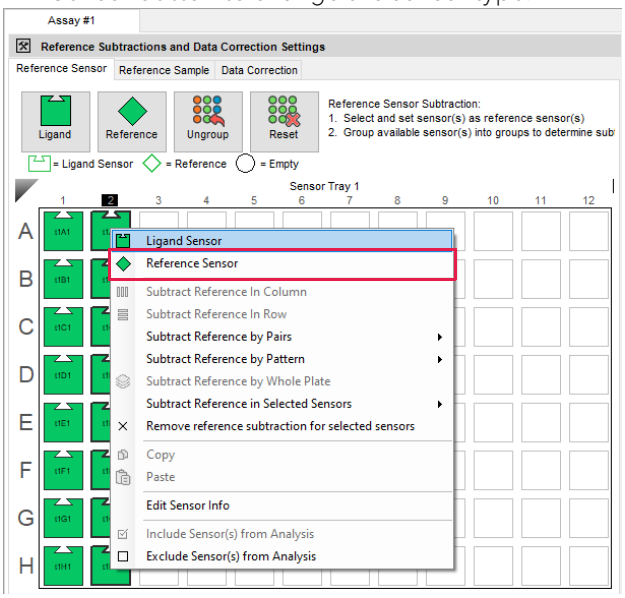


Figure 4-17: Setting Reference Sensors

The selected biosensors display as diamonds, indicating they are set as reference biosensors. They'll also be identified in the Full Traces table (Figure 4-18).

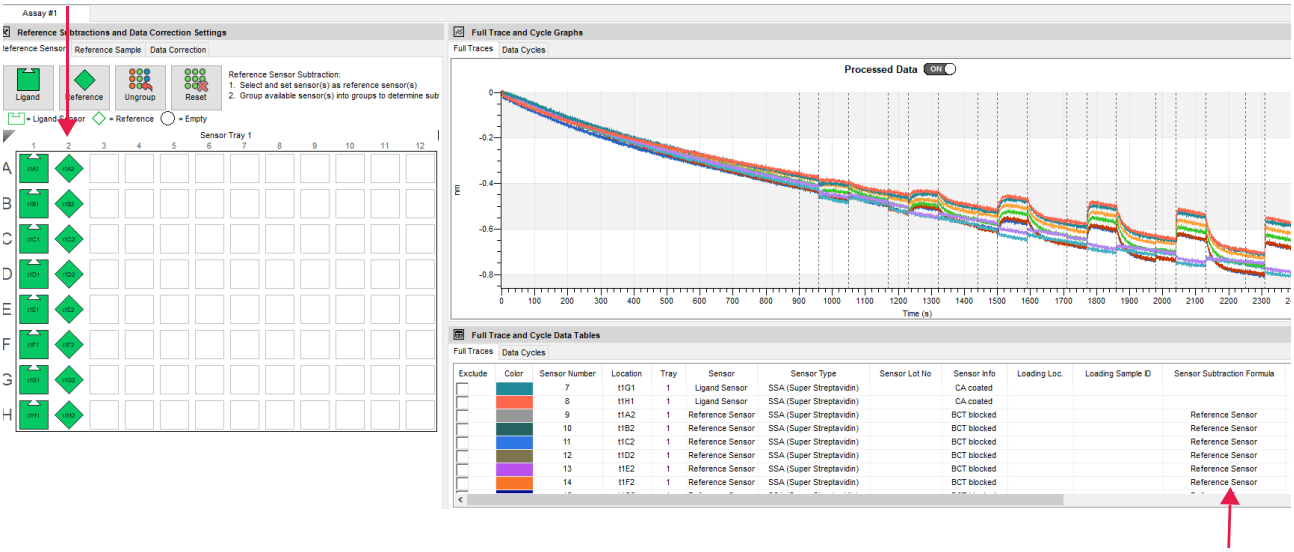


Figure 4-18: Reference Sensors Set

Click 

(**Reset**) to clear the settings for all sensors and revert to the original ligand and reference sensor assignments.

- To remove designations for specific biosensors, select the sensor(s) then right click and select **Ligand Sensor**.

3. **To subtract specific ligand and reference sensors** - select the sensors, right-click anywhere on the biosensor tray and select **Subtract Reference in Selected Sensors by Average** (Figure 4-19). Use this option when there is no pattern or you want to apply different types of referencing on the sensor tray rather than applying one subtraction option to the whole sensor tray.

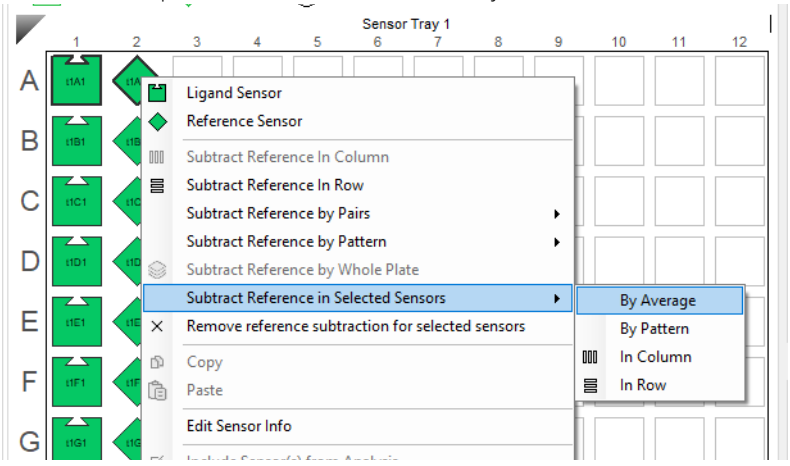


Figure 4-19: Subtracting Specific Wells

To group ligand and reference biosensors for subtraction - right click anywhere on the biosensor tray and select one of the **Subtract Reference** options (Figure 4-20):

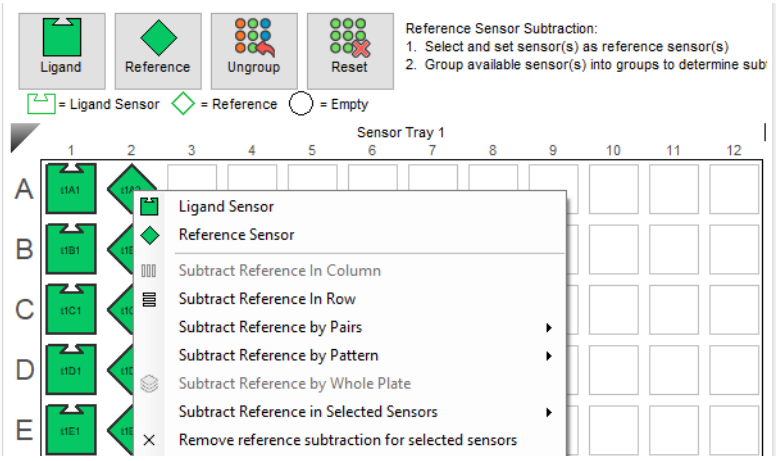
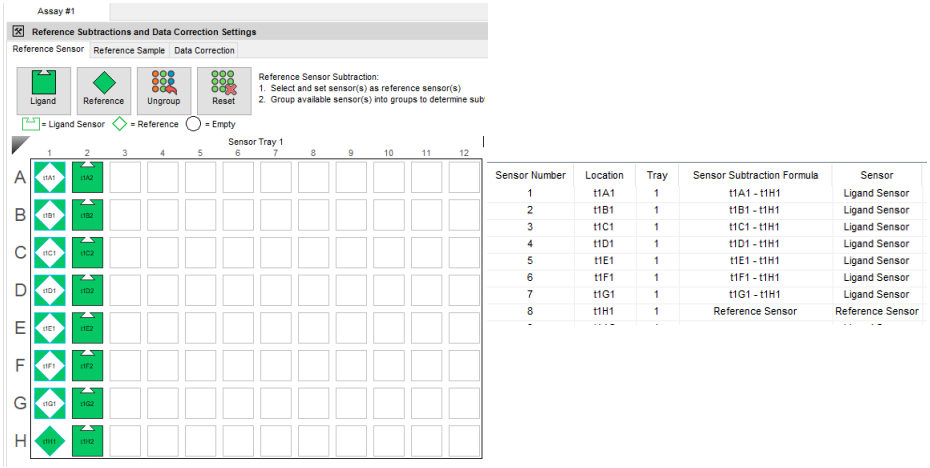


Figure 4-20: Group Reference Options

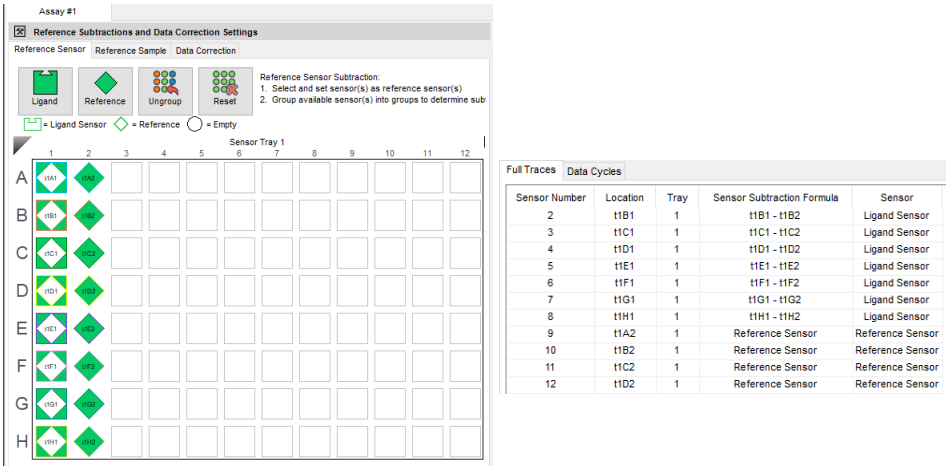
- **Subtract Reference in Column** - Groups reference and ligand biosensors in a column. For example, if your ligand biosensors are A1-G1 in column 1 and your reference biosensor is H1, this subtracts H1 from each sensor in the column, H1 from A1, H1 from B1 and so on (Figure 4-21).



Sensor Number	Location	Tray	Sensor Subtraction Formula	Sensor
1	t1A1	1	t1A1 - t1H1	Ligand Sensor
2	t1B1	1	t1B1 - t1H1	Ligand Sensor
3	t1C1	1	t1C1 - t1H1	Ligand Sensor
4	t1D1	1	t1D1 - t1H1	Ligand Sensor
5	t1E1	1	t1E1 - t1H1	Ligand Sensor
6	t1F1	1	t1F1 - t1H1	Ligand Sensor
7	t1G1	1	t1G1 - t1H1	Ligand Sensor
8	t1H1	1	Reference Sensor	Reference Sensor

Figure 4-21: Group Reference In Column Tray View and Sensor Subtraction Formula

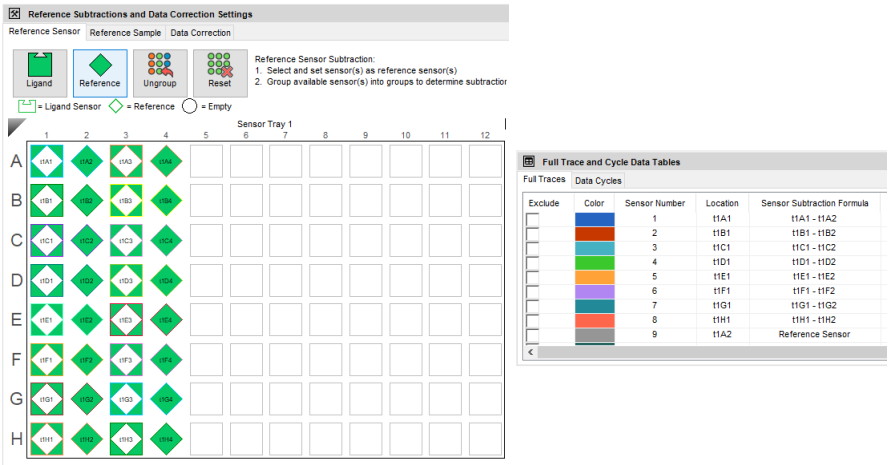
- **Subtract Reference in Row** - Groups reference and ligand biosensors in a row. For example, if your ligand biosensors are in column 1 and reference biosensors are in column 2 this subtracts A2 from A1, B2 from B1, and so on (Figure 4-22).



Sensor Number	Location	Tray	Sensor Subtraction Formula	Sensor
2	t1B1	1	t1B1 - t1B2	Ligand Sensor
3	t1C1	1	t1C1 - t1C2	Ligand Sensor
4	t1D1	1	t1D1 - t1D2	Ligand Sensor
5	t1E1	1	t1E1 - t1E2	Ligand Sensor
6	t1F1	1	t1F1 - t1F2	Ligand Sensor
7	t1G1	1	t1G1 - t1G2	Ligand Sensor
8	t1H1	1	t1H1 - t1H2	Ligand Sensor
9	t1A2	1	Reference Sensor	Reference Sensor
10	t1B2	1	Reference Sensor	Reference Sensor
11	t1C2	1	Reference Sensor	Reference Sensor
12	t1D2	1	Reference Sensor	Reference Sensor

Figure 4-22: Group Reference In Row Tray View and Full Traces Table Subtraction Formula

- **By Pairs Left** - Groups reference and ligand biosensors in pairs and assumes that the reference biosensors are to the right of the ligand sensors. For example, if your ligand biosensors are in column 1 and 3, and the reference biosensors are in column 2 and 4, this subtracts A2 from A1, A4 from A3, and so on (Figure 4-23).



Exclude	Color	Sensor Number	Location	Sensor Subtraction Formula
		1	t1A1	t1A1 - t1A2
		2	t1B1	t1B1 - t1B2
		3	t1C1	t1C1 - t1C2
		4	t1D1	t1D1 - t1D2
		5	t1E1	t1E1 - t1E2
		6	t1F1	t1F1 - t1F2
		7	t1G1	t1G1 - t1G2
		8	t1H1	t1H1 - t1H2
		9	t1A2	Reference Sensor

Figure 4-23: Group Reference By Pairs Left and Full Traces Table Subtraction Formula

- **By Pairs Right** - This function groups sensors similarly to By Pairs Left but it assumes that the reference sensors (A1) are to the left of the ligand sensors (A2) so A1 gets subtracted from A2, A3 from A4 and so on.
- **By Pattern Right** - Groups reference and ligand biosensors in a pattern and assumes that the reference biosensors are to the left of the ligand sensors. This option is typically used when you're running all the reference sensors first and then all the ligand sensors as shown in Figure 4-24 where the experiment was run in 16-channel mode. For example, if your ligand biosensors are in columns 3 and 4, and the reference biosensors are in columns 1 and 2, this subtracts A1 from A3, A2 from A4, and so on.

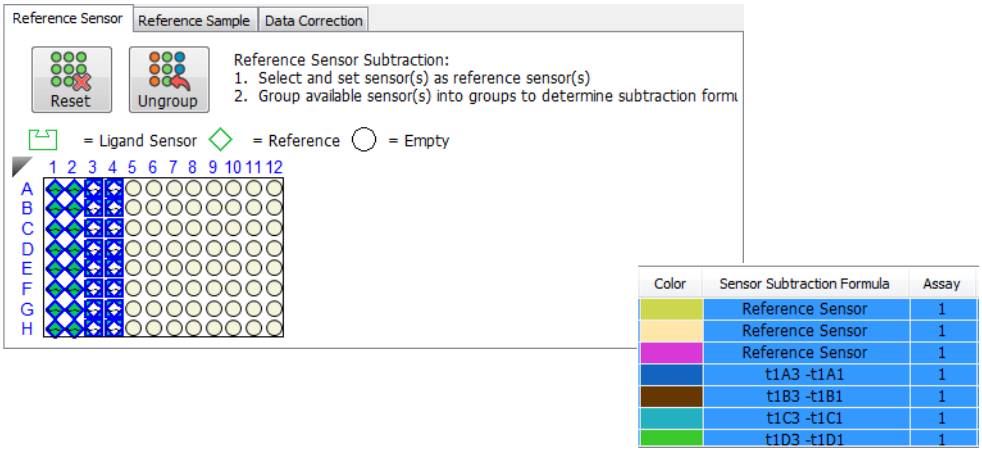


Figure 4-24: Group Reference By Pattern Right and Full Traces Table Subtraction Formula

- **By Pattern Left** - Groups reference and ligand biosensors in a pattern and assumes that the reference biosensors are to the right of the ligand sensors. This option is typically used when you're running all the ligand sensors first and then all the reference sensors. For example, if your ligand biosensors are in columns 1 and 2 and reference biosensors are in columns 3 and 4, this subtracts A3 from A1, A4 from A2, and so on.
- **By Whole Plate** - Use this option if you've run multiple trays of ligand sensors with one entire tray of reference sensors during an experiment or mega-experiment. After assigning the reference sensor type to all sensors in the reference tray, right-click the ligand tray, choose the By Whole Plate option, and then choose the reference tray. More than one tray can be a reference tray. For example, tray 1 could be subtracted from tray 2, and tray 3 could be subtracted from tray 4. Ligand and reference sensors are grouped by corresponding positions on the plate. For example, reference sensor A1 is subtracted from ligand sensor, A1; B1 from B1, B2 from B2.

- **Subtract Reference Across Trays** - Use this option if your workspace contains multiple sensor trays. The selected sensors in each tray will be grouped together so that the reference sensor is subtracted from the other selected ligand sensors. If more than one reference sensor is included in the selection, their responses will be averaged before subtracting from the ligand sensors.
- **Copy and Paste** - If multiple plates and sensor trays were overlaid to create a mega-experiment where all experiments were run in an identical manner, set the reference sensors and grouping on one sensor tray, and then copy and paste the configuration to the other sensor tray using the right-click menu.
  The corrected data displays in the Full Traces and Data Cycles tabs (Figure 4-25).

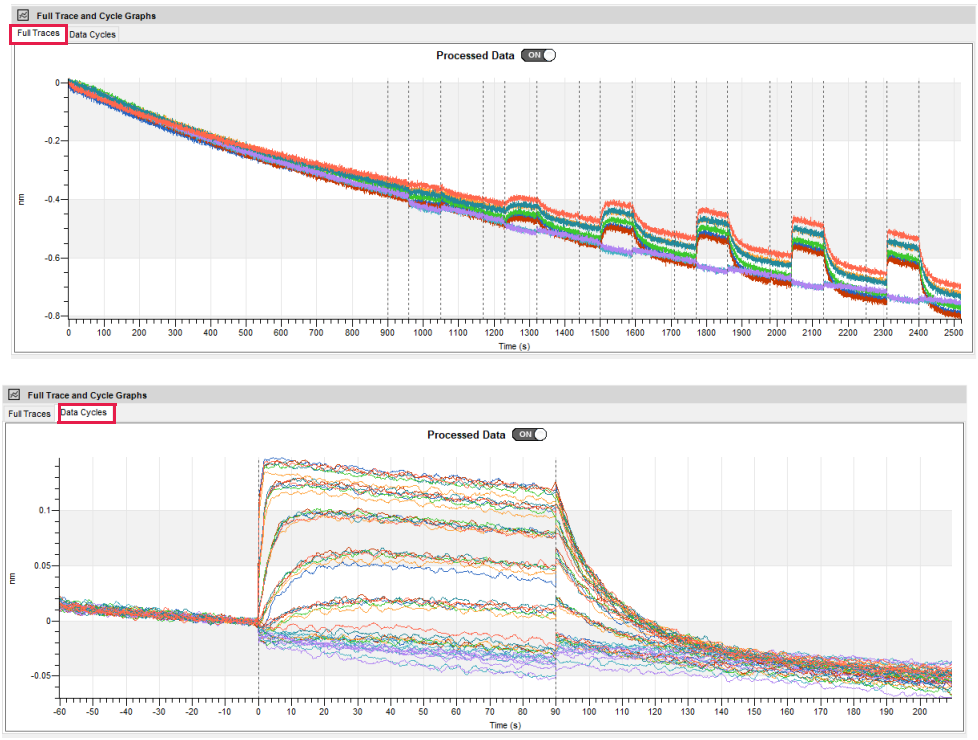


Figure 4-25: Corrected Data

When corrected data is displayed, the Processed Data toggle at the top of the graph is ON. To view uncorrected data, click the button to toggle it OFF (Figure 4-26).

NOTICE: *When sensor referencing has not been configured, the Full Trace uncorrected and processed data is identical.*

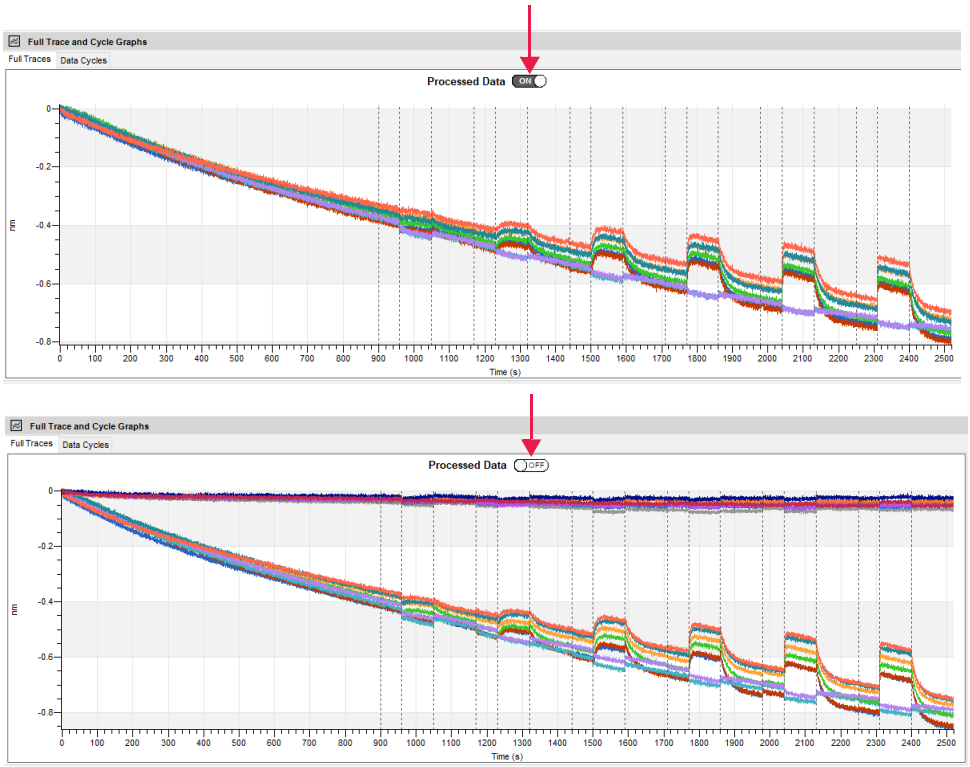


Figure 4-26: Processed Data ON (Top) and OFF (Bottom)

The biosensor subtraction formula also displays in the Full Traces table (Figure 4-27).

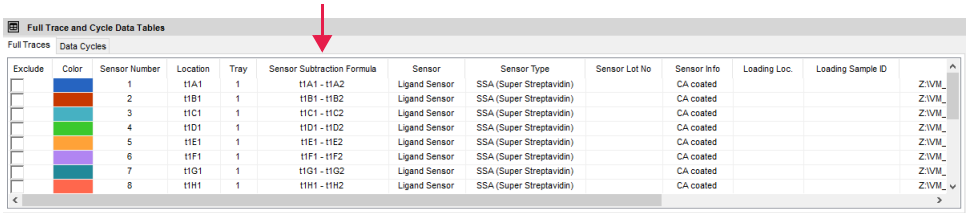


Figure 4-27: Full Traces Table Showing Sensor Subtraction Formula

- Click 

(**Ungroup**) to remove all ligand and reference biosensor groupings.
- To ungroup specific biosensors, select those sensors then right click and select **Remove Reference Subtraction for Selected Sensors**.

- Click 

(**Reset**) to clear all reference biosensor and group settings for all wells.

4. If there is more than one assay in your experiment or combined dataset, click the next **Assay** tab and repeat the prior steps as needed.

Excluding Biosensors from the Analysis

You can exclude specific biosensor(s) from your analysis.

NOTICE: *Excluding biosensors from your analysis clears all reference sensor selections and group settings you've already set.*

- **In the Reference Sensor tab -** Select wells in the biosensor tray you want to exclude. Right-click the tray and select **Exclude Sensors From Analysis** (Figure 4-28).

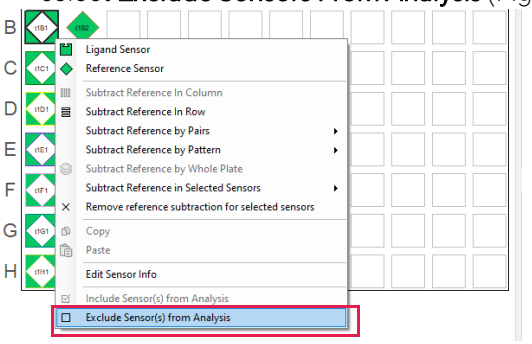


Figure 4-28: Excluding Biosensors in Reference Sensor Tab.

- **In the Full Traces table -** select the check box in the **Exclude** column for the biosensor row(s) (Figure 4-29).

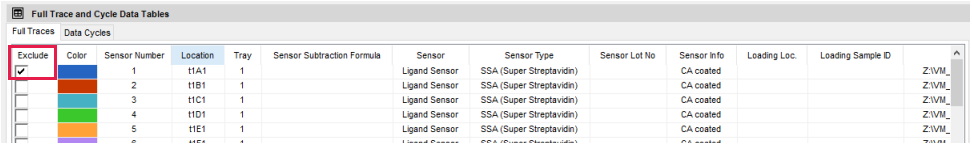


Figure 4-29: Excluding Biosensors in Full Traces Table.

Excluded biosensors are identified in the biosensor tray with horizontal lines, a check mark in the Exclude column in the Full Traces table, and the data no longer displays in the Full Traces and Data Cycles graphs (Figure 4-30).

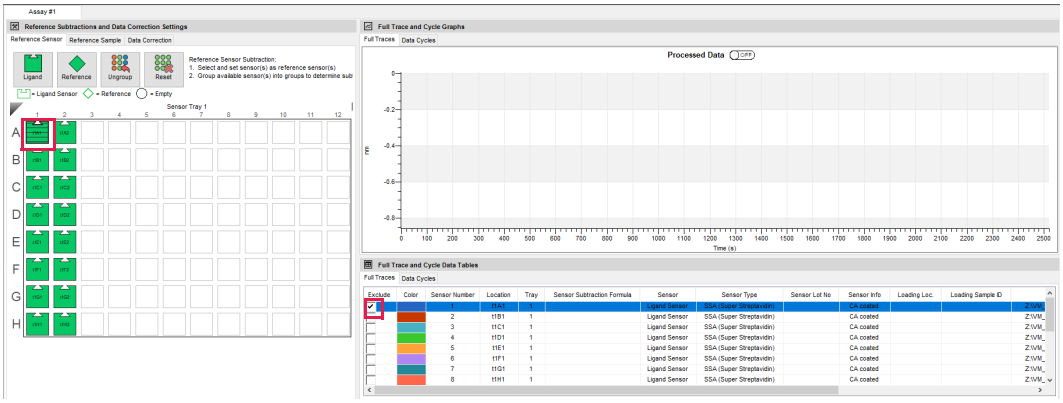


Figure 4-30: Excluded Biosensors

To re-include the biosensor(s) in the analysis, right-click the tray and select **Include Sensors For Analysis** or deselect the box in the **Exclude** column for the biosensor row(s) in the Full Traces table.

Editing Biosensor Information

You can edit biosensor information in the Reference Sensor tab.

1. Select the well or wells you want to edit in the biosensor plate.
2. Right-click the well and select **Edit Sensor Info** (Figure 4-31).

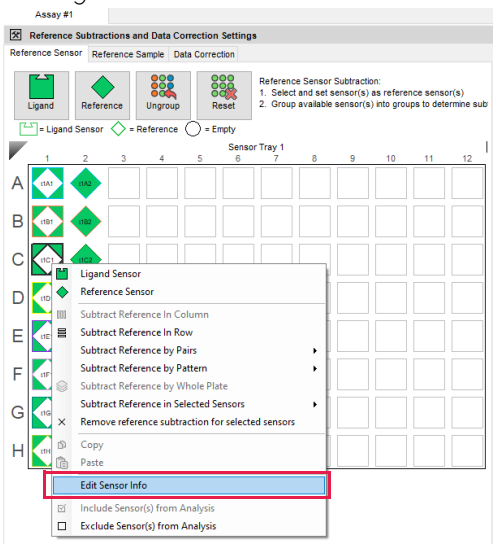


Figure 4-31: Selecting Edit Sensor Info

3. Update the information in the Edit Sensor Info window, then click **OK** (Figure 4-32). Only the fields marked with a check are updated.

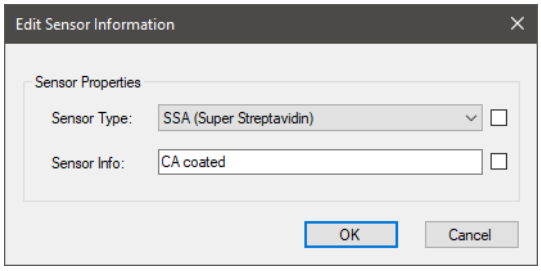


Figure 4-32: Edit Sensor Info Window.

Reference Sample Well Subtraction

To subtract any baseline drift in your experiment using reference sample wells that contained ligand but no analyte, click the **Reference Sample** tab in the Reference Subtractions and Data Corrections window (Figure 4-33).

NOTICE: *The Reference Sample tab is only available for kinetic analysis, it won't display for epitope binning analysis.*

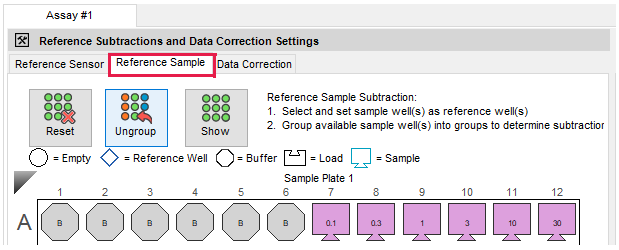


Figure 4-33: Reference Sample Tab

Viewing Data

To view data for different assays in the experiment or combined data set, click the **Assay** tab above the Reference Subtractions and Data Corrections window (Figure 4-34).

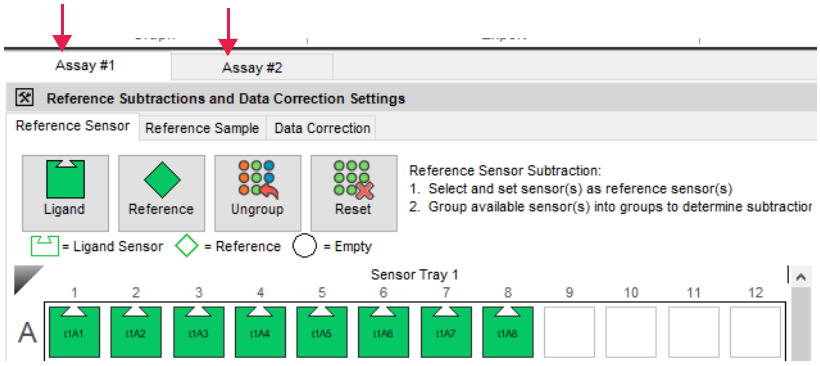


Figure 4-34: Assay Tabs

Click 

(**Show**) to display both the biosensor tray and the sample plate (Figure 4-35). The default view shows only the sample plate.

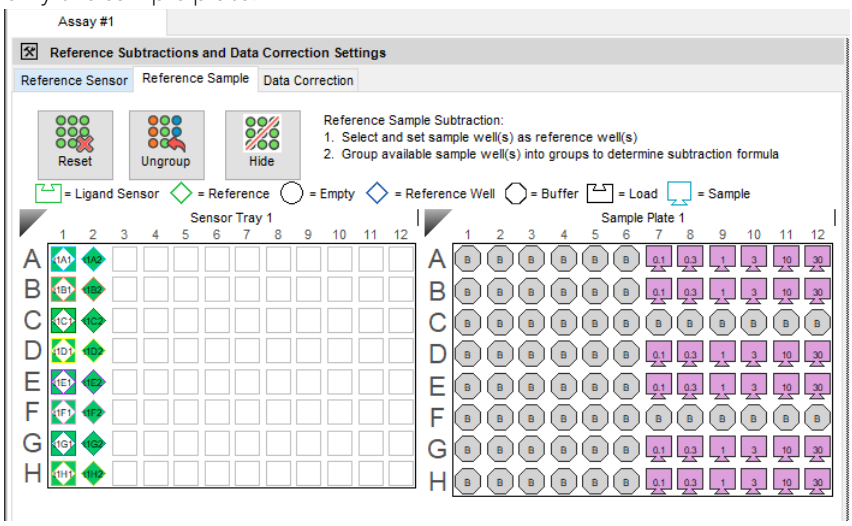


Figure 4-35: Biosensor Tray Display After Show is Selected

Click 

(**Hide**) to hide the biosensor tray.

If you used more than one biosensor tray or sample plate in the experiment or combined dataset, use the scrollbars to move between the plates (Figure 4-36).

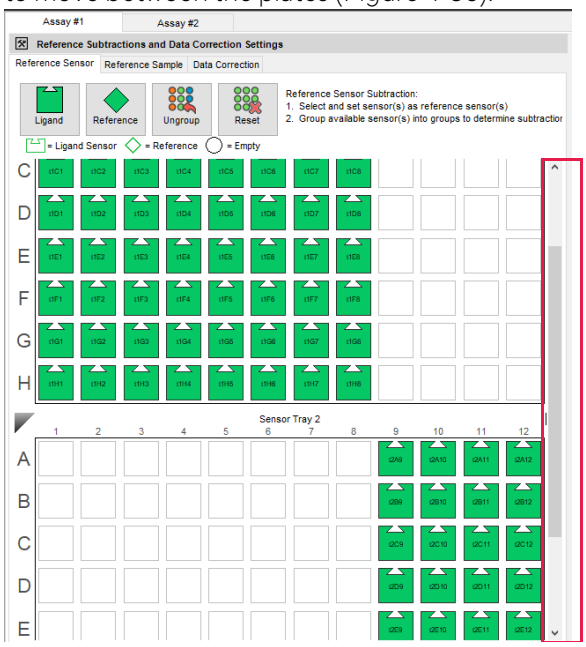


Figure 4-36: Scrolling Between Sample Plates

- Biosensors used in the experiment are green in the tray map and are set to ligand sensors by default, all other wells display empty. If you set reference and ligand biosensors and grouped them for non-specific binding subtraction in the Reference Sensor tab, those designations also display here (Figure 4-35).
- Biosensors are identified by their tray number and well number. For example t1A3 is the biosensor in well A3 on tray 1, t2H5 is the biosensor in well H5 on tray 2 (Figure 4-35).
- Selecting columns, rows or wells in the biosensor tray map also highlights the associated wells in the sample plate map and vice-versa (Figure 4-37).

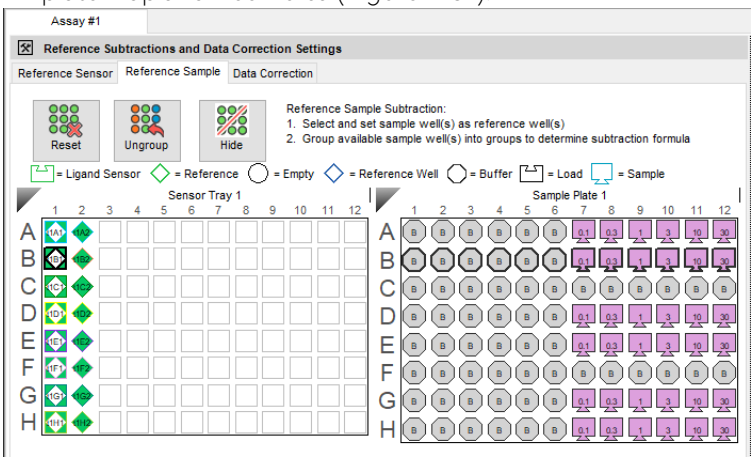


Figure 4-37: Biosensors and Associate Sample Wells Highlighted

- Sample analyte concentrations display in the sample/load wells on the sample plate (Figure 4-38).

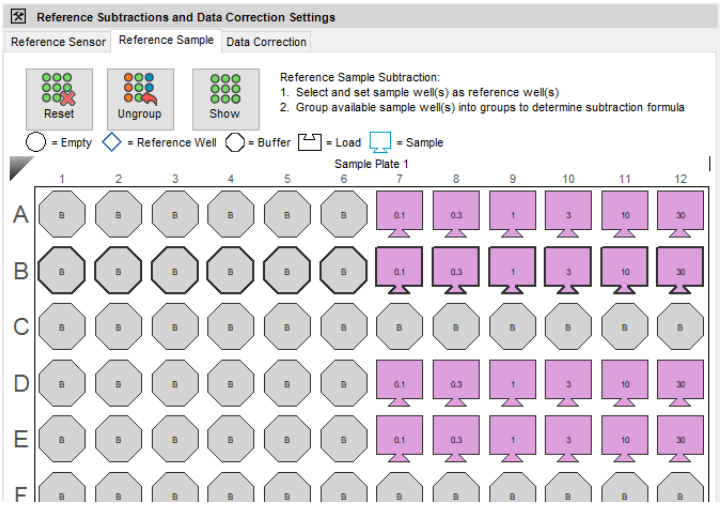


Figure 4-38: Sample Plate Showing Concentrations for Sample/Load Wells

To display date in the Full Traces and Data Cycles graphs (Figure 4-39) do one of the following:

- Click rows, columns or specific well in the biosenor or tray or sample plate in the Reference Senor or Reference Sample tabs
- Click rows in the Full Traces and Data Cycles table.

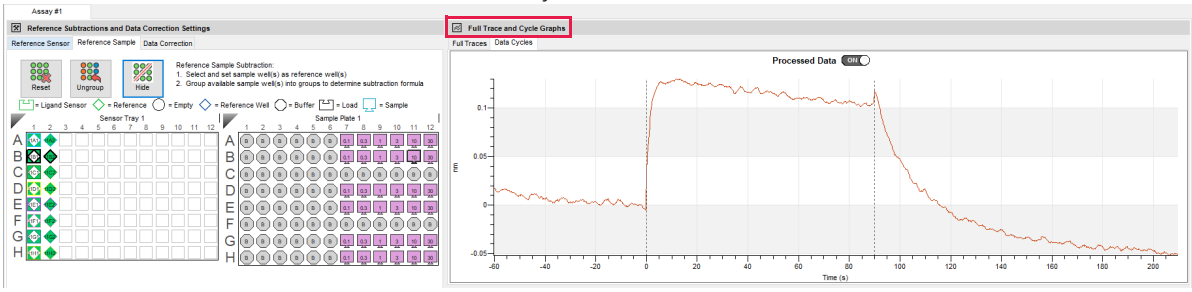


Figure 4-39: Well Selection Data Display

The Full Traces tab shows the raw or reference sensor-corrected data for all steps of the experiment including initial baseline, loading, baseline and association/dissociation (Figure 4-40).

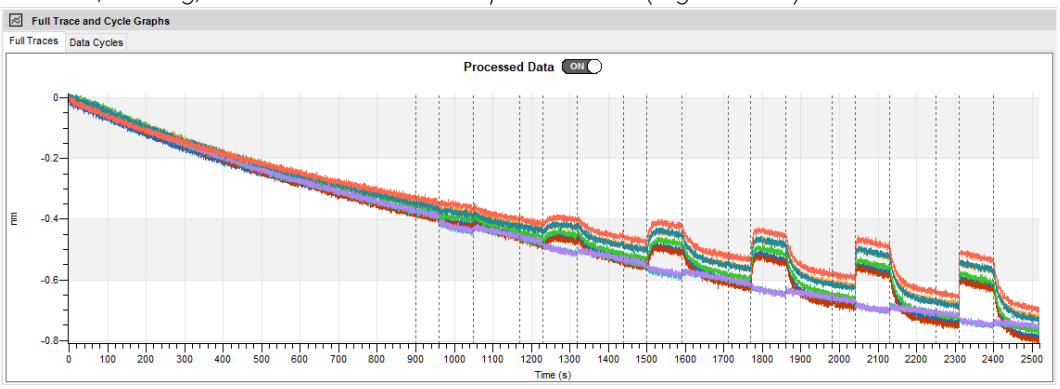


Figure 4-40: Full Traces Tab

The **Data Cycles** tab shows only the raw or reference sensor-corrected baseline after ligand loading and the association/dissociation steps (Figure 4-41).

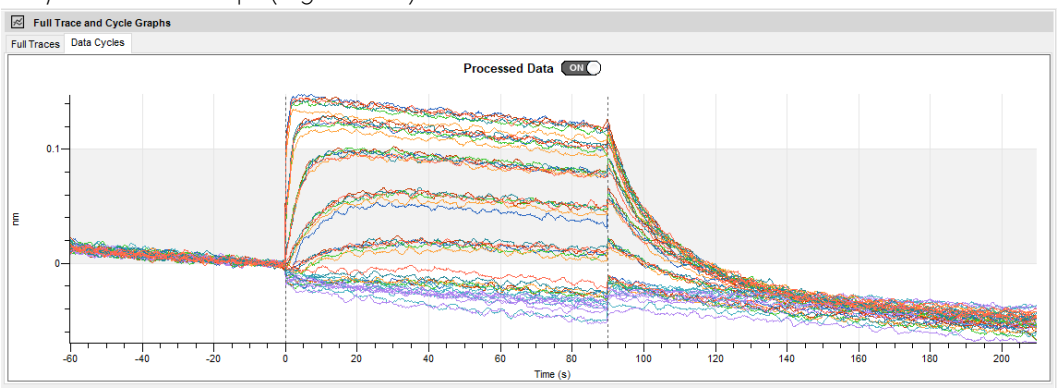


Figure 4-41: Data Cycles Tab When corrected data is displayed, the Processed Data toggle at the top of the graph is ON.

To view uncorrected data, click the button to toggle it OFF (Figure 4-42).

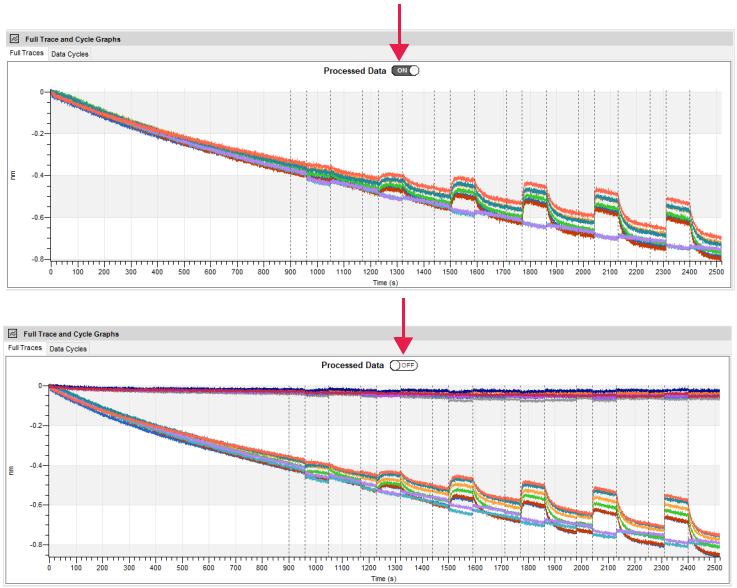


Figure 4-42: Processed Data ON (Top) and OFF (Bottom)

The Full Traces table shows biosensor-specific information (Figure 4-43), including the sensor subtraction formula and reference biosensors if non-specific binding data corrections were made. Sample subtraction formulas display in the Data Cycles table after reference sample subtraction is performed (Figure 4-44).

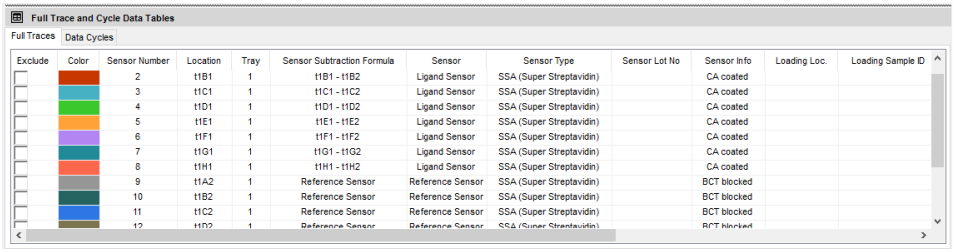


Figure 4-43: Full Traces Table

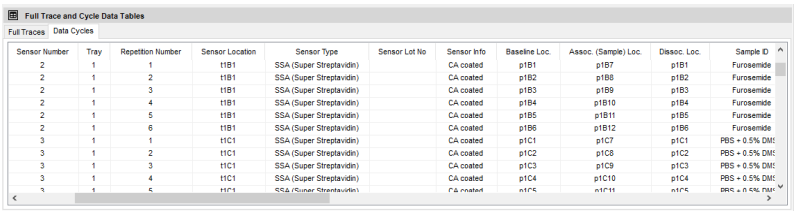


Figure 4-44: Data Cycles Table

- To view a single row or column of biosensor data, click the number above the column or the letter next to the row in the biosensor tray or sample plate (Figure 4-45).

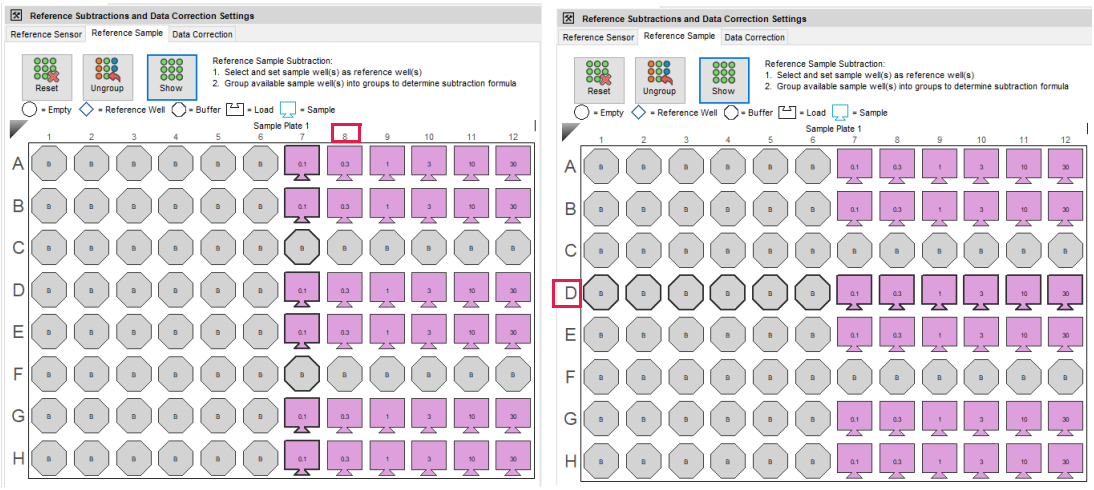


Figure 4-45: Selecting a Single Row or Column

- To view data for multiple sequential rows or columns, click the letter of the first row or the number of the first column you want to view in the biosensor tray or sample plate, then hold the mouse and drag it across all the other row/columns you want to view (Figure 4-46).

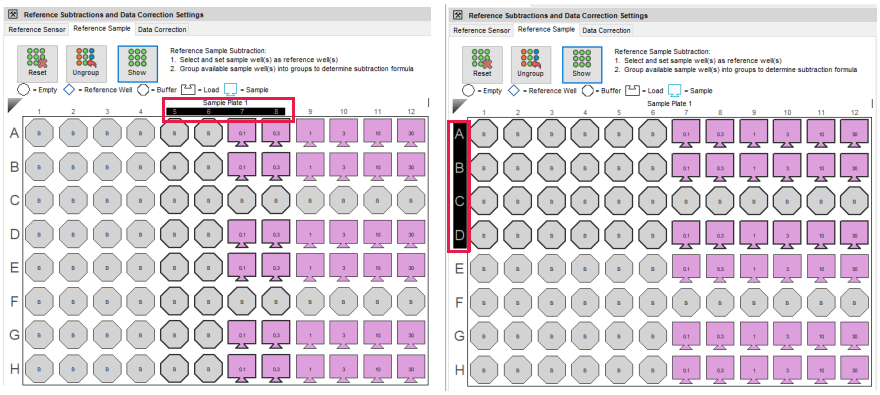


Figure 4-46: Selecting Multiple, Sequential Rows or Columns

- To view data for multiple, non-sequential rows or columns, click the number above the first column or letter next to the first row you want to view in the biosensor tray or sample plate, hold **Ctrl** and select the other rows/ columns you want to view.
- To view data for multiple sequential wells, use your mouse to draw a box around the wells in the biosensor tray or sample plate (Figure 4-47).

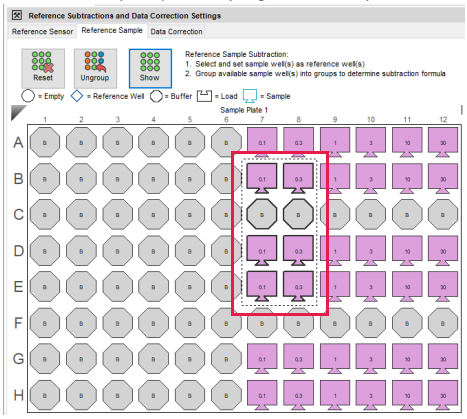


Figure 4-47: Selecting Multiple, Sequential Wells

- To view data for multiple, non-sequential wells, click the first well you want to view, hold **Ctrl** and select the others you want to view.
- To view data for multiple wells according to the instrument read-head pattern, hold the **Shift** button while clicking on a well.

Subtracting Baseline Drift

The experimental data shown for this example uses analyte concentrations from 0.1-30 µg/mL in sample/load wells, and well C7 is the reference sample well containing only buffer (Figure 4-48). Samples were run with the biosensors in column 1 and non-specific binding was subtracted in the Reference Sensor Tab using the reference biosensors in column 2

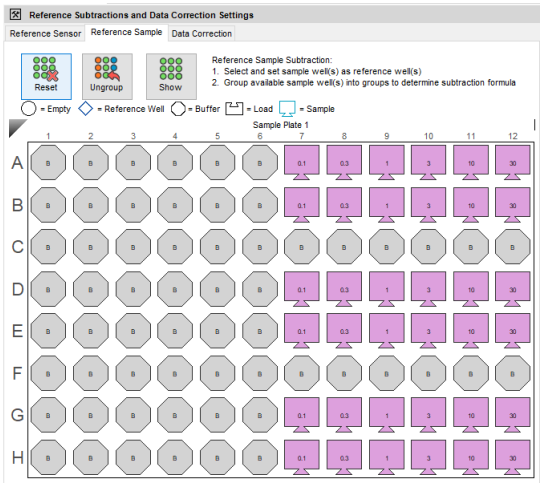


Figure 4-48: Example Experiment for Baseline Drift Subtraction.

1. Select the sample/load well(s) you want to use as your reference sample well(s) to correct for baseline drift. This should be a well that contained only buffer (Figure 4-49). In this example only one zero analyte well was run in the experiment.

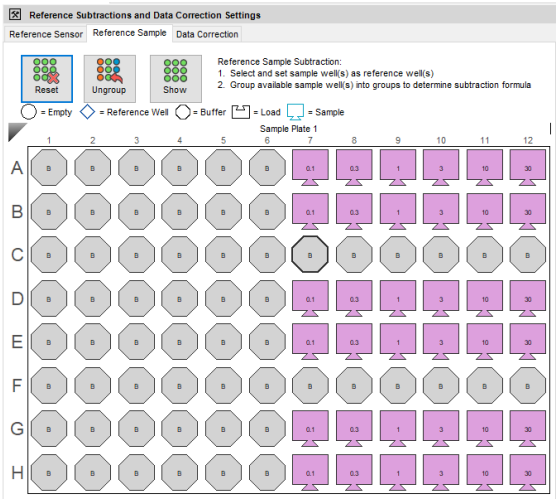


Figure 4-49: Selecting a Reference Sample Well

2. Right-click the well and select **Reference Well** (Figure 4-50).

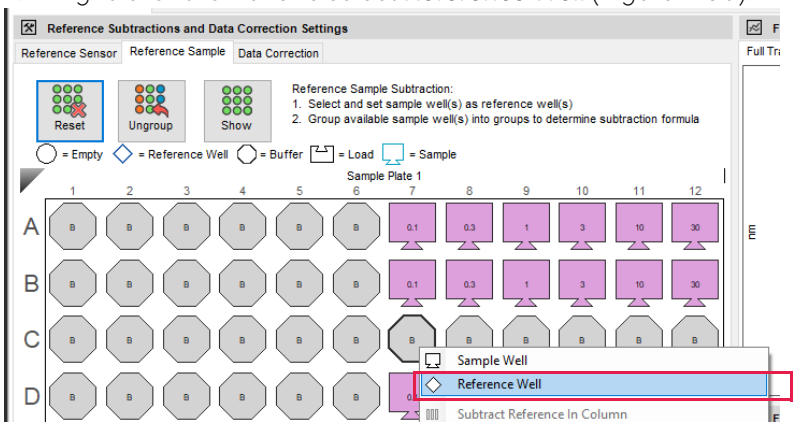


Figure 4-50: Setting the Reference Sample Well

The selected wells appear as a diamond, to indicate it is set as reference well (Figure 4-51).

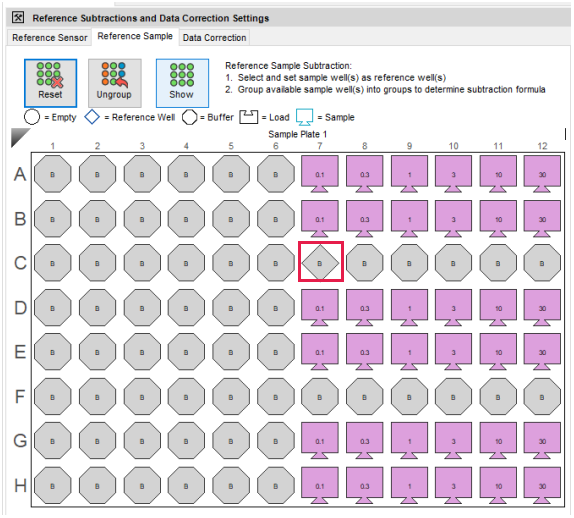


Figure 4-51: Reference Well Set

- Click 

(**Reset**) to clear reference well settings for all wells.
- To remove reference well designations for specific wells, select the well(s) then right click and select **Sample Well**.

3. **To subtract specific reference and load/sample wells** - select the wells, right click anywhere on the sample plate and select **Subtract Reference in Selected Wells by Average** (Figure 4-52). Use this option when there is no pattern or you want to apply different types of referencing on the plate rather than applying one subtraction option to the whole plate.

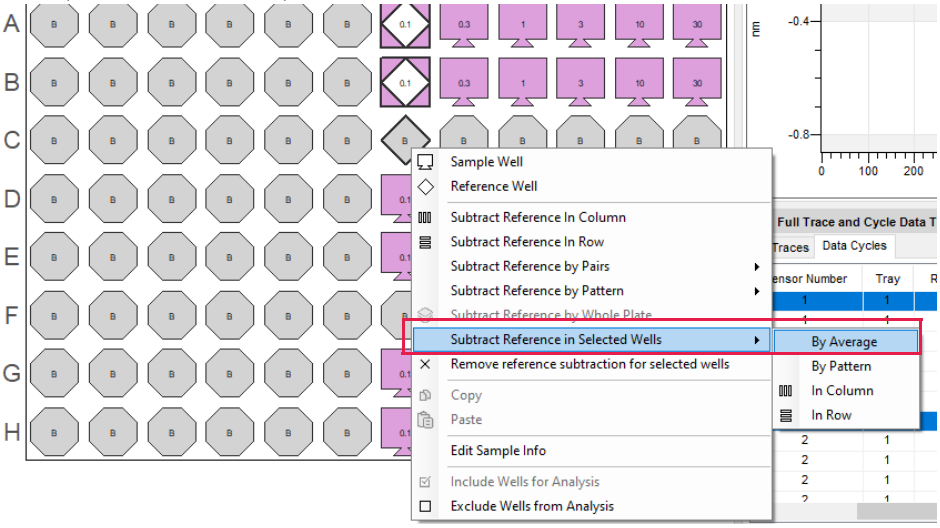


Figure 4-52: Subtracting Specific Wells

To group reference and sample/load wells for subtraction - right click anywhere on the sample plate and select one of the following:

- **Subtract Reference in Column** - – Groups reference and sample/load wells in a column. For example, if you have analyte in wells A7, B7, D7, E7, G7, and H7, and your reference wells are C7 and F7, this subtracts the average of C7 and F7 from A7, the average of C7 and F7 from B7, an so on (Figure 4-53).

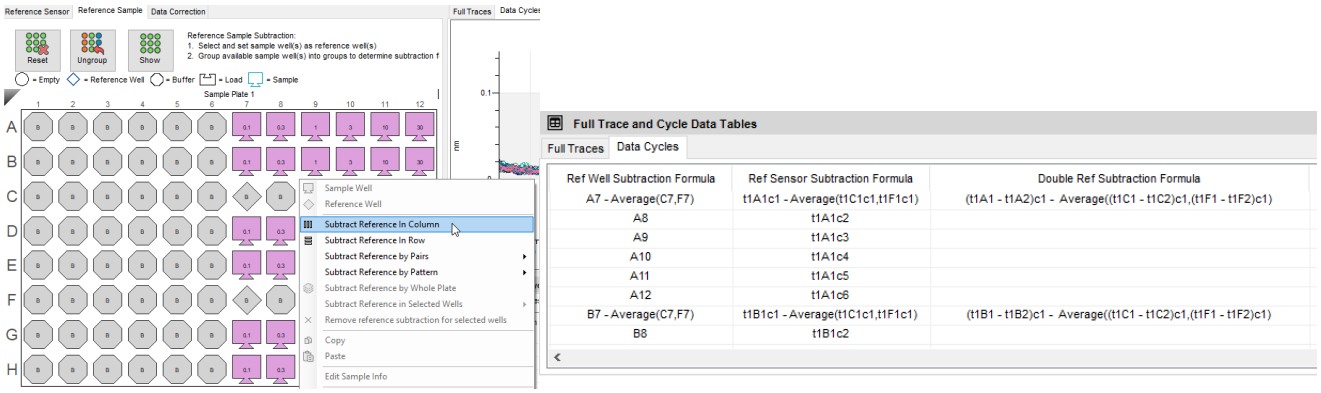


Figure 4-53: Group Reference In Column Plate View and Data Cycles Table Subtraction Formula

NOTICE: *The reference sensor subtraction formula indicates the tray (t), sensor position, and cycle number (c) within the sensor assay. For example, t1A3c1 would indicate the first cycle of sensor A3 from the sensor tray 1. t2B3c2 would indicate the second cycle of sensor B3 from tray 2.*

- **Subtract Reference in Row** - Groups reference and sample/load wells in a row. For example, if your sample/load wells are in are in column 3 and reference wells are in column 4, this subtracts A4 from A3, B4 from B3, and so on.
- **By Pairs Left** - Groups reference and sample/load wells in pairs and assumes that the reference wells are to the right of the sample/load wells. For example, if your sample/load wells are in column 1 and 3, and the reference wells are in column 2 and 4, this subtracts A2 from A1, A4 from A3, and so on.
- **By Pairs Right** - This function groups sensors similarly to By Pairs Left but it assumes that the reference wells (A1) are to the left of the sample/load wells (A2) so A1 is subtracted from A2, A3 from A4 and so on.
- **By Pattern Right** - Groups reference and sample/load wells in a pattern and assumes that the reference wells are to the left of the sample/load wells. This option is typically used when you're running all reference wells first and then all the sample/load wells. For example, if your sample/load wells are in columns 3 and 4, and the reference wells are in columns 1 and 2, this subtracts A1 from A3, A2 from A4, and so on.
- **By Pattern Left** - Groups reference and sample/load wells in a pattern and assumes that the reference wells are to the right of the sample/load wells. This option is typically used when you're running all the sample/load wells first and then all the reference wells. For example, if your sample/load wells are in columns 1 and 2 and reference wells are in columns 3 and 4, this subtracts A3 from A1, A4 from A2, and so on.
- **By Quadrant** - This option is for 384 well plates only. The four sample wells that are accessed by the same sensor are treated as a quadrant. Any of the four wells can be the reference well and is subtracted from the other three wells. This option is applied to the entire plate. For example, if well B1 is a reference well, it will be subtracted from A1, A2 and B2.

- **By Whole Plate** - Use this option if you've run multiple plates of sample/loaded wells with one entire plate of reference wells during an experiment or mega-experiment. After assigning the reference wells in the reference plate, right-click the other sample plate, choose the By Whole Plate option, and then choose the reference plate. More than one plate can be a reference plate. For example, plate 1 could be subtracted from plate 2, and plate 3 could be subtracted from plate 4. Reference and sample/load wells are grouped by corresponding positions on the plate. For example, reference well A1 is subtracted from sample well A1; B1 from B1, B2 from B2, etc.

NOTICE: *When using referencing by Whole Plate, the reference well subtraction formula displays the well names with a "p" prefix and the plate number. For example, p2A3 indicates well A3 on plate number 2.*

  If multiple plates and sensor trays were overlaid to create a mega-experiment where all experiments were run in an identical manner, set the reference wells and grouping on one plate, then copy and paste the configuration to the other plate using the right-click menu.

- **Subtract Reference Across Plates** - Use this option if your workspace contains multiple sample plates. The selected wells in each plate will be grouped together so that the reference well is subtracted from the other selected sample wells. If more than one reference well is included in the selection, their responses will be averaged before subtracting from the sample wells.

The corrected data displays in the Full Traces and Data Cycles tabs (Figure 4-54).

NOTICE: *Data corrections are cumulative, so if you corrected for non-specific binding on the Reference Sensor tab, that is also included in the corrected data displayed.*

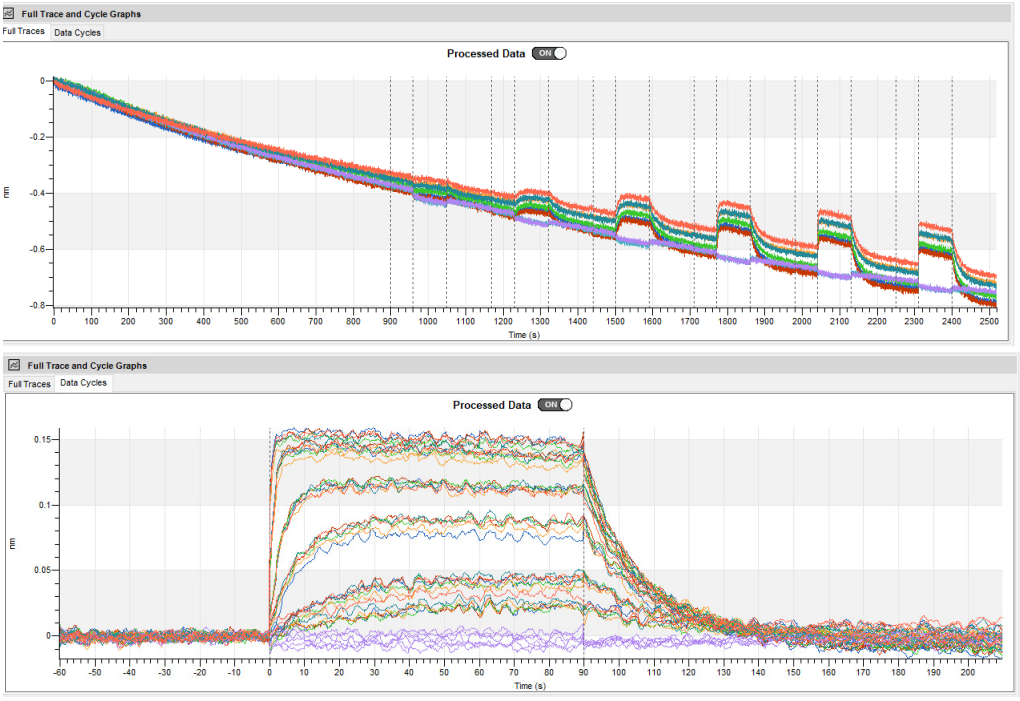


Figure 4-54: Corrected Data

When corrected data is displayed, the Processed Data toggle at the top of the graph is ON. To view uncorrected data, click the button to toggle it OFF (Figure 4-55).

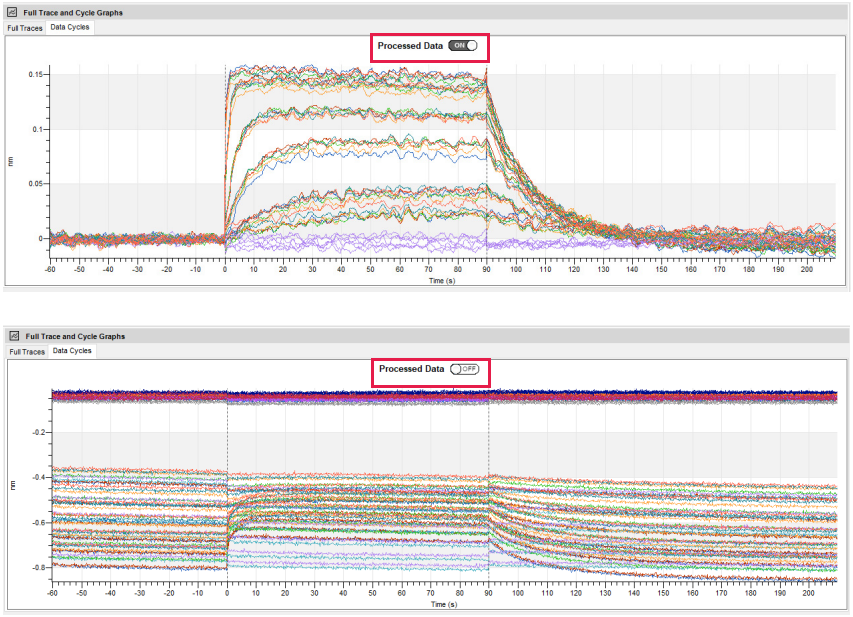


Figure 4-55: Processed Data ON (Top) and OFF (Bottom)

The reference well subtraction formula displays in the Data Cycles table. This table also displays the double reference subtraction formula if you did subtractions to correct for non-specific binding on the Reference Sensor tab (Figure 4-56).

Ref Well Subtraction Formula	Ref Sensor Subtraction Formula	Double Ref Subtraction Formula
A11 - C11	t1A1c5 - t1C1c5	(t1A1 - t1A2)c5 - (t1C1 - t1C2)c5
A12 - C12	t1A1c6 - t1C1c6	(t1A1 - t1A2)c6 - (t1C1 - t1C2)c6
B7 - C7	t1B1c1 - t1C1c1	(t1B1 - t1B2)c1 - (t1C1 - t1C2)c1
B8 - C8	t1B1c2 - t1C1c2	(t1B1 - t1B2)c2 - (t1C1 - t1C2)c2
B9 - C9	t1B1c3 - t1C1c3	(t1B1 - t1B2)c3 - (t1C1 - t1C2)c3
B10 - C10	t1B1c4 - t1C1c4	(t1B1 - t1B2)c4 - (t1C1 - t1C2)c4
B11 - C11	t1B1c5 - t1C1c5	(t1B1 - t1B2)c5 - (t1C1 - t1C2)c5
B12 - C12	t1B1c6 - t1C1c6	(t1B1 - t1B2)c6 - (t1C1 - t1C2)c6
C7	t1C1c1	

Figure 4-56: Data Cycles Table Showing Reference Well and Double Reference Subtraction Formulas

- Click 

(**Ungroup**) to remove all reference well groupings. You can also right-click the sample plate and select **Remove Reference Subtraction** > **Remove All Reference Subtraction**.
- To ungroup specific wells, select those wells then right click and select **Remove Reference Subtraction** > **Remove Selected Reference Subtraction Only**.
- Click 

(**Reset**) to clear all reference well and group settings for all wells.

4. If there's more than one assay in your experiment or combined dataset, click the next **Assay** tab and repeat the prior steps as needed.

Excluding Sample/Load Wells

- **In the Reference Sample tab -** Select the sample/load well locations in the sample plate. Right-click the plate and select **Exclude Wells From Analysis** (Figure 4-57).

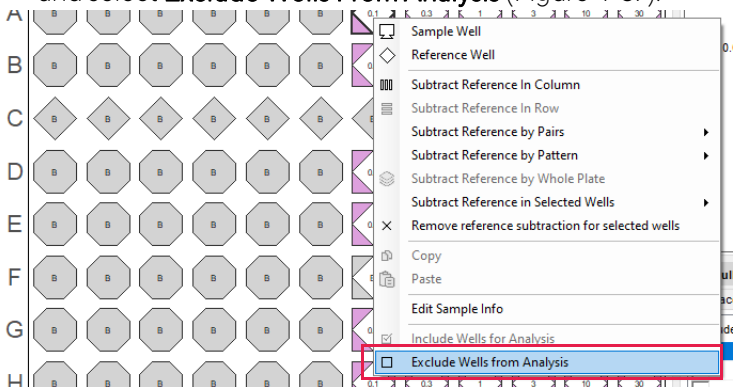
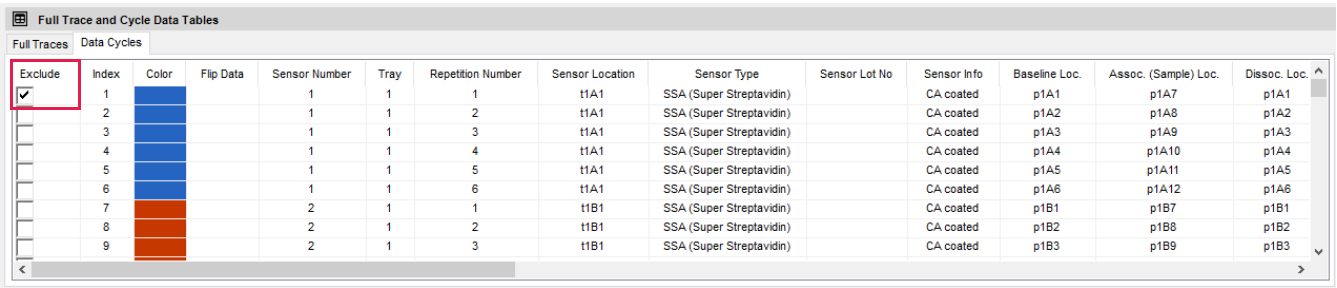


Figure 4-57: Excluding Sample/Load Wells in Sample Plate

- **In the Data Cycles table -** select the check box in the **Exclude** column for the sample/load well row(s) (Figure 4-58).



Exclude	Index	Color	Flip Data	Sensor Number	Tray	Repetition Number	Sensor Location	Sensor Type	Sensor Lot No	Sensor Info	Baseline Loc.	Assoc. (Sample) Loc.	Dissoc. Loc.
☑	1			1	1	1	t1A1	SSA (Super Streptavidin)		CA coated	p1A1	p1A7	p1A1
	2			1	1	2	t1A1	SSA (Super Streptavidin)		CA coated	p1A2	p1A8	p1A2
	3			1	1	3	t1A1	SSA (Super Streptavidin)		CA coated	p1A3	p1A9	p1A3
	4			1	1	4	t1A1	SSA (Super Streptavidin)		CA coated	p1A4	p1A10	p1A4
	5			1	1	5	t1A1	SSA (Super Streptavidin)		CA coated	p1A5	p1A11	p1A5
	6			1	1	6	t1A1	SSA (Super Streptavidin)		CA coated	p1A6	p1A12	p1A6
	7			2	1	1	t1B1	SSA (Super Streptavidin)		CA coated	p1B1	p1B7	p1B1
	8			2	1	2	t1B1	SSA (Super Streptavidin)		CA coated	p1B2	p1B8	p1B2
	9			2	1	3	t1B1	SSA (Super Streptavidin)		CA coated	p1B3	p1B9	p1B3

Figure 4-58: Excluding Sample/Load Wells in Data Cycles Table.

Excluded wells are identified in the sample plate with horizontal lines, a check mark in the Exclude column in the Data Cycles table, and the data no longer displays in the Full Traces and Data Cycles graphs (Figure 4-59).

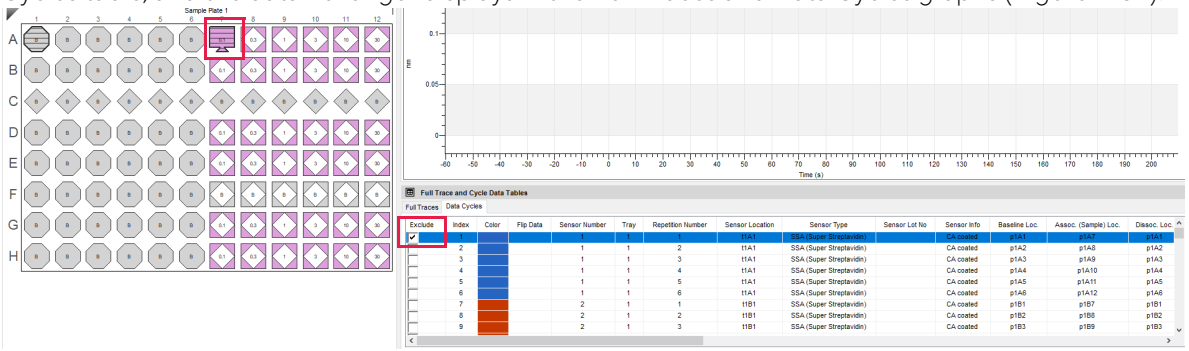


Figure 4-59: Excluded Sample/Load Wells

To re-include the well(s) in the analysis, right-click the sample plate and select **Include Wells For Analysis** or deselect the box in the **Exclude** column for the well row(s) in the Data Cycles table.

Editing Sample Information

You can edit loading sample and association sample information in the Reference Sample tab.

1. Select the well you want to edit in the sample plate.
2. Right-click the well and select **Edit Sample Info** (Figure 4-60).

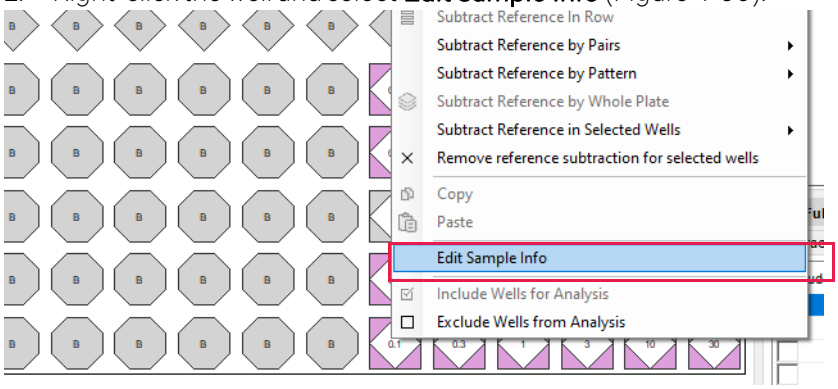


Figure 4-60: Selecting Edit Sample Info

3. Update the information in the Edit Sample Info window, then click **OK**. **Only the fields with a check next to them update (**Figure 4-61)**.**

   You can also double click in the cells in the Loading Sample ID, Loading Concentration, Sample ID and Concentration columns in the Data Cycles table to change this information.

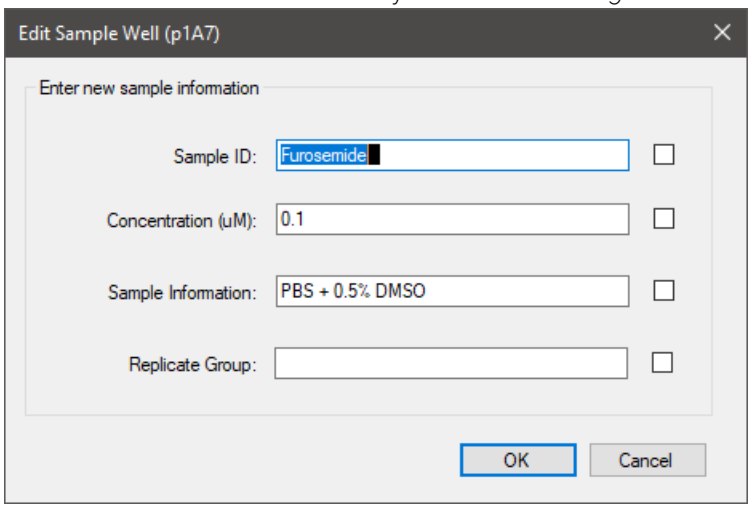


Figure 4-61: Edit Sample Info Window.

Data Correction

The Data Correction tab has options for correcting measurement artifacts before you perform curve fitting.(Figure 4-62).

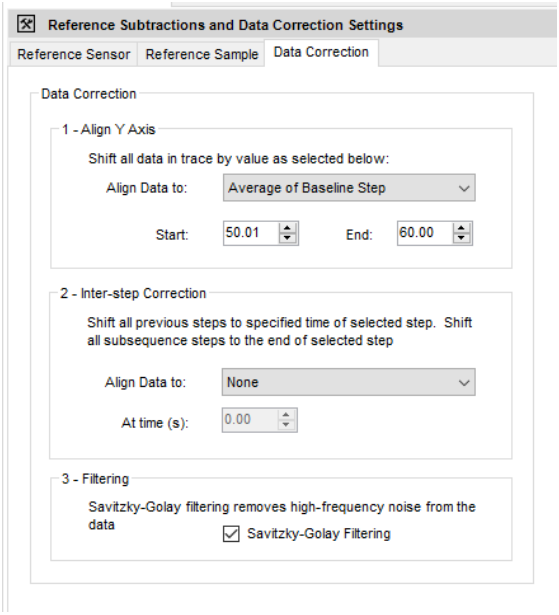


Figure 4-62: Data Correction Tab

Step 1: Aligning the Y Axis

To fit curves correctly, they need to be aligned to a common reference point on both the X and Y axes. Curves are aligned to the X-axis automatically during the assay as biosensors move in parallel. The Align Y Axis function lets you apply a Y-axis adjustment to the baseline in your dataset. Most kinetic fitting models require a zero baseline for fitting, so this step is almost always a preparatory step in kinetic evaluation.

Click the **Align Data to** box to select a step option (Figure 4-63).

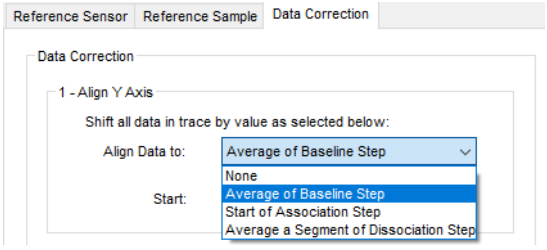


Figure 4-63: Align Data To Selections

Enter a **Start** and **End** time range (in seconds) or use the default times for the section of the curve to be treated as baseline. The software calculates an average from that time frame which then sets to Y=0 (Figure 4-64).

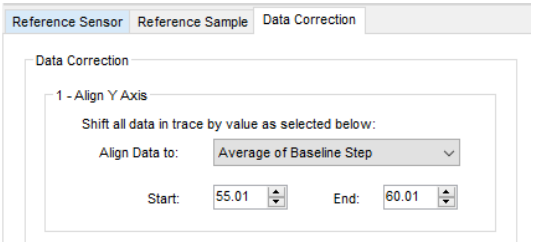


Figure 4-64: Setting Start and End Time Ranges

- **Average of Baseline Step** - Use this option to adjust the baseline step so the y-axis shows zero, prior to fitting the kinetic data. Use it to align kinetic data where the time interval chosen are the last 5-10 seconds of the baseline step to align the Y-axis. An example of data aligned to the average of the baseline step is shown in Figure 4-65.

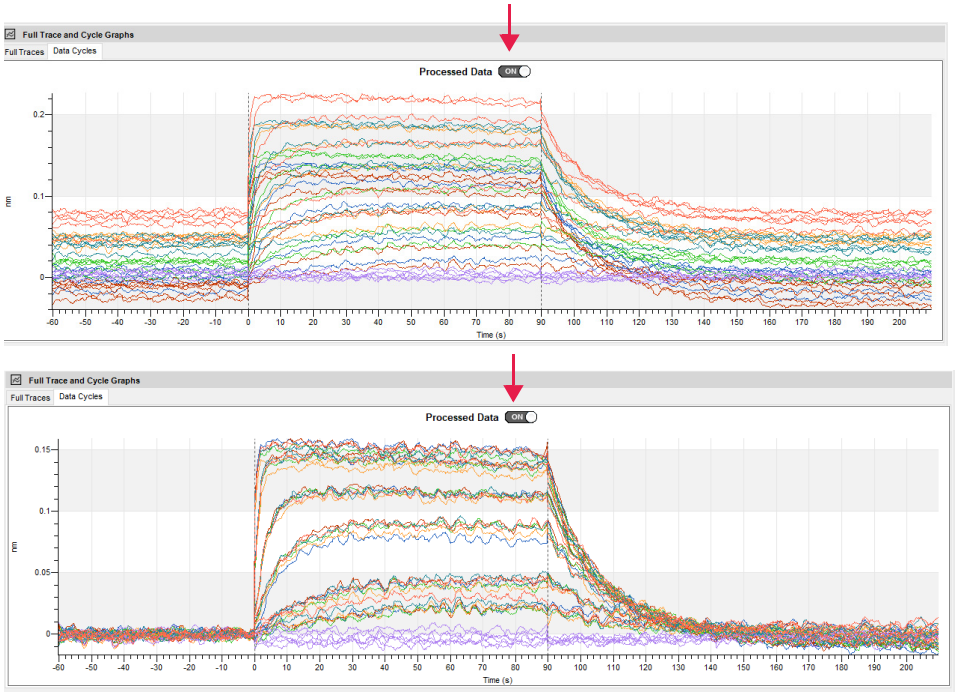


Figure 4-65: Y-axis aligned to Average of Baseline Step Uncorrected (Top) and Corrected Data (Bottom)

- **Start of Association Step** - This option is only used if there is a jump between the baseline and the association step due to a buffer mismatch or artifacts and the y-axis needs to be aligned to zero prior to fitting the data. An example of data aligned to the start of the association step is shown in Figure 4-66.

NOTICE: *The time window should be minimized at the beginning of the association. The data within this window is set to an average of zero and is included in the final curve fit.*

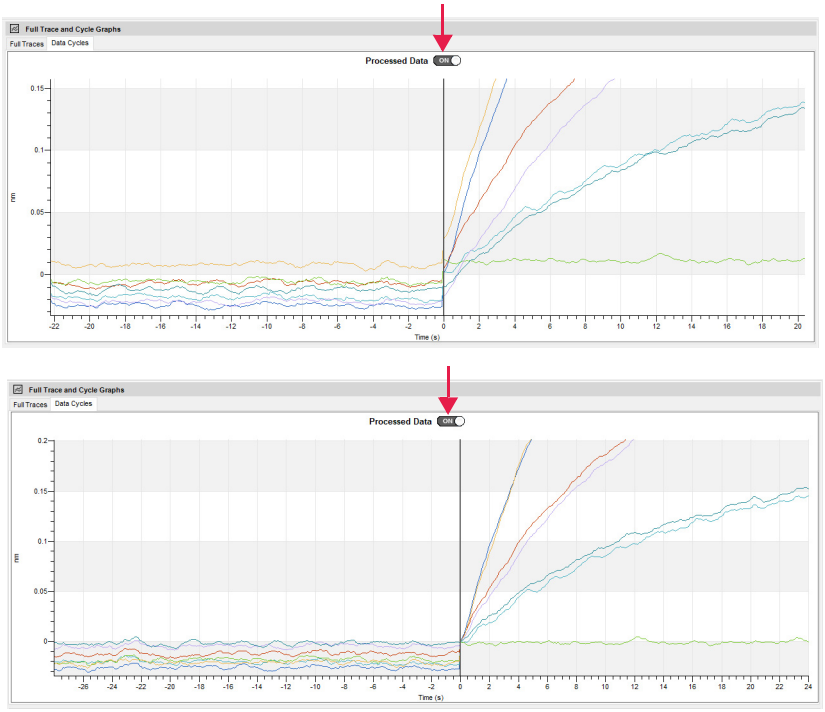


Figure 4-66: Y-axis aligned to Start of Association Step Uncorrected (Top) and Corrected Data (Bottom)

- **Average a Segment of Dissociation Step** - This option is used to adjust the end of the dissociation step to Y=0 to demonstrate complete dissociation. An example of data aligned to the average of a segment of dissociation step is shown in Figure 4-67.

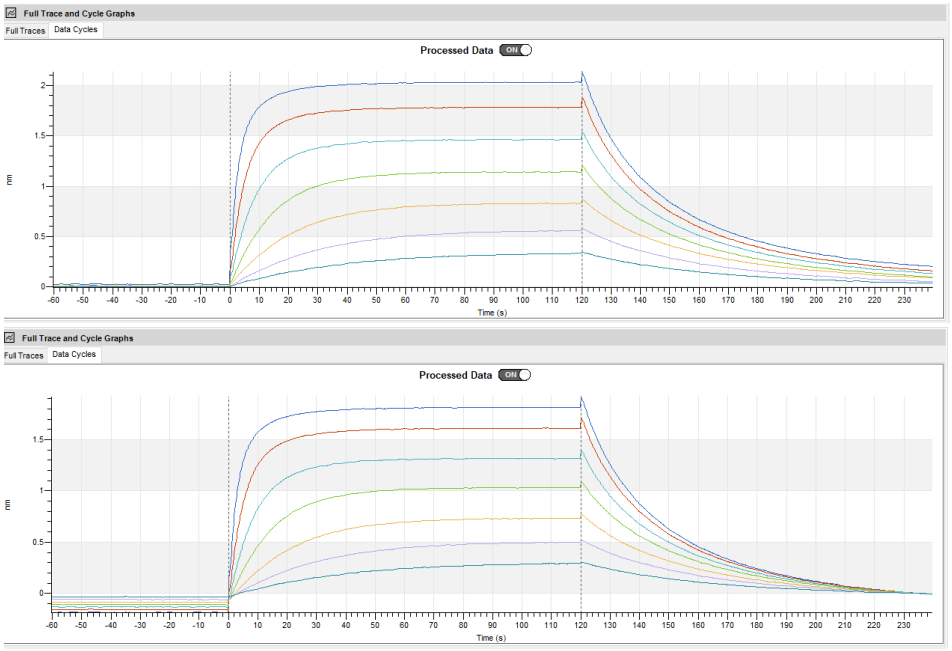


Figure 4-67: Y-axis aligned to Average of Segment of Dissociation Step Uncorrected (Top) and Corrected Data (Bottom)

If there is more than one assay in your experiment or combined dataset, click the next **Assay** tab and apply Y axis alignment as needed.

Step 2: Inter-step Correction

This option lets you correct misalignment between steps that can occur when biosensors move between the wells of the plate.

NOTICES:

Inter-step correction should only be used for experiments where the baseline and dissociation steps were done in the same well.

We don't recommend using the inter-step correction with very fast kinetics as some kinetic information can be lost.

Click the **Align Data to** box to select a step option (Figure 4-68).

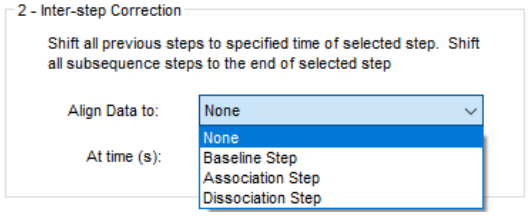


Figure 4-68: Align Data To Selections

Enter the time (in seconds) to apply the inter-step correction (Figure 4-69).

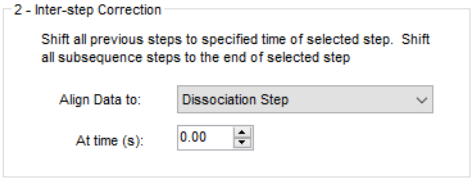


Figure 4-69: Setting Time for Inter-Step Correction

- **Dissociation Step** - Aligns the end of the association step on the Y-axis with the beginning of the adjacent dissociation step and the end of the baseline step to the beginning of the adjacent association step. If the default time used in the inter-step correction does not fix the bulk shifts or signal jumps between the association and dissociation steps, then modify the timing to remove these artifacts before fitting.

An example is shown in Figure 4-70.

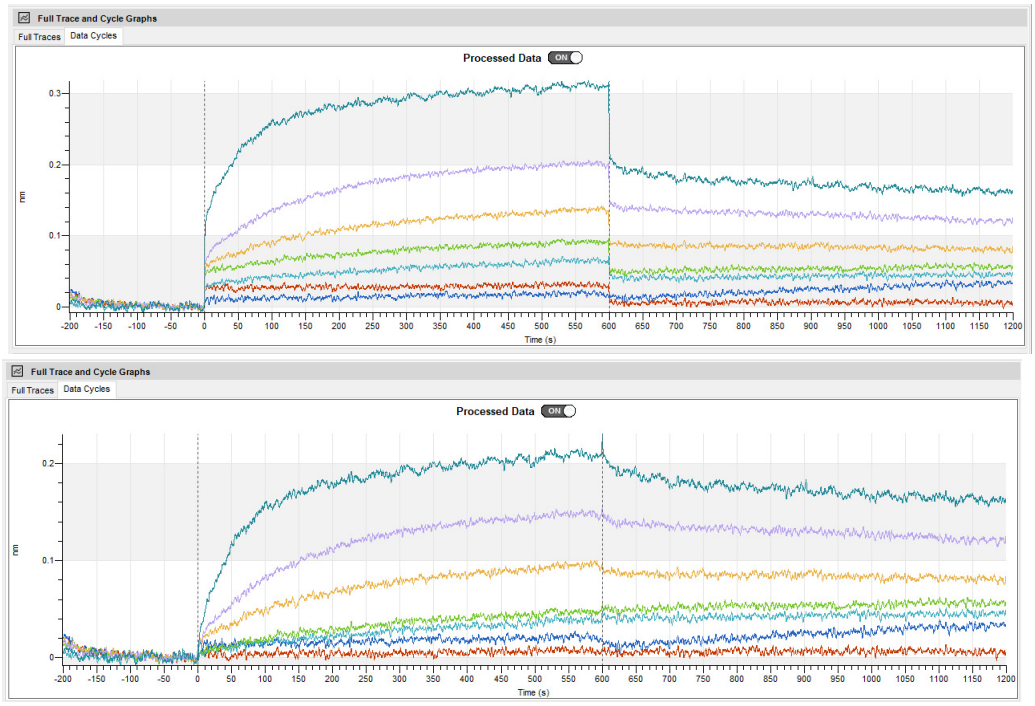


Figure 4-70: Inter-step Correction aligned to Dissociation Step Uncorrected (Top) and Corrected Data (Bottom)

- **Baseline Step** - Aligns the beginning of the association step on the Y axis with the end of the adjacent baseline step. An example is shown in Figure 4-71.

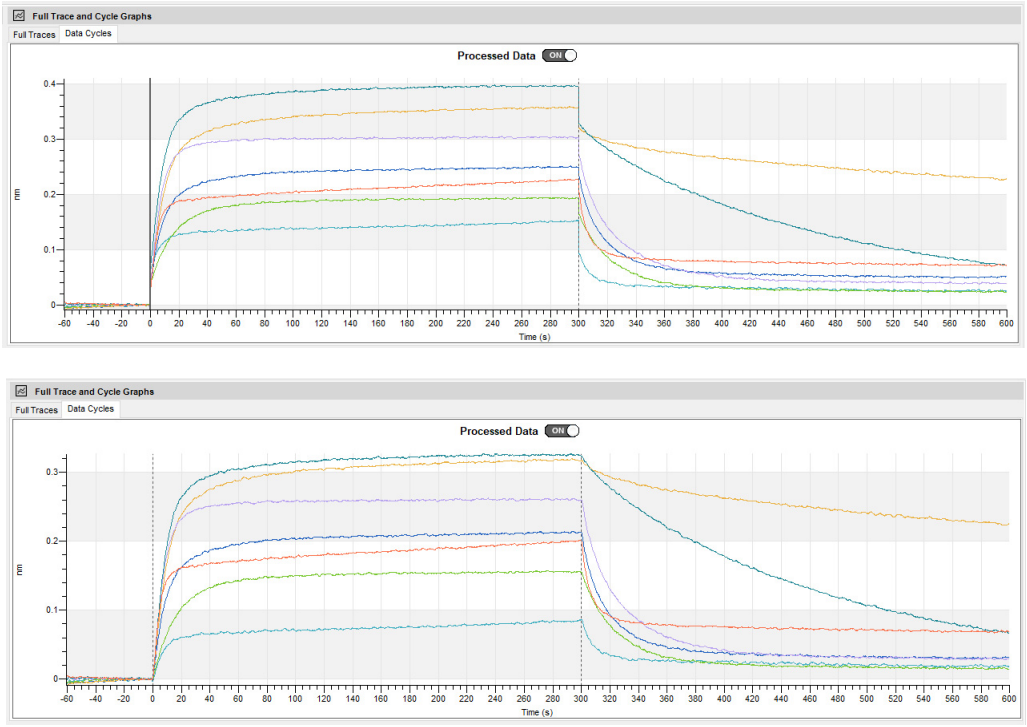


Figure 4-71: Inter-step Correction aligned to Baseline Step Uncorrected (Top) and Corrected Data (Bottom)

- **Association Step** - Aligns the end of the baseline step on the Y axis with the beginning of the adjacent association step. An example is shown in Figure 4-72.

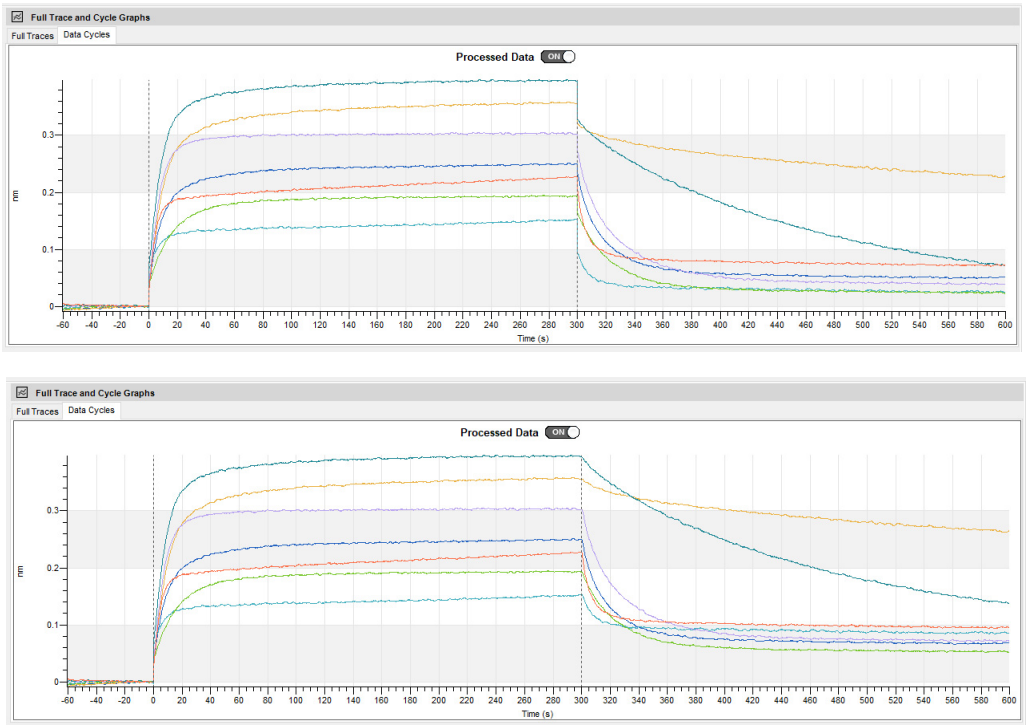


Figure 4-72: Inter-step Correction aligned to Association Step Uncorrected (Top) and Corrected Data (Bottom)

Step 3: Noise Filtering

Savitzky-Golay filtering removes high-frequency noise from the data. We recommend using this filtering unless the data you're analyzing has less than 20 data points in a step (Figure 4-73).

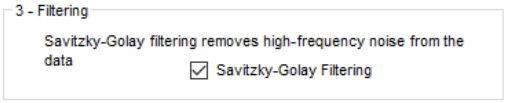


Figure 4-73: Data Filtering

Filtering is selected (on) by default. To turn it off, click the **Savitzky-Golay Filtering** check box. Examples of filtered and unfiltered data are shown in Figure 4-74.

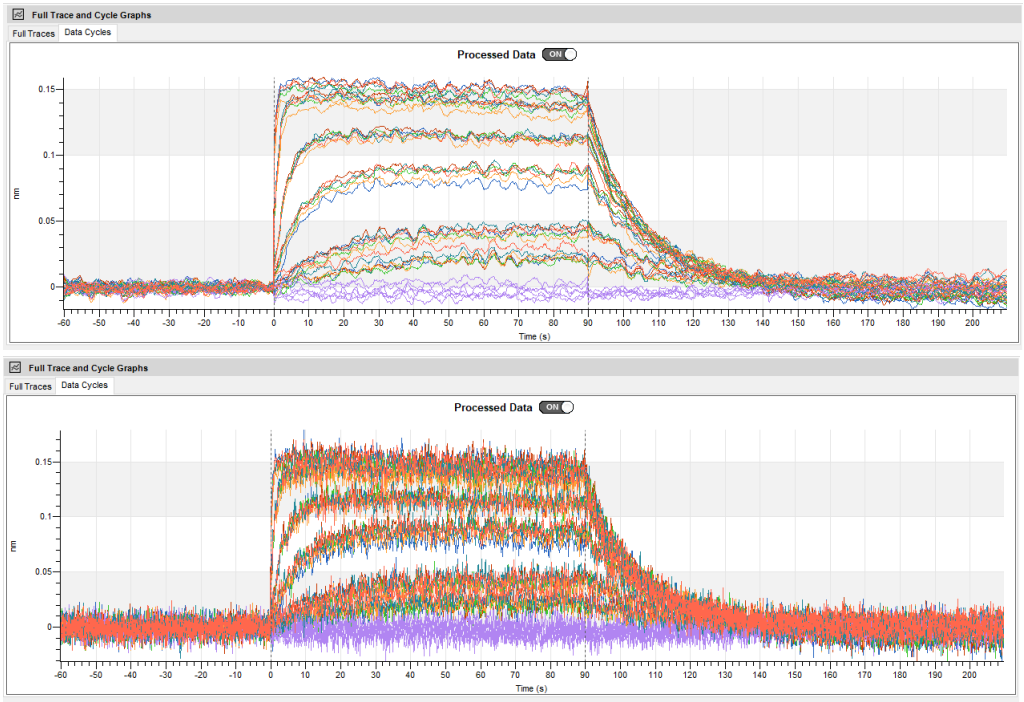


Figure 4-74: Savitzky-Golay Filtering On (Top) and Off (Bottom)

Quantitating Steps in a Kinetic Dataset

1. Click the **Preprocess Data** tab.
2. Select the step in the Full Traces graph that you want to perform quantitation analysis on (Figure 4-75).

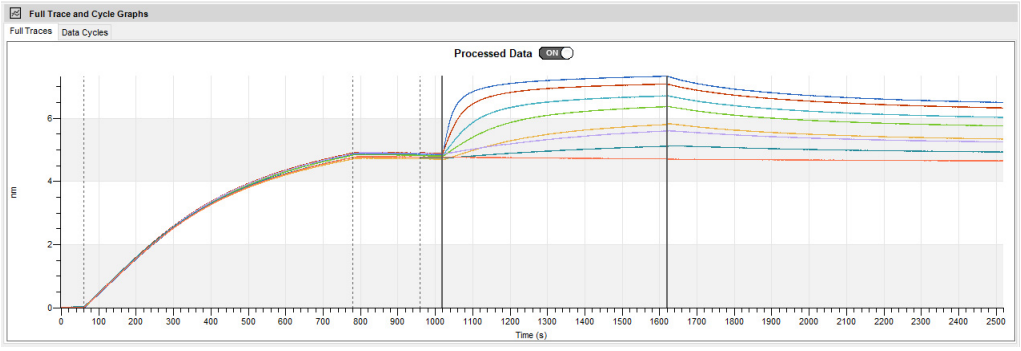


Figure 4-75: Selecting a Kinetic Step for Quantitation

3. Click the **arrow** next to **K to Q** in the Operations section of the icon bar.

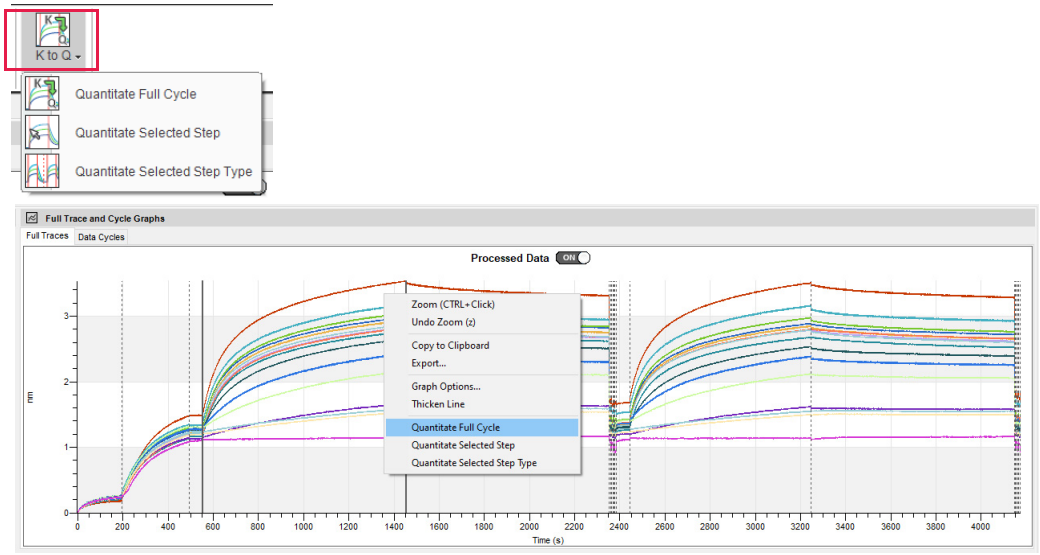


Figure 4-76: K to Q Menu

4. Select an option from the drop down menu to change to quantitation mode.
   - If only one step in the assay is being quantitated as shown in Figure 4-75, select **Quantitate Selected Step**.
   - In kinetic datasets where regeneration was performed, often the same step in each regeneration cycle needs to be moved to quantitation mode as shown in Figure 4-77. In this case, select **Quantitate Selected Step Type**
   - Choose **Quantitate Full Cycle** to convert all steps in the Kinetic cycle to a Quantitation cycle. This option is useful if you need to see a loading step in addition to the quantitation step.

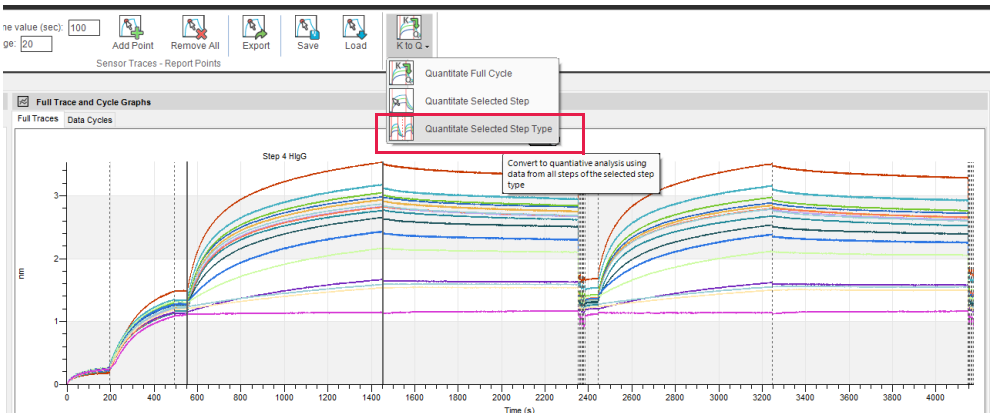


Figure 4-77: Kinetic Dataset with Regeneration, Select Quantitate Step Type

5. The software moves the raw or unprocessed data into quantitation mode in the Preprocess Data screen (Figure 4-78). You should now perform any reference subtraction or data correction before you move to the Quantitation Analysis screen. For details on preprocessing quantitation datasets, see "Preprocessing Quantitation Datasets" on page 189.

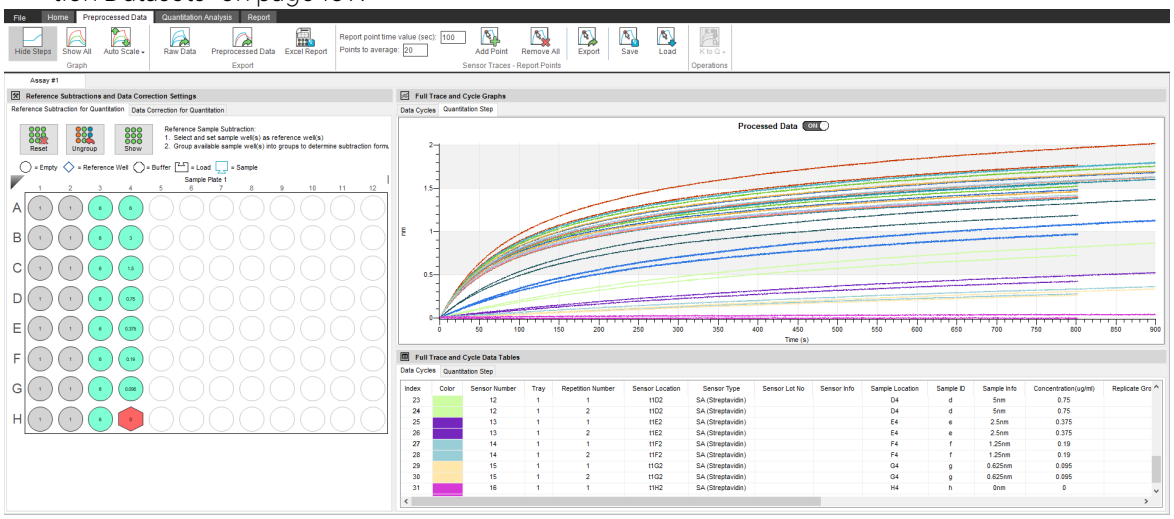


Figure 4-78: Kinetic Step Moved into Quantitation Mode in the Preprocess Data Screen

6. Click the **Quantitation Analysis** tab. You can now perform routine quantitation analysis on the kinetic step you selected. For full details, see "Quantitation Analysis" on page 217.

7. To return to kinetic mode, go back to the Home screen and click the Kinetic Analysis button.

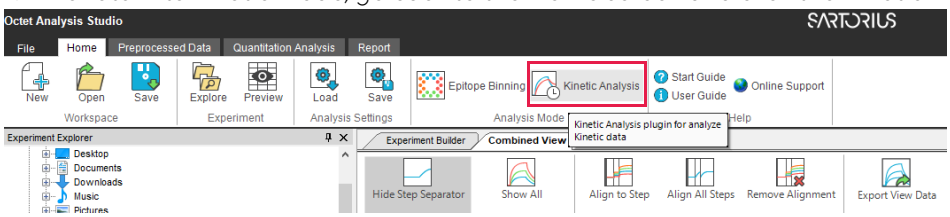


Figure 4-79: Selecting Kinetic Analysis

Report Points

Adding Report Points

Some applications, like screening, rely on single-point measurements called report points that can be placed at the end of loading or association steps, or at the early or late phase of the dissociation step. Report points can be added to the entire sensor trace in the Preprocess Data Screen or just to the kinetic cycles (baseline, association and dissociation steps) in the Kinetics Screen.

1. In the Full Traces - Report Points menu at the top of the Preprocess Data screen, enter a time (in seconds) in the **Report point time value (sec)** box (Figure 4-80).

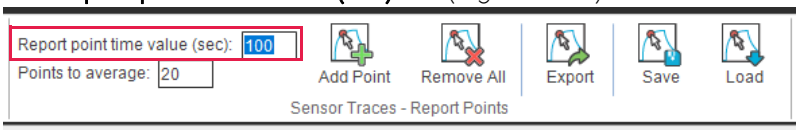


Figure 4-80: Entering a Report Point Time Value

2. Enter a value in the **Points to average box** (Figure 4-81). This takes an average of the data points centered around the report point time value. For example, if the report point value you entered was 100 seconds and the points to average is 20, the software will average the combined 10 data points just before the 100 second mark and 10 data points at and just after the 100 second mark. If the report point time is set to the beginning or end of the assay step, the software will average the first or last 20 data points of the assay step.

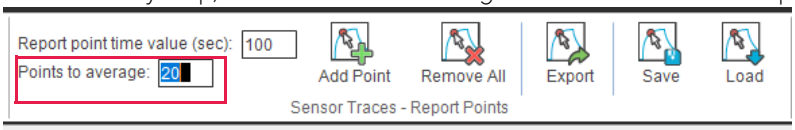
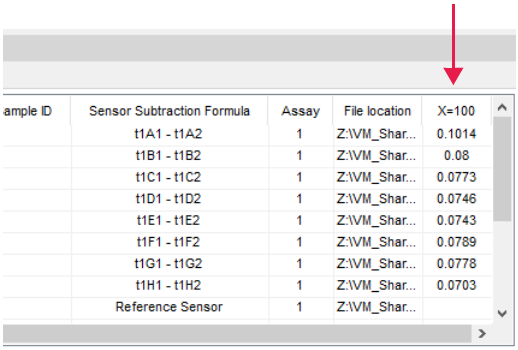


Figure 4-81: Entering the Points to Average

3. Click  (**Add Point**). A new column is added to the Full Traces table. Its header becomes X=time entered in the report point time value (sec) box. For example if you entered 100 seconds, the new column becomes X=100 with the signal (nm shift) of all traces at the X=100 time point for each ligand biosensor in the experiment (Figure 4-82).



ample ID	Sensor Subtraction Formula	Assay	File location	X=100
	t1A1 - t1A2	1	Z:\VM_Shar...	0.1014
	t1B1 - t1B2	1	Z:\VM_Shar...	0.08
	t1C1 - t1C2	1	Z:\VM_Shar...	0.0773
	t1D1 - t1D2	1	Z:\VM_Shar...	0.0746
	t1E1 - t1E2	1	Z:\VM_Shar...	0.0743
	t1F1 - t1F2	1	Z:\VM_Shar...	0.0789
	t1G1 - t1G2	1	Z:\VM_Shar...	0.0778
	t1H1 - t1H2	1	Z:\VM_Shar...	0.0703
	Reference Sensor	1	Z:\VM_Shar...	

Figure 4-82: Report Point Column in Full Traces Table

- Click  (**Remove All**) to remove all report points.

- Click (**Save**) to save the report points as a .csv file.
- Click (**Load**) to load a previously saved report file and automatically add those report points to your current experiment.

Exporting Report Points

Click (**Export**) to export the report points for your current experiment. The Full Trace Report Points Export window displays:

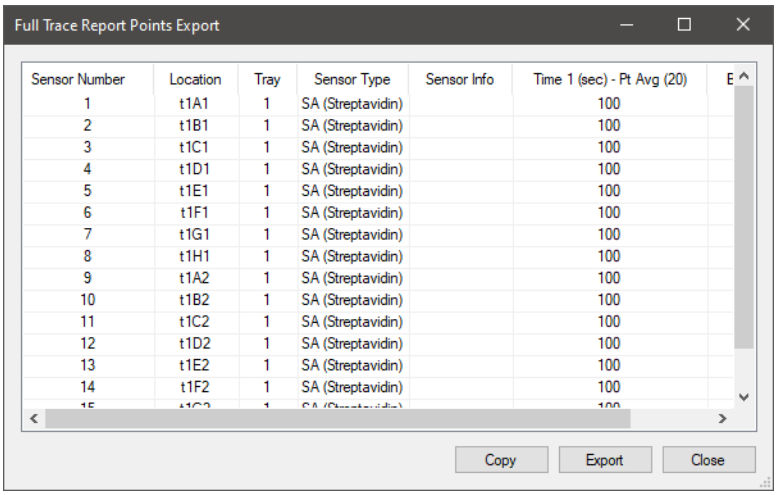


Figure 4-83: Report Points Export

- Select **Copy** to copy and paste all the report point data for the current experiment into another program like Microsoft® Word® or Excel®.
- Select **Export** to export and save all the report point data for the current experiment as a .csv file.

Changing the Graph View

To switch between viewing corrected and uncorrected data, click the **Processed Data toggle** at the top of the graph (Figure 4-84).

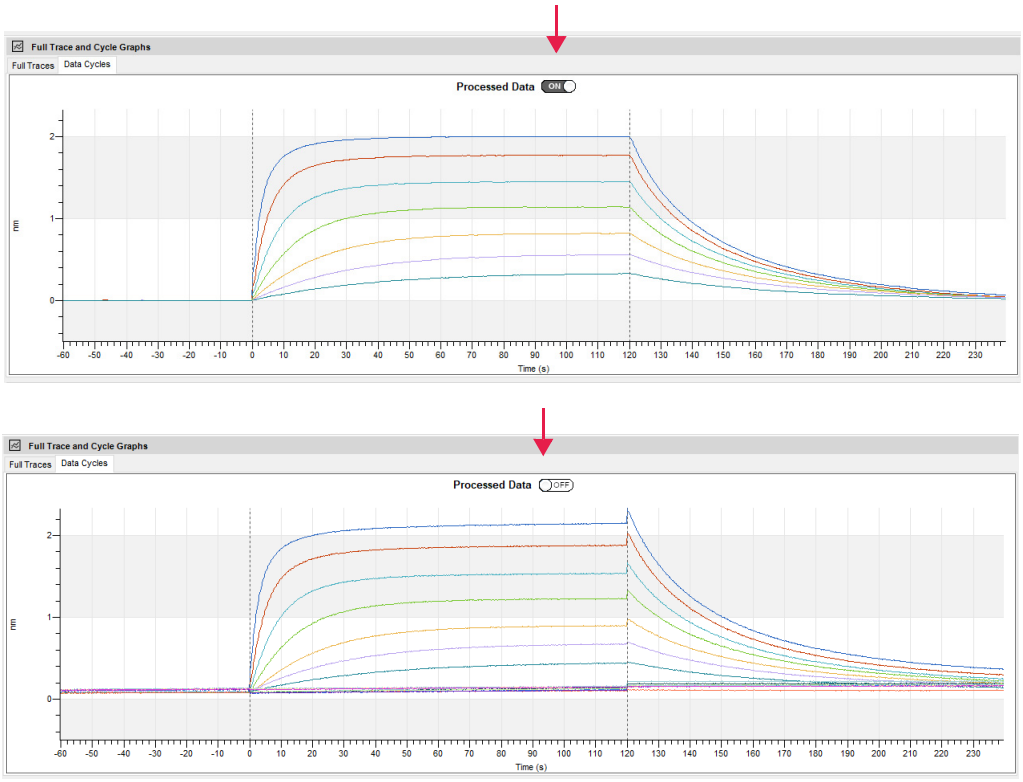


Figure 4-84: Processed Data ON (Top) and OFF (Bottom)

Export Options

To export the graphs, right-click the graph and select **Export**. The Export window displays (Figure 4-85).

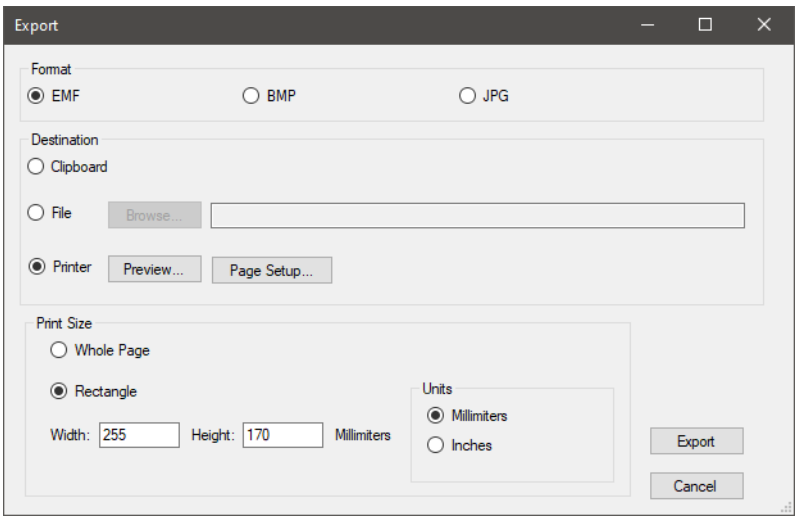


Figure 4-85: Export Window

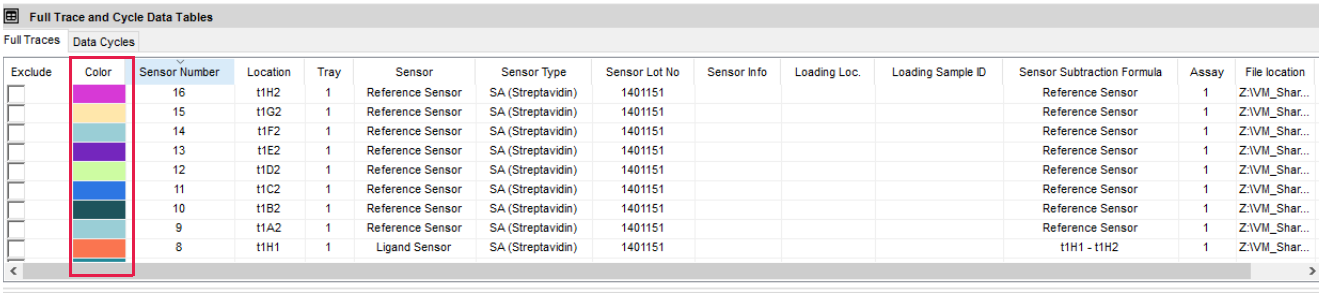
Chose a format (EMF, BMP or JPG), an export destination (Clipboard, save to File, or Printer), set print size parameters if needed then click **Export**.

Changing the Table View

You can sort data in the Full Traces and Data Cycles tables by clicking the column header (Figure 4-86). When a column header is blue, it indicates the table data is currently sorted by that parameter (Figure 4-86).

- Clicking a header for a column that contains numbers or letters sort the data alpha-numerically lowest to highest or A-Z. Clicking the header again resorts the data highest to lowest or Z-A.
- Clicking **Exclude** sorts the data so excluded biosensors and cycles are listed first, clicking **Exclude** again resorts the data to show included biosensors and cycles first.
- Clicking **Tray** sorts the data by tray first to last starting at 1, clicking **Tray** again resorts the data by trays last to first.
- Clicking **Sensor Number** sorts the data by sensor number in sensor trays 1-4 starting with A1 and sensor tray 1. Clicking on **Sensor Number** again sorts the data by sensor number in sensor trays 4-1, starting with A1 and sensor tray 4.

- Clicking **Color** groups all data with the same color together.

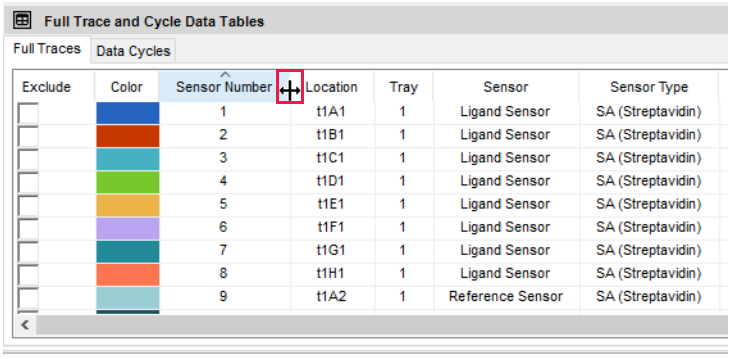


Exclude	Color	Sensor Number	Location	Tray	Sensor	Sensor Type	Sensor Lot No	Sensor Info	Loading Loc.	Loading Sample ID	Sensor Subtraction Formula	Assay	File location
		16	t1H2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		15	t1G2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		14	t1F2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		13	t1E2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		12	t1D2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		11	t1C2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		10	t1B2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		9	t1A2	1	Reference Sensor	SA (Streptavidin)	1401151				Reference Sensor	1	Z:\VM_Shar...
		8	t1H1	1	Ligand Sensor	SA (Streptavidin)	1401151				t1H1 - t1H2	1	Z:\VM_Shar...

Figure 4-86: Sorting Data Table Columns

- Clicking **Assay** sorts the data by assay number in the run, starting with the first assay. Clicking **Assay** again resorts the data to so the last assay run is listed first.

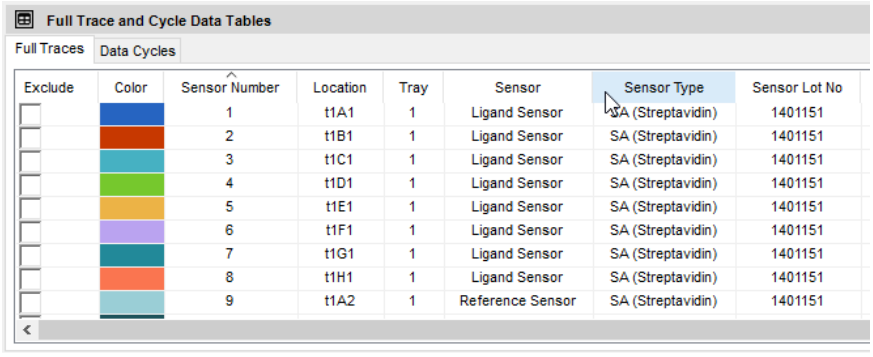
To resize the table columns, roll the cursor over the border between the column headers until the resizing cursor displays until the resize cursor appears (Figure 4-87).



Exclude	Color	Sensor Number	Location	Tray	Sensor	Sensor Type
		1	t1A1	1	Ligand Sensor	SA (Streptavidin)
		2	t1B1	1	Ligand Sensor	SA (Streptavidin)
		3	t1C1	1	Ligand Sensor	SA (Streptavidin)
		4	t1D1	1	Ligand Sensor	SA (Streptavidin)
		5	t1E1	1	Ligand Sensor	SA (Streptavidin)
		6	t1F1	1	Ligand Sensor	SA (Streptavidin)
		7	t1G1	1	Ligand Sensor	SA (Streptavidin)
		8	t1H1	1	Ligand Sensor	SA (Streptavidin)
		9	t1A2	1	Reference Sensor	SA (Streptavidin)

Figure 4-87: Column Resizing Cursor

Then click and drag the column to resize (Figure 4-88).



Exclude	Color	Sensor Number	Location	Tray	Sensor	Sensor Type	Sensor Lot No
		1	t1A1	1	Ligand Sensor	SA (Streptavidin)	1401151
		2	t1B1	1	Ligand Sensor	SA (Streptavidin)	1401151
		3	t1C1	1	Ligand Sensor	SA (Streptavidin)	1401151
		4	t1D1	1	Ligand Sensor	SA (Streptavidin)	1401151
		5	t1E1	1	Ligand Sensor	SA (Streptavidin)	1401151
		6	t1F1	1	Ligand Sensor	SA (Streptavidin)	1401151
		7	t1G1	1	Ligand Sensor	SA (Streptavidin)	1401151
		8	t1H1	1	Ligand Sensor	SA (Streptavidin)	1401151
		9	t1A2	1	Reference Sensor	SA (Streptavidin)	1401151

Figure 4-88: Resized Column

Exporting Datasets

Use the Export options menu at the top of the Preprocess Data screen to export your datasets (Figure 4-89).

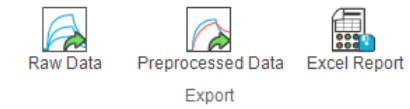


Figure 4-89: Export Options.

- Click (Raw Data) to export the raw, uncorrected data for your experiment or combined data set in .csv format.
- Click (Processed Data) to export the processed (corrected) data for your experiment or combined data set in .csv format.
- Click (Excel Report) to export the processed (corrected) data for your experiment or combined data set in .xlsx format. Select which experimental components to export, use the default report name or click ... to specify another name/location, then click Export (Figure 4-90).

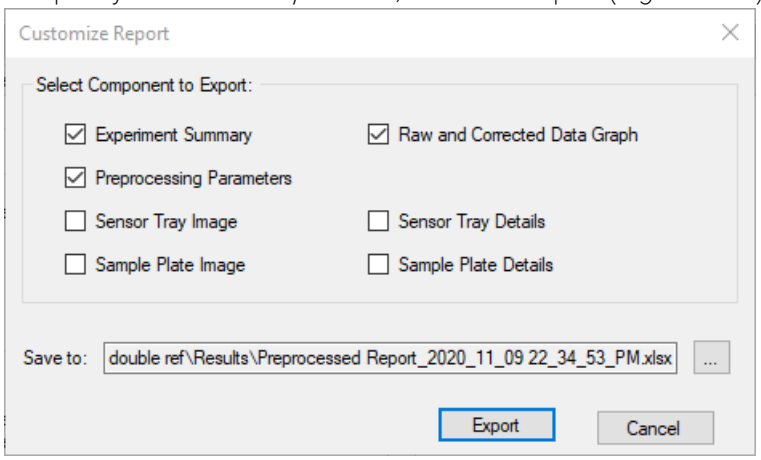


Figure 4-90: Excel Report Export Options

Chapter 5:

Kinetic Analysis

Kinetic Analysis

Binding kinetic sensorgrams can give you a lot of information on the interaction between molecules. They can answer simple questions on the presence or absence of a binding interaction, or let you do complex analyses to fully characterize the interaction and extract binding rates (k_a, k_d) and equilibrium binding constants.

Kinetic data are interpreted based on a mathematical model of the interaction, from which kinetic and equilibrium binding constants can be calculated based on rates of association and dissociation. You can perform this type of analysis:

- **Locally -** where kinetic parameters are determined based on a single analyte concentration or
- **Globally -** where constants are derived simultaneously from all analyte concentrations available

The equilibrium dissociation constant (K_D) can also be determined using data at equilibrium from each available analyte concentration using steady state analysis.

Kinetic Analysis Screen Overview

The Kinetic Analysis Screen lets you determine the association and dissociation rates of a molecular interaction. Once you've made your reference sensor, reference sample well and data corrections in the Preprocess Data screen, click the **Kinetic Analysis** tab to start your analysis. For information on how to pre-process your data before proceeding to quantitation, please refer to "Preprocessing Kinetic Datasets" on page 39. The software automatically analyzes the data using default settings, but you'll be able to modify analysis parameters as needed (Figure 5-1).

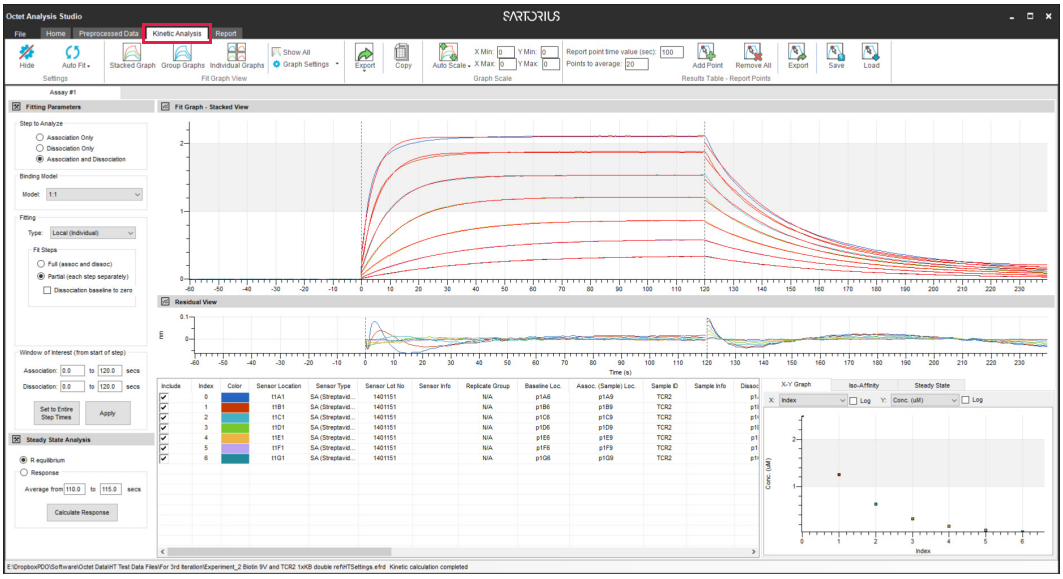


Figure 5-1: Kinetic Analysis Screen

Auto-fitting of kinetics data can be paused until all the settings and included traces have been identified. This may be useful when handling large datasets where multiple experiments have been overlaid or appended as auto-fitting triggers after every setting change, increasing the analysis time. Auto Fit can be toggled off by clicking **Suspend Fit** (Figure 5-2).

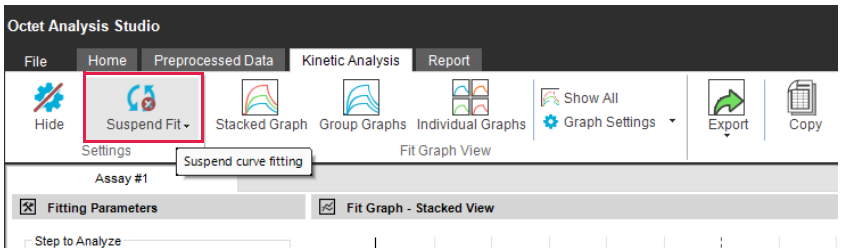


Figure 5-2: Pausing Auto Fit

To view or analyze data for different assays in the experiment, click the **Assay** tab above the Fitting Parameters window (Figure 5-3). Click the **All Assays** tab to view the summary data for the combined dataset - the graphs and kinetic data table.

NOTICE: *Analysis settings cannot be modified in the All Assays tab.*

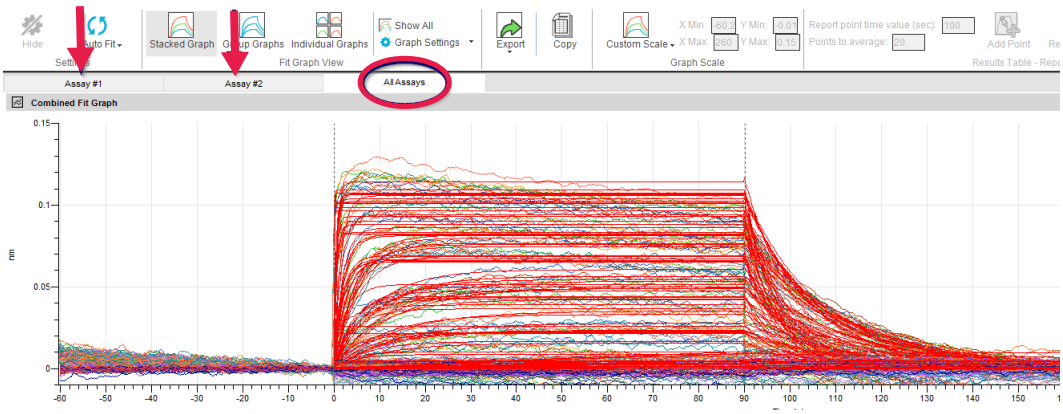


Figure 5-3: Assay Tabs

Viewing Settings

You can view kinetic analysis settings for your experiment in the Fitting Parameters and Steady State Analysis windows (Figure 5-4). Step-by-step details for performing kinetic analysis on your data can be found in "Analyzing Your Kinetic Data" on page 101.

- Click (**Settings**) to hide the panel.

- Click (**Settings**) to show the panel again.

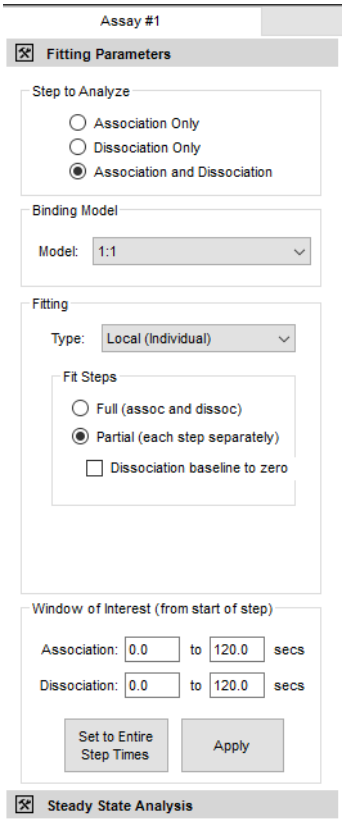


Figure 5-4: Kinetic Analysis Settings Panel

NOTICE: *See "Resizing, Hiding and Closing Windows" on page 11. for information about working with the software windows.*

Changing the Graph View

NOTICE: *See "Resizing, Hiding and Closing Windows" on page 11. for information about displaying graphs and the display options.*

To change the graph display, right-click the graph and select **Graph Options**. The Graph Options window displays (Figure 5-5).

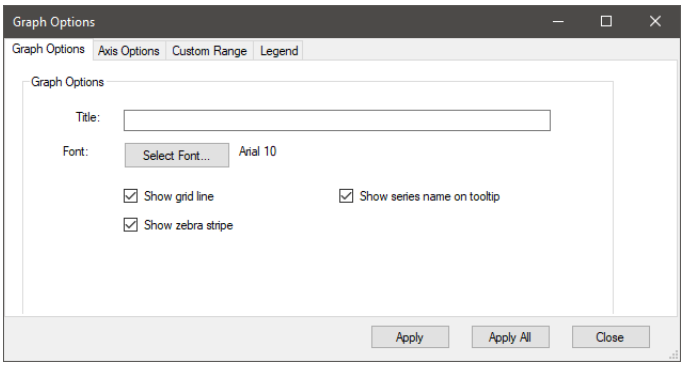


Figure 5-5: Graph Options Window

After fitting is performed, select **Options** in the Fit Graph View section of the icon bar. In the Legend Option box, select **Show Distinct Colors** (Figure 5-6)

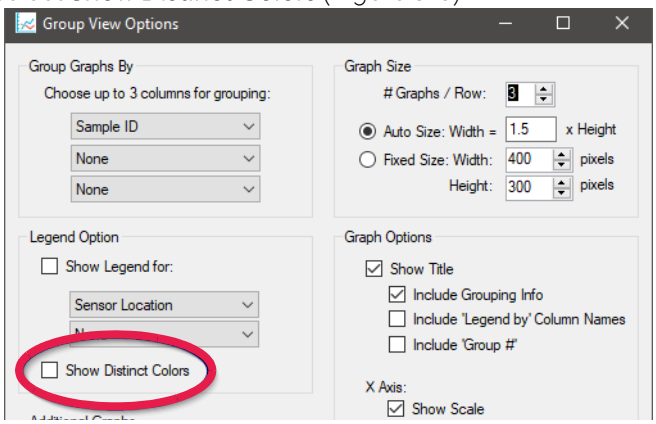


Figure 5-6: Group View Options

Viewing Fit Graph and Residual Data

The Fit Graph window displays the corrected, processed binding data and the fitted binding curve (red) for all analyzed biosensors. The Residual View displays the difference between the raw binding data and the fitted curve for analyzed biosensors (Figure 5-7).

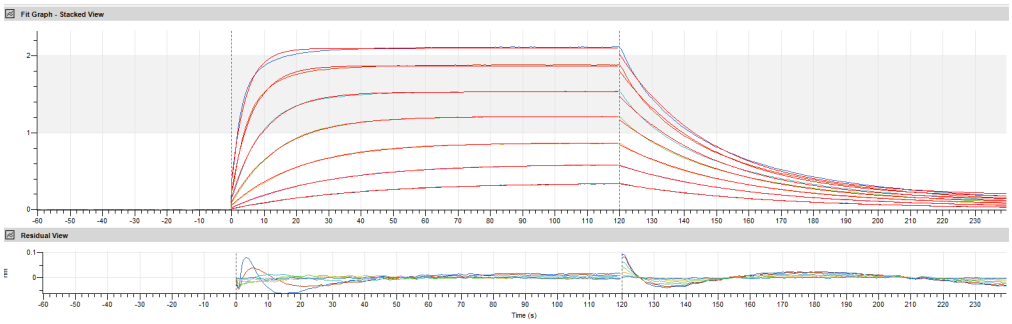


Figure 5-7: Fit Graph and Residual View

To view individual or overlay multiple graphs, click the Stacked option in the Fit Graph View section (Figure 5-8).

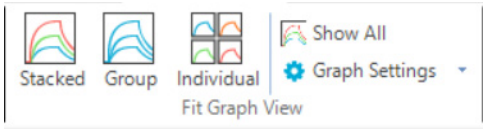


Figure 5-8: Selecting the Stacked Fit Graph View

- To view one graph, just click its row in the Analysis Results table (Figure 5-9).

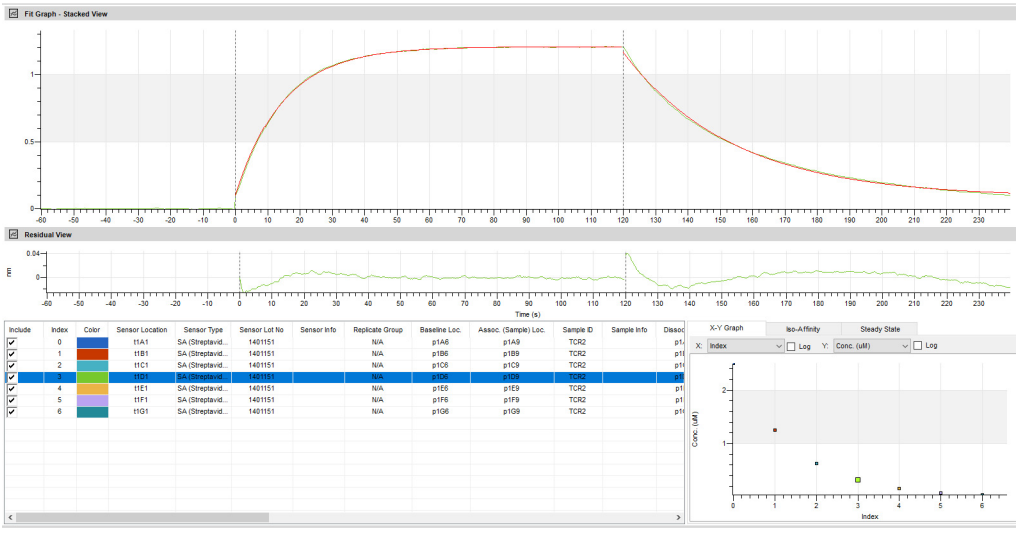


Figure 5-9: Viewing Individual Graphs

- To overlay multiple graphs, press and hold **Ctrl**, then select the rows in the table you want to overlay (Figure 5-10).

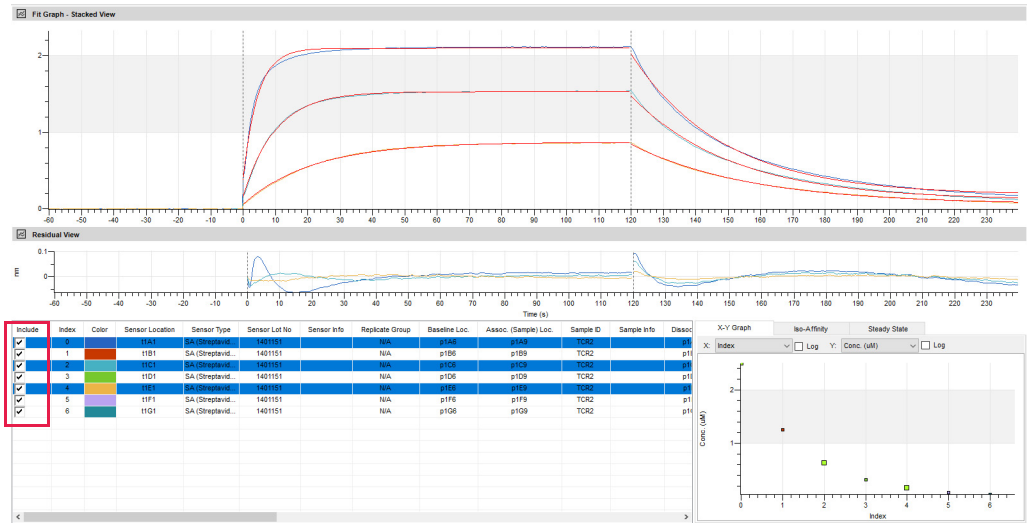


Figure 5-10: Overlaying Multiple Graphs

Click  (**Show All**) to view all graphs in the dataset again.

To view data in individual graph mode select the Individual icon in the Fit Graph View section (Figure 5-11).

Figure 5-11: Selecting the Individual Fit Graph View

All graphs in the experiment display (Figure 5-12):

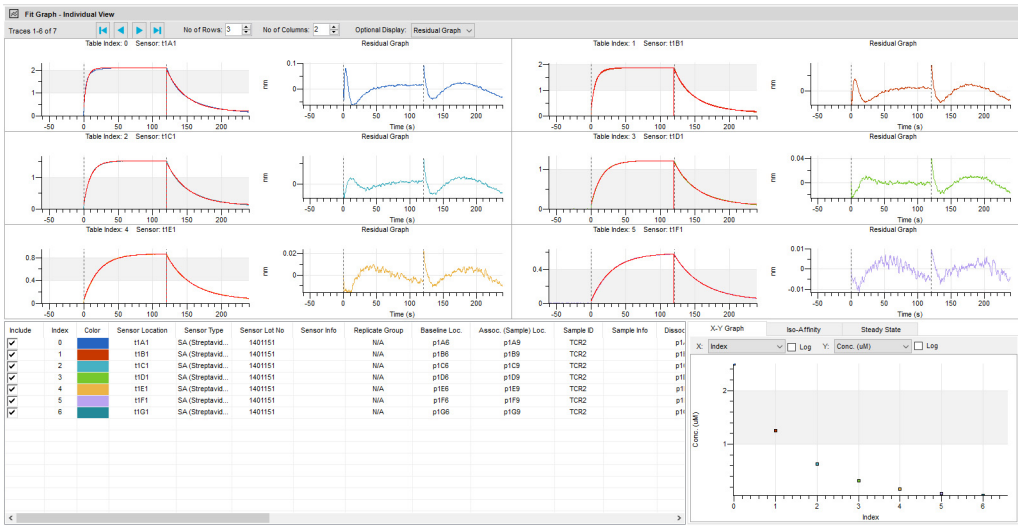


Figure 5-12: Fit Graph Individual View

The tools at the top of the window let you change the display (Figure 5-13):

Figure 5-13: Fit Graph Individual View Toolbar

- Use the **arrow buttons** to page through the sensorgrams.
- To change how many graphs display in the window, adjust the **No of Rows** or **No of Columns**.
- Change what displays by clicking the **Optional Display** dropdown menu (Figure 5-14):
  - **None** - displays the individual data graphs
  - **Residual Graph** - displays the individual data and residual graphs
  - **Fit Results** - displays the individual data graphs and calculated kinetic values

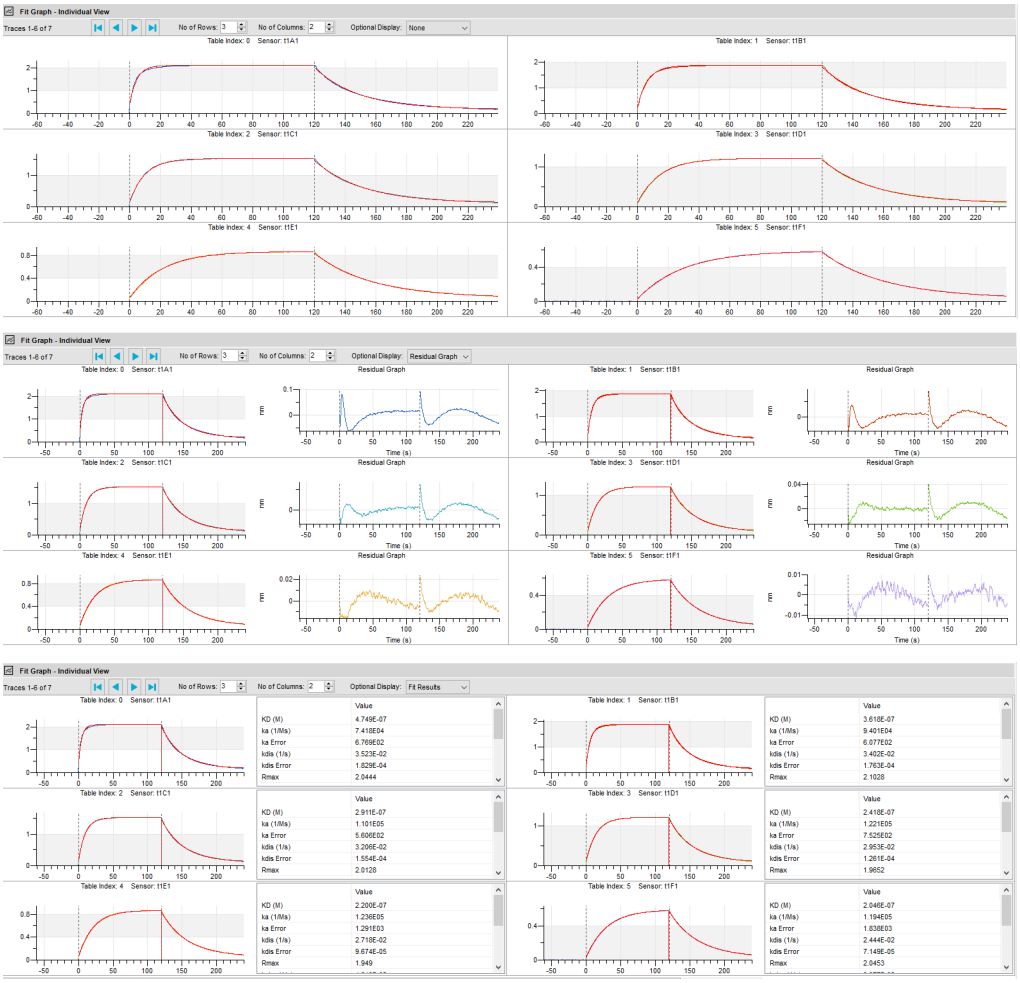


Figure 5-14: Individual View Options: None (Top), Residual Graph (Middle), Fit Results (Bottom)

To view graphs grouped by specific options, click **Graph Settings** in the Fit Graph View section (Figure 5-15).

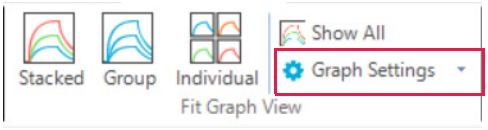


Figure 5-15: Selecting the Group View

Open the **Graph Setting** menu and select **Group Graph options**.

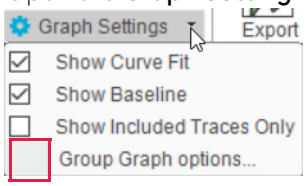


Figure 5-16: Graph Settings - Group Graph options

Set your grouping options in the **Group View Options** screen (Figure 5-17).

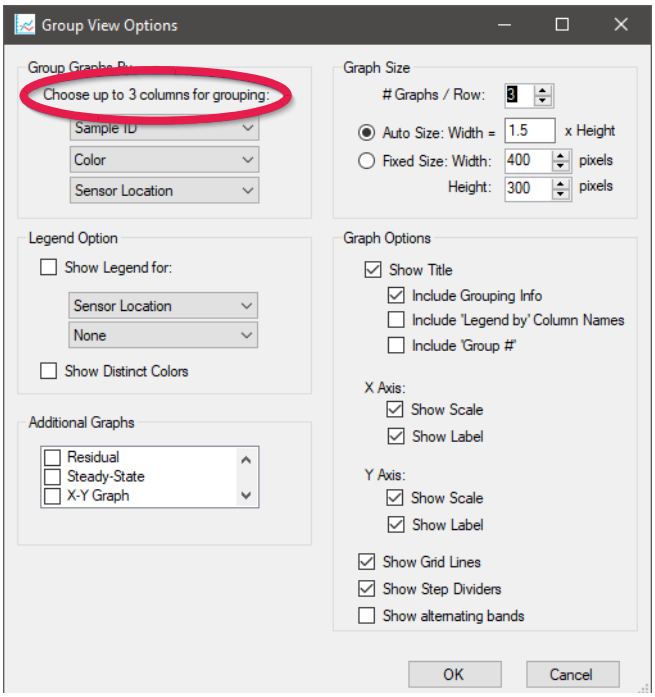


Figure 5-17: Group View Options

- **Group Graphs By** - Lets you select up to three categories for grouping the data across three independent parameters.
- **Legend Option** - Check the **Show Legend for:** box then select up to two categories to include in the graph legends.
- **Data Options** - Check the **Show Included Traces Only** option to remove the excluded traces from the graph display.
- **Additional Graphs** - Lets you select other graphs to display with the analyzed (fitted) data.
- **Graph Size** - Options for the number of graphs to display per row and the graph size.
- **Graph Options** - Lets you choose options for graph labels and other graph display features.

After you've made your selections, click **OK**. Figure 5-18 is an example.

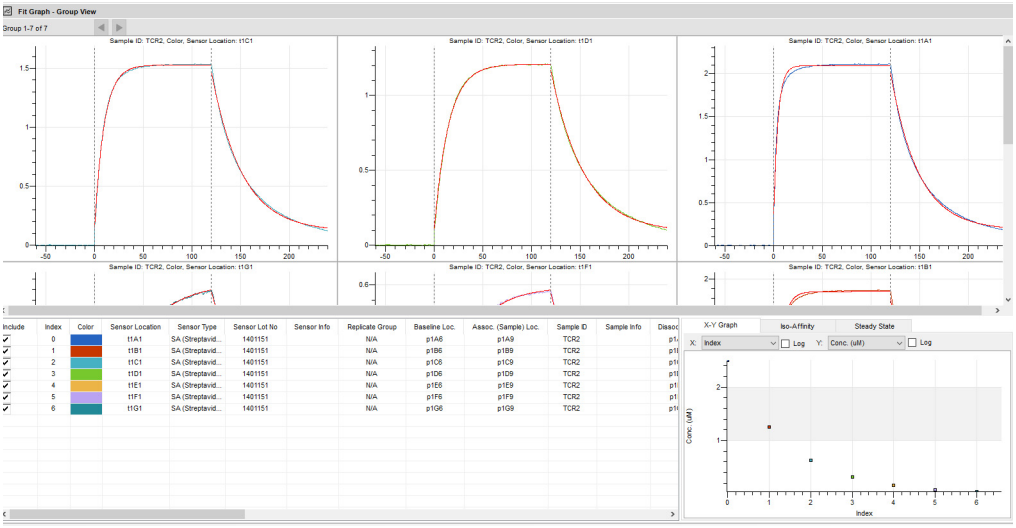


Figure 5-18: Grouped View

Viewing Analysis Results Table Data

Results for your kinetic analysis are shown in the Analysis Results table (Figure 5-19). Each row displays the results for one set of association/dissociation data. Sensor and Sample Well properties are entered in the Octet® BLI Discovery software, but they can be edited in the Preprocess Data tab if necessary.

Include	Index	Flip	Plate	Color	Sensor Location	Sample Location	Sample ID	Type	Binding Rate	Known Conc(µg/ml)	Well Conc.	Dilution Factor	Calc Conc.
☑	1		1		t1A1	A4	hIgG at 15...	J...	1.25591	N/A	1458.94002		1458.94002
☑	2		1		t1B1	B4	hIgG at 15...	J...	1.18986	N/A	1299.89796		1299.89796
☑	3		1		t1C1	C4	hIgG at 15...	J...	1.22031	N/A	1373.21950		1373.21950
☑	4		1		t1D1	D4	hIgG at 15...	J...	1.23435	N/A	1407.01044		1407.01044
☑	5		1		t1E1	E4	hIgG at 15...	J...	1.23754	N/A	1414.70678		1414.70678
☑	6		1		t1F1	F4	hIgG at 15...	J...	1.27859	N/A	1516.38284		1516.38284
☑	7		1		t1G1	G4	hIgG at 15...	J...	1.23920	N/A	1418.70099		1418.70099
☑	8		1		t1H1	H4	hIgG at 15...	J...	1.24532	N/A	1433.42102		1433.42102
☑	9		1		t1A1	A5	hIgG at 10...	J...	0.01749	N/A	9.71759		9.71759
☑	10		1		t1B1	B5	hIgG at 10...	J...	0.01721	N/A	9.56197		9.56197
☑	11		1		t1C1	C5	hIgG at 10...	J...	0.01716	N/A	9.53235		9.53235
☑	12		1		t1D1	D5	hIgG at 10...	J...	0.01736	N/A	9.64691		9.64691
☑	13		1		t1E1	E5	hIgG at 10...	J...	0.01732	N/A	9.62375		9.62375
☑	14		1		t1F1	F5	hIgG at 10...	J...	0.01758	N/A	9.77028		9.77028
☑	15		1		t1G1	G5	hIgG at 10...	J...	0.01704	N/A	9.46285		9.46285
☑	16		1		t1H1	H5	hIgG at 10...	J...	0.01742	N/A	9.67871		9.67871
☑	17		1		t1A12	A9	hIgG at 15...	J...	1.27203	N/A	1497.73341		1497.73341
☑	18		1		t1B12	B9	hIgG at 15...	J...	1.25787	N/A	1463.65849		1463.65849
☑	19		1		t1C12	C9	hIgG at 15...	J...	1.26430	N/A	1479.13585		1479.13585

Figure 5-19: Analysis Results Table

- **Include** - When this box is checked, the data is included in the analysis. If this box is blank, the data is excluded from the analysis.
- **Index** - This is the numbered order of the curves processed. The index is useful if you need to sort back to the original order and in the X-Y, Iso-Affinity and Steady-State Analysis graphs.
- **Color** - The color of the biosensor binding curve in the Fitting and Residual views.
- **Sensor Location** - Location of the biosensor in the sensor tray.
- **Sensor Type** - Type of biosensor used in the experiment.

- **Sensor Lot No** - The lot number of the biosensors used in the experiment. This is entered in Octet® BLI Discovery software.
- **Sensor Info** - Custom information about the biosensor used in the experiment.
- **Replicate Group** - Displays if the biosensor is part of a replicate group.
- **Baseline Loc.** - The well location in the sample or sample plate where the baseline step was performed.
- **Assoc. (Sample) Loc.** - Well location in the sample plate where the association step was performed.
- **Sample ID** - The sample ID entered during assay setup in Octet® BLI Discovery software.
- **Sample Info** - Custom information about the association sample well.
- **Dissoc. Loc.** - The well location in the sample well or sample plate where the dissociation step was performed.
- **Loading Loc** - Location of the sample well used during the loading step of the experiment.
- **Loading Sample ID** - The sample ID of the loading sample well.
- **Loading Conc.** - The concentration of the loading sample well .
- **Loading Response -** The binding shift of the loading step for this cycle.
- **Cycle** - Number of biosensor regeneration cycles.
- **Conc (nM)** - The molar concentration of the sample used in the association step. This is either entered by you or computed by the molarity calculator during experiment setup.
- **Response** - Response calculated from the time window entered in the Steady State Analysis parameters window.
- **KD (M)** - Affinity constant. For the 2:1 and 1:2 models, the software computes two K_D values.
- **ka (1/Ms)** - Rate of association. For the 2:1 and 1:2 models, the software computes two k_a values.
- **ka Error** - Standard error of the rate of association.
- **kd (1/s)** - Rate of dissociation. For the 2:1 and 1:2 models, the software computes two k_d values.
- **kd Error** - Standard error of the rate of dissociation.
- **Rmax** - The R_{max} is also calculated during the fit and reflects the maximum response when all ligand is occupied.
- **kobs (1/s)** - Observed binding rate. For the 2:1 and 1:2 models, the software computes two k_{obs} values.
- **kobs Error** - Standard error of the observed binding rate.
- **Req** - The calculated response at equilibrium that is determined from a fit of the binding data.
- **Req/Rmax(%)** - Ratio of R_{eq} to R_{max}.
- **Assoc RSS, Dissoc RSS, Full RSS** - RSS is the residual sum of squares, also known as the sum of squared residuals (SSR) or sum of squared estimate of errors (SSE). If the Step to Analyze option was set to both Association and Dissociation, the header will display Full RSS. If only fitting the association step or dissociation step, the header will display Assoc. RSS or Dissoc. RSS.
- **Full R^2** - The R^2 value indicates how well the fit and the experimental data correlate. In general, R^2 values above 0.95 are considered as a good fit for large molecule kinetics.
- **File location** - Location of the data file.
- **Report point X=time** - Report points can be added to the Analysis Results table using the Report Points feature. Its header becomes X=time entered in the report point time value (sec) box. For example, if you entered 100 seconds, the new column becomes X=100 with the signal (nm shift) of all traces at the X=100 time point for each ligand biosensor in the experiment. For more information, see "Report Points" on page 111.

Viewing Analysis Graphs

Results for your kinetic analysis are also shown in the X-Y, Iso-Affinity and Steady State Analysis graphs on the bottom right of the Kinetics Analysis Screen.

X-Y Graph

The X-Y graph is a scatter plot of analysis results based on x and y variables you can choose from (Figure 5-20).

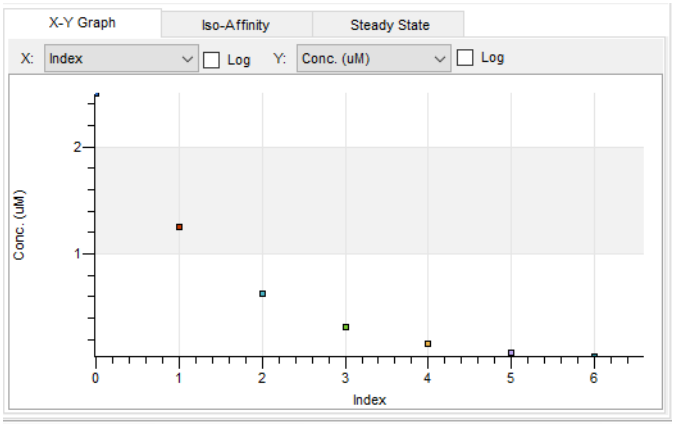


Figure 5-20: X-Y Graph

- **Changing the x/y variables** - You can choose which variables to plot by selecting the drop down menu next to either the x or y axis and selecting an option (Figure 5-21).

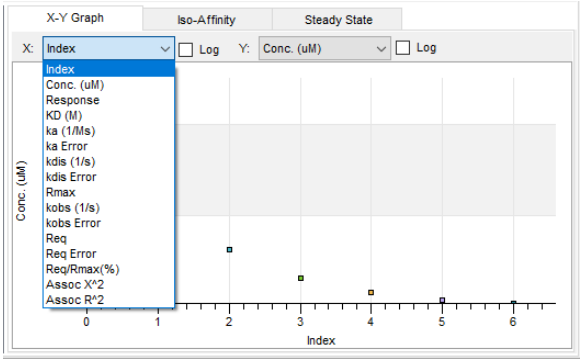


Figure 5-21: Variable Selection for X and Y Axis

In the example shown in Figure 5-22, the concentration is plotted against K_D.

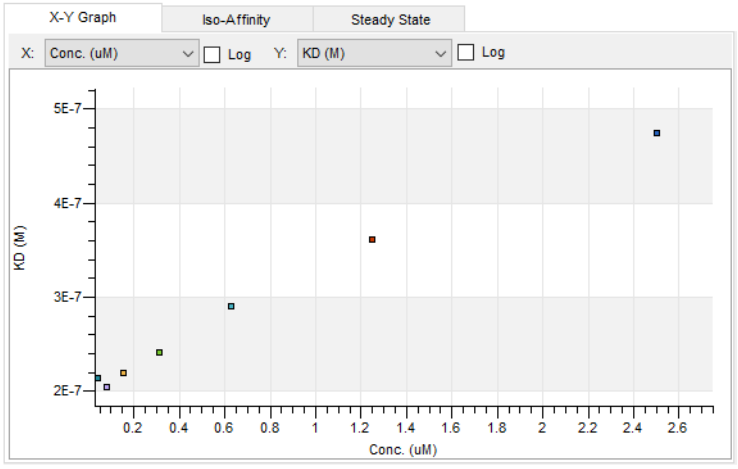


Figure 5-22: X-Y Graph of Concentration vs. K_D

- **Changing the scales** - A linear scale is displayed by default. Select one or both of the **Log** check boxes to display either axes in a logarithmic scale.

Iso-Affinity Graph

The Iso-Affinity graph (Figure 5-23) lets you view the continuum of k_d and k_a values that generate a single value of K_D, which is a convenient way to view both kinetic and affinity data. The value of the affinity constant, K_D, is the ratio of the dissociation rate k_d and association rate k_a. A single value of K_D can be obtained from varying values of k_a and k_d. For example, a K_D value of 1 µM can be the result of k_d=1x10^{-3} 1/s and k_a=1x10^{+3} 1/Ms or k_d=1x10^{-2} 1/s and k_a=1x10^{+4} 1/Ms.

Each Iso-Affinity plot has two red lines that correspond to a single K_D value. The position of the K_D lines is determined by taking the average of all K_D values and plotting one red line 10-fold lower than the average and one red line 10-fold higher than the average.

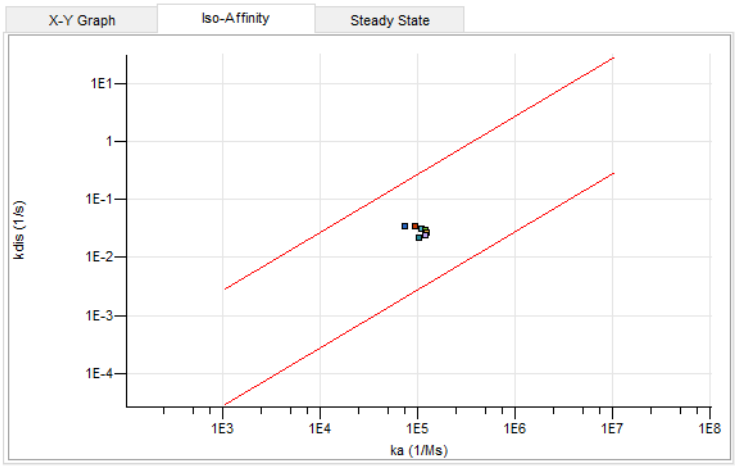


Figure 5-23: Iso-Affinity graph—X Axis = k_a, Y Axis = k_d

Steady State Analysis Graph

The Steady State Analysis graph (Figure 5-24) displays the results from the steady state analysis. The graph plots the R equilibrium or Response vs. concentration and the curve fit, and displays the calculated affinity constant K_D and R_{Max}. For more information, see "Step 6: Steady State Analysis (Optional)" on page 109.

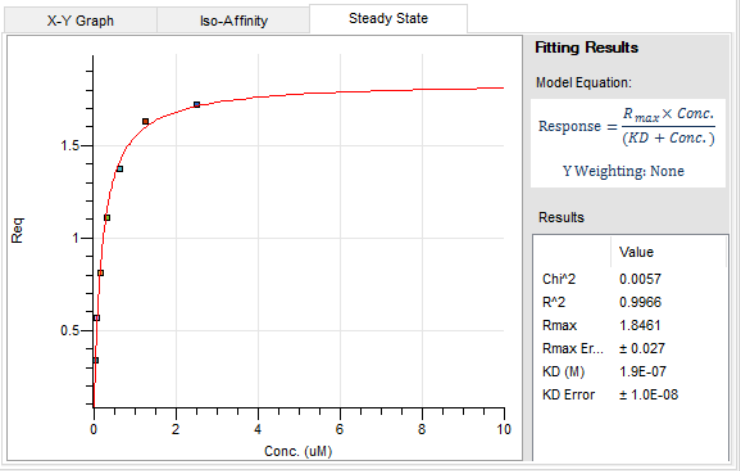


Figure 5-24: Steady State Analysis Graph

Analyzing Your Kinetic Data

You can select options for kinetic data analysis in the Fitting Parameters and Steady State Analysis windows. Before you get started, first make sure you've made any reference sensor, reference sample well and data corrections needed for your experiment in the Preprocess Data screen.

NOTICE: *If you do not need to do curve fitting and are only doing steady state analysis, skip to "Step 6: Steady State Analysis (Optional)" on page 109.*

Step 1: Choose the Step(s) to Analyze

In the Step to Analyze box in the Fitting Parameters window, select the step(s) you'd like to include in your analysis (Figure 5-25):

- Association only - Generates k_{obs}
- Dissociation only - Generates k_d
- Association and Dissociation - Generates k_{obs}, k_a, k_d, and K_D. Select this option if you are doing Local (individual) fitting with Full or Partial fit steps, or if you'll be doing Global (group) fitting.

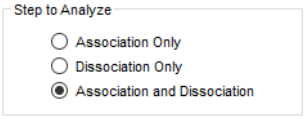


Figure 5-25: Selecting the Step to Analyze

Step 2: Choose a Binding Model

We recommend you use the 1:1 model unless the interaction is known to be more complex, or the goal is to attempt to understand a mechanism of interaction. You should choose an appropriate kinetic binding model for an interaction based on knowledge of the interaction and the molecules involved, valency and predicted stoichiometry of binding or estimated binding constants. For more in-depth information on binding models and fitting calculations, see "Binding Models" on page 117.

In the Binding Model box in the Fit Parameters window, click the drop down menu and choose a binding model (Figure 5-26).

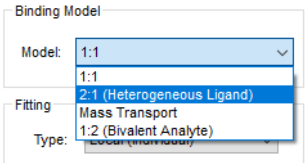


Figure 5-26: Binding Model Selection

NOTICE: *The new 1:1 and 2:1 model algorithms implemented in the software were optimized for speed and efficiency and may result in some variation in fitting results between Octet® Analysis Studio and Octet® BLI Analysis software. For well-fitting data, the new Octet® Analysis Studio software algorithms generally give better fits and may diverge by 1-5%. For noisy data or data that does not fit well the results may diverge by >5%.*

We recommend you analyze your data with both Octet® Analysis Studio and Octet® BLI Analysis software to ensure you're comfortable with the results before migrating to Octet® Analysis Studio software.

- **1:1** - This is an updated version of the prior 1:1 model that lets you processes data faster. Fits one analyte in solution binding to one binding site on the surface. Assumes the interaction between ligand and analyte follows pseudo-first-order kinetics.
- **2:1 (Heterogeneous Ligand)** - This is an updated version of the prior 2:1 model that lets you processes data faster. Fits the binding of one analyte in solution to two different binding sites on the surface. Kinetic parameters are calculated for two interactions (k_{a1}, k_{a2}, k_{d1}, k_{d2}, K_{D1}, K_{D2}). You can assign %K_{D1} and K_{D2} contributions to interactions in this model.
- **Mass Transport** - A Mass Transport model that fits the binding of the analyte taking into account two steps: 1) transport of the analyte from the bulk solution to the surface, and 2) molecular interaction of the analyte with the ligand.
- **1:2 (Bivalent Analyte)** - Fits the binding of one bivalent analyte to a monomeric immobilized ligand. Kinetic parameters are calculated for two interactions (k_{a1}, k_{a2}, k_{d1}, k_{d2}, K_{D1}, K_{D2}).

Data in the Analysis Results table and the Analysis Graphs update after the model is applied.

For example purposes, we processed both the association and dissociation steps in our experiment using the 1:1 model as shown in Figure 5-27.

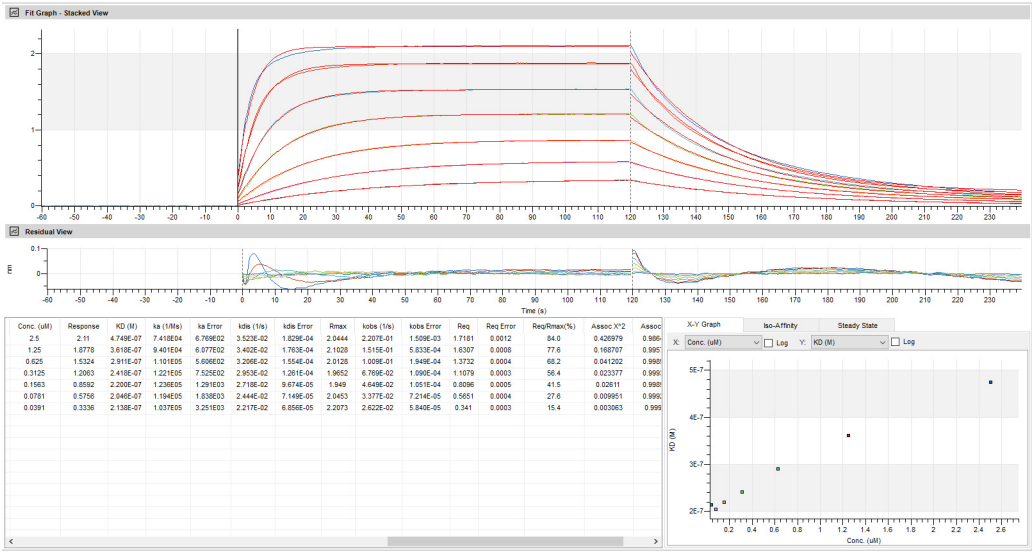


Figure 5-27: Kinetic Data Analysis Using the 1:1 Model

Step 3: Set Fitting Parameters

You can choose to do local or global fitting of your data.

Local Fitting

When local fitting is performed, kinetic parameters are derived individually for each analyte concentration.

The full fit option assumes that an interaction is fully reversible, so that as the dissociation step time approaches infinity, all of the analyte bound to the ligand dissociate. Since the dissociation curve eventually reaches the pre-association baseline, the rate of dissociation is extrapolated until it reaches zero signal on the Y-axis. The full dissociation option is recommended for data with a very low dissociation rate.

The partial dissociation model does not assume the signal reaches pre-association baseline. Only a portion of the analyte bound dissociates even as the step time approaches infinity, and the rate of dissociation is fit to the measured data only. Partial dissociation can be used to fit portions of curves in data sets with significant or biphasic dissociation, however partial fitting may tend to give higher k_d values. The curve fitting assumptions do not include dissociation signal decaying to zero.

Analyzing Data Using Local Fitting

Local (individual) fitting can be performed on the association step only, the dissociation step only or both the association and dissociation steps.

1. In the Fitting box in the Fitting Parameters window, select **Local (Individual)** as the Type (Figure 5-28):

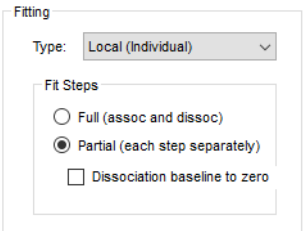


Figure 5-28: Selecting Local (Individual) Fit

2. Select how you'd like to fit the steps:
   - **Full (assoc and dissoc)** - Assumes that the off rate eventually reaches the pre-association baseline and forces the curve fit to that point. This option is only available if you've selected **Association and Dissociation** in the Step to Analyze box.
   - **Partial (each step separately)** - Does not assume the dissociation reaches the pre-association baseline. This option is only available if you've selected **Association and Dissociation** in the Step to Analyze box.
   - **Dissociation baseline to zero** - Select this box to have the curve fit assume the dissociation signal is decaying to zero. This option is only available if you've selected **Association and Dissociation** in the Step to Analyze box and **Partial (each step separately)**.

   Data in the Analysis Results table and the Analysis Graphs updates after the type and fit steps are applied.

   For example purposes, we processed both the association and dissociation steps in our experiment using the 1:1 model with the Partial (each step separately) option and Dissociation baseline to zero selected as shown in Figure 5-29.

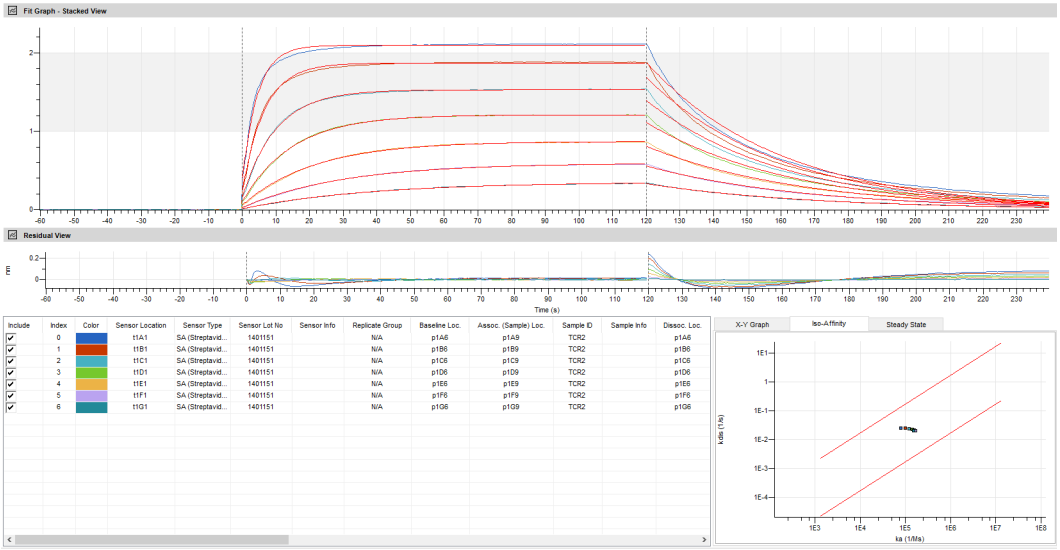


Figure 5-29: Kinetic Data Analysis Using the 1:1 Model, Partial (Each Step Separately) and Dissociation Baseline to Zero Parameters

Global Fitting

The most accurate kinetic and affinity constants are obtained when performing a global fit using several analyte concentrations. Global fit analysis includes all binding curve data in the group using a full fit option. Fitting several curves to one set of results yields more robust and reliable curve fits. The kinetic constants that are calculated depend upon the binding model selected.

R_{max} should remain unlinked by biosensor when separate biosensors are used for each individual analyte concentration. When R_{max} is linked, the theoretical maximum response is calculated assuming equal binding capacity between biosensors. Different biosensors have slight variability in surface capacity. R_{max} can be linked if the same biosensor is used for every analyte concentration in the series. This strategy is typically used in small molecule analyses, where dissociation is rapid and complete and enables re-use of the same biosensor for a new sample concentration. In standard large molecule kinetics assays where each sample is run on a new or regenerated biosensor, R_{max} should be unlinked to enable calculation of separate R_{max} for each sample.

Analyzing Data Using Global Fitting

Global (group) fitting can only be performed on the association and dissociation steps together.

1. Make sure you've selected **Association and Dissociation** in the Step Type box.
2. In the Fitting box under Fit Steps, select **Full (assoc and dissoc)**, as shown in Figure 5-30.
3. Select **Global (Group)** as the Type (Figure 5-30):

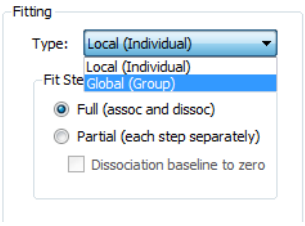


Figure 5-30: Selecting Global (Group) Fit

The Group By and Rmax Values options display (Figure 5-31):

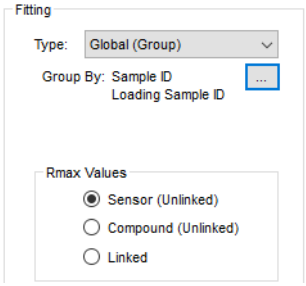


Figure 5-31: Global (Group) Fit Options

4. Select a Group By option. The software groups all data with the chosen selection together and applies a global fit to the group.

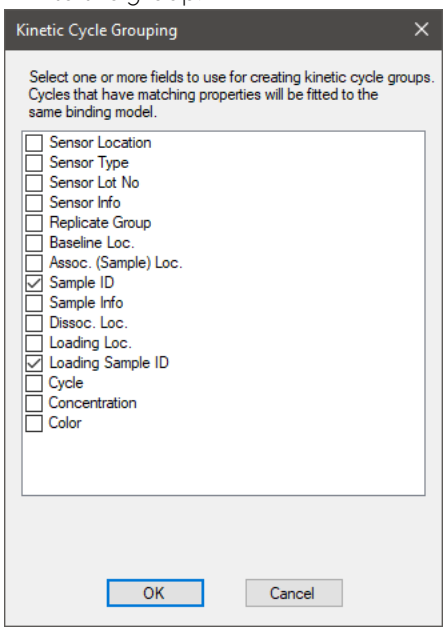


Figure 5-32: Group By Options

Data in the Analysis Results table and the Analysis Graphs update after the Group By option is applied.

NOTICE: *Previous versions of Octet® Analysis Studio only allowed fitting groups by a single field, and grouping by Color was suggested for more complex grouping scenarios. Version 12, enables you to create fitting groups based on multiple fields, for example Sample ID and Loading Sample ID. Grouping by color is retained for compatibility purposes but is no longer recommended.*

In the example shown in Figure 5-33, the data is grouped by Sample ID.

NOTICE: *For more information on defining colors by sample attributes, see "Color-Coding Data" on page 114.*

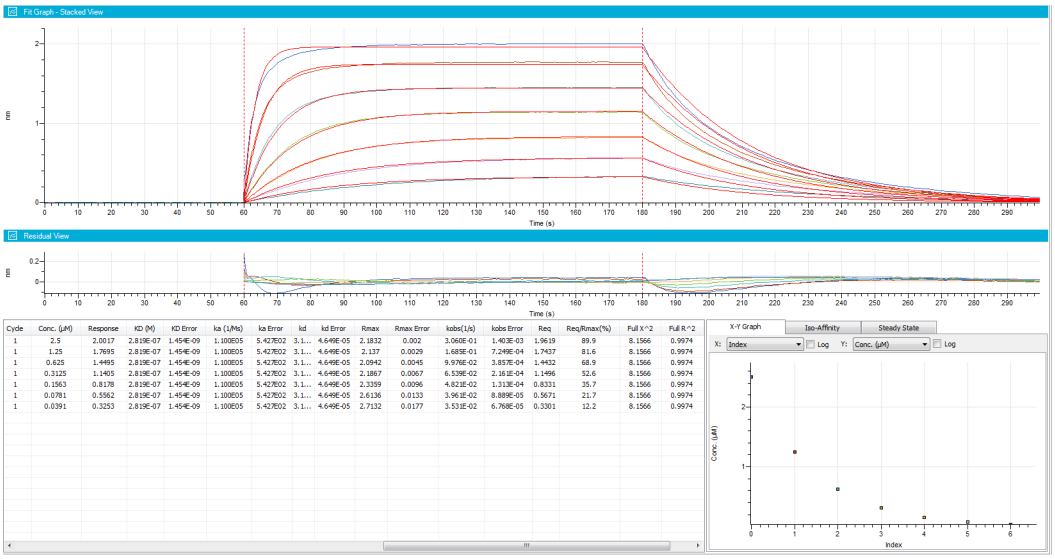


Figure 5-33: Kinetic Data Grouped by Sample ID.

5. Select an Rmax Values option (Figure 5-34). When fitting data, the theoretical response maximum (Rmax) can be calculated with:
   - **Sensor (Unlinked)** - Assumes non-equivalent surface capacity between biosensors.
   - **Compound (Unlinked)** - Assumes non-equivalent surface capacity between binding molecules (different sample compounds).
   - **Linked** - Assumes equivalent surface capacity between biosensors and binding molecules (assumes the same biosensor and same sample compound).

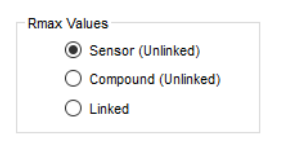


Figure 5-34: Rmax Values

Data in the Analysis Results table and the Analysis Graphs update after the Rmax Values option is applied.

Step 4: Set a Window of Interest (Optional)

You can set a Window of Interest (from start of step) if you'd only like to analyze a specific portion of the association or dissociation steps, which is useful for biphasic interactions. The default values are the full step durations for each step (Figure 5-35).

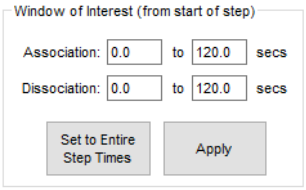


Figure 5-35: Window of Interest

To change the window of analysis, enter a from and/or to time in seconds for either or both steps, then click **Apply**. The software analyzes only the time range entered for either step.

To reset the time values back to the full step durations, click **Set to Entire Step Times**.

Data in the Analysis Results table and the Analysis Graphs update after the Group By option is applied. In the example shown in Figure 5-36, both the association and dissociation windows of interest were set from 0 to 90 seconds.

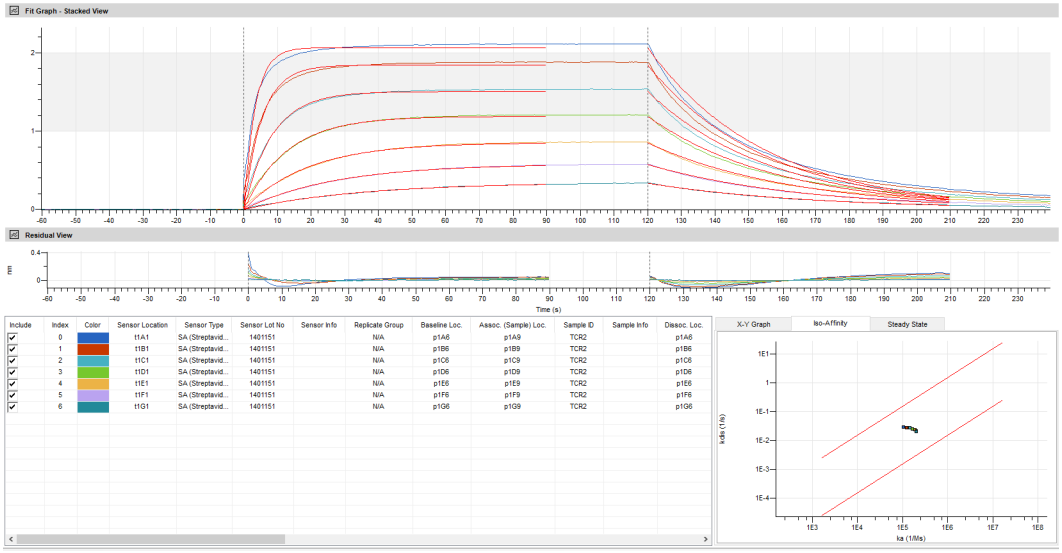


Figure 5-36: Kinetic Data With Window of Interest Set.

Step 5: Review the Data Fit Quality

Once you're done curve fitting, it's best to evaluate both the quality of the fit and the reliability of calculated binding and affinity constants. To assess how well the fitted curves adhere to the experimental data, here are a few general guidelines:

- Visually inspect the data and determine if the fit lines conform well to the data traces. If the fit lines are far from the actual data, this indicates the fit is not ideal.

- Look at the highest and lowest concentrations of your analyte. Is the behavior of one or both of these curves different from the other concentrations? If the highest concentration(s) show greater deviation in fitting, it (they) may be excluded from the analysis. Likewise, if the lowest concentration shows little or weak response, these data may be excluded as well. Exclusion of concentrations outside of the working range of the analyte titration improve fitting in a global analysis. See "Excluding Data from the Analysis" on page 109 for more information.
- Confirm that the apparent kinetic constants are consistent with expectations based on literature or previous experimental knowledge of the interaction.
- Residuals are calculated in the Analysis Results table and plotted in the Residual Graph, and can be examined to validate the fit. Residual values should not be greater than ±10% of the maximum response of the fitted curve. The shape of the residual plot corresponds to differences between the fit curve and the experimental data and should show signals that are equally distributed above and below the mean. Higher values indicate inaccurate fitting.
- Error values are provided in the analysis table for k_a and k_d. These errors are considered acceptable if they are within one order of magnitude of the rate constant values.
- The R^2 value indicates how well the fit and the experimental data correlate. In general, R^2 values above 0.95 are considered as a good fit for large molecule kinetics.
- Examine RSS values. RSS is a measure of error between the experimental data and the fitted line. A smaller RSS indicates a better fit.

Excluding Data from the Analysis

To remove specific data from the analysis, deselect the box in the **Include** column for that row of data. The software updates the Analysis Results table automatically (Figure 5-37).

Include	Index	Color	Sensor Location	Sensor Type	Sensor Lot No	Sensor Info	Replicate Group	Baseline Loc.	Assoc. (Sample) Loc.	Sample ID	Sample Info	Dissoc. Loc.
☐	0		t1A1	SA (Streptavid...	1401151		N/A	p1A6	p1A9	TCR2		p1A6
☑	1		t1B1	SA (Streptavid...	1401151		N/A	p1B6	p1B9	TCR2		p1B6
☑	2		t1C1	SA (Streptavid...	1401151		N/A	p1C6	p1C9	TCR2		p1C6
☑	3		t1D1	SA (Streptavid...	1401151		N/A	p1D6	p1D9	TCR2		p1D6
☑	4		t1E1	SA (Streptavid...	1401151		N/A	p1E6	p1E9	TCR2		p1E6
☑	5		t1F1	SA (Streptavid...	1401151		N/A	p1F6	p1F9	TCR2		p1F6
☑	6		t1G1	SA (Streptavid...	1401151		N/A	p1G6	p1G9	TCR2		p1G6

Figure 5-37: Excluding Data

Step 6: Steady State Analysis (Optional)

You can perform steady state, or equilibrium analysis, when full kinetic analysis is not possible or required. This analysis option is useful for analyzing interactions that are of low affinity or with very fast on-rates. If the initial slope of the binding curve is steep, it can be difficult for the software to perform accurate curve fitting. Steady state analysis is often used with protein-small molecule interactions, where on and off rates tend to be very fast and the signal fairly low. For accurate steady state analysis results, the association binding curves must reach equilibrium for every analyte concentration in a titration. Extended association step times of 30–60 minutes may be required to reach equilibrium binding. This approach is feasible only for well-behaved binding pairs exhibiting 1:1 binding kinetics.

When the Response option is selected, binding rate and affinity constants calculate based on the average signal response (nm) during the assay time specified. If the R equilibrium option is selected, rate and affinity constants calculate based on the theoretical R_{eq} value based on the curve fits. If all curves have reached equilibrium, these two sets of values should match.

1. In the Steady State Analysis window, select an analysis option (Figure 5-38):

- **R equilibrium** - Fits the binding curve to a 1:1 model and uses the calculated Req to determine the steady state affinity. If this option is selected, you first must perform a curve fitting kinetic analysis.
- **Response** - Takes the average response in the Average from time window and uses it to calculated the steady state affinity.

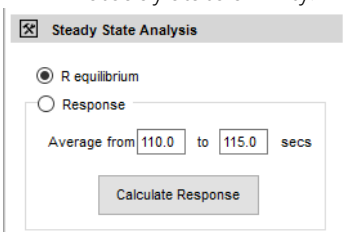


Figure 5-38: Steady State Analysis Parameters

2. If you chose Response, enter the **Average from** times. This is the amount of equilibrium state data to analyze, from the time equilibrium was reached to the time at which the response should be calculated.
3. Click **Calculate Response**. Calculated equilibrium information displays in the Steady State graph.

   In the example shown in Figure 5-39, the data was analyzed using the R equilibrium option.

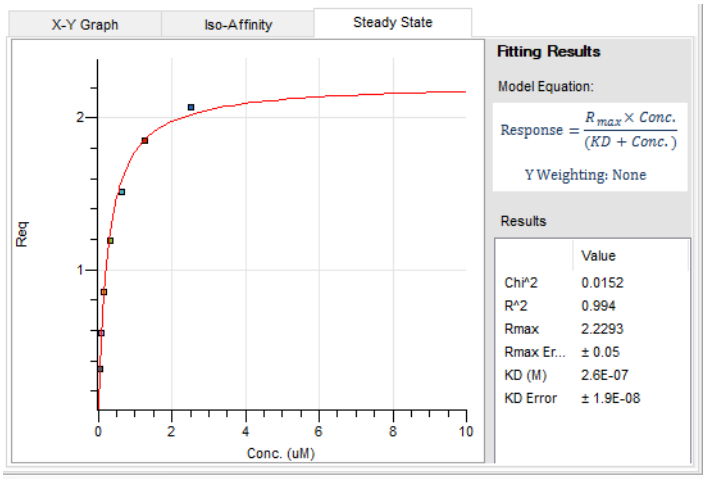


Figure 5-39: Stead State Analysis Using R Equilibrium.

Step 7: Analyze the Next Assay

If your data includes more than one experiment or combined data set, repeat Steps 1-6 on the next Assay tab. Click the **All Assays** tab to view the summary data for the combined dataset (all assays) - the graphs and kinetic data table.

NOTICE: *Analysis settings cannot be modified in the All Assays tab.*

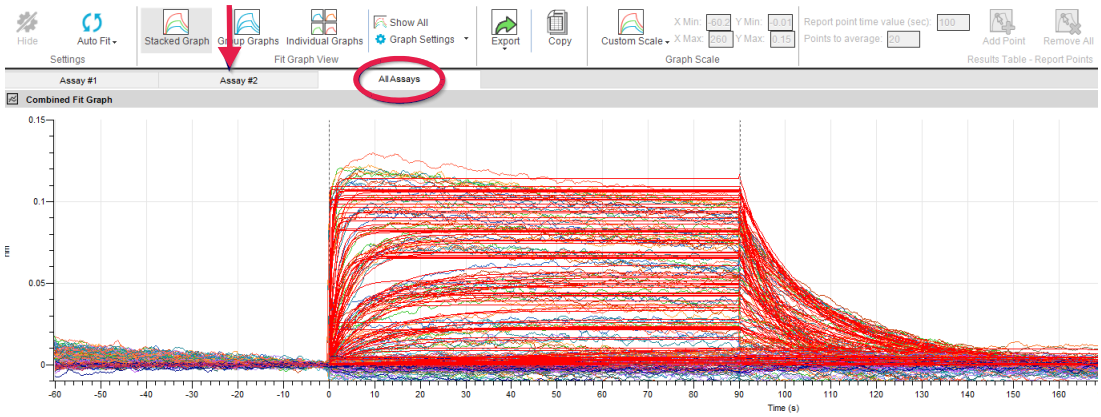


Figure 5-40: Assay Tabs

Report Points

Adding Report Points

Some applications like screening rely on single-point measurements called report points that can be placed at the end of loading or association steps, or at the early or late phase of the dissociation step. Report points can be added to just to the kinetic cycles (baseline, association and dissociation steps) in the Kinetic Analysis Screen.

1. In the Report Points menu at the top the screen, enter a time (in seconds) in the **Report point time value (sec)** box (Figure 5-41).

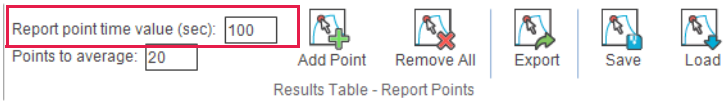


Figure 5-41: Entering a Report Point Time Value

2. Enter a value in the **Points to average box** (Figure 5-42). This takes an average of the data points centered around the report point time value. For example, if the report point value you entered was 100 seconds and the points to average is 20, the software will average the combined 10 data points just before the 100 second mark and 10 data points at and just after the 100 second mark. If the report point time is set to the beginning or end of the assay step, the software will average the first or last 20 data points of the assay step.

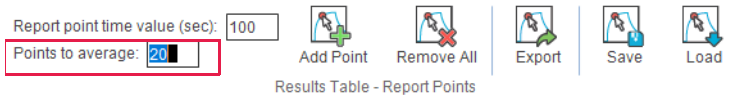
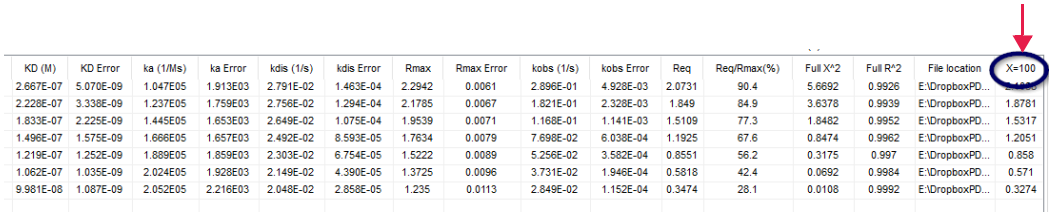


Figure 5-42: Entering the Points to Average

3. Click (**Add Point**). A new column is added to the end of the Analysis Results table. Its header becomes X=time entered in the report point time value (sec) box. For example if you entered 100 seconds, the new column becomes X=100 with the signal (nm shift) of all traces at the X=100 time point for each ligand biosensor in the experiment (Figure 5-43).



KD (M)	KD Error	ka (1/Ms)	ka Error	kdis (1/s)	kdis Error	Rmax	Rmax Error	kobs (1/s)	kobs Error	Req	Req/Rmax(%)	Full X^2	Full R^2	File location	X=100
2.667E-07	5.070E-09	1.047E05	1.913E03	2.791E-02	1.463E-04	2.2942	0.0061	2.896E-01	4.928E-03	2.0731	90.4	5.6692	0.9926	E:\DropboxPD...	
2.228E-07	3.338E-09	1.237E05	1.759E03	2.756E-02	1.294E-04	2.1785	0.0067	1.821E-01	2.328E-03	1.849	84.9	3.6378	0.9939	E:\DropboxPD...	1.8781
1.833E-07	2.225E-09	1.445E05	1.653E03	2.649E-02	1.075E-04	1.9539	0.0071	1.168E-01	1.141E-03	1.5109	77.3	1.8482	0.9952	E:\DropboxPD...	1.5317
1.496E-07	1.575E-09	1.666E05	1.657E03	2.492E-02	8.593E-05	1.7634	0.0079	7.698E-02	6.038E-04	1.1925	67.6	0.8474	0.9962	E:\DropboxPD...	1.2051
1.219E-07	1.252E-09	1.889E05	1.859E03	2.303E-02	6.754E-05	1.5222	0.0089	5.256E-02	3.582E-04	0.8551	56.2	0.3175	0.997	E:\DropboxPD...	0.858
1.062E-07	1.035E-09	2.024E05	1.928E03	2.149E-02	4.390E-05	1.3725	0.0096	3.731E-02	1.946E-04	0.5818	42.4	0.0692	0.9984	E:\DropboxPD...	0.571
9.981E-08	1.087E-09	2.052E05	2.216E03	2.048E-02	2.858E-05	1.235	0.0113	2.849E-02	1.152E-04	0.3474	28.1	0.0108	0.9992	E:\DropboxPD...	0.3274

Figure 5-43: Report Point Column in Analysis Results Table

- Click (**Remove All**) to remove all report points.
- Click (**Save**) to save the report points as a .csv file.
- Click (**Load**) to load a previously saved report file and automatically add those report points to your current experiment.

Exporting Report Points

Click (**Export**) to export the report points for your current experiment. The Report Points Export window displays:

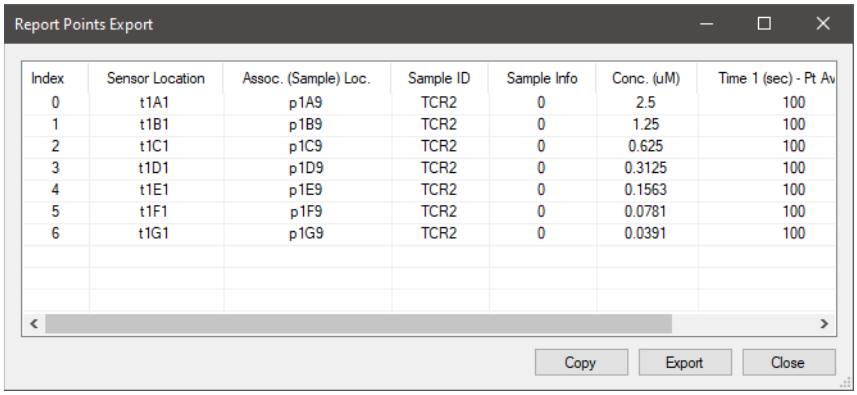


Figure 5-44: Report Points Export

- Select **Copy** to copy and paste all the report point data for the current experiment into another program like Microsoft® Word® or Excel®.
- Select **Export** to export and save all the report point data for the current experiment as a .csv file.

Analysis Results Table Options

The Analysis Results table has several options to let you change the display.

- To sort data in the table alphabetically or numerically, just click the column header.
- To view table display options, right-click a populated table row or column to see the menu (Figure 5-45).

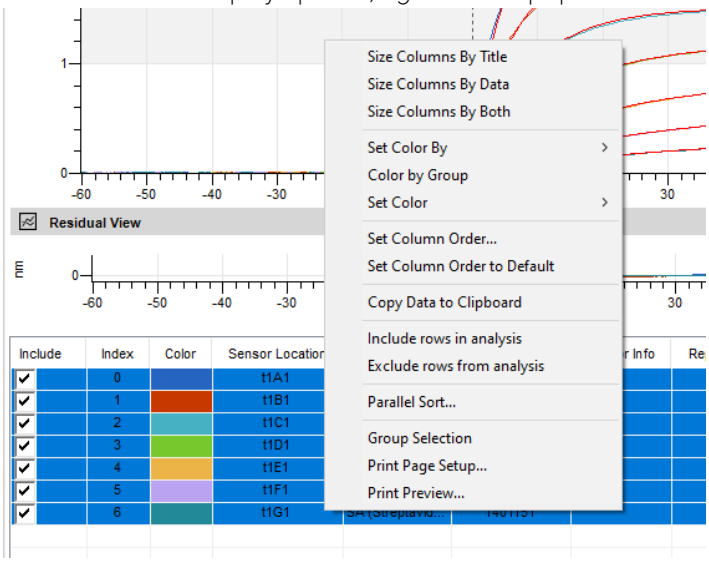


Figure 5-45: Analysis Results Table Menu

Sizing Columns

The following automatic column sizing options are available in the table's right-click menu:

- **Size Columns by Title** - Sets all column widths to fit the column titles.
- **Size Columns by Data** - Sets all column widths to fit the data.
- **Size Columns by Both** - Sets all column widths to best fit both the column titles and the data.

To manually resize the columns, roll the cursor over the border between the column headers until the resize cursor appears (Figure 5-46). Then just click and drag the column to resize.

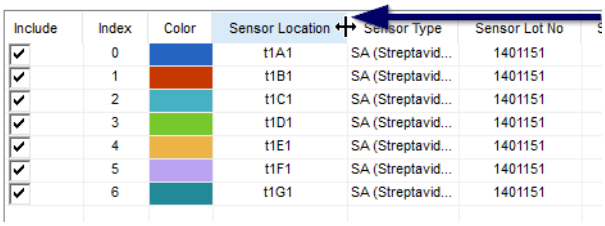


Figure 5-46: Column Resize Cursor

Color-Coding Data

You can assign specific colors to data as needed. Results can also be color-coded by category to group them for a global fit (for example, colored by compound), then fit using Global Fit by Color.

Select one or more rows in the Analysis Results table, right-click and choose one of the following options:

- **Set Color By** - Lets you color-code data by a table column variable (Figure 5-47). Once you select a variable, the binding curve and table row colors automatically update.

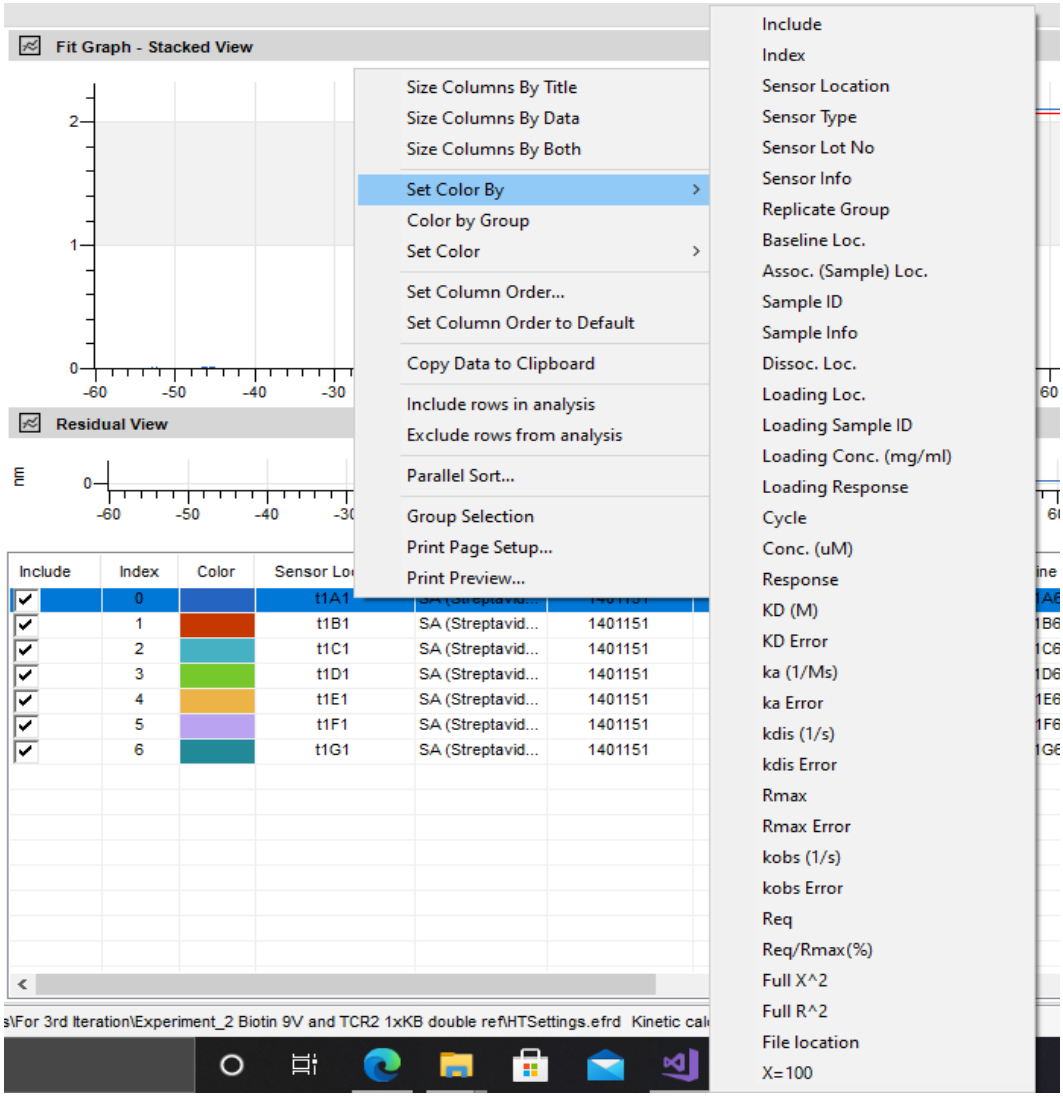


Figure 5-47: Set Color By

- **Set Color By Group** - Color-codes the data per the Group View Options. For more information on viewing data by groups and setting group view options, see page 95.

- **Set Color** - Opens the color palette so you can change the color for the table row(s) currently selected (Figure 5-48). Select a color from the palette or define a custom color then click **OK**. The binding curve and table row colors automatically update.

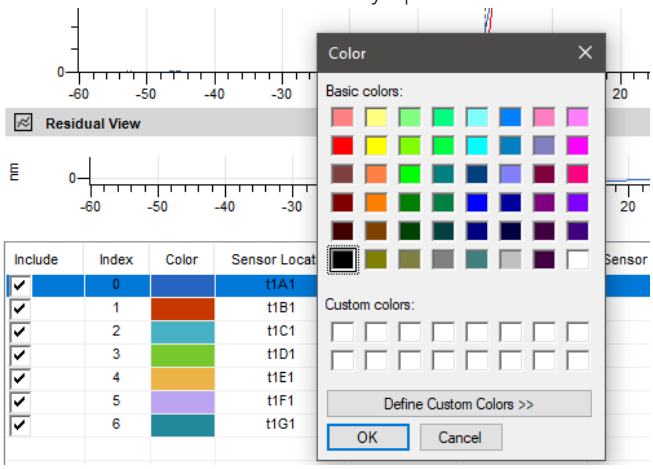


Figure 5-48: Set Color

Changing the Column Order

To change the order of columns shown in the Analysis Results table, right-click the table and select **Set Column Order**. The window that displays lets you shuffle columns (Figure 5-49).

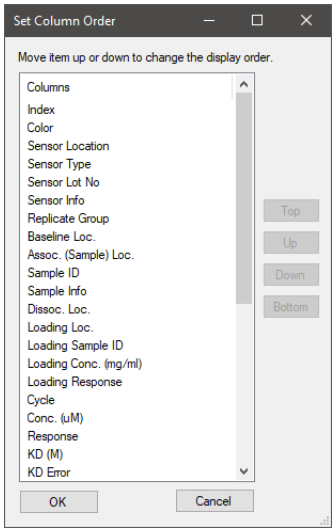


Figure 5-49: Set Column Order Window

Select one or more column header(s), then click **Top**/**Bottom** to make them the first or last columns in the table, or click **Up**/**Down** to move them one position up or down at a time.

To restore the table column order to default settings, select **Set Column Order to Default**.

Copying the Table

To copy the data in the Analysis Results table so you can paste it into other programs (Microsoft® Excel®, Microsoft Word®, electronic notebooks, etc.), right-click the table and select **Copy to Clipboard** (Figure 5-50).

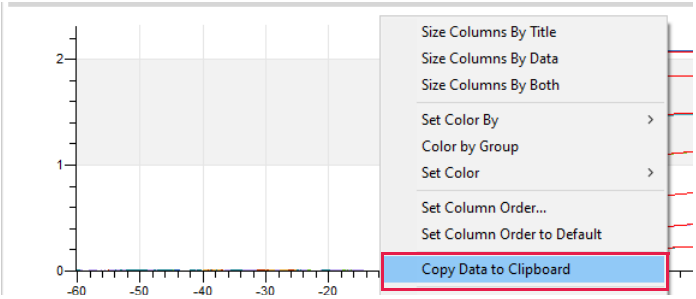


Figure 5-50: Copying the Analysis Results Table

Exporting Datasets

You can use the Export options menu at the top of the Kinetics Analysis screen to export your datasets (Figure 5-51).

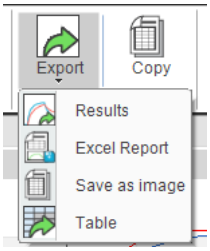


Figure 5-51: Export Options.

- Click (**Excel Report**) to export the processed (corrected) data for your experiment or combined data set in .xlsx format. Select which experimental components to export, use the default report name or click **...** to specify another name/location, then click **Export** (Figure 5-52).

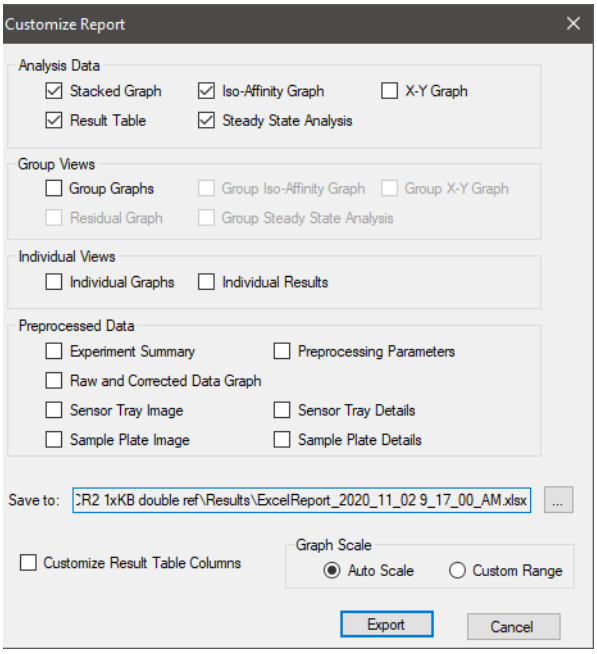


Figure 5-52: Excel Report Export Options

- Click (**Results**) to export the fit results in .csv format. You can export to a single csv file or one csv file per sensor.
- Click (**Table**) to export results in the Analysis Results table in .csv format.
- Click (**Copy**) to copy the results in the Analysis Results table and paste into another program like Microsoft® Excel®.

Binding Models

Fitting the experimental data to a model involves some consideration. Many interactions studied do not fit a simple 1:1 binding model. It can be tempting to choose a model based upon best empirical fit to the data. More complicated models feature more variables and degrees of freedom, and tend to offer statistically better fits.

1:1 Model

In a 1:1 bimolecular interaction, both the association and dissociation phases display a time-resolved signal that is described by a single exponential function. Analyte molecules bind at the same rate to every ligand binding site. The association curve follows a characteristic hyperbolic binding profile, with exponential increase in signal followed by a leveling off to plateau as the binding reaches equilibrium. The dissociation curve follows single exponential decay with signal eventually returning to baseline. The full fitting solution for a 1:1 binding is:

Association phase:

$$y = R_{max} \frac{1}{1 + \frac{k_d}{k_a * [Analyte]}} (1 - e^{-(k_a * [Analyte] + k_d)x})$$

Dissociation phase:

$$y = y_0 e^{-k_d (x - x_0)}$$

$$y_0 = R_{max} \frac{1}{1 + \frac{k_d}{k_a * [Analyte]}} (1 - e^{-(k_a * [Analyte] + k_d)x_0})$$

An example of kinetic data following a 1:1 interaction is shown in Figure 5-53.

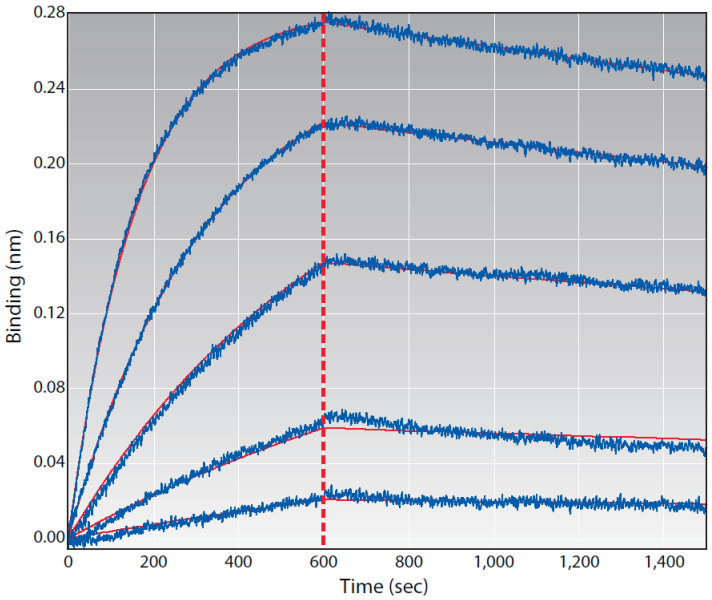


Figure 5-53: Analyzed Data Illustrating a 1:1 Binding Interaction. Blue Lines Represent Data Traces, Curve Fits are Red.

2:1 Heterogeneous Ligand Model

The 2:1 heterogeneous ligand model assumes analyte binding at two independent ligand sites. Each ligand site binds the analyte independently and with a different rate constant. Two sets of rate constants are given, one for each interaction where A represents the analyte and B represents the immobilized ligand:

$$A + B1 \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AB1$$

$$A + B2 \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} AB2$$

Mathematically, the equation used to fit a 2:1 binding interaction is a combination of two 1:1 curve fits, with an additional parameter to account for percentage of binding contributed by each interaction. Figure 5-54 shows an example of fitted data using the 2:1 heterogeneous ligand binding model.

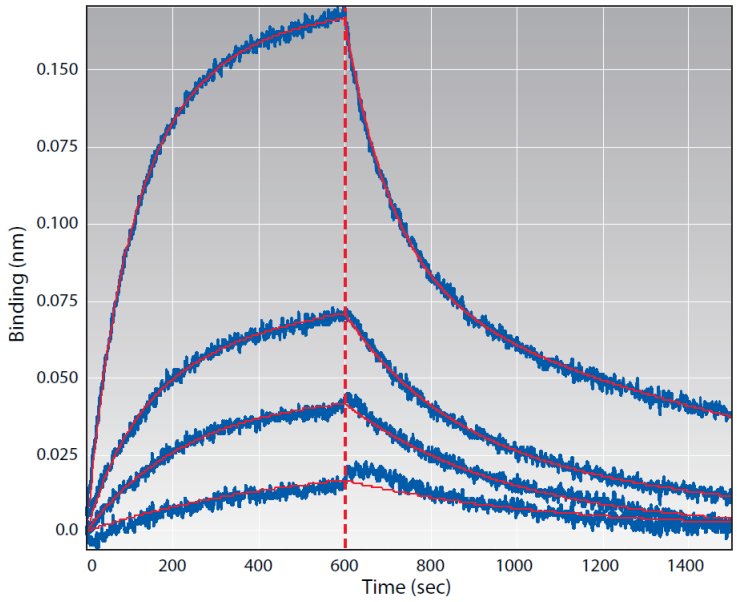


Figure 5-54: Analyzed Data Fit Using the 2:1 Heterogeneous Ligand Binding Model. Curves are Biphasic, Indicating that More than One Interaction is Occurring.

NOTICE: *When the 2:1 Heterogenous Ligand model is used, the fitting results are presented such that binding site 1 will be the higher affinity site, and binding site 2 will be the lower affinity site.*

Mass Transport Model

In a fluidics-based system, samples pass over the biosensor surface via laminar flow, where frictional forces from the sides of the tubing and the surface of the biosensor slow the velocity of the liquid close to the surface. In such a system, the decreased flow rate immediately adjacent to the biosensor inhibits efficient exchange of analyte molecules from the surface to the bulk solution. At low concentrations, analyte molecules present near the biosensor surface can bind to the ligand faster than they can be replaced in the surrounding solution. When this occurs, the binding rate becomes dependent on supply of analyte molecules rather than the actual kinetics of the interaction. The shape of the binding curve are determine by the rate at which the analyte diffuses to the surface, and change with flow rate. As flow increases, so does the apparent binding rate, as the supply of analyte molecules available to bind increases.

$$A_{bulk} \underset{k_m}{\overset{k_m}{\rightleftharpoons}} A_{surf} + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

Mass transport limited curves are often less steep than expected, and can appear as straight lines as in Figure 5-55.

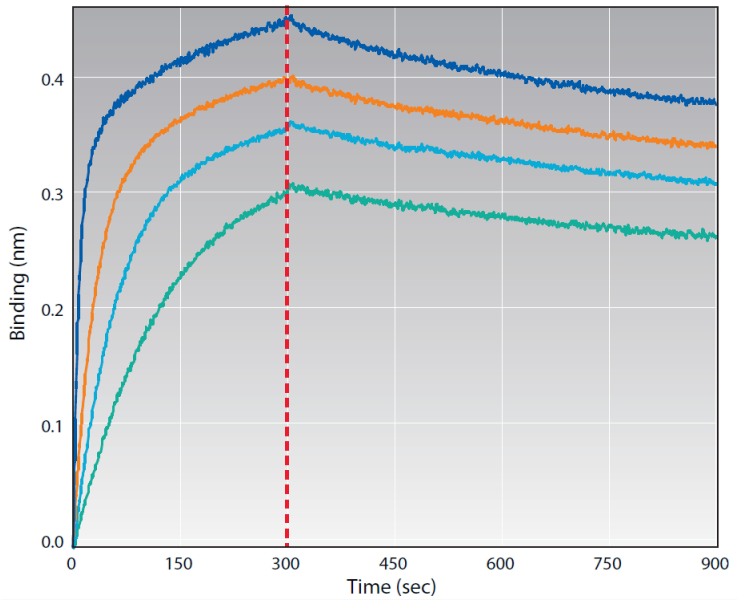


Figure 5-55: Range of Analyte Concentrations is too Low, and Data is Limited by Mass Transport.

Mass transport limited data can be fit using the Mass Transport binding model, which fits the binding of the analyte taking into account two steps: 1) rate of transport of the analyte from the bulk solution to the surface (k_m), and 2) interaction of the analyte with the ligand. Diffusion limitation occurs when k_m is much smaller than k_a[B]:

$$\frac{dR}{dt} = \frac{k_a * [Analyte]}{1 + \frac{k_a}{k_m}(R_{max} - R)}(R_{max} - R) - \frac{k_d}{1 + \frac{k_a}{k_m}(R_{max} - R)}R$$

Agitating the sample plate in the Dip and Read format creates a turbulent flow over the biosensor which is not subject to laminar forces and is highly efficient at replacing volume close to the surface of the biosensor. If mass transport effects are an issue, the supply of analyte to the surface must effectively be raised. This can be accomplished by reducing the level of immobilized ligand, or increasing the shaking speed during the assay to increase flow rate.

1:2 Bivalent Analyte Model

The 1:2 Bivalent Analyte model fits the binding of one bivalent analyte to a monomeric immobilized ligand. Kinetic parameters are calculated for two interactions (k_{a1}, k_{a2}, k_{d1}, k_{d2}, K_{D1}, K_{D2}).

$$A+B \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AB \longrightarrow AB+B \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} AB_2$$

This model assumes that because of limited distance between two adjacent binding sites on the surface, the bivalent analyte can form a bridged complex. This interaction is linked, meaning that the formation of AB_2 complex cannot occur before the formation of AB, and AB cannot dissociate before the dissociation of AB_2. This avidity effect results in a slower apparent dissociation rate than would be expected if the interaction followed a 1:1 binding profile.

Two sets of rate constants and K_D values are reported using the 1:2 bivalent analyte model. The first set of values reflects the binding due to the affinity of the interaction. The second set of values represents binding due to avidity. Three equations are used to fit bivalent analyte curves, the first describing association of A to B, the second is the association of AB to B, and the third describes dissociation of the AB_2 complex.

$$\frac{dB}{dt} = -(2k_{a1} * [A] * B - k_{d1} * AB) - (k_{a2} * AB * B - 2k_{d2} * AB_2)$$

$$\frac{dAB}{dt} = -(2k_{a1} * [A] * B - k_{d1} * AB) - (k_{a2} * AB * B - 2k_{d2} * AB_2)$$

$$\frac{dAB_2}{dt} = (k_{a2} * AB * B - 2k_{d2} * AB_2)$$

Changes in the assay format and conditions can reduce avidity effects in a binding interaction. One approach is to lower the density of the immobilized ligand by decreasing ligand concentration or decreasing the loading step time. A lower ligand density effectively increases the distance between molecules on the surface, minimizing the likelihood of a bound analyte reaching adjacent molecules. This approach may require some optimization because lowering the ligand density decreases the sensitivity of the assay. Alternatively, reversing the assay orientation so that the bivalent molecule is immobilized on the surface eliminates the possibility of analyte bridging. The 1:2 binding model is useful when the bivalent molecule cannot be captured due to issues related to instability under conditions of immobilization, lack of sensitivity in the opposite assay orientation, reagent availability, or if an interaction needs to be tested in multiple formats.

Chapter 6:

Preprocessing Epitope Binning Datasets

Epitope binning segments a panel of monoclonal antibodies (mAbs) into 'bins' based on the antigen region, or epitope, bound by each antibody. Grouping is performed using cross-competition assays where the competitive binding of antibody pairs to a specific antigen is characterized. 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 2D matrix of pairwise binders and blockers. Epitope binning experiments are run as kinetic experiments with many repeating stepsin the Octet® BLI Discovery software. Epitope binning analysis in Octet® Analysis Studio software lets you compare cycles of the same pattern, for example repeating Loading and Association steps, with monitoring of baselines or Dissociation steps for binding stability.

Processing of datasets for Epitope Binning analysis shares many features with Kinetic data processing. The preprocess data tab lets you subtract reference sensors and identify the specific antibody binding steps in your experiment(s). You can also make changes to sample information if needed.

Reference Sensor Subtraction

Reference sensor subtraction is used in experiments where reference biosensors were used for analyte binding with no ligand present.

If all experiments in the workspace contain the same sequence of steps, then reference sensor subtraction options can be found under the Assay #1 tab, Reference Sensor.

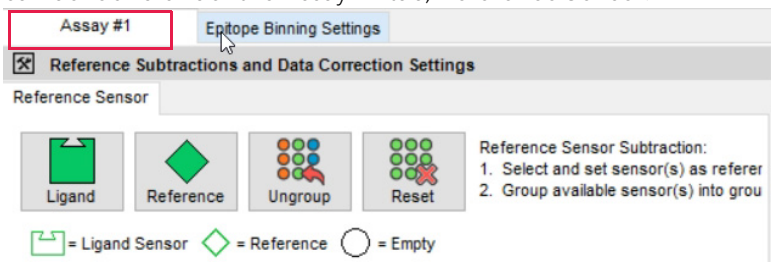


Figure 6-1: Reference Sensor subtraction with identical experiment step sequences

Depending on the assay format, it may be necessary to combine experiments that contain a different numbers of steps. For example, some experiments may contain four cycles - testing the first antibodies against four different second-antibody steps, and some experiments may contain three cycles - testing the first antibodies against three different second-antibody steps. In those cases, experiments with matching step sequences is placed into their own Assay tab for purposes of reference sensor subtraction. Reference sensor subtraction should be configured for each Assay tab before continuing to the Epitope Binning settings.

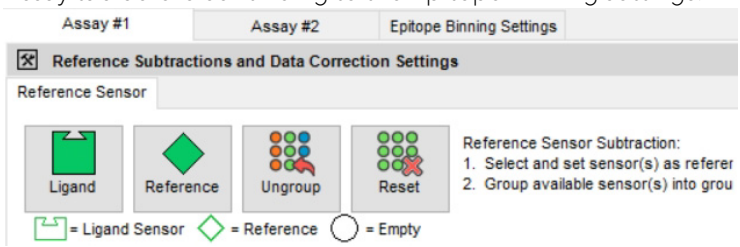


Figure 6-2: Reference sensor subtraction where experiments do not have identical step sequences

Reference sensor subtraction for Epitope Binning is configured in the same way as for Kinetic experiments. See "Reference Sensor (Non-specific Binding) Subtraction" on page 47.

After subtracting the reference sensor, the full traces are split into epitope binning cycles and combined into a single group for analysis.

Epitope Binning Cycle Settings

After you confirm your reference sensor subtractions, click the Epitope Binning Settings tab to identify the cycle pattern in preparation for analysis. The software automatically searches for the binning cycle pattern in the dataset (repeating sequences of baseline, loading and/or association steps) and overlays them in the Epitope Binning Cycle Data graph.

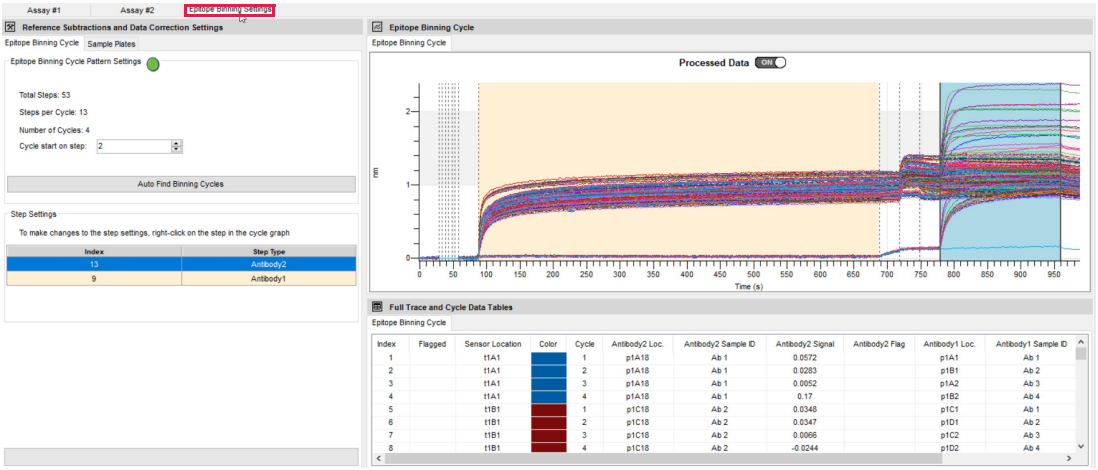


Figure 6-3: Epitope Binning Settings Screen

The software also scans the dataset and selects the Antibody 1 and Antibody 2 steps. Once the scan is complete, the Step Settings table indicates which steps were chosen. The antibody steps can be modified if needed, and other steps such as baseline and dissociation can be added manually.

Epitope Binning Cycle Pattern Settings

This area shows the total number of steps found, the number of cycles of repeating steps, and the number of steps found per cycle for the experiment.

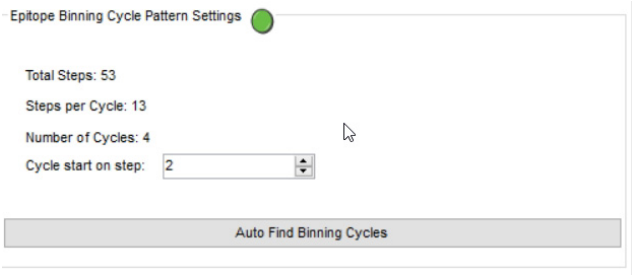


Figure 6-4: Epitope Binning Cycle Pattern Settings

The Cycle start on step option can be used for assays where a number of initial assay steps are followed by a set of repetitive cycles, such as when you load the first antibody in one experiment, then follow with the second experiment where it iterates through all the combinations of the second antibodies.

When comparing repeating steps in a binning experiment, the option lets you tell the software to ignore steps that do not need to be overlaid for processing the binning results.

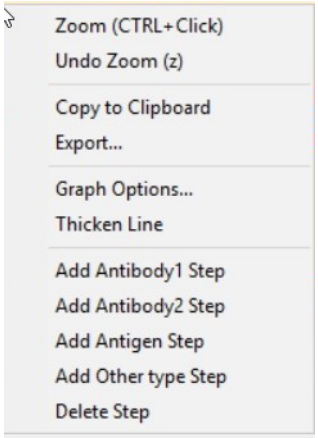
Change the Cycle start on step value to the step you want to start analysis. The software then reprocesses the data-set ignoring any initial non-repeating steps present only in the first cycle.

Step Settings

This table shows the currently defined steps in the cycle pattern. For epitope binning analysis, the Antibody 1 and Antibody 2 steps must be defined.

Figure 6-5: Epitope Binning Step Settings

If the cycle pattern could not be automatically identified, right-click the cycle graph and choose one of the **Add step** options to identify the correct step type. The step type identification can also be removed by selecting the **Delete step** option. This does not delete data.



You can add Antigen or Buffer/Dissociation steps if needed to assess data quality for reproducibility of antigen loading or to highlight antibodies with high off rates.

If you need to add other steps:

1. Right-click the step you want to add in the Epitope Binning Cycle Data graph.
2. Click **Add Other type Step**.

Editing Sample Information

Sample information for wells used in the Antibody 1, Antibody 2 or 'Other' steps can be edited. Click the **Sample plates** tab to see a combined view of the sample plates from all experiments in the workspace.

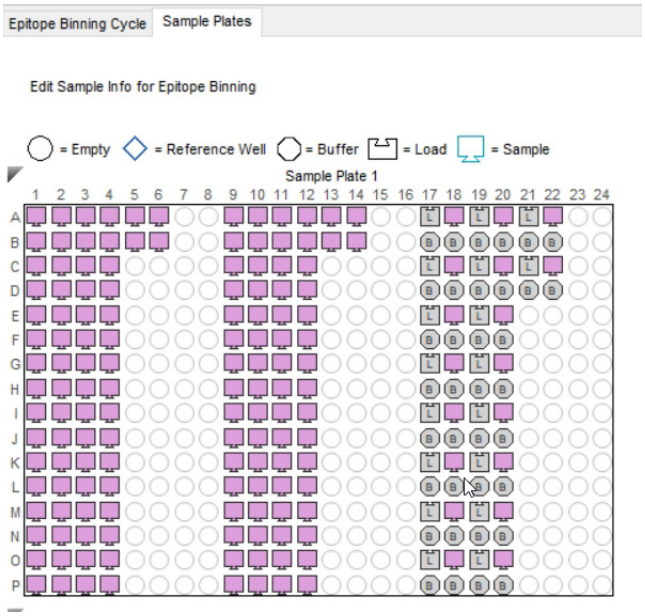


Figure 6-6: Sample Plates Display

To update sample information, click one or more sample wells, right-click and select **Edit sample info**. The following dialog displays. Make any edits as needed. Only the fields that are checked update. This allows you to batch edit multiple wells at the same time and only change a specific field.

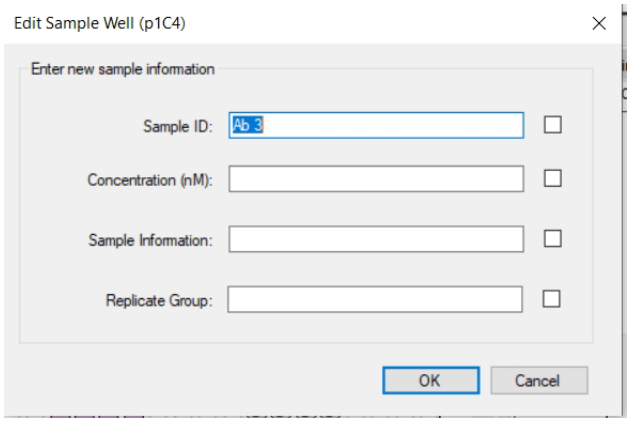


Figure 6-7: Editing sample information for Epitope Binning Cycles

You can also click the cells in the Sample ID columns in the Epitope Binning Cycle table to change this information.

Chapter 7:

Epitope Binning

Epitope Binning Screen Overview

NOTICE: *If you need to change sample IDs or exclude sensors or samples from your analysis, do this in the Preprocess Data screen first before starting your analysis. For details see "Preprocessing Epitope Binning Datasets" on page 123.*

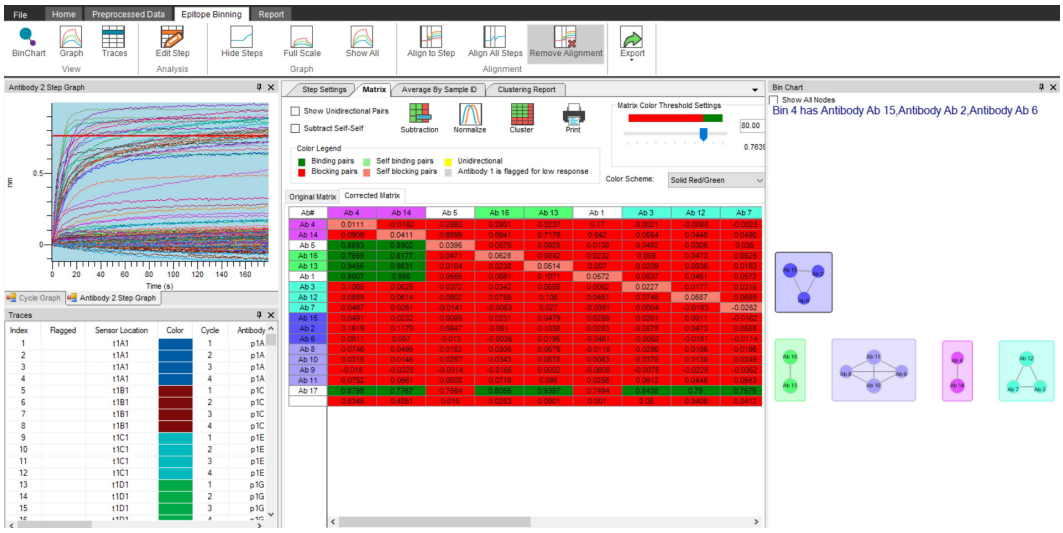


Figure 7-1: Epitope Binning Screen

Epitope Binning Cycle Data

The Cycle Graph displays the sensorgrams for the biosensors in the dataset. You can view individual sensorgrams, overlay multiple sensorgrams and zoom in on specific assay steps. Antibody 2 Step graph displays the current Antibody2 data and the red horizontal line is the threshold value. When the threshold is updated, the line is also updated accordingly (Figure 7-2).

NOTICE: *See "Resizing, Hiding and Closing Windows" on page 11. for information on working with the software windows.*

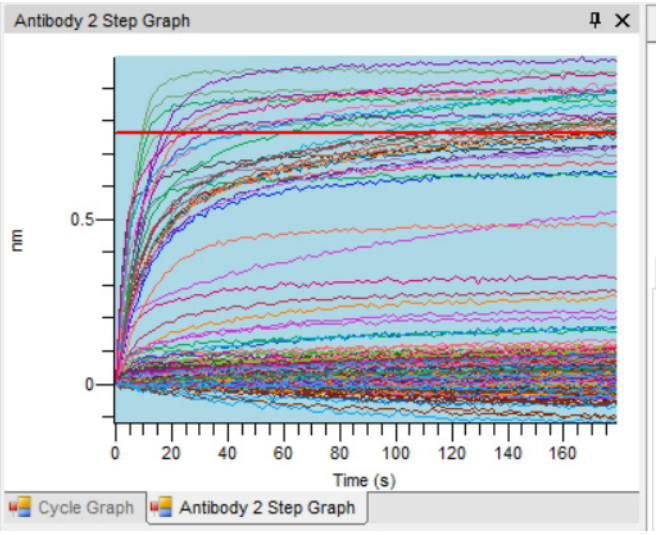


Figure 7-2: Antibody 2 Step Graph

To view individual or overlay multiple sensorgrams, click the **Traces** tab.

- To view one sensorgram, click a biosensor row in the table (Figure 7-3).

Traces

Index	Flagged	Sensor Location	Color	Cycle	Antibody
1		t1A1		1	p1A
2		t1A1		2	p1A
3		t1A1		3	p1A
4		t1A1		4	p1A
5		t1B1		1	p1C
6		t1B1		2	p1C
7		t1B1		3	p1C
8		t1B1		4	p1C

Figure 7-3: Viewing Individual Sensorgrams

- To overlay multiple sensorgrams, press and hold **Ctrl**, then select the biosensor rows in the table you want to overlay (Figure 7-4).

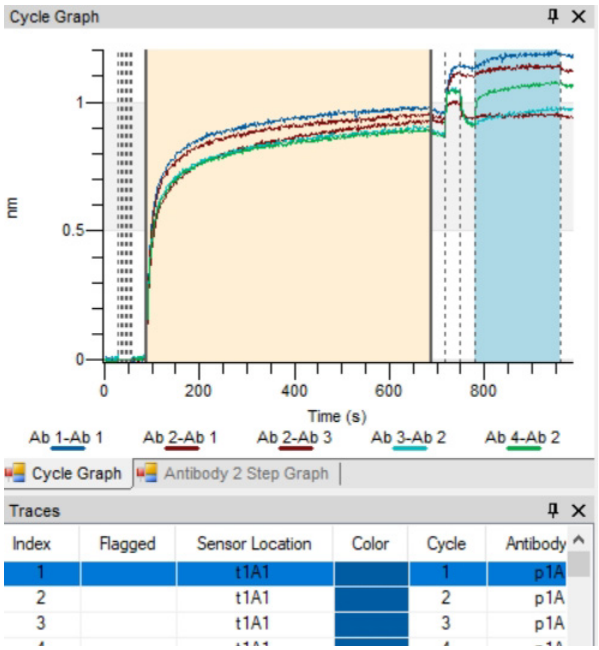


Figure 7-4: Overlaying Multiple Sensorgrams

Click (**Show All**) to view all sensorgrams in the dataset again.

Click (**Hide Step Dividers**) or (**Show-Steps-Dividers**) to hide or show step dividers.

Click (**Align To Step**) to align traces to start of currently selected step.

Click (**Remove Alignment**) to remove the trace alignment and return to the default view.

Click (**Align All Steps**) to align traces to the beginning of each step.

To make the sensorgram traces thicker, right-click the graph and select **Thicken Line**.

To zoom in on a specific area, right-click the graph and select **Zoom** (Figure 7-5), or press **CTRL** and click.

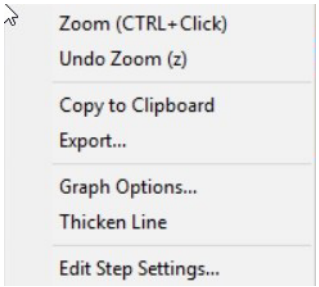


Figure 7-5: Selecting Zoom

Draw a box around the area you want to zoom in on with your mouse (Figure 7-6):

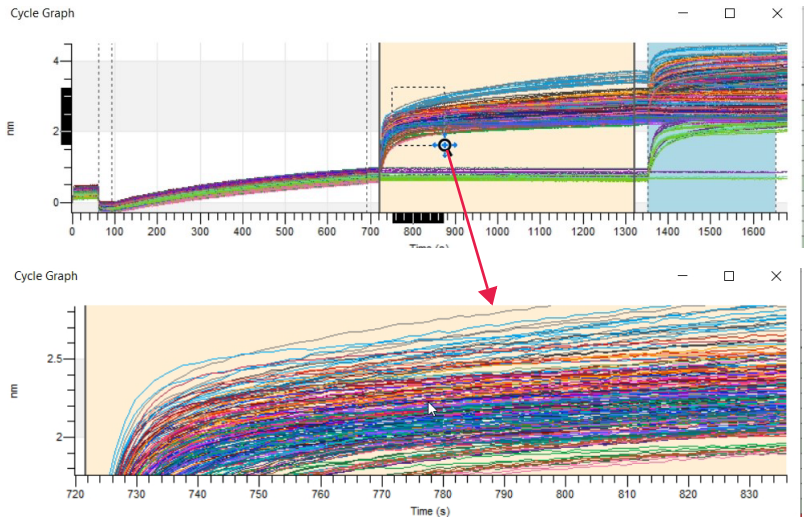


Figure 7-6: Selecting a Zoom Area

Right-click the Epitope Binning Cycle Data area and select **Undo Zoom** or press **Z** on your keyboard to return to the full view.

Step Settings Tab

This tab lets you set and select Antibody 1 and Antibody 2 assay steps to build step settings and calculate parameters for the epitope binning matrix. It also allows you to flag problematic traces when adding new steps or editing existing ones (Figure 7-7).

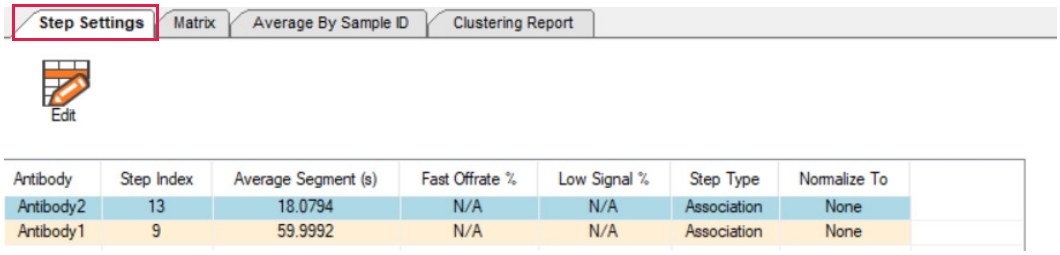
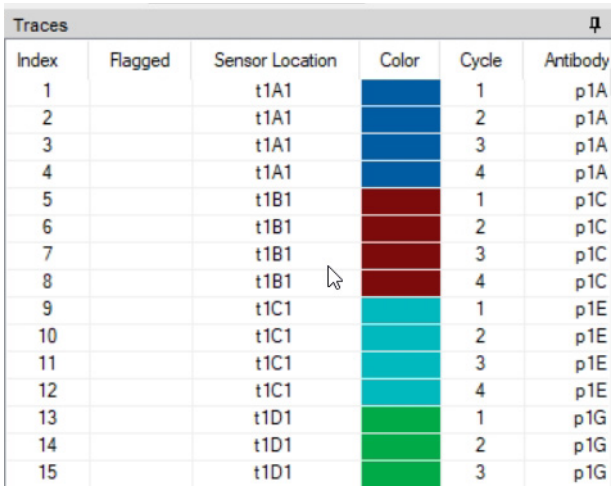


Figure 7-7: Step Settings Tab

Traces Tab

This tab contains individual cycle data for each sensor. If any step in the cycle for a biosensor has a flag, it displays in the Flagged column in the table (Figure 7-8). To view the details on the type and location (step number) of the flag, scroll to the right across the row. See "Step 1: Review Step Settings" on page 142 and "Step 2: Check for Flagged Traces" on page 143 for more information.



Traces

Index	Flagged	Sensor Location	Color	Cycle	Antibody
1		t1A1		1	p1A
2		t1A1		2	p1A
3		t1A1		3	p1A
4		t1A1		4	p1A
5		t1B1		1	p1C
6		t1B1		2	p1C
7		t1B1		3	p1C
8		t1B1		4	p1C
9		t1C1		1	p1E
10		t1C1		2	p1E
11		t1C1		3	p1E
12		t1C1		4	p1E
13		t1D1		1	p1G
14		t1D1		2	p1G
15		t1D1		3	p1G

Figure 7-8: Traces Tab

Table Display Options

Right-click a populated table row or column to display the menu (Figure 7-9).

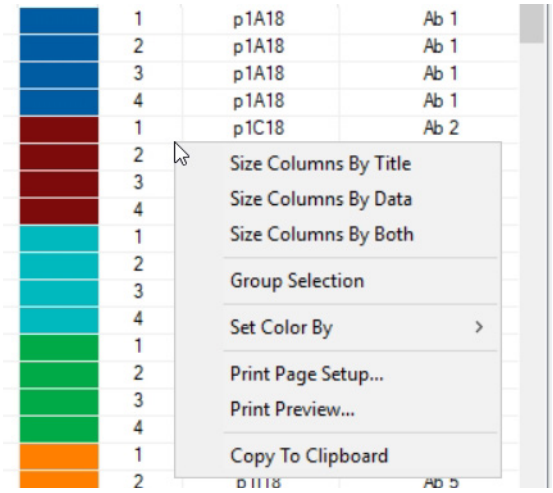


Figure 7-9: Traces Display Menu

- **Size Columns by Title**—Sets all column widths to fit the column titles.
- **Size Columns by Data**—Sets all column widths to fit the data.
- **Size Columns by Both**—Sets all column widths to best fit both the column titles and the data.
- **Group Selection**—Lets you manually group selected rows together and move them to the top of the table.
- **Set Color By** – Sets trace colors for epitope binning cycles.
- **Print Page Setup**—Lets you adjust the print settings (before printing the table).
- **Print Preview**—Shows a print preview of the table. You can modify how many pages it prints to, and then print the table (Figure 7-10):

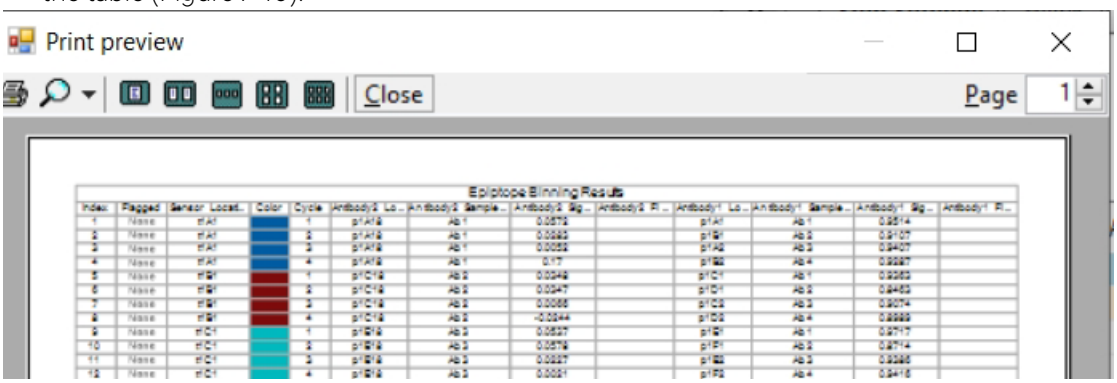


Figure 7-10: Print Preview

- **Copy to Clipboard**—Copies the table to the clipboard so you can paste it into other programs (Microsoft® Excel®, Microsoft Word®, electronic notebooks, etc.).

Changing the Table View

You can sort data in the table alphabetically or numerically by clicking the column header.

To resize the table columns, roll the cursor over the border between the column headers until the resizing cursor displays (Figure 7-11). Then just click and drag the column to resize.

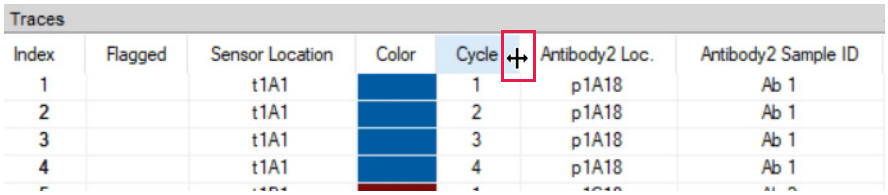


Figure 7-11: Column Resize Cursor

Matrix Tab

The matrix displays the nm shift, or normalized data, of the Second Antibody competitively binding to the antigen in the presence of the First Antibody. In the 2-D matrix (Figure 7-12):

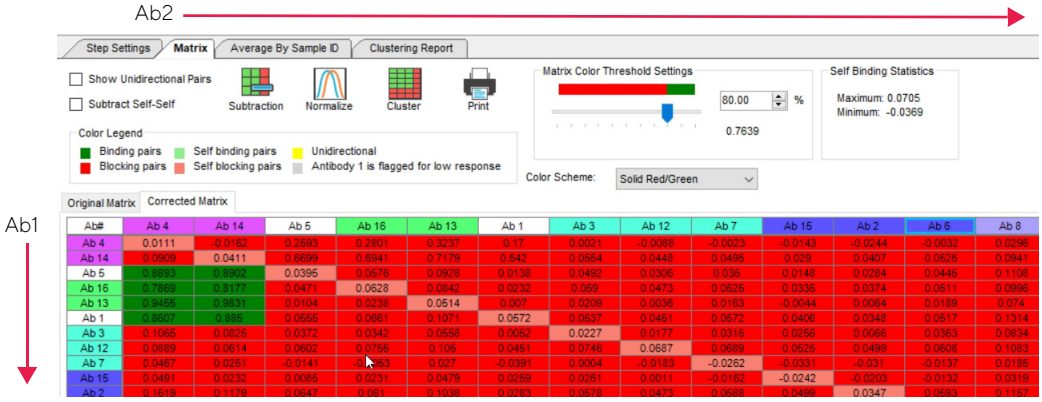


Figure 7-12: Matrix Tab

- Antibody 1 is shown in the first column on the left
- Antibody 2 is shown across the top row

In the Matrix tab, you can:

- Generate a heat map showing blocking and sandwiching antibody interactions
- Cluster antibodies into bins using custom algorithms
- Normalize data to selected controls
- Highlight unidirectional antibody pairs

For more information on matrix analysis, see “Step 3: Determine Threshold Settings” on page 144.

Matrix Display Options

Right-click the matrix to display the menu (Figure 7-13).

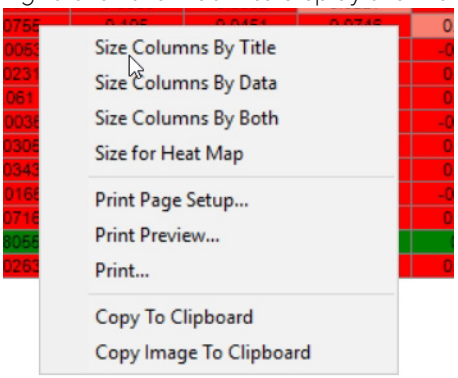


Figure 7-13: Matrix Display Menu

- **Size Columns by Title**—Sets all column widths to fit the column titles.
- **Size Columns by Data**—Sets all column widths to fit the data.
- **Size for Heat Map**—Resizes the matrix into a full-window heat map for easier viewing (Figure 7-14).

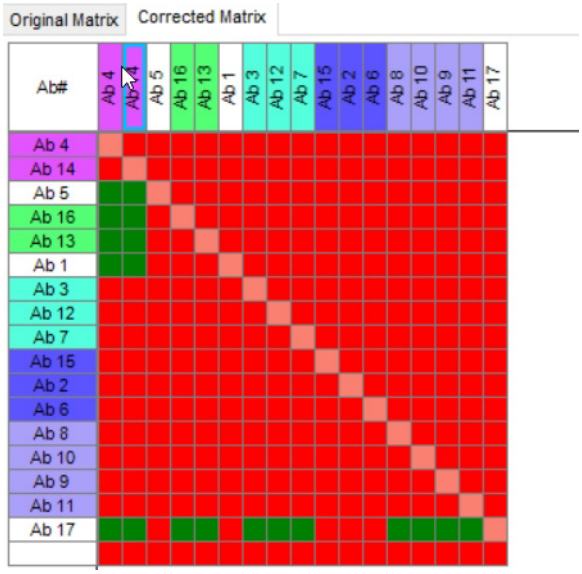


Figure 7-14: Heat Map

- **Print Page Setup**—Lets you adjust the print settings (before printing the matrix).
- **Print Preview**—Shows a print preview of the matrix. You can modify how many pages it prints to, and then print the matrix.
- **Print**—Lets you print the matrix.
- **Copy to Clipboard**—Copies the matrix to the clipboard so you can paste it into other programs (Microsoft® Excel®, Microsoft Word®, electronic notebooks, etc.).

Color Scheme Selection

The matrix can be displayed in a variety of color schemes.

- **Solid Red/Green** - Responses below the threshold (blocking behavior) are shown in red. Responses above the threshold (non-blocking behavior) are shown in green.
- **Gradient Red/Green** - Responses are shaded according to their distance from the threshold value. Responses at exactly the threshold value are shown in white. Responses below the threshold (blocking behavior) are shaded in red, with solid red used for the response furthest from the threshold. Responses above the threshold (non-blocking behavior) are shaded in green, with solid green indicating the maximum response above the threshold.
- **Solid Orange/Blue** - An alternative solid color scheme. Responses below the threshold are shown in orange, and responses above the threshold are shown in blue.
- **Gradient Orange/Blue** - An alternative gradient color scheme similar to the gradient red/green scheme. Responses below the threshold are shaded in orange, and responses above the threshold are shaded in blue.
- **No Color** - Displays the matrix in black text on a white background.

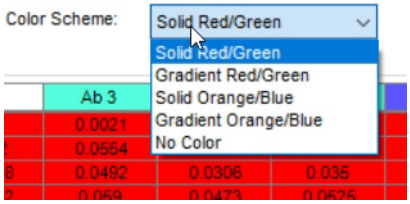
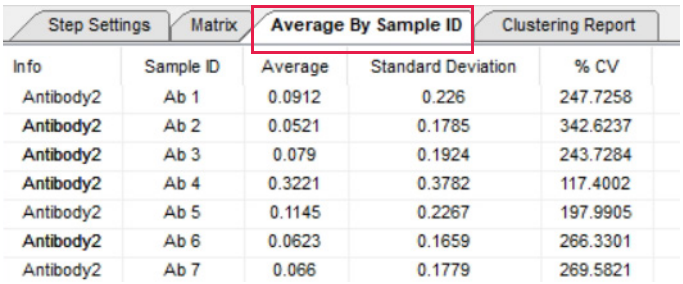


Figure 7-15: Color Scheme Selection

Average by Sample ID Tab

This tab shows the average nm shift, standard deviation and coefficient of variation (% CV) for each sample ID (Antibody, in this case) across different sensors for each step. (Figure 7-16). For more information on using this tab, see “Step 4: Review Data Quality” on page 152.



Info	Sample ID	Average	Standard Deviation	% CV
Antibody2	Ab 1	0.0912	0.226	247.7258
Antibody2	Ab 2	0.0521	0.1785	342.6237
Antibody2	Ab 3	0.079	0.1924	243.7284
Antibody2	Ab 4	0.3221	0.3782	117.4002
Antibody2	Ab 5	0.1145	0.2267	197.9905
Antibody2	Ab 6	0.0623	0.1659	266.3301
Antibody2	Ab 7	0.066	0.1779	269.5821

Figure 7-16: Average by Sample ID Tab

Table Display Options

Display options here are the same as the Traces table. See “Table Display Options” on page 135 for more details.

Changing the Table View

Display options here are also the same as the Traces table. See “Changing the Table View” on page 135 for more details.

BinChart

The BinChart is a graphical display of the information contained in the Matrix. Antibodies with the same blocking pattern group together as a cluster of nodes. The cluster colors in the BinChart synchronize with the matrix heading colors for easy identification.

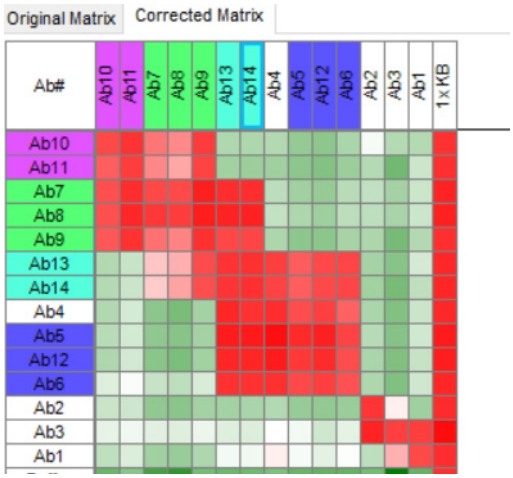


Figure 7-17: Matrix with clusters identified by color

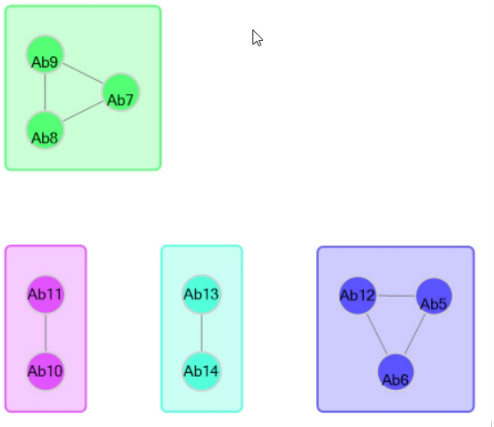


Figure 7-18: BinChart representation of the matrix from Figure 7-17

By default, the BinChart only displays antibodies that belong to a cluster. To display all antibodies, select the **Show All Nodes** option. Antibodies outside of a cluster display as single nodes with no connections.

Clustering Report tab

The clustering report tab provides a table of the clusters identified on the Matrix and in the BinChart. Each cluster is assigned a Bin ID. The antibodies in the cluster and the cluster color are provided. The clustering table can be included in an Excel report, or a custom Report as defined in the Report tab.

Bin ID	AB #	Color	Unidirectional
1	Ab10		No
1	Ab11		No
2	Ab7		No
2	Ab8		No
2	Ab9		No
3	Ab13		No
3	Ab14		No
4	Ab5		No
4	Ab12		No
4	Ab6		No

Figure 7-19: Clustering report for the matrix from Figure 7-17

Analyzing Your Binning Data

Step 1: Review Step Settings

Review that the settings for each assigned step in your dataset is correct. If you need to change which steps are marked as Antibody 1 or Antibody 2, return to the Preprocessing tab and make corrections there.

1. Double click the Step Settings table. The Edit Step Settings box displays (Figure 7-20).

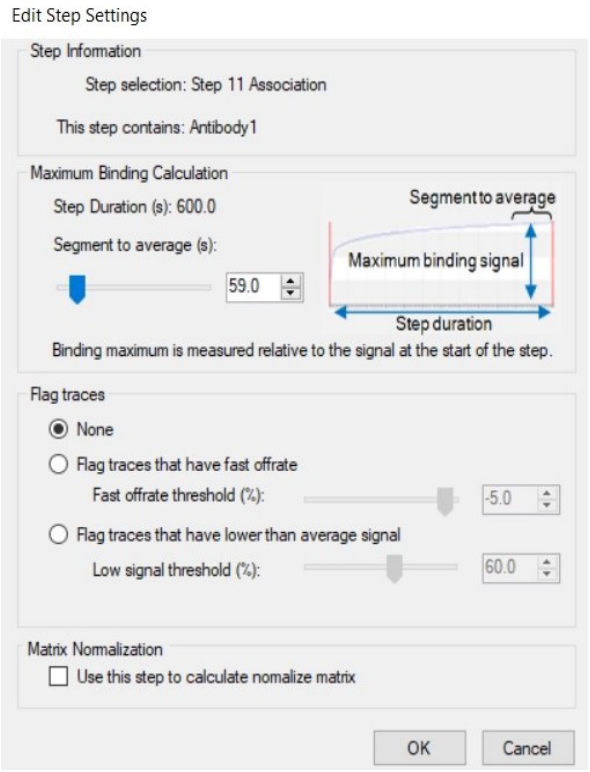


Figure 7-20: Edit Step Settings

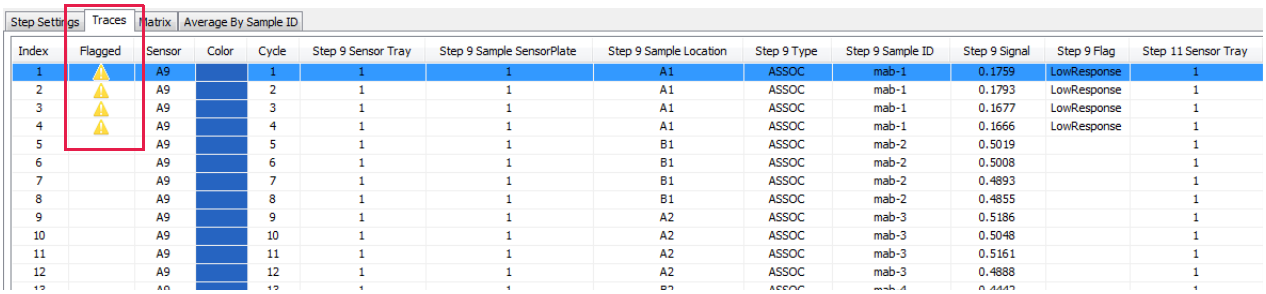
2. Change the following settings as needed:
   - **Maximum Binding Calculation** — Determines the segment used to average and calculate the maximum binding signal (nm shift) relative to the signal at the start of the step. The default selection is using the last 10% of the step duration to calculate the binding maximum nm shift. If the binding signal changes significantly towards the end of step, we recommend changing the maximum binding calculation to only include the last 5-7% of the step duration.
   - **Flag traces, none** — Default setting.
   - **Flag traces that have fast offrate** — This setting flags traces that have a drop in amplitude (negative change) relative to the first data point of the step in the Traces table. This is most relevant for baseline and dissociation steps.
   - **Flag traces that have lower than average signal** — This setting flags traces that have a lower than average signal relative to the other traces in the selected step in the Traces table. This is most relevant for loading and association steps.

- **Matrix Normalization** – Select **Use this step to normalize the matrix** box to normalize the second antibody step data. This is used in cases when the same Antibody 1 and antigen are loaded on column(s) of biosensors, and then an array of Antibody 2s are associated. The Antibody 2 responses can be normalized to minimize any effects from variations in Antibody 1 or antigen loading between the biosensors.

3. Click **OK**.
4. Repeat these steps to modify step settings for other antibody steps as needed.

Step 2: Check for Flagged Traces

1. Click the **Traces** tab.
2. If you set flags in Step 2, scroll through the table to check for flag icons in the Flagged column for any steps in the cycle for a biosensor (Figure 7-21).



Index	Flagged	Sensor	Color	Cycle	Step 9 Sensor Tray	Step 9 Sample SensorPlate	Step 9 Sample Location	Step 9 Type	Step 9 Sample ID	Step 9 Signal	Step 9 Flag	Step 11 Sensor Tray
1	⚠	A9		1	1	1	A1	ASSOC	mab-1	0.1759	LowResponse	1
2	⚠	A9		2	1	1	A1	ASSOC	mab-1	0.1793	LowResponse	1
3	⚠	A9		3	1	1	A1	ASSOC	mab-1	0.1677	LowResponse	1
4	⚠	A9		4	1	1	A1	ASSOC	mab-1	0.1666	LowResponse	1
5		A9		5	1	1	B1	ASSOC	mab-2	0.5019		1
6		A9		6	1	1	B1	ASSOC	mab-2	0.5008		1
7		A9		7	1	1	B1	ASSOC	mab-2	0.4893		1
8		A9		8	1	1	B1	ASSOC	mab-2	0.4855		1
9		A9		9	1	1	A2	ASSOC	mab-3	0.5186		1
10		A9		10	1	1	A2	ASSOC	mab-3	0.5048		1
11		A9		11	1	1	A2	ASSOC	mab-3	0.5161		1
12		A9		12	1	1	A2	ASSOC	mab-3	0.4888		1

Figure 7-21: Flagged Traces

3. If flags are present, scroll to the right across the row to view details on the type (low response or fast off-rate) and location (step number) of the flag.

NOTICE: *If the Antibody 1 step is configured to show the low response flag, this flag also displays in the Matrix tab.*

Step 3: Determine Threshold Settings

Set a threshold to discriminate between Antibody 2s that are blocked or not blocked (those that bind to antigen) by Ab1 by color-coding them in bright red and dark green, respectively.

1. Click the **Matrix** tab. In the 2-D matrix, Antibody 1 is shown in the first column on the left, and Antibody 2 is shown across the top row (Figure 7-22).

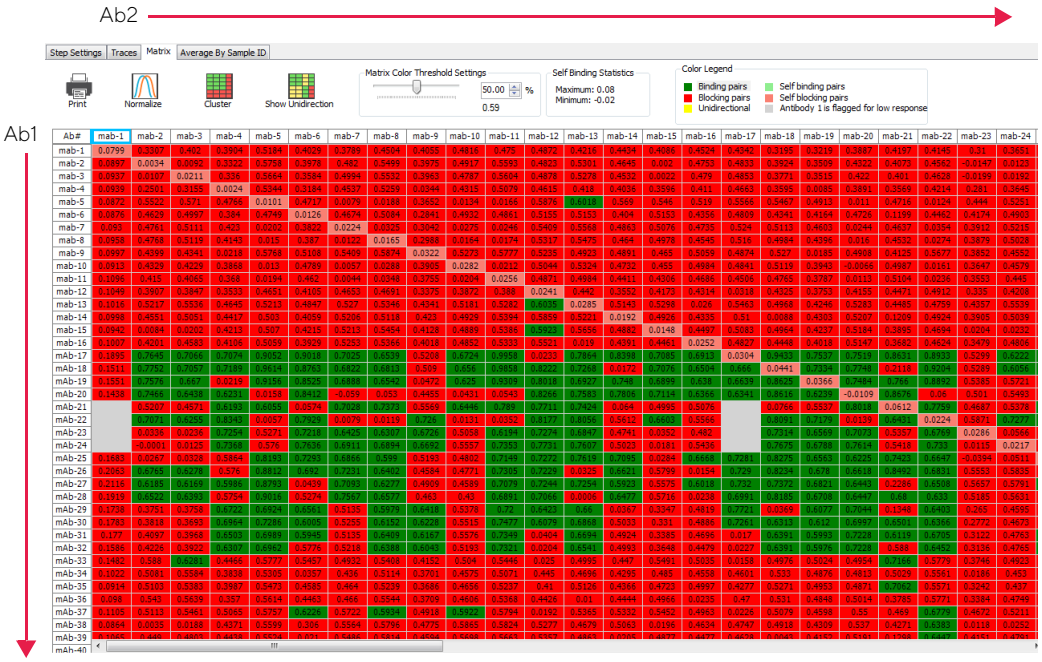


Figure 7-22: Matrix Tab

2. For all binning assay formats, the threshold is generally set as the highest self-binding signal in the panel, which is displayed as the **Maximum** value in the Self Binding Statistics box (Figure 7-23). Self-binding signal is referred to the Antibody 2 binding signal (nm shift) when the same antibody is presented as Antibody 1 and Antibody 2.

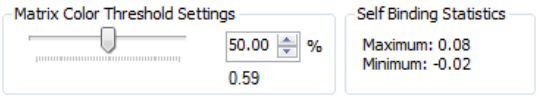


Figure 7-23: Matrix Color Threshold Settings

Change the threshold settings by moving the slider left or right of 50% in the Matrix Color Threshold Settings box (Figure 7-23). This changes how the matrix is colored (more green or red, Figure 7-24).

NOTICE: *Assays should be optimized to ensure all immobilized antibodies (classical sandwich and premix binning formats) are still able to bind to the antigen, self binding signals for all antibodies are low, and any additional Antibody 2 binding signal is antigen-dependent. If these criteria are satisfied, then the self binding signal threshold is appropriate*

.

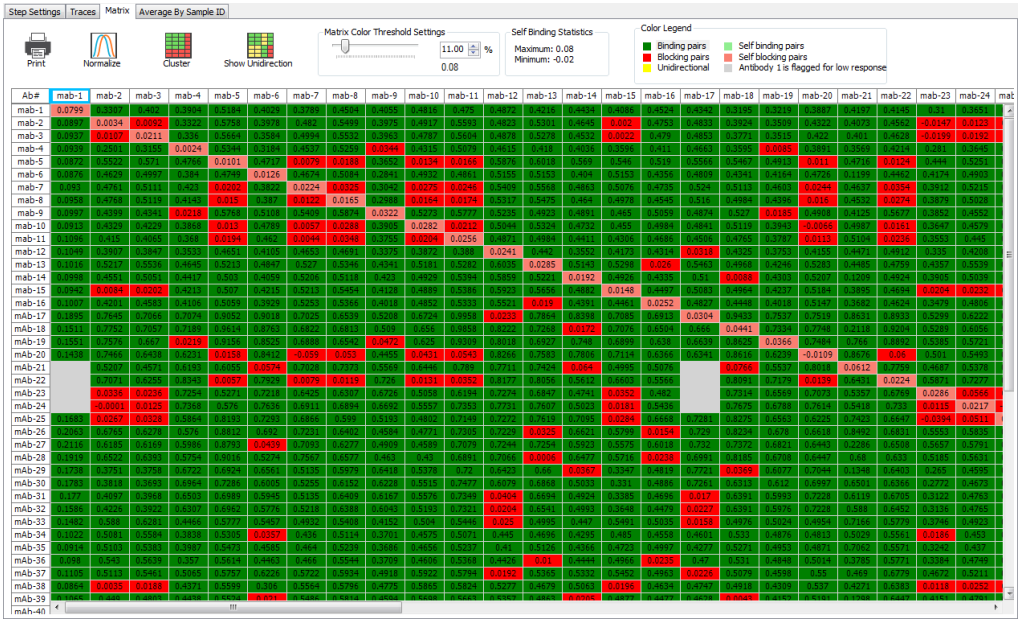


Figure 7-24: Matrix Color Threshold Settings Set to Self Binding Statistics Maximum

Once the threshold is set, the colors in the matrix illustrate binding, blocking, unidirectional binning and flagged antibody pairs as shown in the color legend (Figure 7-25).

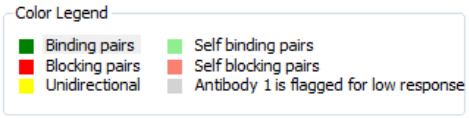


Figure 7-25: Color Legend

Self-binding pairs are shown diagonally in the matrix and are colored pink or light green if they are blocking or binding pairs, respectively.

3. Adjust the matrix as needed using the optional tools described in the next section.

Matrix Viewing Options

Viewing the Heat Map

Right click the matrix and select **Size for Heat Map** to resize the matrix into full-window heat map for easier viewing (Figure 7-26).

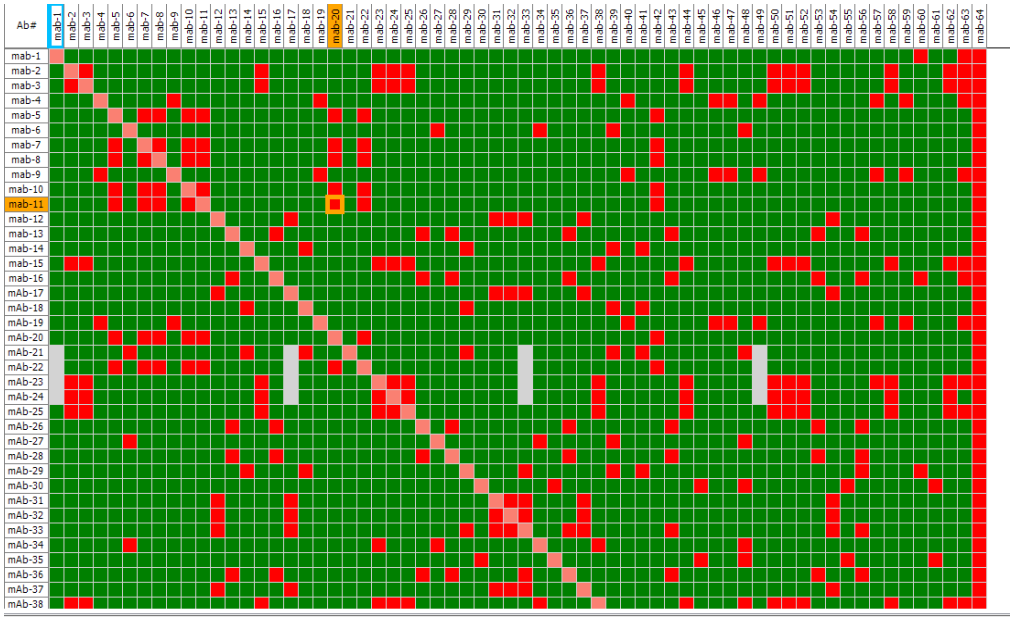


Figure 7-26: Heat Map

Showing Unidirectional Binning Pairs

Blocking and binding pairs are colored bright red and dark green, respectively, to clearly distinguish and identify the epitope bins. But in some cases, mAb pairs may appear as blocking in one orientation and binding in the other. For example in Figure 7-27, when mAb-1 is presented as Antibody 1 and mAb-2 is presented as Antibody 2, it appears that mAb-2 is able to bind to the antigen in the presence of Antibody 1 and is colored dark green in the matrix. However, when the orientation is switched (when mAb-2 is presented as Antibody 1 and mAb-1 is presented as Antibody 2), mAb-2 is not able to bind to the antigen in the presence of mAb-1 and is colored bright red in the matrix (Figure 7-28).

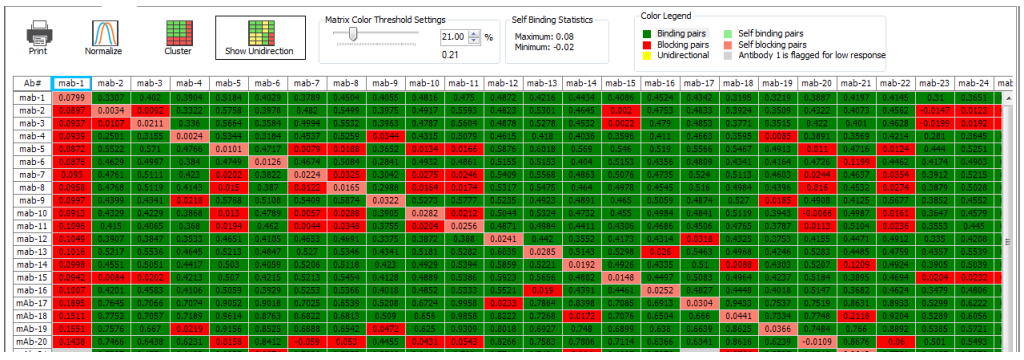


Figure 7-27: mAb Pairs Blocking and Binding

To highlight unidirectional binning pairs, click **Show Unidirectional Pairs.**

Unidirectional binning pairs are highlighted in yellow (Figure 7-28).

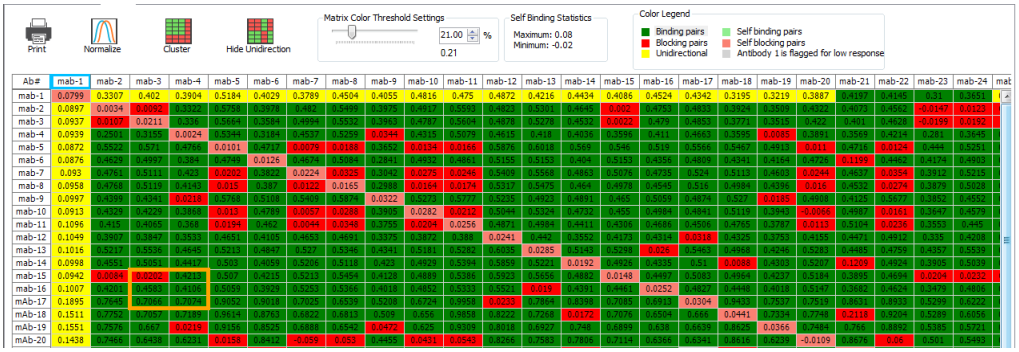


Figure 7-28: Show Unidirectional Pairs

Deselect the option to use the regular matrix color scheme for unidirectional pairs.

Adding Antibody 1 Low Response Flags

You can flag any mAbs, when presented as Antibody 1, that do not bind to the biosensor (in classical or pre-mix binning formats) or to the antigen (in-tandem binning format) as low responders. When this happens, it often means that any Antibody 2 data associated with that biosensor, for example when the entire row is ambiguous and should be re-evaluated.

To flag low responders:

1. Click the **Step Settings** tab.
2. Select the **Antibody 1** step in the table.

3. Click **Edit**. The Edit Step Setting box displays (Figure 7-29):

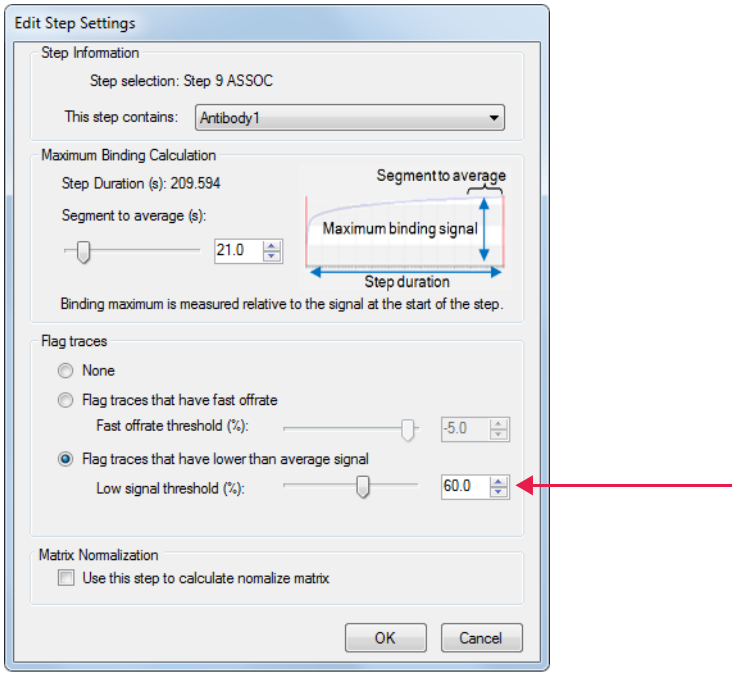


Figure 7-29: Edit Step Settings

4. Click **Flag traces that have lower than average signal** (60% of average signal or 60% of the average nm shift values for all traces for this step) and set a low signal threshold.
5. Click **OK**.
6. Click the **Matrix** tab. Low responders display in gray in the matrix (Figure 7-30).

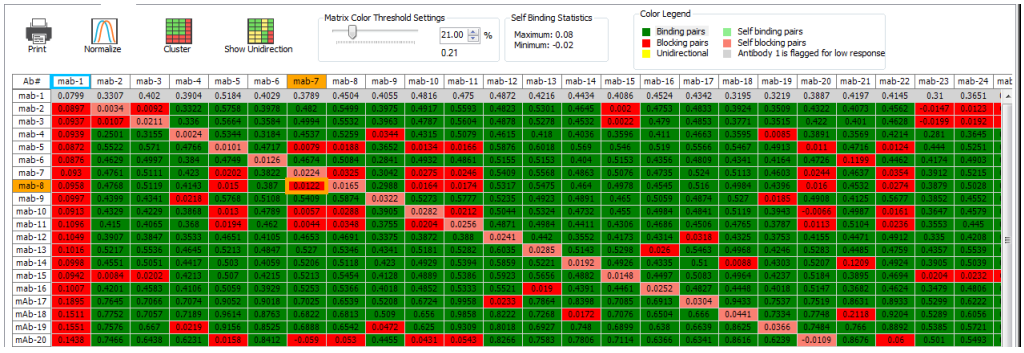
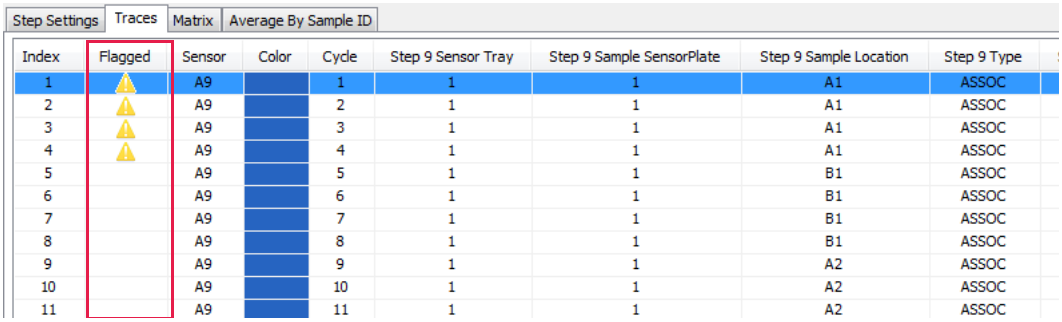


Figure 7-30: Low Responses Flagged for Antibody 1 in Matrix Tab

Biosensors with low responses are also flagged in the Traces tab (Figure 7-31).



Index	Flagged	Sensor	Color	Cycle	Step 9 Sensor Tray	Step 9 Sample SensorPlate	Step 9 Sample Location	Step 9 Type
1	⚠	A9		1	1	1	A1	ASSOC
2	⚠	A9		2	1	1	A1	ASSOC
3	⚠	A9		3	1	1	A1	ASSOC
4	⚠	A9		4	1	1	A1	ASSOC
5		A9		5	1	1	B1	ASSOC
6		A9		6	1	1	B1	ASSOC
7		A9		7	1	1	B1	ASSOC
8		A9		8	1	1	B1	ASSOC
9		A9		9	1	1	A2	ASSOC
10		A9		10	1	1	A2	ASSOC
11		A9		11	1	1	A2	ASSOC

Figure 7-31: Low Responses Flagged for Antibody 1 in Traces Tab

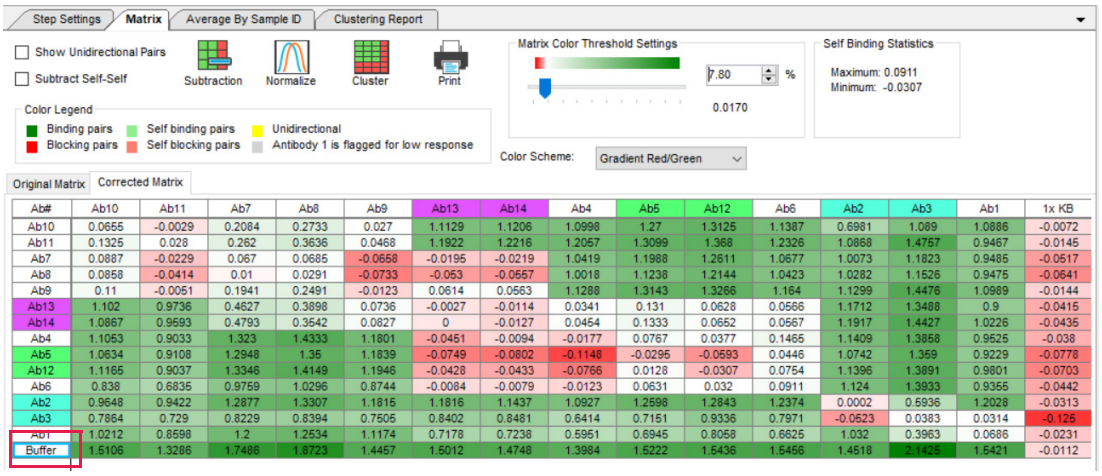
Normalizing the Matrix

Data in the matrix can be normalized by row or column based on the maximal binding signal of Antibody 2s, especially when the distinction between binding and blocking pairs is unclear. This function is most relevant for in-tandem binning datasets when a control is run where all Antibody 2s are exposed to the antigen in the absence of Antibody 1.

Normalization is performed by dividing each Antibody 2 binding signal by the Ab2-only control signal (buffer as Ab1/no Ab1) to make a clear distinction between Ab2s that are or are not blocked by Ab1.

To normalize the matrix:

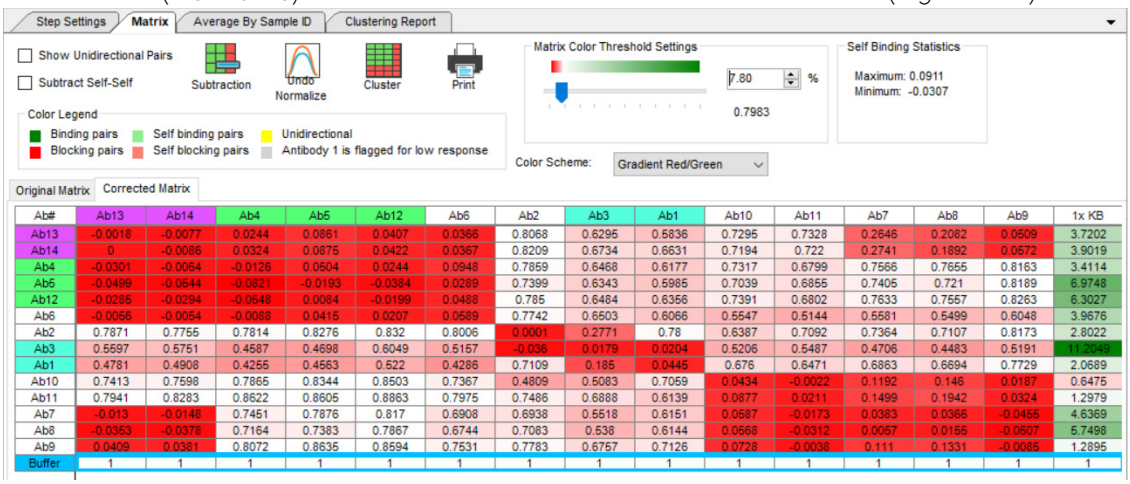
1. Select the row or column to normalize against. In the example in Figure 7-32, the selected row is in the blue box, and it is the last row.



Ab#	Ab10	Ab11	Ab7	Ab8	Ab9	Ab13	Ab14	Ab4	Ab5	Ab12	Ab6	Ab2	Ab3	Ab1	1x KB
Ab10	0.0655	-0.0029	0.2084	0.2733	0.027	1.1129	1.1206	1.0998	1.27	1.3125	1.1387	0.6981	1.089	1.0886	-0.0072
Ab11	0.1325	0.028	0.262	0.3636	0.0468	1.1922	1.2216	1.2057	1.3099	1.368	1.2326	1.0868	1.4757	0.9467	-0.0145
Ab7	0.0887	-0.0229	0.067	0.0685	-0.0658	-0.0195	-0.0219	1.0419	1.1988	1.2611	1.0677	1.0073	1.1823	0.9485	-0.0517
Ab8	0.0858	-0.0414	0.01	0.0291	-0.0733	-0.053	-0.0557	1.0018	1.1238	1.2144	1.0423	1.0282	1.1526	0.9475	-0.0641
Ab9	0.11	-0.0051	0.1941	0.2491	-0.0123	0.0614	0.0563	1.1288	1.3143	1.3266	1.164	1.1299	1.4476	1.0989	-0.0144
Ab13	1.102	0.9736	0.4627	0.3898	0.0736	-0.0027	-0.0114	0.0341	0.131	0.0628	0.0566	1.1712	1.3488	0.9	-0.0415
Ab14	1.0867	0.9593	0.4793	0.3542	0.0827	0	-0.0127	0.0454	0.1333	0.0652	0.0567	1.1917	1.4427	1.0226	-0.0435
Ab4	1.1053	0.9033	1.323	1.4333	1.1801	-0.0451	-0.0094	-0.0177	0.0767	0.0377	0.1465	1.1409	1.3858	0.9525	-0.038
Ab5	1.0634	0.9108	1.2948	1.35	1.1839	-0.0749	-0.0802	-0.1148	-0.0295	-0.0593	0.0446	1.0742	1.359	0.9229	-0.0778
Ab12	1.1165	0.9037	1.3346	1.4149	1.1946	-0.0428	-0.0433	-0.0766	0.0128	-0.0307	0.0754	1.1396	1.3891	0.9801	-0.0703
Ab6	0.838	0.6835	0.9759	1.0296	0.8744	-0.0084	-0.0079	-0.0123	0.0631	0.032	0.0911	1.124	1.3933	0.9355	-0.0442
Ab2	0.9648	0.9422	1.2877	1.3307	1.1815	1.1816	1.1437	1.0927	1.2598	1.2843	1.2374	0.0002	0.5936	1.2028	-0.0313
Ab3	0.7864	0.729	0.8229	0.8394	0.7505	0.8402	0.8481	0.6414	0.7151	0.9336	0.7971	-0.0523	0.0383	0.0314	-0.125
Ab1	1.0212	0.8598	1.2	1.2534	1.1174	0.7178	0.7238	0.5951	0.6945	0.8058	0.6625	1.032	0.3963	0.0686	-0.0231
Buffer	1.5106	1.3286	1.7486	1.8723	1.4457	1.5012	1.4748	1.3984	1.5222	1.5436	1.5456	1.4518	2.1425	1.5421	-0.0112

Figure 7-32: Selecting a Row to Normalize Against

2. Click  (**Normalize**). The data normalize to the row or column selected (Figure 7-33).



Ab#	Ab13	Ab14	Ab4	Ab5	Ab12	Ab6	Ab2	Ab3	Ab1	Ab10	Ab11	Ab7	Ab8	Ab9	1x KB
Ab13	-0.0018	-0.0077	0.0244	0.0861	0.0407	0.0366	0.8068	0.6295	0.5836	0.7295	0.7328	0.2646	0.2082	0.0609	3.7202
Ab14	0	-0.0086	0.0324	0.0875	0.0422	0.0367	0.8209	0.6734	0.6631	0.7194	0.722	0.2741	0.1892	0.0672	3.9019
Ab4	-0.0301	-0.0064	-0.0126	0.0504	0.0244	0.0948	0.7859	0.6468	0.6177	0.7317	0.6799	0.7566	0.7655	0.8163	3.4114
Ab5	-0.0499	-0.0544	-0.0821	-0.0193	-0.0384	0.0289	0.7399	0.6343	0.5985	0.7039	0.6855	0.7405	0.721	0.8189	6.9748
Ab12	-0.0285	-0.0294	-0.0548	0.0084	-0.0199	0.0488	0.785	0.6484	0.6356	0.7391	0.6802	0.7633	0.7557	0.8263	6.3027
Ab6	-0.0056	-0.0054	-0.0088	0.0415	0.0207	0.0589	0.7742	0.6503	0.6066	0.5547	0.5144	0.5581	0.5499	0.6048	3.9676
Ab2	0.7871	0.7755	0.7814	0.8276	0.832	0.8006	0.0001	0.2771	0.78	0.6387	0.7092	0.7364	0.7107	0.8173	2.8022
Ab3	0.5597	0.5751	0.4587	0.4698	0.6049	0.5157	-0.036	0.0179	0.0204	0.5206	0.5487	0.4706	0.4483	0.5191	11.2049
Ab1	0.4781	0.4908	0.4255	0.4563	0.522	0.4286	0.7109	0.185	0.0445	0.676	0.6471	0.6863	0.6694	0.7729	2.0689
Ab10	0.7413	0.7598	0.7865	0.8344	0.8503	0.7367	0.4809	0.5083	0.7059	0.0434	-0.0022	0.1192	0.146	0.0187	0.6475
Ab11	0.7941	0.8283	0.8622	0.8605	0.8863	0.7975	0.7486	0.6888	0.6139	0.0877	0.0211	0.1499	0.1942	0.0324	1.2979
Ab7	-0.013	-0.0148	0.7451	0.7876	0.817	0.6908	0.6938	0.5518	0.6151	0.0587	-0.0173	0.0383	0.0366	-0.0455	4.6369
Ab8	-0.0353	-0.0378	0.7164	0.7383	0.7867	0.6744	0.7083	0.538	0.6144	0.0568	-0.0312	0.0057	0.0155	-0.0507	5.7498
Ab9	0.0409	0.0381	0.8072	0.8635	0.8594	0.7531	0.7783	0.6757	0.7126	0.0728	-0.0038	0.111	0.1331	-0.0085	1.2895
Buffer	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Figure 7-33: Normalized Data

To remove the normalization, click  (**Undo Normalize**).

Cluster Analysis

Clustering finds groups of antibodies such that the antibodies within one group are similar (or related) to one another and different from (or unrelated to) the antibody in other groups. In the software, grouping is primarily performed using hierarchical clustering where an appropriate metric (a measure of distance between pairs of observations), and a linkage criterion (measure of dissimilarity of sets as a function of the pairwise distances of observations in the sets) needs to be selected.

To change clustering settings:

1. Click  (**Cluster**). The Hierarchical Clustering settings box appears (Figure 7-34).

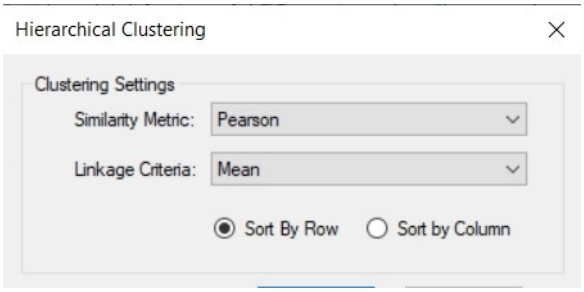


Figure 7-34: Hierarchical Clustering Settings

2. Select a Similarity Metric. Commonly used similar metrics between two sets of observations A and B are available (Figure 7-35). Euclidean or Pearson and more details for all clustering formulas are summarized in “Hierarchical Clustering Formulas” on page 155. The choice of an appropriate metric influences the shape of the clusters.

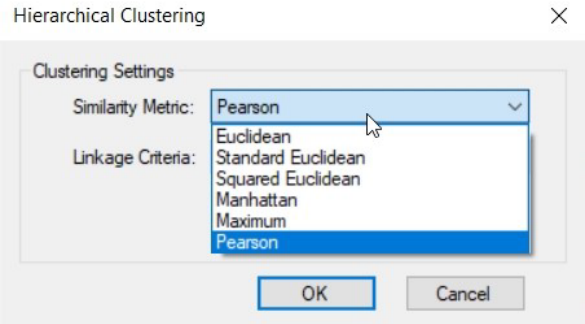


Figure 7-35: Similarity Metric Options

3. Select Linkage Criteria. Linkage criterion determines the distance between sets of observations as a function of the pairwise distances between observations. Some commonly used linkage criteria between two sets of observations A and B are available (Figure 7-36) and are summarized in “Hierarchical Clustering Formulas” on page 155.

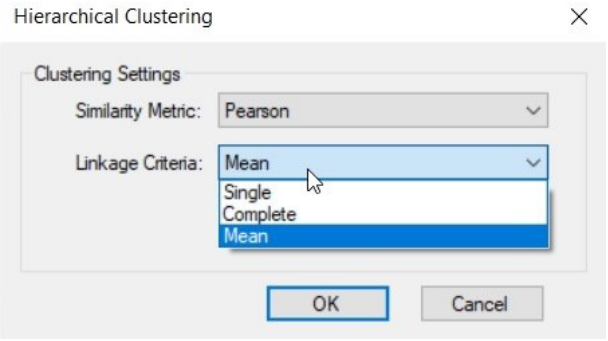


Figure 7-36: Linkage Criteria Options

4. Select **Sort by Row** or **Sort by Column** (Figure 7-34). Click **OK**. The matrix cluster per the settings you select (Figure 7-37).

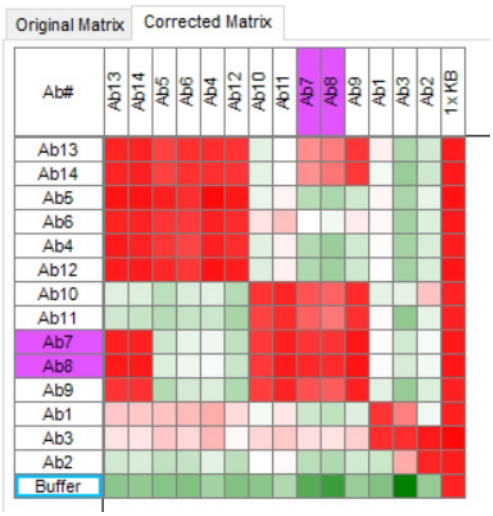


Figure 7-37: Matrix Clustered Using Maximum, Mean and Sort by Row

Step 4: Review Data Quality

Click the **Average by Sample ID** tab to review data quality by looking at loading levels of antigen and each sample ID per step. The table contains the nm shift, standard deviation and coefficient of variation (% CV) for each sample ID (Antibody) across different sensors for each step. For example, in the in-tandem 64x64 dataset shown, mAb-10 was loaded 64 times as saturating mAb (Ab 1) on antigen-bound biosensors. The average loading signal of mAb-3 was 0.5452 nm and the %CV across 64 different associations was low at 5%.

Clicking on a row in the table shows all the traces associated with that sample ID (Figure 7-38).

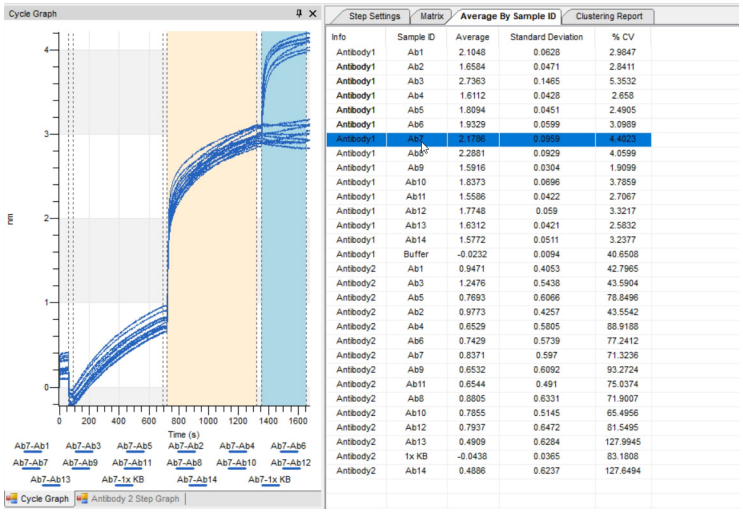
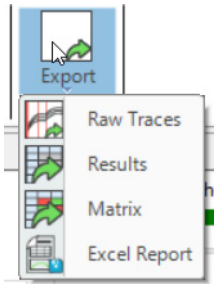


Figure 7-38: Average by Sample ID Tab

Step 5: Export Data (Optional)

To export data from the Epitope Binning Screen:

Select an option from the **Export** menu.



- Click **Raw Traces** to export the raw sensorgrams in .csv format.
- Click **Results** to export results in the Traces table and the Average By Sample ID table in .csv format.
- Click **Matrix** to export the matrix in .csv format.
- Click **Excel** to export into Excel format and the following dialog opens:

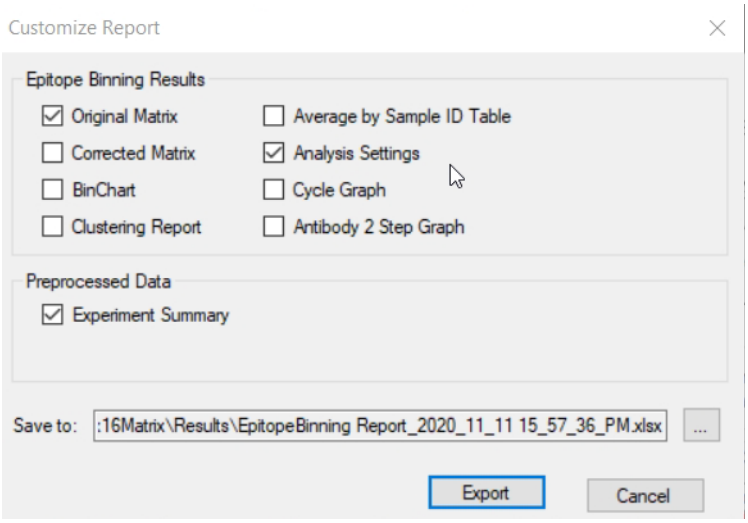


Figure 7-39: Excel Report Options

To export the sensorgrams, right-click the Epitope Binning Cycle Data graph and select **Export** (Figure 7-40).

Chose a format (EMF, BMP or JPG), an export destination (Clipboard, save to File, or Printer), set print size parameters if needed then click **Export**.

All sensorgrams, selected sensorgrams, sensorgrams zoomed in to a selected area, sensorgrams aligned to a selected step or aligned to all steps can be exported using this feature.

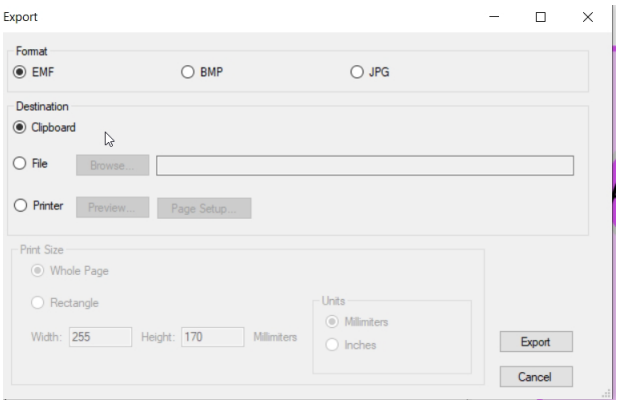


Figure 7-40: Export Dialog Box

Positioning the BinChart

The BinChart panel can be rearranged on screen. Click and drag the grey border at the top of the window (Figure 7-41).

Figure 7-41: Bin Chart Dialog

The BinChart can be dragged off of the main screen and placed on a second monitor if needed. Click the **X** to close the BinChart window.

Printing Matrix Data

Click (**Print**). Select the printer name and set properties to print the matrix directly, or use Print Preview (Figure 7-42) to view and modify pages before printing.

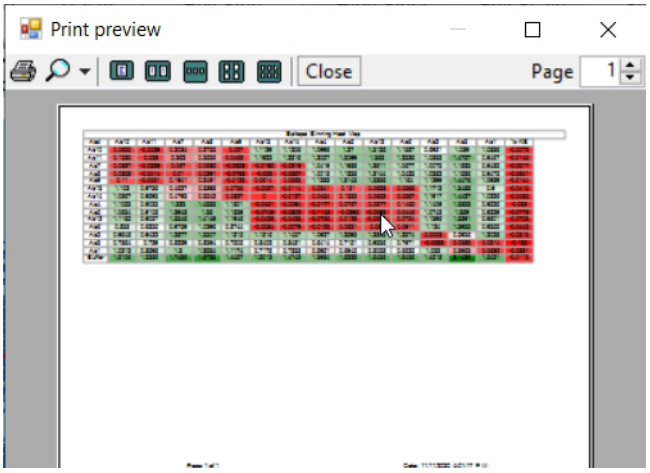


Figure 7-42: Matrix Print Preview

Hierarchical Clustering Formulas

The hierarchical clustering algorithm is a popular clustering algorithm used in data mining. Descriptions and examples can be found online, for example, an introduction is given in Wikipedia at http://en.wikipedia.org/wiki/Hierarchical_clustering

The algorithm puts data points (or vectors) into multi-dimensional space in clusters according to the closeness of:

1. One data point to the other or
2. One data point to a group of data points or
3. One group of data points to the other group of data points.

The closeness, distance, or similarity of two data points is given by so-called Metric functions, while the closeness of two groups is given by so-called Linkage functions.

Metric Functions

Six metric functions are used for the distance between n-dimensional vectors:

$$\vec{x} = (x_1,\ x_2, \ldots, x_n), \text{ and vector } \vec{y} = (y_1,\ y_2, \ldots, y_n)$$

1. Euclidean distance:

$$\|\vec{x} - \vec{y}\| = \sqrt{\sum_{1}^{i=n} (x_i - y_i)^2}$$

2. Standard Euclidean. This is different from Euclidean in that weight,

$$\overrightarrow{w} = (w_1,\ w_2, \ldots, w_n)$$

for each dimension is needed to be given:

$$\|\overrightarrow{x} - \overrightarrow{y}\| = \sqrt{\sum_{1}^{i=n} \frac{(x_i - y_{i)^2}}{w_i}}$$

The ith component, the square of standard deviation, serves as a normalization.

3. Squared Euclidean:

$$\|\overrightarrow{x} - \overrightarrow{y}\| = \sum_{1}^{i=n} (x_i - y_i)^2$$

4. Manhattan, or, City Block:

$$\|\overrightarrow{x} - \overrightarrow{y}\| = \sum_{1}^{i=n} |x_i - y_i|$$

$|a|$ is absolute value of a.

5. Maximum:

$$\|\overrightarrow{x} - \overrightarrow{y}\| = \max_{i=1,n} |x_i - y_i|$$

6. Pearson:

$$S_x = \sum_{1}^{i=n} x_i$$

$$S_y = \sum_{1}^{i=n} y_i$$

$$S_{xx} = \sum_{1}^{i=n} x_i x_i$$

$$S_{xy} = \sum_{1}^{i=n} x_i y_i$$

$$S_{yy} = \sum_{1}^{i=n} y_i y_i$$

Pearson distance is defined as:

$$\|\overrightarrow{x} - \overrightarrow{y}\| = 1 - \frac{S_{xy} - \frac{S_x S_y}{n}}{\sqrt{S_{xx} - \frac{S_x S_x}{n}}\sqrt{S_{yy} - \frac{S_y S_{xy}}{n}}}$$

Linkage Functions

There are four Linkage functions: G_1 and G_2 with two groups of vectors or data points. G_1 has m data points, G_2 has n data points, and the G_{1j} j^{th} data point is in the G_1 group.

1. Single. The distance of two groups is defined as the shortest distance between one vector in one group to the other:

$$\|G_1 - G_2\| = \min_{i=1,m;j=1,n} \|G_{1i} - G_{2j}\|$$

2. Complete. Complete is opposite of Single:

$$\|G_1 - G_2\| = \max_{i=1,m;j=1,n} \|G_{1i} - G_{2j}\|$$

3. Mean, or Average:

$$\|G_1 - G_2\| = \frac{1}{m * n} \sum_{=1,m;j=1,n} \|G_{1i} - G_{2j}\|$$

Chapter 8:

Dose Response

Dose Response Analysis Overview

Octet® Analysis Studio software provides easy determination of common dose response metrics such as EC_{50}, IC_{50}, and Hill coefficient to support measurement of drug potencies or activity. In this chapter we will demonstrate how to extract these values and highlight some of the additional features that allow you to analyze less than optimal or unusual data.

In an Octet® BLI dose response experiment, what is typically measured is the binding response 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 saturating higher concentrations, 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 (response vs. log concentration) as shown in Figure 8-1. The mid-point is the Half-maximal effective concentration, or EC_{50}.

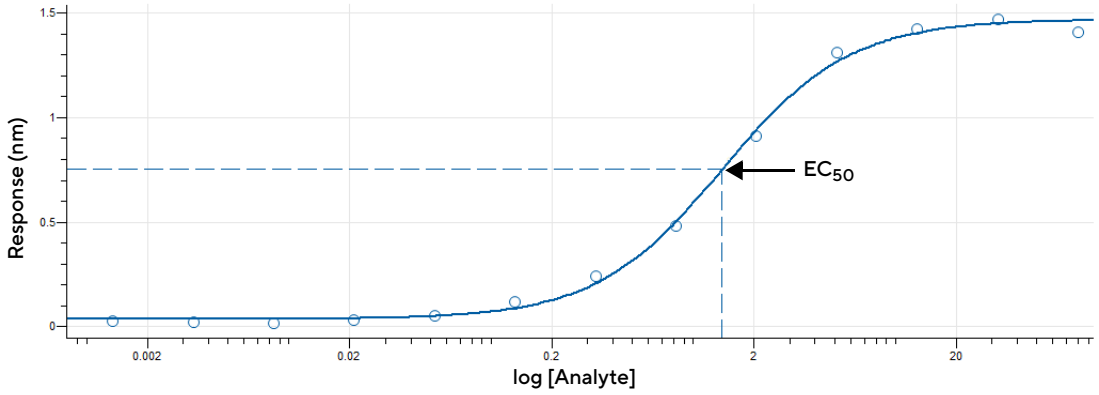


Figure 8-1: Determining EC_{50} from the Dose Response Curve

Similarly, we can design an assay where a second molecule can inhibit or block a binding interaction. Typically, a series dilution of the inhibitor is used where the binding complex is at a fixed concentration. In this case, the mid-point is called the Half maximal inhibitory concentration or IC_{50} (see Figure 8-2).

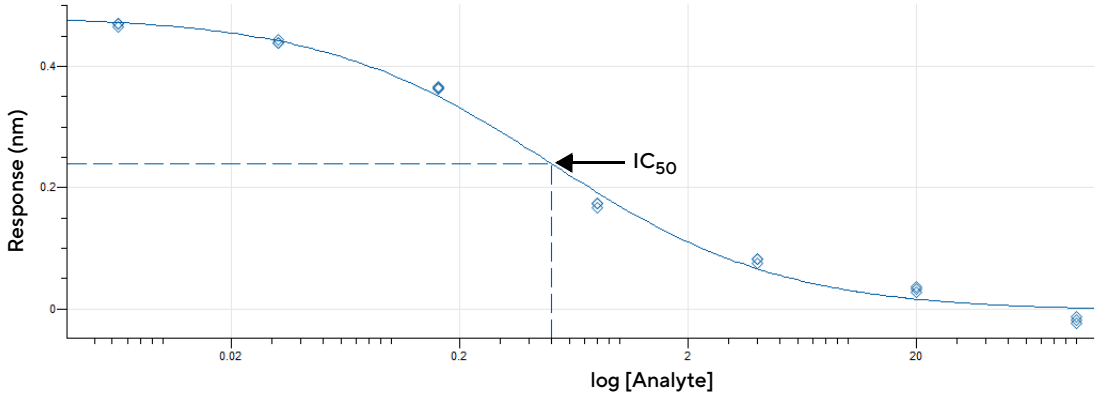


Figure 8-2: IC_{50} Curve

Mathematically, the response curves can be modeled with the Hill equation:

$$y = R_{Bot} + \frac{(R_{Top} - R_{Bottom})}{1 + \left(\frac{x}{A}\right)^{h}}$$

Where:

y and *x* are plotting coordinates

R_{Top} is the upper asymptote

R_{Bottom} is the lower asymptote

A is the x-value at the mid-range concentration inflection point

h is the Hill coefficient

EC_{50} is the mid point concentration between the upper and lower asymptotes

The Hill coefficient can be useful for quality control purposes. Repeated dose response measurements of the same molecules in the same or different lots will yield similar sigmoidal plots but they may vary in the maximal response, E_{max}, or the EC_{50}/IC_{50} values. However, they should all have very similar Hill Coefficients. A pronounce deviation in the Hill coefficient of a sample can indicate degradation of the sample or contamination.

In the following sections we'll demonstrate a dose response analysis by analyzing a simple EC_{50} determination with an anti-HER2 and HER2 (human epidermal growth factor receptor 2) pair and an IC_{50} inhibition assay with competitive binding of anti-hTNFα and TNFα-receptor (tumor necrosis factor alpha) to Biotin-TNFα.

EC_{50} Analysis Using a 2-Step Dose Response Assay

In this experiment, we will be looking at AHC2 biosensors ligated with various concentrations of anti-HER2 and examining the equilibrated binding curve response of HER2 bound to anti-HER2.

In the preview view on the Home screen, this experiment run on an Octet® RH16 instrument uses 16 biosensors and a 384-well sample plate. The assay has four steps:

1. Buffer (pre-sample)
2. Immobilize anti-HER2
3. Buffer
4. Detection with HER2

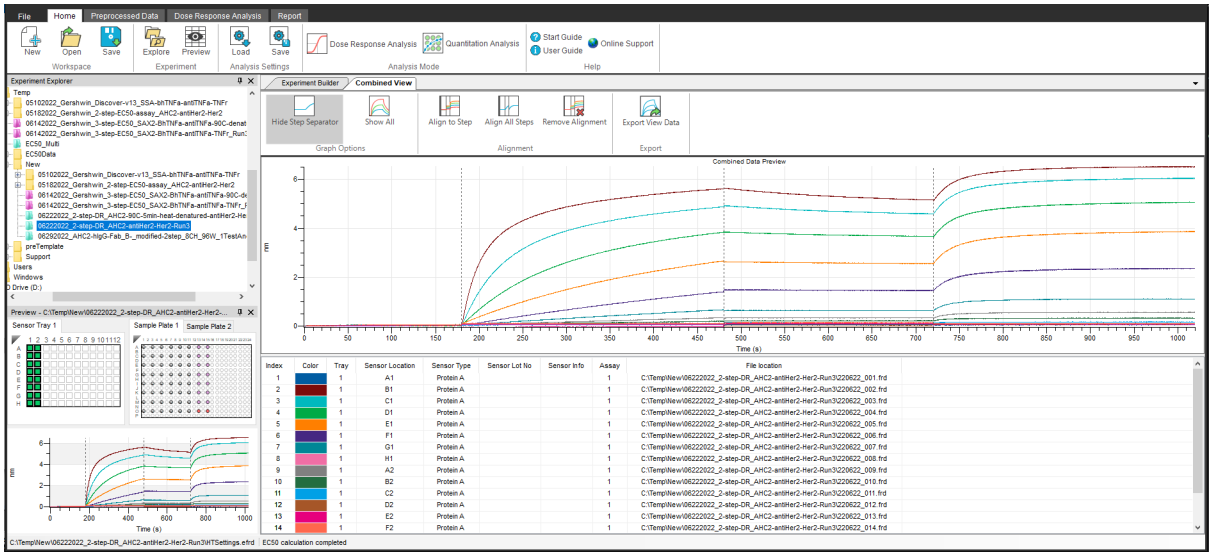


Figure 8-3: Experiment Preview

Preprocessing: 2-Step Assay

Click on the **Preprocessed Data** tab.

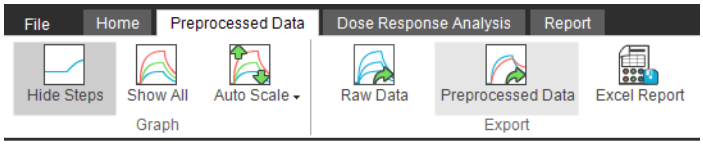


Figure 8-4: Preprocessed Data Tab

In the sample plate there are four columns of buffer in columns 1, 3, 9, and 11. In the first step, biosensors dip into the buffer wells labeled **B**. After immobilizing anti-HER2 onto the biosensor, the biosensors dip into the second set of buffer wells labeled **2** (Figure 8-5).

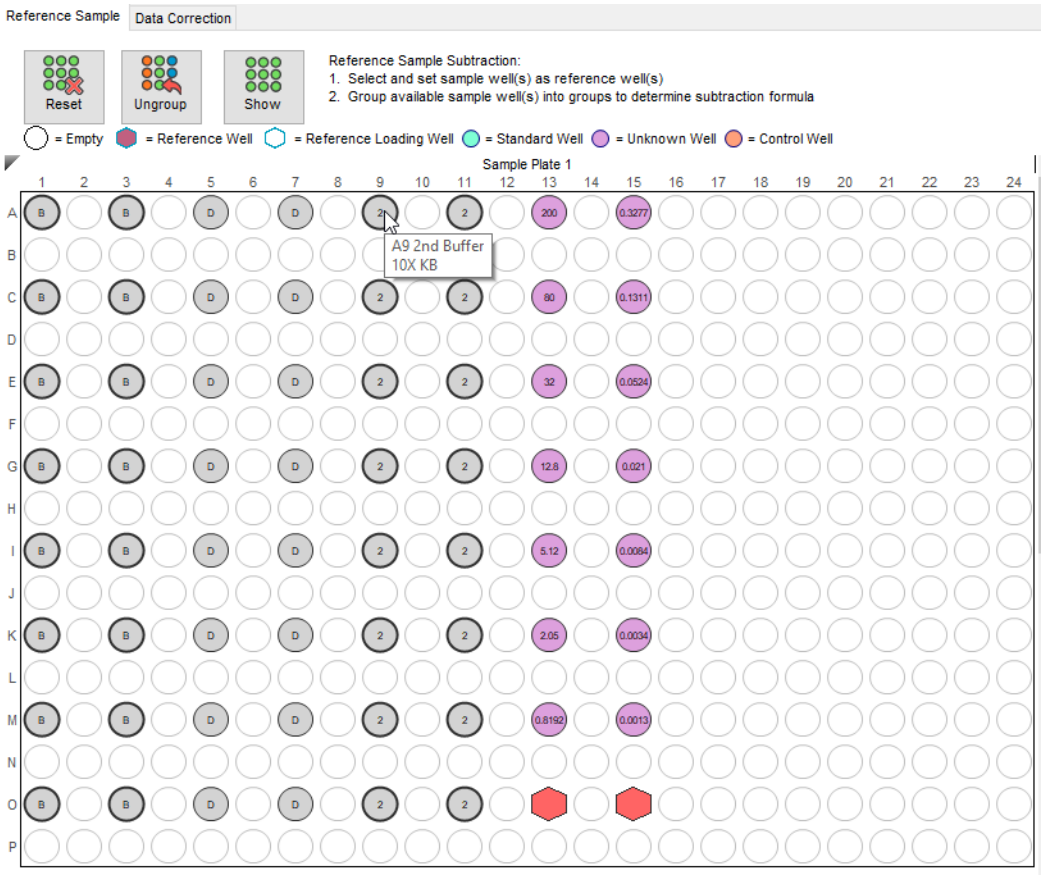


Figure 8-5: Example Sample Plate

In columns 13 and 15 (except for wells O13 and O15), are anti-HER2 in concentrations ranging from 200 µg/mL to 0.0013 µg/mL. As a holdover from the quantitation analysis feature of software, these wells with a series dilution for dose-response can be of the types **Standard**, **Control**, or **Unknown/Sample** (as shown in Figure 8-6). Only these types of wells should be used as the dose-response algorithm will look for these well types to determine the concentrations to use for the dose response curve fits.

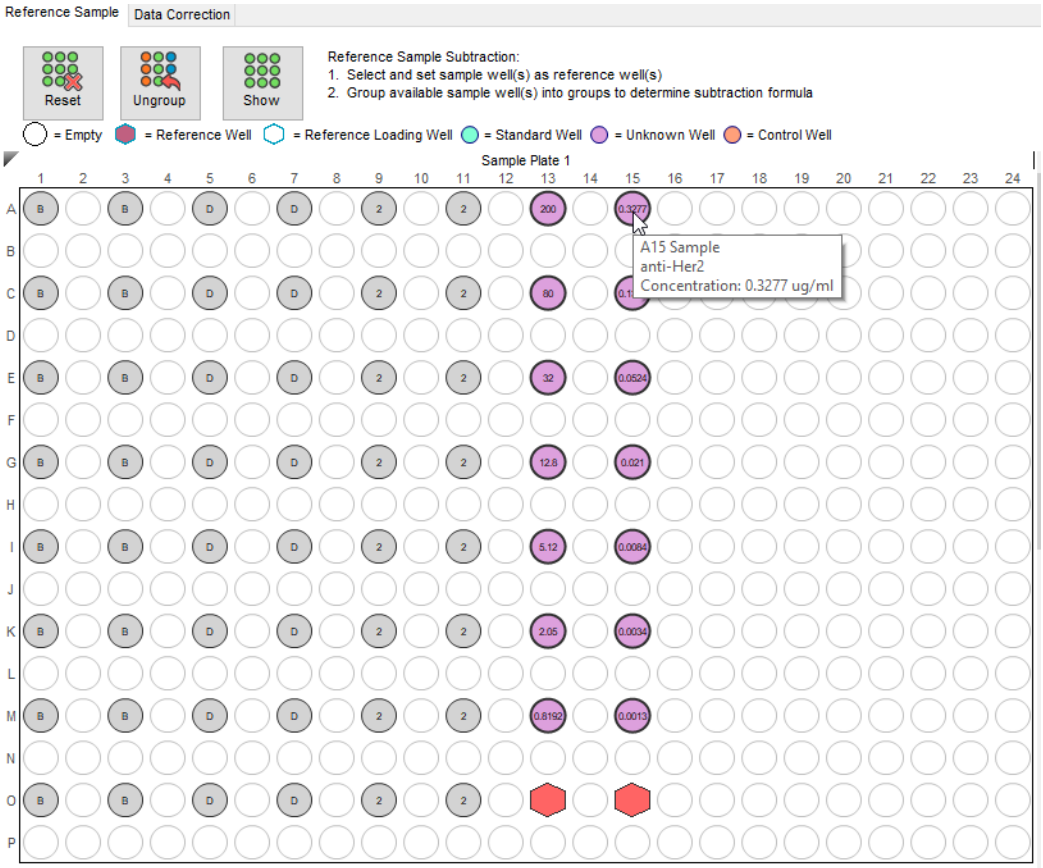


Figure 8-6: Sample Wells

Wells O13 and O15 are reference wells filled with buffer. These will be used for reference subtraction.

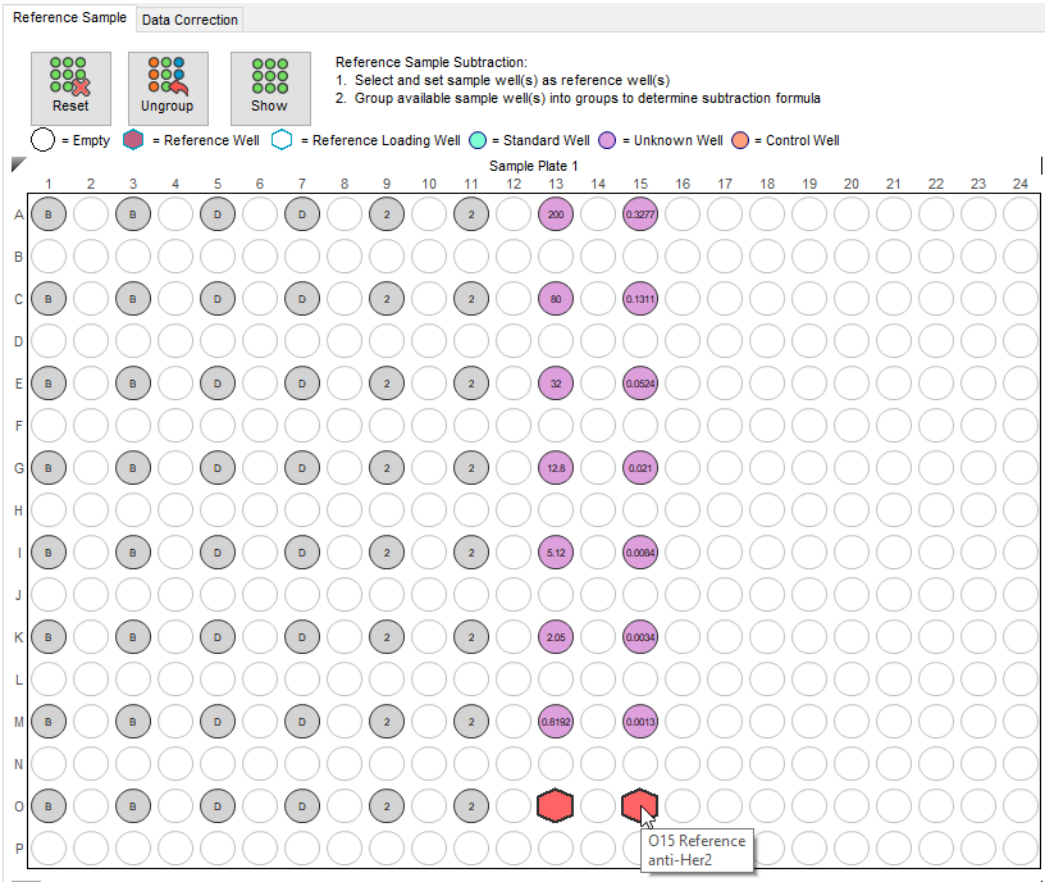


Figure 8-7: Reference Wells

Columns 5 and 7 contain the HER2. All wells have the same concentration of 10 µg/mL. The wells here are labeled **D** for detection as the binding response curve measured in these wells provide the response value in the dose response curve.

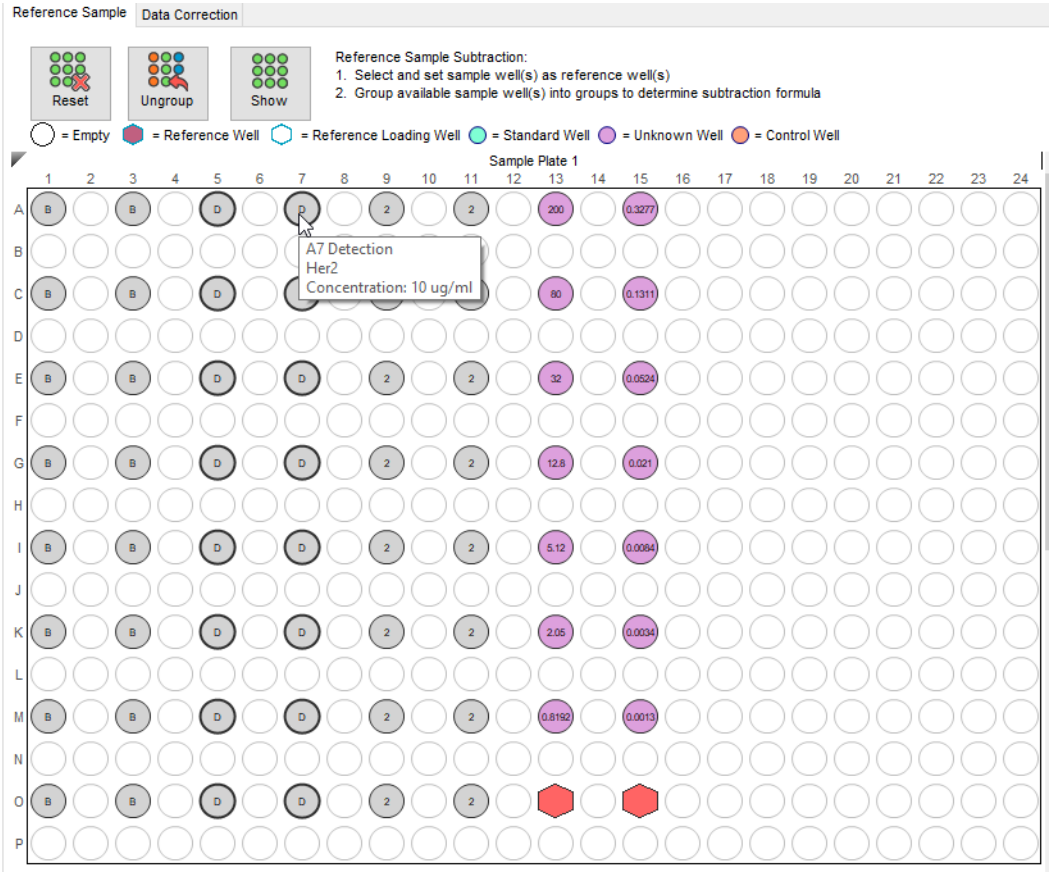


Figure 8-8: Detection Wells

On the right are the binding response curves overlaid by cycles. In this example, four steps of a cycle (buffer, anti-HER2, buffer, HER2) are shown with the sample step highlighted in blue and the detection step in yellow. Clicking on a step will display a title confirming the assignment.

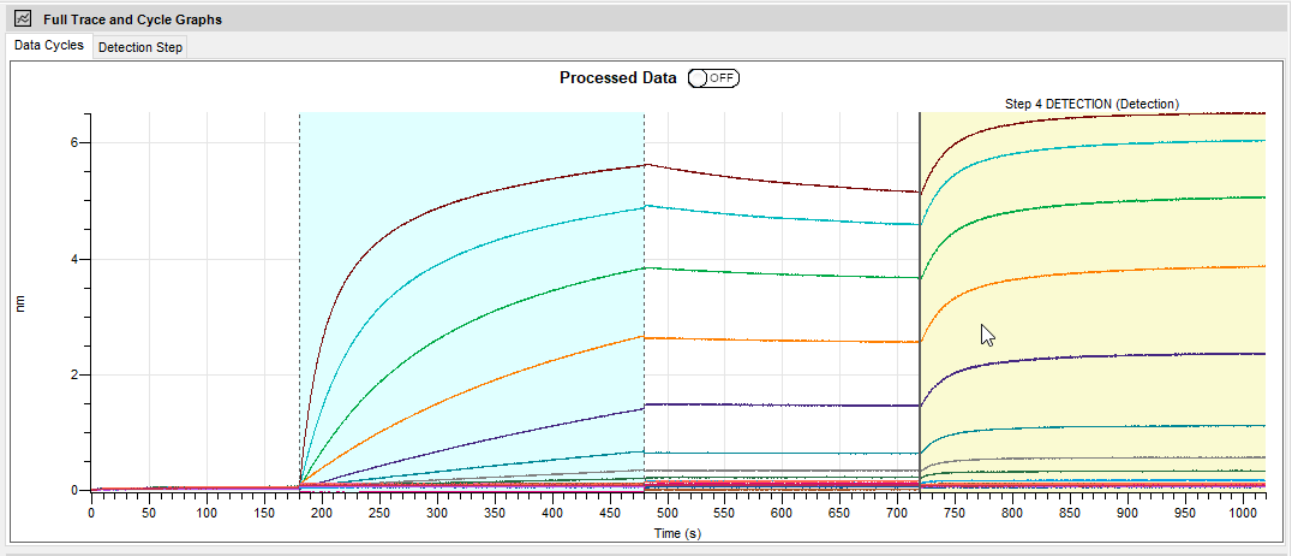


Figure 8-9: Colored Step Assignments

The software should correctly identify these steps, but depending on the experiment you may be required to explicitly assign sample or detection steps. To assign a step type, right click on the step in the plot. Then set the step type assignment from the menu to either **Loading**, **Sample**, or **Detection**.

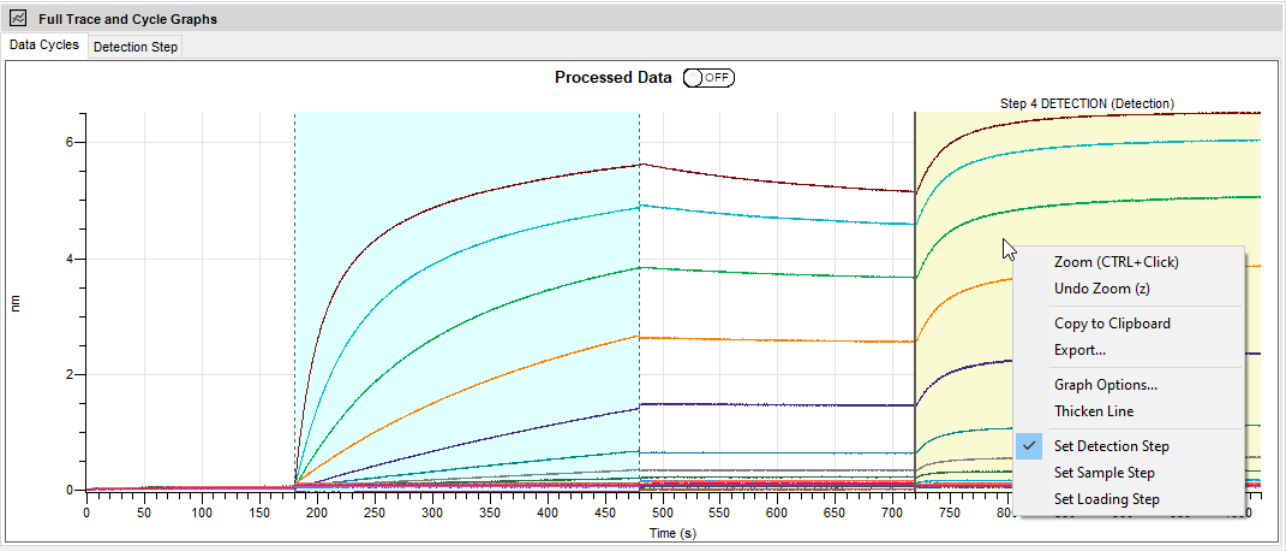


Figure 8-10: Setting Step Assignment

The most important task to perform in Preprocessed Data tab is reference sample and/or reference sensor subtraction. In this experiment, there are no reference sensors but there are two reference wells in the sample plate.

In the sample plate, we want to subtract the signal of row **O** from all the others. To do this, click on reference well **O13** or **O15** to highlight it then right click on the well. **Select Subtract Reference In Column**.

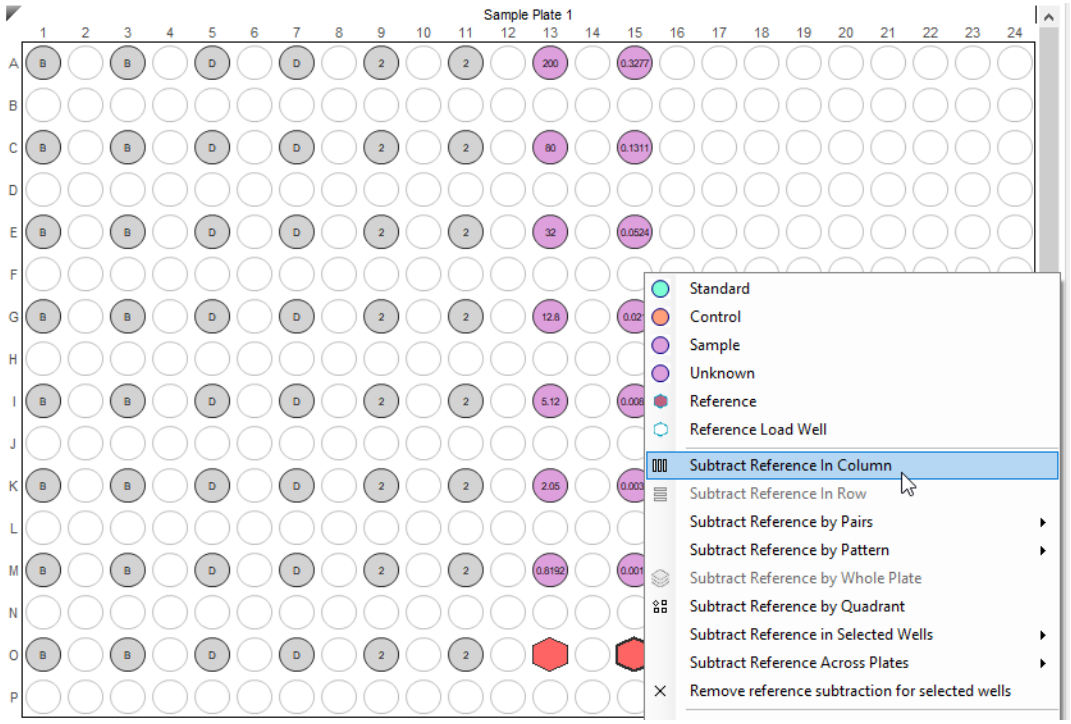


Figure 8-11: Subtracting a Reference Well

After reference subtraction, the plate will look like this:

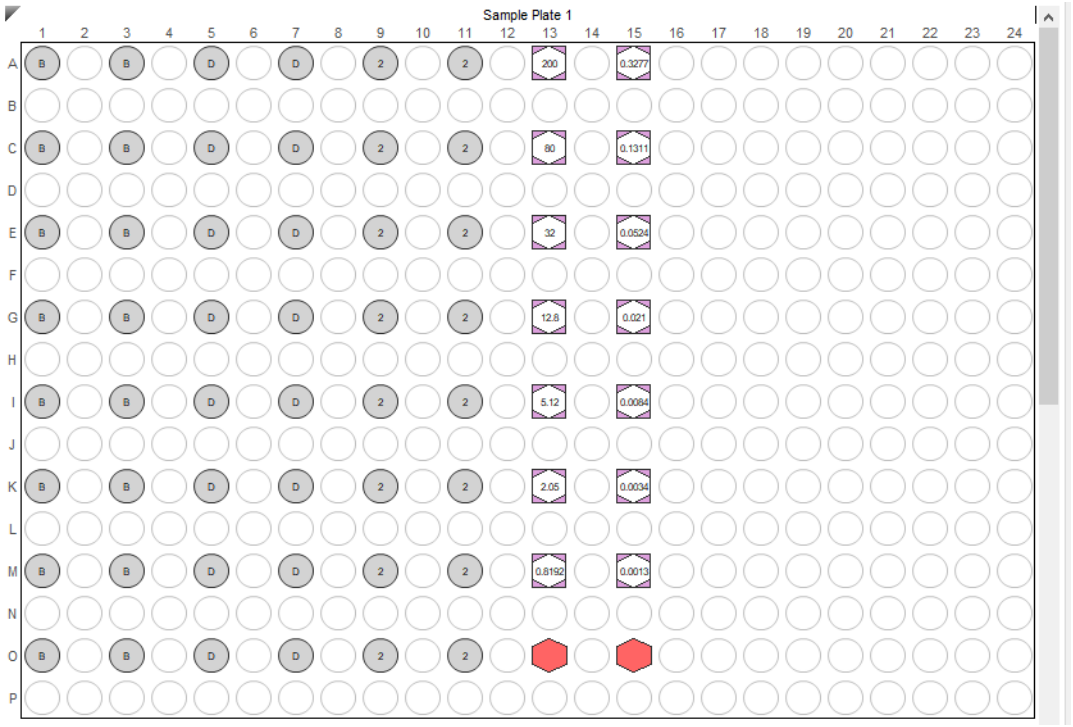
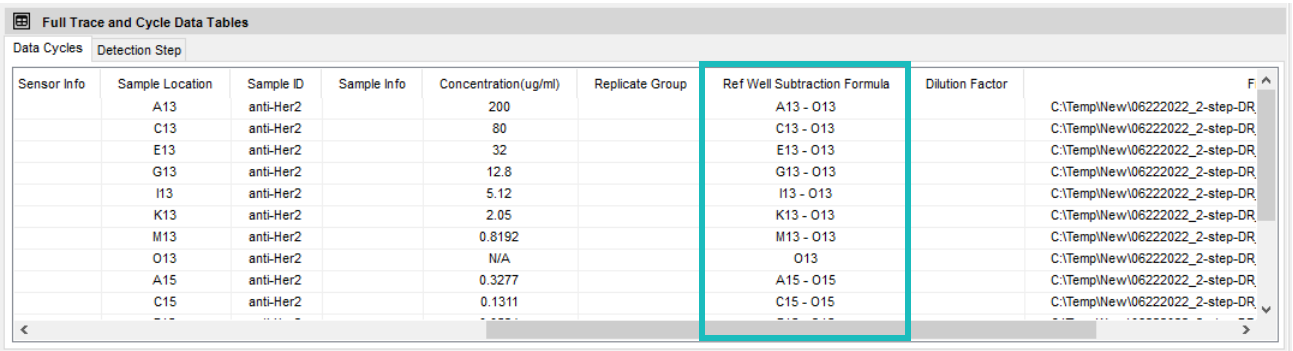


Figure 8-12: Reference Subtraction Applied

The result is that the binding response curve of O13 is subtracted from A13...M13, and O15 is subtracted from A15...M15. This is confirmed in the table data in the Ref Well Subtraction Formula column.



Sensor Info	Sample Location	Sample ID	Sample Info	Concentration(ug/ml)	Replicate Group	Ref Well Subtraction Formula	Dilution Factor	F
	A13	anti-Her2		200		A13 - O13		C:\Temp\New\06222022_2-step-DR
	C13	anti-Her2		80		C13 - O13		C:\Temp\New\06222022_2-step-DR
	E13	anti-Her2		32		E13 - O13		C:\Temp\New\06222022_2-step-DR
	G13	anti-Her2		12.8		G13 - O13		C:\Temp\New\06222022_2-step-DR
	I13	anti-Her2		5.12		I13 - O13		C:\Temp\New\06222022_2-step-DR
	K13	anti-Her2		2.05		K13 - O13		C:\Temp\New\06222022_2-step-DR
	M13	anti-Her2		0.8192		M13 - O13		C:\Temp\New\06222022_2-step-DR
	O13	anti-Her2		N/A		O13		C:\Temp\New\06222022_2-step-DR
	A15	anti-Her2		0.3277		A15 - O15		C:\Temp\New\06222022_2-step-DR
	C15	anti-Her2		0.1311		C15 - O15		C:\Temp\New\06222022_2-step-DR

Figure 8-13: Reference Well Subtraction in Data Cycles Table

In addition to the column reference subtraction, the same subtraction pattern is applied in the other columns. For example, in detection column 5, O5 is subtracted from A5...M5.

As the reference wells do not contain anti-HER2, this reference subtraction scheme will, ideally, remove non-specific binding (NSB) signal in the detection step. In addition, since the reference row is measured at the same time as the other signal, this will reduce the effect of baseline drift and evaporation. See Chapter 9, "Preprocessing Quantitation Datasets" on page 189 for other sample referencing options.

Align X: Use this option if you need to remove artifacts at the beginning of the binding curves.

Flip Data: The Flip Data function inverts binding curves from positive to negative or from negative to positive (see Figure 9-32). Select this option when the observed nm shift goes negative due to presence of large analytes such as phage, cells, and lipoparticles on the biosensor surface.

Dose Response Analysis

Click on the **Dose Response Analysis** tab.

On the left are the data analysis options described next.

Report point settings

The dose response curve is the analyte binding response curve value at a chosen time value along the binding curve. The time is set with **Time(s)** settings, averaging a number of data points set in **# Points to Average**. The **Time Range** shows the effective span of data that is averaged. The number of points to average is symmetric around the time setting if possible. If the time setting is at or near the beginning or end of the binding curve, the time range is adjusted to ensure the set number points is averaged.

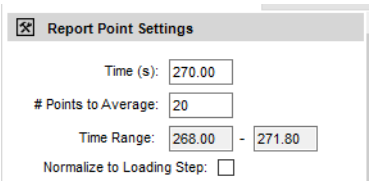


Figure 8-14: Report Point Settings

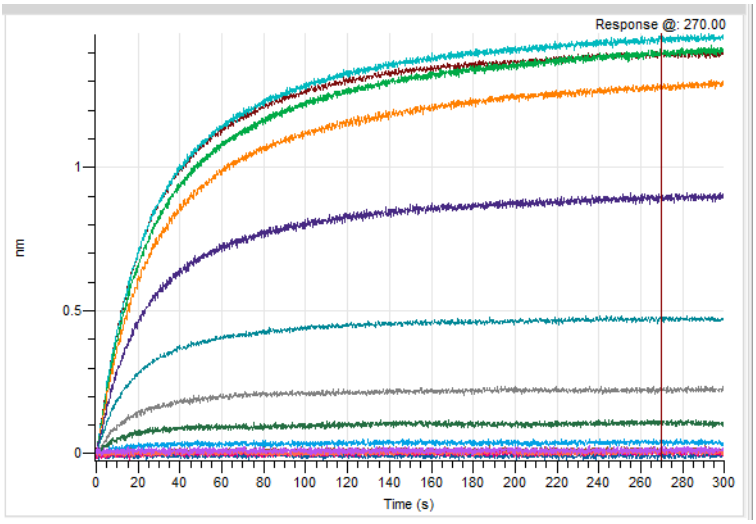


Figure 8-15: Center of Averaging Window in Binding Response Curve Plot

In the binding response curve plot on the far right, a vertical bar shows the center of this averaging window. Note that the report point bar in this graph can be moved by clicking anywhere on the graph.

Grouping, selecting standard groups, and normalization

Grouping allows you to organize the data for fitting, designating which data groupings to fit in one model versus data which are distinct, and fit to independent models.

As an example of how to use this option, let us assume we have an experiment where there are three replicates organized in columns in the sample plate. In the experiment, all the data have the same Sample ID. Click the **Group By...** button and select **Sample ID** only in the pop up window.

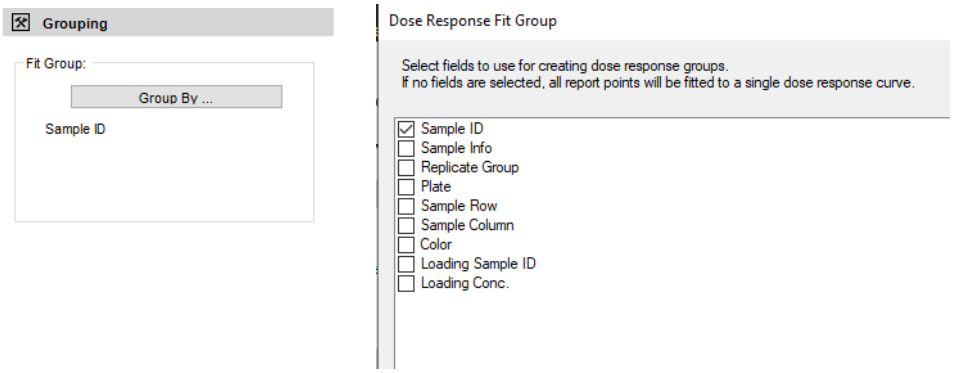


Figure 8-16: Grouping by Sample ID

Then in a dose response plot, there will be a single fitted dose response curve with three replicates at each concentration.

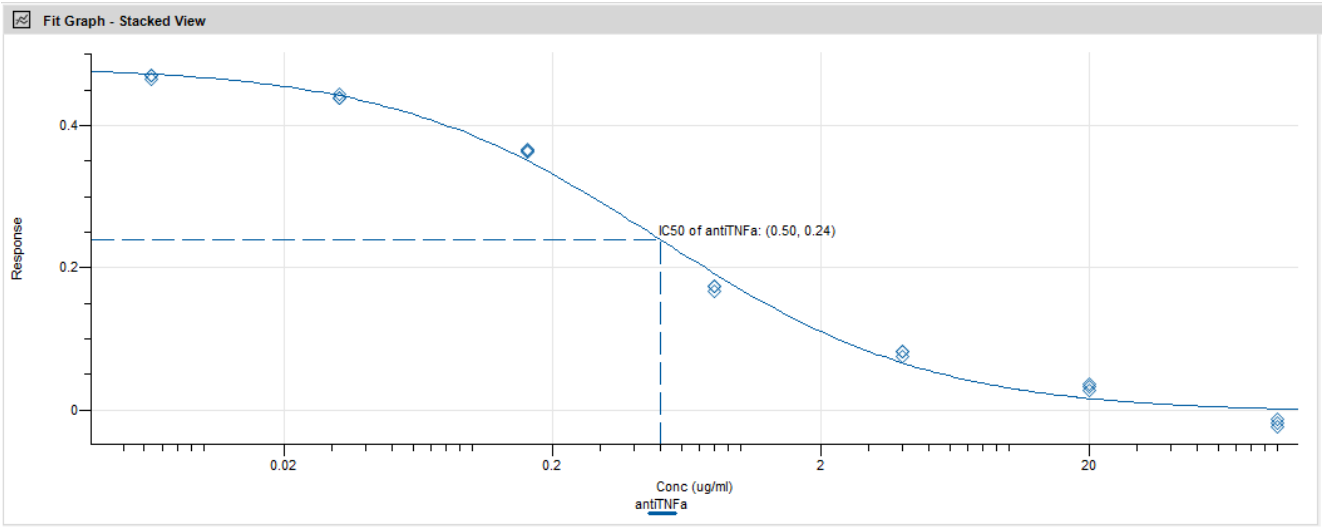


Figure 8-17: Dose Response Plot of Grouped Replicates by Sample ID

If we click **Group By...** and instead select **Sample ID** and **Sample Column** in the dose response plot, there would be three sets of points with three independent dose response curve fits.

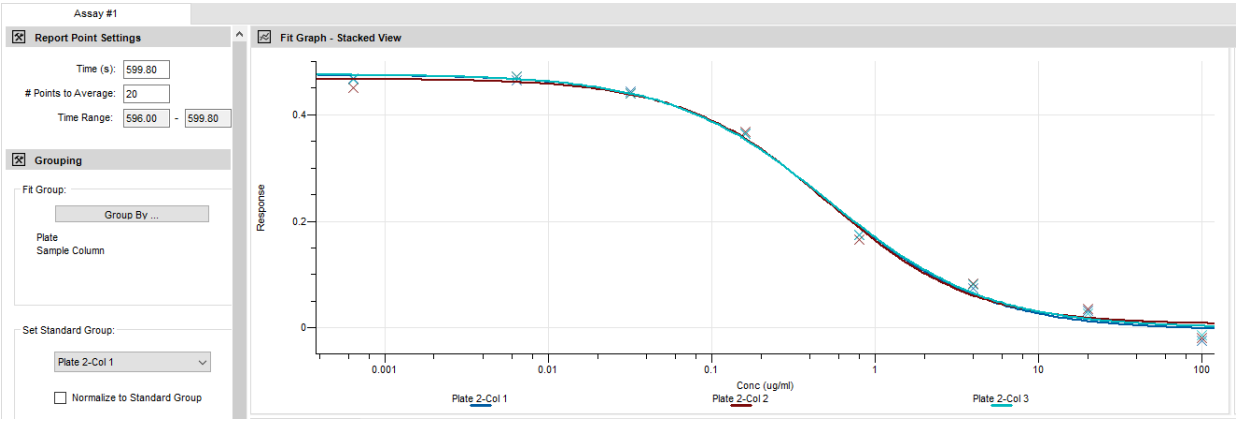


Figure 8-18: Dose Response Plot of Grouped Replicates by Sample ID and Sample Column

If the choice of grouping options have created multiple dose response curves, it is possible to set one as a standard by selecting one group in the **Set Standard Group** option.

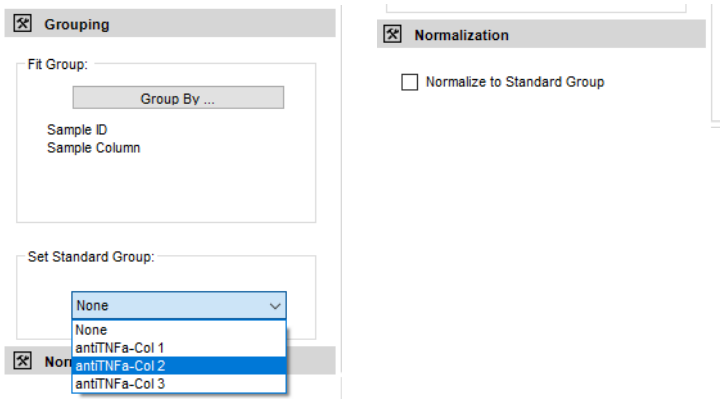


Figure 8-19: Setting a Standard Group

Choosing one standard enables the Pair View where you can see individual dose response curves overlaid on the standard curve. Choosing a standard also enables the Normalize to Standard Group checkbox. This normalization option rescales the response. Rescaling sets the minimum response of the standard group to 0 and the maximum response is set to 100. All other response values are scaled according to this transformation.

Curve fitting

The Curve Fitting panel collects the options for tailoring the dose response model fit to achieve the best statistics.

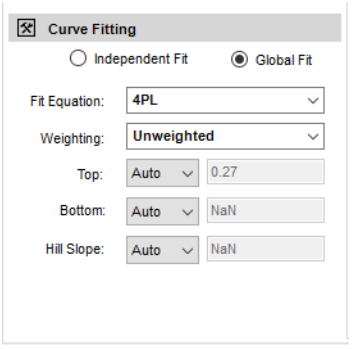


Figure 8-20: Curve Fitting

Fit Equation: There are five model functions.

- **4PL**

$$y = R_{Bot} + \frac{(R_{Top} - R_{Bottom})}{1 + \left(\frac{x}{A}\right)^{h}}$$

Where:

y and *x* are plotting coordinates

R_{Top} is the upper asymptote

R_{Bottom} is the lower asymptote

A is the x-value at the mid-range concentration inflection point

h is the Hill coefficient

EC_{50} is the mid point concentration between the upper and lower asymptotes

- **5PL:** Like 4PL with additional asymmetry term

$$y = R_{Bot} + \frac{(R_{Top} - R_{Bottom})}{\left(1 + \left(\frac{x}{A}\right)^{h}\right)^{b}}$$

Where:

y and *x* are plotting coordinates

R_{Top} is the upper asymptote

R_{Bottom} is the lower asymptote

A is the x-value at the mid-range concentration inflection point

h is the Hill coefficient

b is the asymmetric factor

EC_{50} is the mid point concentration between the upper and lower asymptotes

- **3PL:** This uses the 4PL model with the Hill Slope fixed to 1.0.
- **Linear:** y= a*x + b
- **Semilog Line:** y = a*log(x) + b

The rule of thumb is to pick the model that fits the data the best with the fewest number of floating parameters. If the data looks like a symmetric sigmoidal curve with clear upper and lower asymptotes, start with the 4PL model.

Weighting:

- **Dose Response (Unweighted):** A symmetrical dose response curve. No points are weighted during the curve fitting.
- **Dose Response (Weighted Y):** A symmetrical dose response curve with weighting applied as 1/Y (as Y increases, weighting decreases).
- **Dose Response (Weighted Y^2):** A symmetrical dose response curve with weighting applied as $1/Y^2$.

Fit Parameters: With the 3PL, 4PL, and 5PL models, you have some control of the independent variables such as the upper and lower asymptotes, the Hill slope, and the asymmetry. For each independent variable you select a fitter option:

- **Auto:** The curve fit code will make an initial estimate of this variable and run the iterative fitter, optimizing this variable to minimize the difference between the model and data.
- **Seed:** If the data is sparse and noisy, the initial estimate of the variable may not be good and the fitter will have trouble optimizing the model. You can override the automatic initial estimation by selecting **Seed** and entering your best guess.
- **Fixed:** Here you can assign a variable to a set value and the fitter will treat it as a constant. This option is useful for the asymptote values if your assay doesn't include controls at very high and very low concentrations.

Independent/Global Fit:

- **Independent:** If there are multiple data sets, each is fit with the same model function, but the variables of all curves are independent of one another.
- **Global:** If there are multiple data sets, each is fit with the same model function with the constraints that all curve fits converge to the same upper and lower asymptotes, the same Hill slope, and same asymmetry parameter. Only the EC_{50}/IC_{50} values differ between data sets. For the linear and semi-log fits, the slope is forced to be the same for each group, while the intercept term is allowed to differ between groups.

Alerts: Enabling the Loading Z-Score alert will flag cycles that may need to be excluded from the analysis due to variability in loading. The z-score is computed with this formula:

$$z = y_{(i)} - \bar{y}/\sigma$$

Where $y_{(i)}$ is the response, or nm shift, of an individual loading step, $\bar{y}$ is the average response of the group of loading steps, and σ is the standard deviation of this group. A group is defined as loading steps that have the same Sample ID and loading concentration so that the data are comparable.

The z-score is a measure of the distance of a data point from the mean in units of standard deviation of the sampled population. If the distribution of values about the mean is normal, also known as Gaussian, 99.7% of random samples should fall withing 3 σ of the mean.

Typically, if a loading step z-score is more than 3 σ, the assay is considered suspect and is flagged for inspection. If a flag is raised, the data is not automatically excluded from the analysis. The process to manually exclude data is explained in the IC_{50} example later in the chapter.

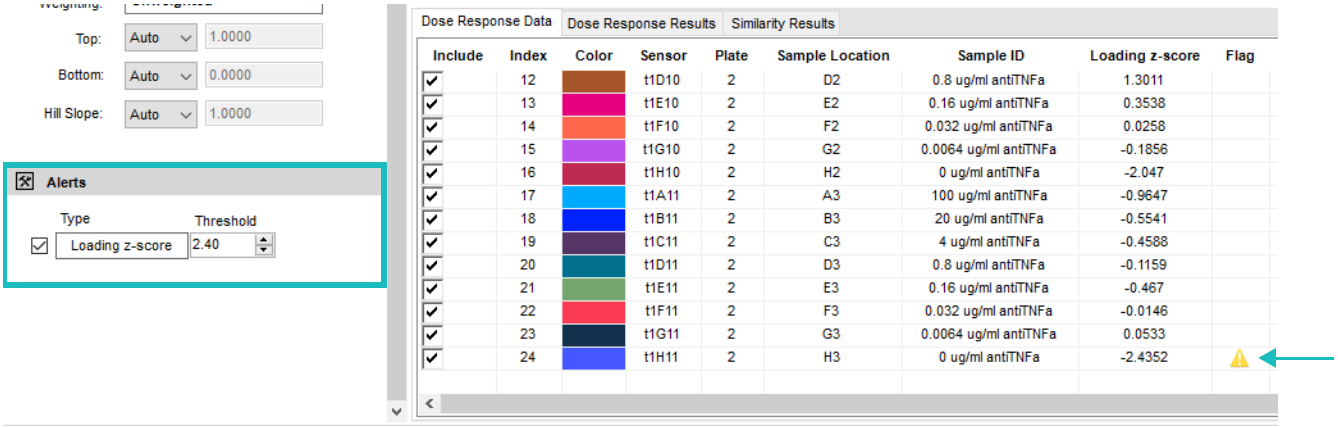


Figure 8-21: Loading Z-Score Alert

To complete our EC_{50} example, we set or uncheck the following options:

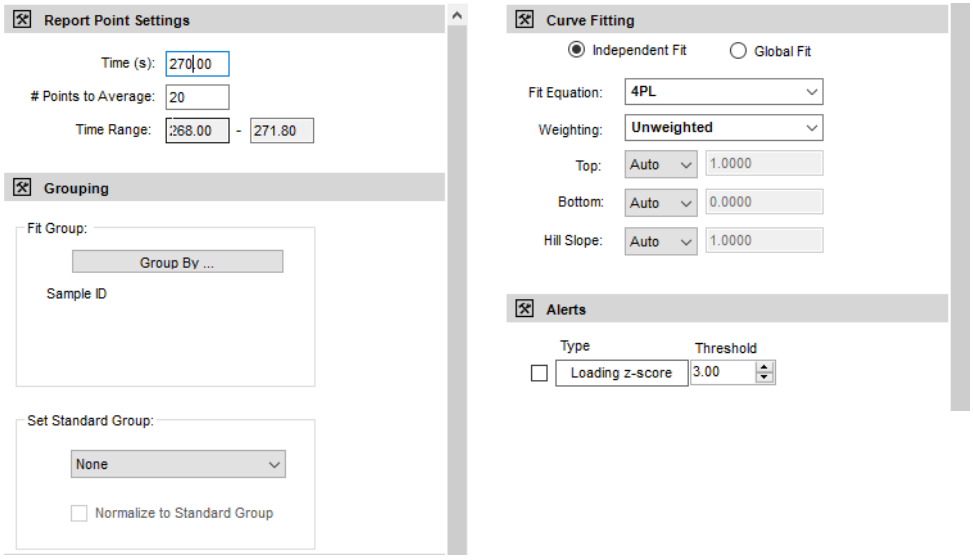


Figure 8-22: Selecting Analysis Options

This is the dose response curve fit:

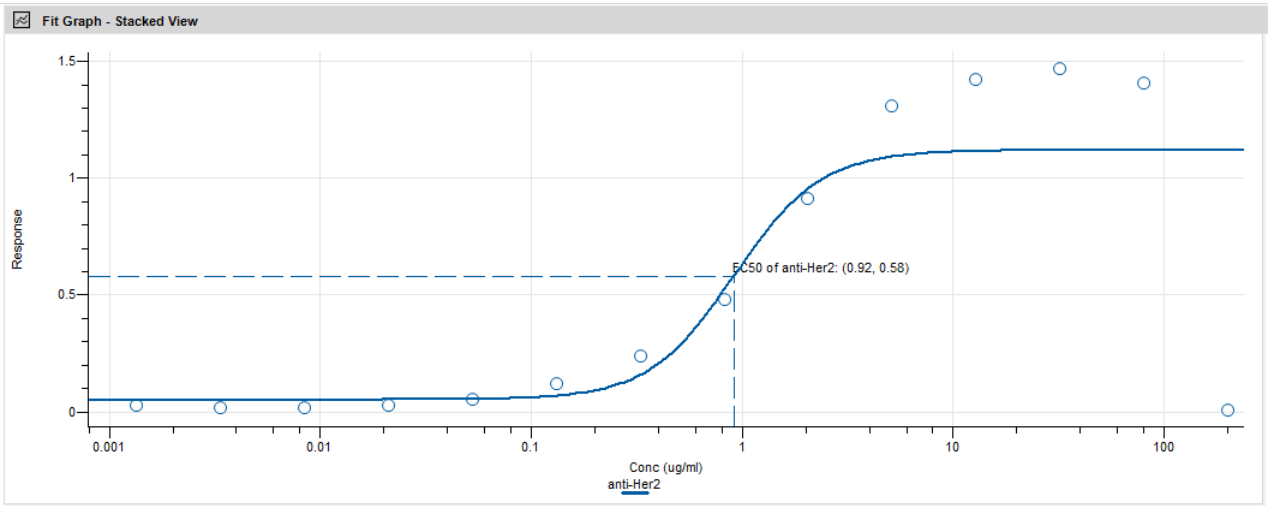


Figure 8-23: Example Dose Response Curve Fit

In this example, the fit in the higher concentrations does not look good. There is a data point at 200 µg/mL with almost no response. If we examine the input data in the table below this graph, the sample at 200 µg/mL has a background subtracted response of -0.0069 nm, essentially zero.

Dose Response Data | Dose Response Results | Similarity Results

Include	Index	Color	Sensor	Plate	Sample Location	Sample ID	Sample Info	Replicate Group	Conc. (ug/ml)	Response
✓	1		t1A1	1	A13	anti-Her2			200	-0.0069
✓	2		t1B1	1	C13	anti-Her2			80	1.3933
✓	3		t1C1	1	E13	anti-Her2			32	1.446
✓	4		t1D1	1	G13	anti-Her2			12.8	1.3994
✓	5		t1E1	1	I13	anti-Her2			5.12	1.2812
✓	6		t1F1	1	K13	anti-Her2			2.05	0.8936
✓	7		t1G1	1	M13	anti-Her2			0.8192	0.4705
✓	8		t1A2	1	A15	anti-Her2			0.3277	0.2229
✓	9		t1B2	1	C15	anti-Her2			0.1311	0.1091
✓	10		t1C2	1	E15	anti-Her2			0.0524	0.0393
✓	11		t1D2	1	G15	anti-Her2			0.021	0.015
✓	12		t1E2	1	I15	anti-Her2			0.0084	0.003
✓	13		t1F2	1	K15	anti-Her2			0.0034	0.0045
✓	14		t1G2	1	M15	anti-Her2			0.0013	0.0118

Figure 8-24: Background Subtracted Response

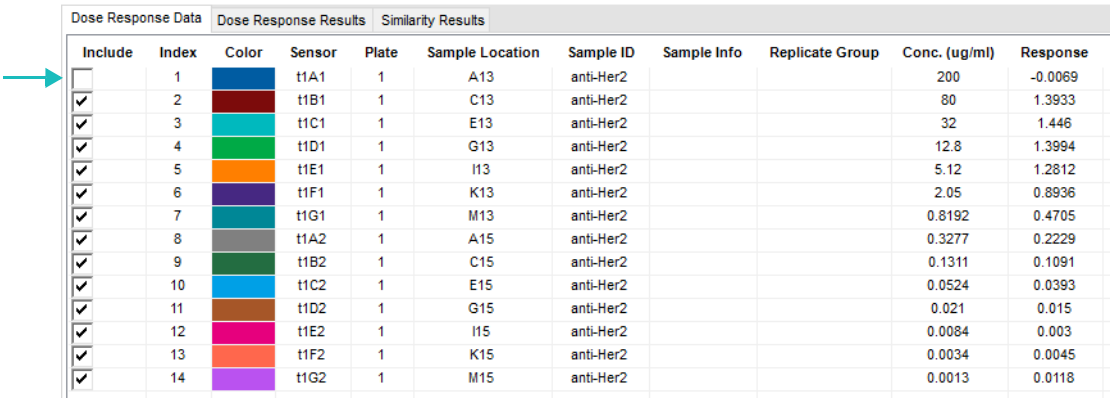
If we look at the Dose Response Results, the R^2 of the fit is 0.6788, which is a very bad fit. Ideally this should be R^2 of 0.98 or better, with 1.0 indicating a perfect fit.

Dose Response Data | Dose Response Results | Similarity Results

Include	Index	Color	Symbol	Line Style	Group Name	Standard	EC50(ug/ml)	Hill Slope	Bottom	Top	R^2	EC50 95% CI	Hill Slope 95% CI	Top 95% CI	Bottom 95% CI
☑	1		■	—	anti-Her2		0.9112	2.116	0.0352	1.0988	0.6788	0.296 to 2.808	-2.435 to 6.667	0.786 to 1.412	-0.241 to 0.311

Figure 8-25: Dose-Response Analysis With a 4-PL Fit That Provides a Non-Optimal R^2

For this example, there was an error in pipetting well A13, so we can legitimately exclude this bad data. To do so, uncheck the row to exclude.



Dose Response Data | Dose Response Results | Similarity Results

Include	Index	Color	Sensor	Plate	Sample Location	Sample ID	Sample Info	Replicate Group	Conc. (ug/ml)	Response
☐	1		t1A1	1	A13	anti-Her2			200	-0.0069
☑	2		t1B1	1	C13	anti-Her2			80	1.3933
☑	3		t1C1	1	E13	anti-Her2			32	1.446
☑	4		t1D1	1	G13	anti-Her2			12.8	1.3994
☑	5		t1E1	1	I13	anti-Her2			5.12	1.2812
☑	6		t1F1	1	K13	anti-Her2			2.05	0.8936
☑	7		t1G1	1	M13	anti-Her2			0.8192	0.4705
☑	8		t1A2	1	A15	anti-Her2			0.3277	0.2229
☑	9		t1B2	1	C15	anti-Her2			0.1311	0.1091
☑	10		t1C2	1	E15	anti-Her2			0.0524	0.0393
☑	11		t1D2	1	G15	anti-Her2			0.021	0.015
☑	12		t1E2	1	I15	anti-Her2			0.0084	0.003
☑	13		t1F2	1	K15	anti-Her2			0.0034	0.0045
☑	14		t1G2	1	M15	anti-Her2			0.0013	0.0118

Figure 8-26: Excluding Well Data

The result is a good fit and R^2 of 0.9979.

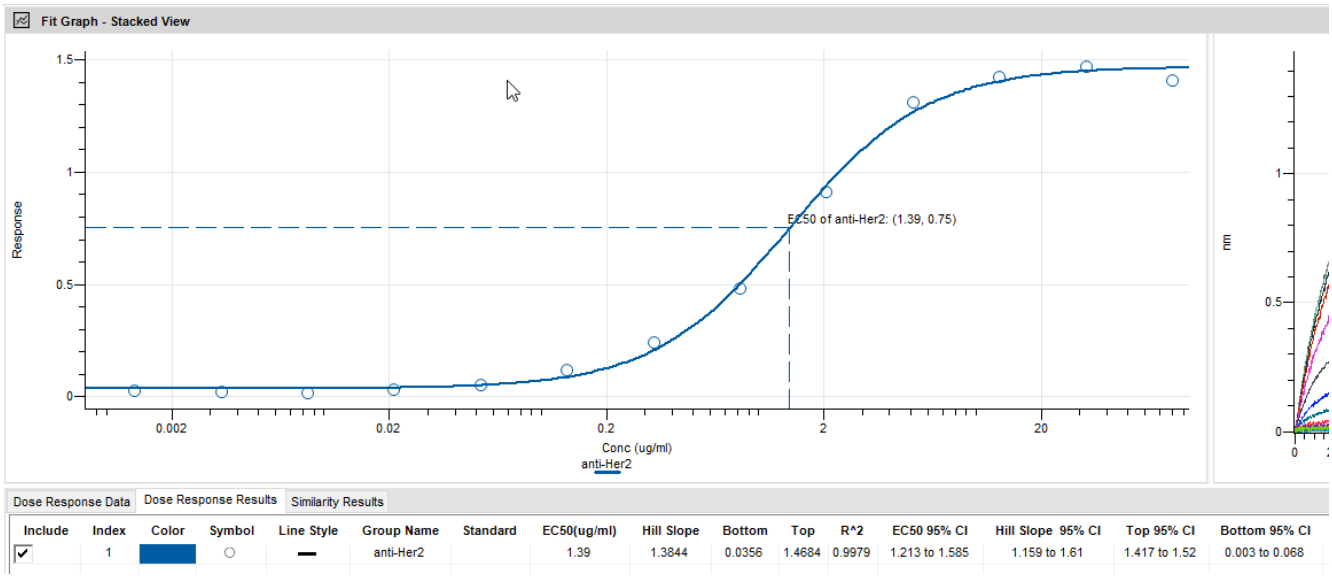


Figure 8-27: Dose-Response Analysis With a 4-PL Fit With Improved R^2

The table shows the EC_{50} = 1.39 µg/mL with a 95% confidence interval spanning 1.213 µg/mL to 1.585 µg/mL.

IC_{50} Analysis Using a 3-Step Dose Response Assay

In this section, we will explore the dose response analysis a little more with a 3-step inhibition assay to determine an IC_{50} value. The analysis is similar to the previous 2-step example, but a 3-step experiment has replicates which presents a few more options.

In this experiment, SAX2 biosensors are ligated with a fixed concentration of Biotin-TNFα. After a buffer wash, the immobilized Biotin-TNFα is dipped into various concentrations (dose) of anti-hTNFα, followed by a second buffer wash and then a dip into fixed concentrations of TNFα-receptor for a detection (response) measurement. The anti-hTNFα is the inhibitor which blocks TNFα + TNFα-receptor binding.

Also of note is that this experiment uses a custom assay based upon a quantitation experiment and was not created with an Octet® BLI Discovery software dose-response method template. For this reason, Octet® Analysis Studio software needs a little help in interpreting the experiment. The experiment is also missing the necessary concentrations so we will also show how to add these data.

When first loading the experiment, click the **Dose Response Analysis** button in the Home screen tab.

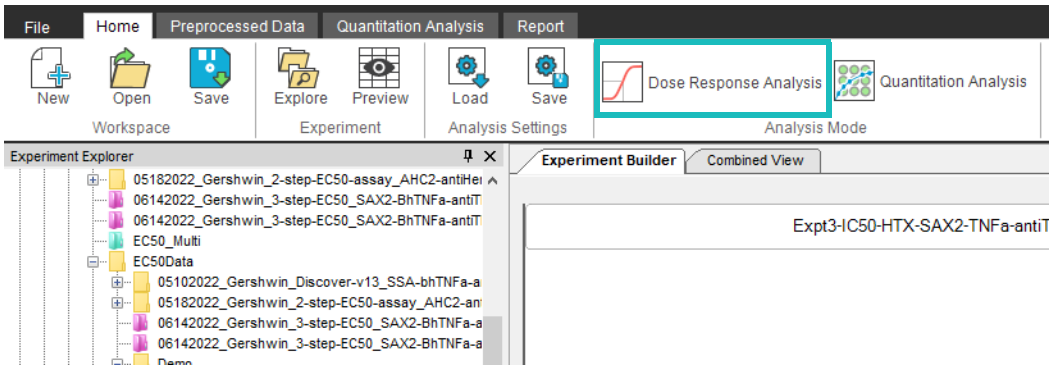


Figure 8-28: Dose Response Analysis Button in Home Tab

Preprocessing: 3-Step Assay

We will examine the plate layout and binding response curve assignments.

The data were collected on an Octet® RH96 system in 24 channel mode, picking up 3 columns of biosensors at a time. There are two 96-well sample plates. On plate 1, there are three sets of buffer wells labeled **B**, **2**, and **3** for the three washing steps and there is one set of loading wells labeled **L** to bind the Biotin-TNFα. Additionally, on plate 2, there are three columns of anti-hTNFα designated as purple sample wells with three red reference wells, and three columns of TNFα receptors in the detection wells labeled **D**.

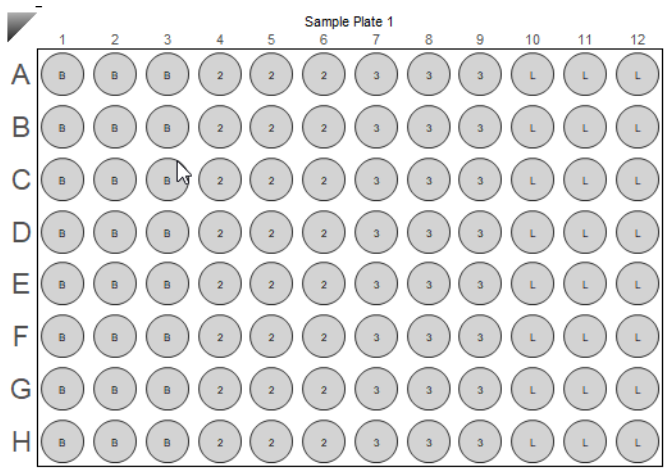


Figure 8-29: Sample Plate 1

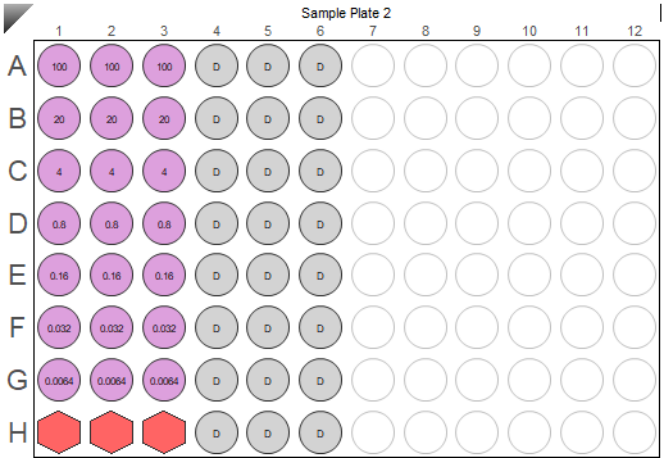


Figure 8-30: Sample Plate 2

In the Data Cycles view we can see how Analysis Studio assigns the step types. For an IC_{50} assay, there should be three steps: Loading (green), Sample (blue), and Detection (yellow).

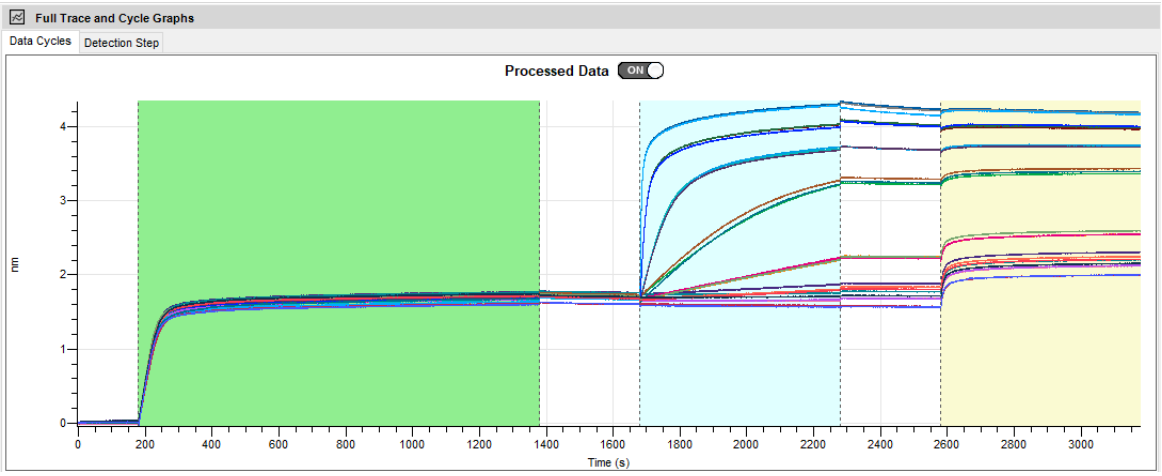


Figure 8-31: Assay Steps

If you have a custom assay that is not following the standard pattern of Loading, Sample, and Detection step types, you can manually assign the step assignment by right clicking a step in the graph and selecting **Set Detection Step**, **Set Sample Step**, or **Set Loading Step**.

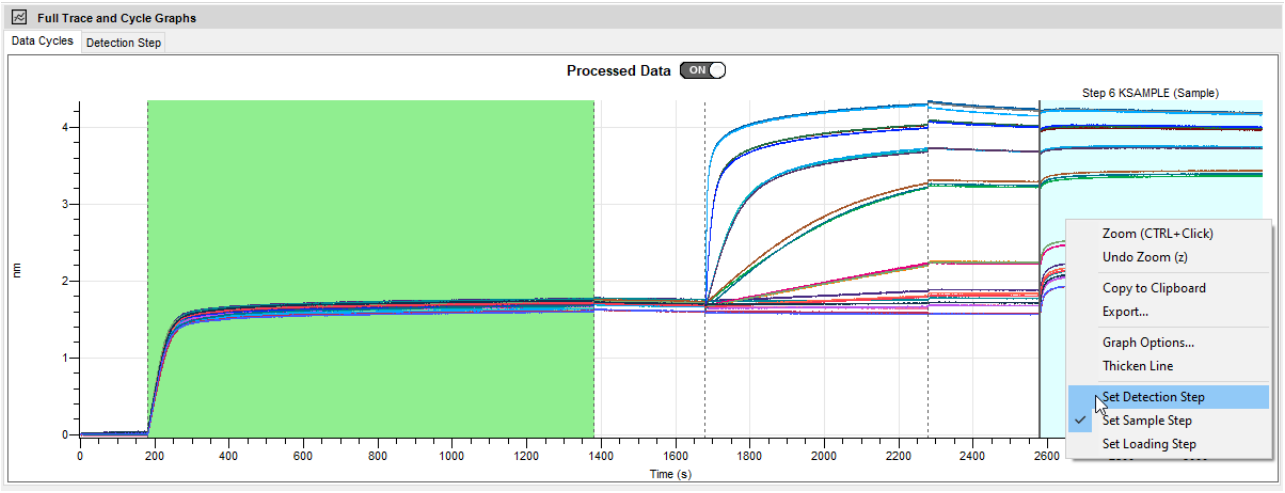


Figure 8-32: Setting the Correct Step Type

In Plate 2, there are three reference wells (Figure 8-30). If this were a typical quantitation or kinetics assay, reference wells would normally be subtracted from the samples. However, the reference wells provide valuable information about one of the dose response curve asymptotes, so we would like to use them in the analysis. If the reference wells are used for background subtraction they are excluded. So for this reason reference subtraction is skipped.

By default, reference wells have a sample concentration of 0 µg/mL. As the concentrations are used in a logarithmic function, and log(0) is an invalid number, to include the reference wells in the analysis they must have a concentration greater than 0 µg/mL. The lowest concentration sample is 0.0064 µg/mL in row G so we can safely set the reference wells 100 times lower at 0.00064 µg/mL.

To assign a new concentration to the reference wells, select a reference well and right click. Select **Edit Sample Info** then enter 0.000064 in the **Sample Concentration** field (Figure 8-33).

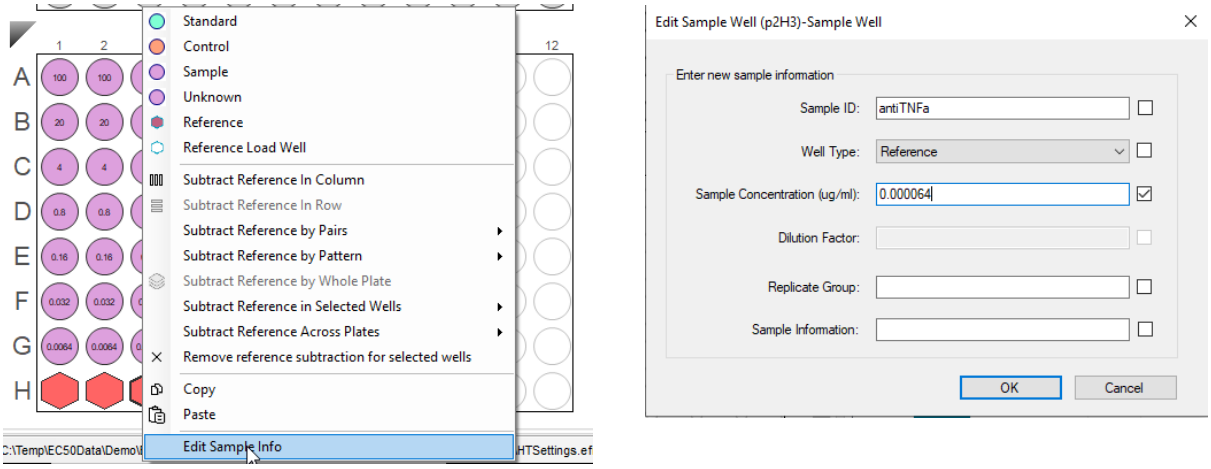


Figure 8-33: Editing Sample Info

Analysis

For the first pass analysis we keep the default settings in the Report Point panel, Normalization panel, and Curve Fitting panel. In the Grouping panel, set grouping by **Plate**. This grouping will organize all the data into a single curve fit with three replicates at each concentration.

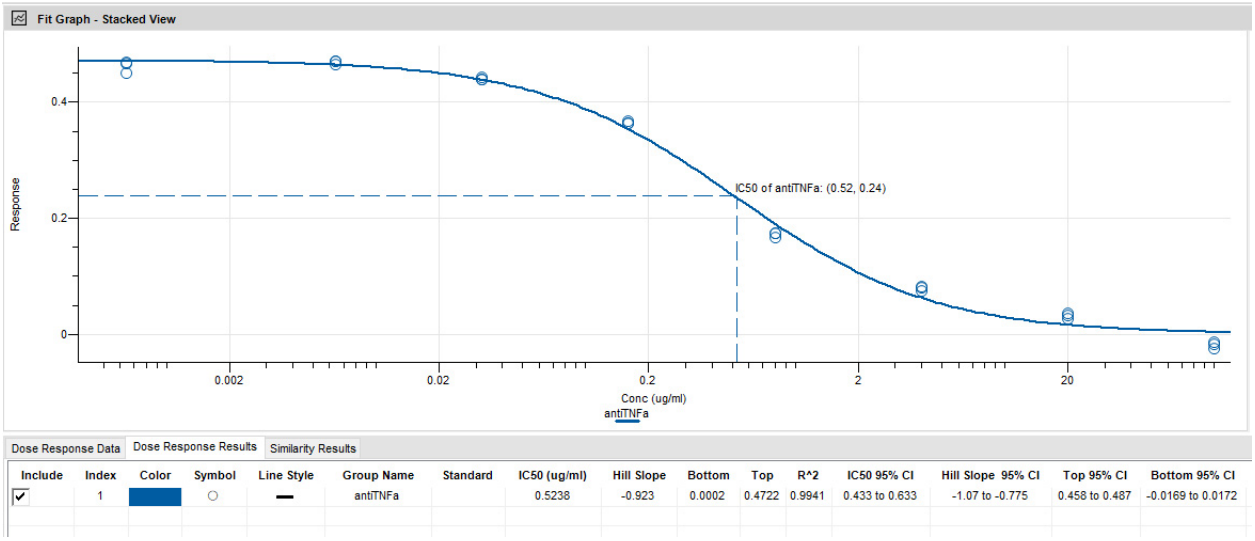


Figure 8-34: Grouped Data by Plate

As the response decreases with increasing anti-hTNFα concentration, the software correctly identifies the mid-point of the curve as an IC_{50} value and not an EC_{50} value. The IC_{50} is 0.5238 µg/mL with a 95% confidence interval spanning 0.433 µg/mL to 0.633 µg/mL.

Now if we go back and change the grouping to include Sample Column:

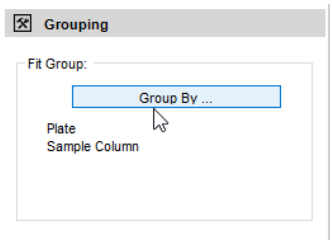


Figure 8-35: Adding Sample Column in Grouped Data

Each set of replicates can then be treated as independent data and we can fit three dose response curves.

One of the data sets is also designated as the Standard Group. This is assigned to the first column of samples:

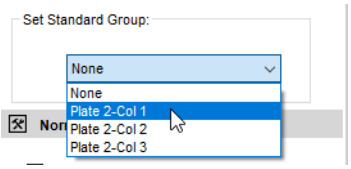
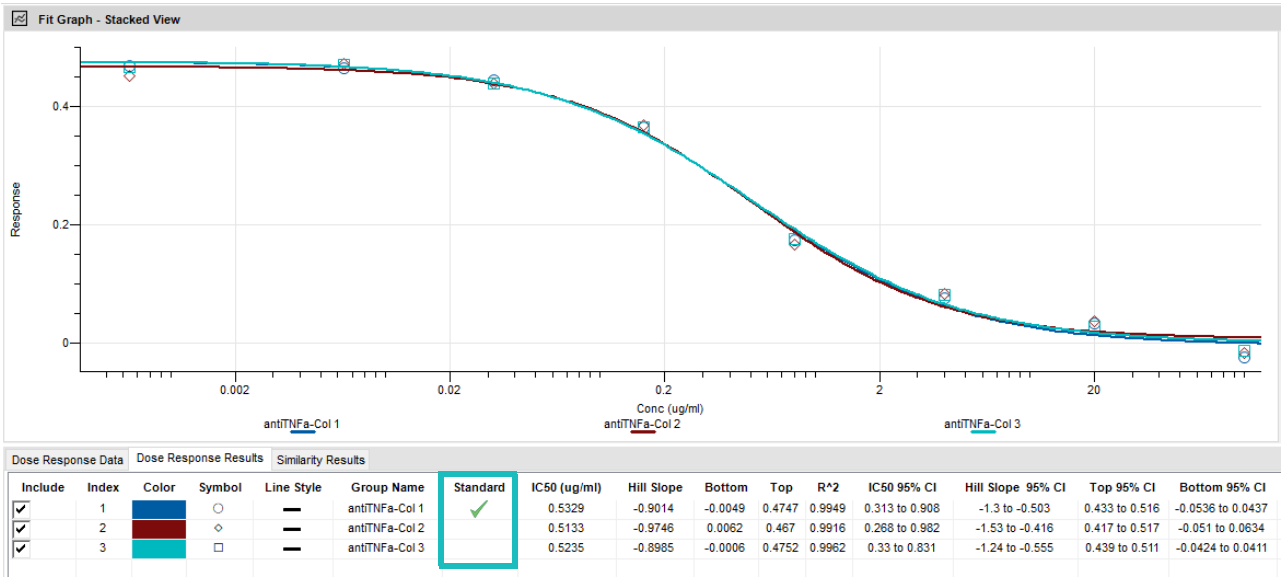


Figure 8-36: Setting the Standard Group

Now we see three dose response curve fits with IC_{50} values of 0.5329, 0.5133, and 0.5235 µg/mL. The check mark in the Standard column indicates which curve is the designated standard.



Include	Index	Color	Symbol	Line Style	Group Name	Standard	IC50 (ug/ml)	Hill Slope	Bottom	Top	R^2	IC50 95% CI	Hill Slope 95% CI	Top 95% CI	Bottom 95% CI
☑	1		○	—	antiTNFa-Col 1	✓	0.5329	-0.9014	-0.0049	0.4747	0.9949	0.313 to 0.908	-1.3 to -0.503	0.433 to 0.516	-0.0536 to 0.0437
☑	2		◇	—	antiTNFa-Col 2		0.5133	-0.9746	0.0062	0.467	0.9916	0.268 to 0.982	-1.53 to -0.416	0.417 to 0.517	-0.051 to 0.0634
☑	3		□	—	antiTNFa-Col 3		0.5235	-0.8985	-0.0006	0.4752	0.9962	0.33 to 0.831	-1.24 to -0.555	0.439 to 0.511	-0.0424 to 0.0411

Figure 8-37: Dose-Response Analysis After Grouping by Plate, and Assigning a Standard Group Curve

In the Similarity Results tab, the software reports the ratio of the dose response curve parameters relative to the standard.

Dose Response Data | Dose Response Results | Similarity Results

Index	Type	Group Name	IC50 Ratio	Hill Slope Ratio	Bottom Ratio	Top Ratio
1	Standard	antiTNFa-Col 1	1	1	1	1
1	Test 1	antiTNFa-Col 2	0.9632	1.0812	-1.2507	0.9837
2	Standard	antiTNFa-Col 1	1	1	1	1
2	Test 2	antiTNFa-Col 3	0.9823	0.9968	0.1288	1.0009

Figure 8-38: Similarity Results

Table data

Input data and analysis results are presented in three tables located on the bottom of the Analysis screen.

Dose Response Data: These are the data included in the analysis, with each row representing one assay cycle. A biosensor can be excluded from the analysis by unchecking the box in the first column. Columns to note for grouping and normalization are Sample Location, Sample ID, Concentration (of the analyte), Response (of the agonist), and Loading Response.

Dose Response Results: The table in Figure 8-39 shows the dose response fit results for one or more curves. Each row contains the optimized parameters and their 95% confidence intervals for each fitted dose response curve. R^2 is also calculated to help with model selection and evaluation.

Dose Response Data | Dose Response Results | Similarity Results

Include	Index	Color	Symbol	Line Style	Group Name	Standard	IC50 (ug/ml)	Hill Slope	Bottom	Top	R^2	IC50 95% CI	Hill Slope 95% CI	Top 95% CI	Bottom 95% CI
☑	1		○	—	antiTNFa-Col 1	✓	0.5329	-0.9014	-0.0049	0.4747	0.9949	0.313 to 0.908	-1.3 to -0.503	0.433 to 0.516	-0.0536 to 0.0437
☑	2		◇	—	antiTNFa-Col 2		0.5133	-0.9746	0.0062	0.467	0.9916	0.268 to 0.982	-1.53 to -0.416	0.417 to 0.517	-0.051 to 0.0634
☑	3		□	—	antiTNFa-Col 3		0.5235	-0.8985	-0.0006	0.4752	0.9962	0.33 to 0.831	-1.24 to -0.555	0.439 to 0.511	-0.0424 to 0.0411

Figure 8-39: Dose Response Results

If there are multiple curves, you can also select the tick mark style and line style to make the dose response curves easier to interpret. Select a row in the table and right click to open the menu of display options.

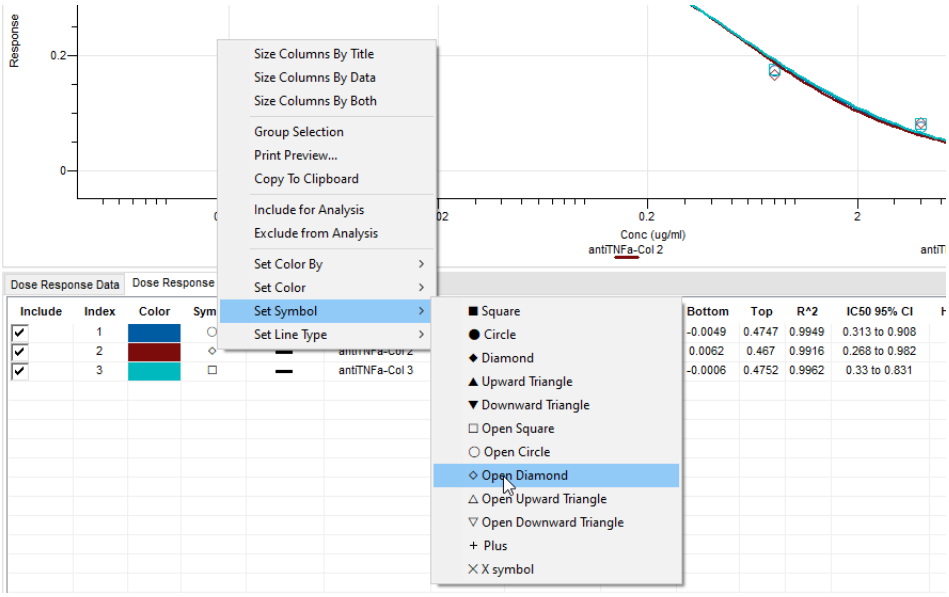


Figure 8-40: Selecting Curve Fit Styles

Similarity Results: If a standard group has been set, this table will show a pair wise comparison of the fit parameters of the curves in the analysis as compared to the standard.

Dose Response Data | Dose Response Results | Similarity Results

Index	Type	Group Name	IC50 Ratio	Hill Slope Ratio	Bottom Ratio	Top Ratio
1	Standard	antiTNFa-Col 1	1	1	1	1
1	Test 1	antiTNFa-Col 2	0.9632	1.0812	-1.2507	0.9837
2	Standard	antiTNFa-Col 1	1	1	1	1
2	Test 2	antiTNFa-Col 3	0.9823	0.9968	0.1288	1.0009

Figure 8-41: Similarity Results

Views and graph options

In the default view, all dose response curves are shown. There are several ways to tailor the format of this graph. You can toggle the concentration axis from linear to log by clicking the **Linear** or **Semilog** buttons in the Dose Response Analysis tab.

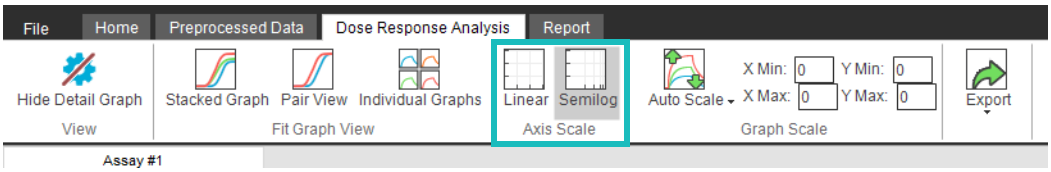


Figure 8-42: Axis Scale Options

Axis scaling and ranges can be set with the options in the Graph Scale controls.

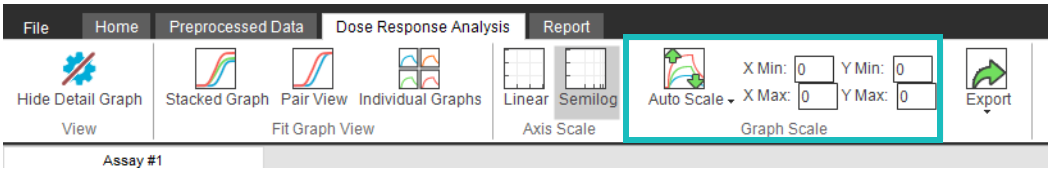


Figure 8-43: Graph Scale Options

And as mentioned before, data point and line styles can be changed in the Dose Response Results table below the graph.

You can view each dose response individually by clicking the **Individual Graphs** button.

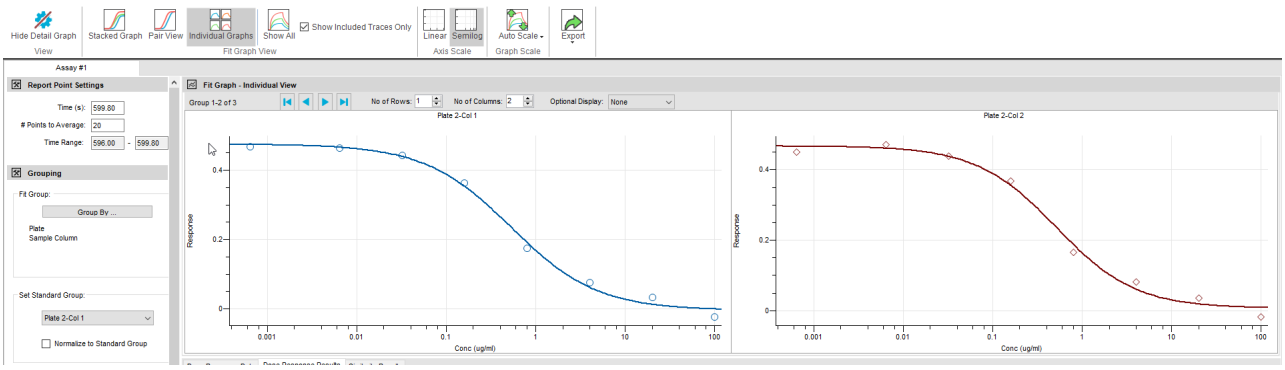


Figure 8-44: Individual Graphs View

Or you can create pair wise comparison plots of a selected Standard Group vs. non-standard groups by clicking the **Pair View** button.

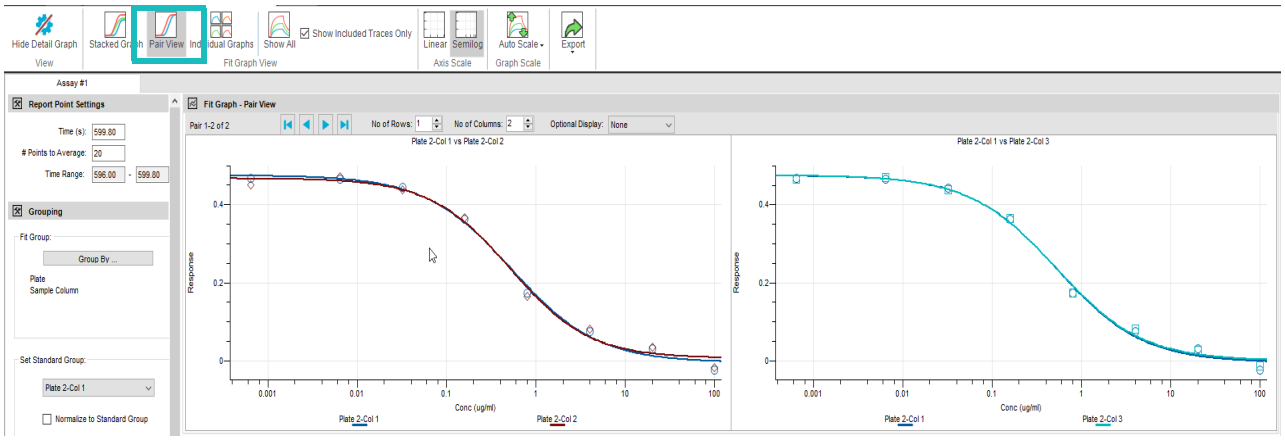


Figure 8-45: Pair View

In the Individual Graphs or Pair View, Option Display allows you to add the analysis results to the graph.

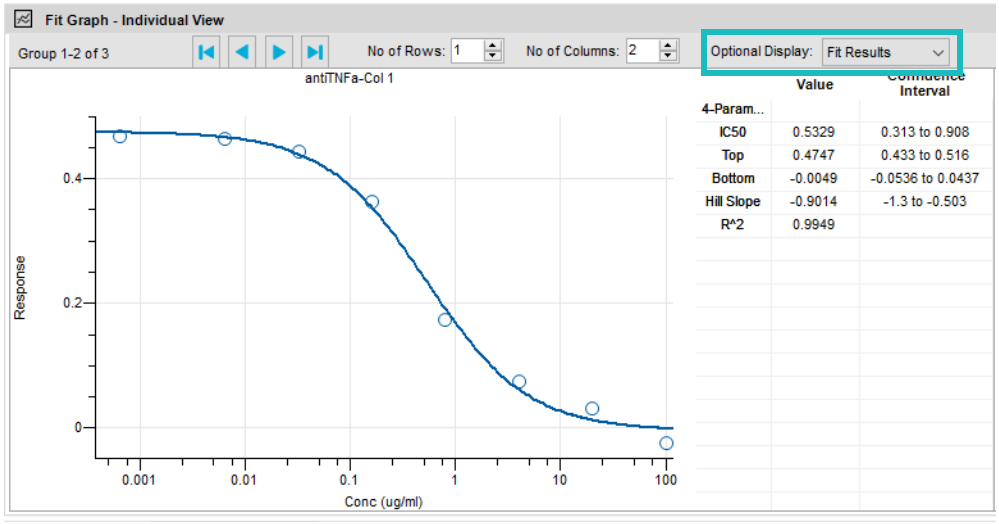


Figure 8-46: Add Optional Results Display

Chapter 9:

Preprocessing Quantitation Datasets

Pre-analysis Data Correction

The Preprocess Data Screen lets you subtract any non-specific binding or baseline drift data and make other data corrections prior to doing analysis if needed. Click the **Preprocess Data** tab (Figure 9-1).

NOTICE:

See "Resizing, Hiding and Closing Windows" on page 11. for information on working with the software windows and for more information on the displaying graphs and display options.

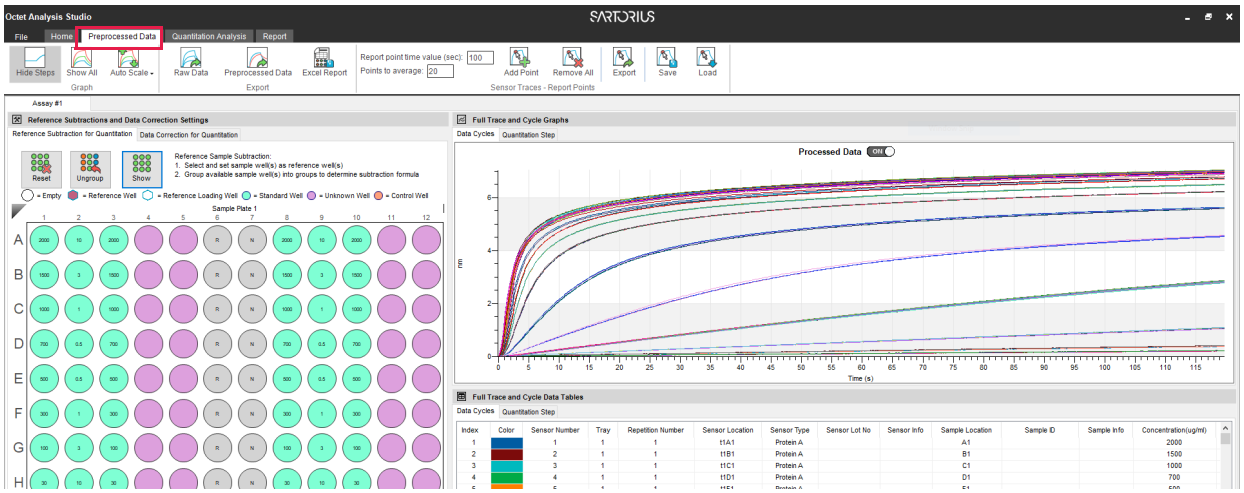


Figure 9-1: Preprocess Data Screen

Right-click a step to open a menu to designate it as a Detection, Sample or Loading step.

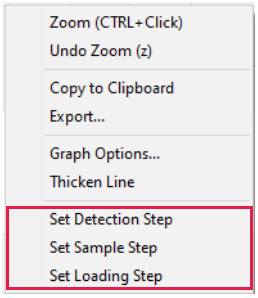


Figure 9-2: Designating a Step

To switch between viewing corrected and uncorrected data, click the **Processed Data toggle** at the top of the graph (Figure 9-3).

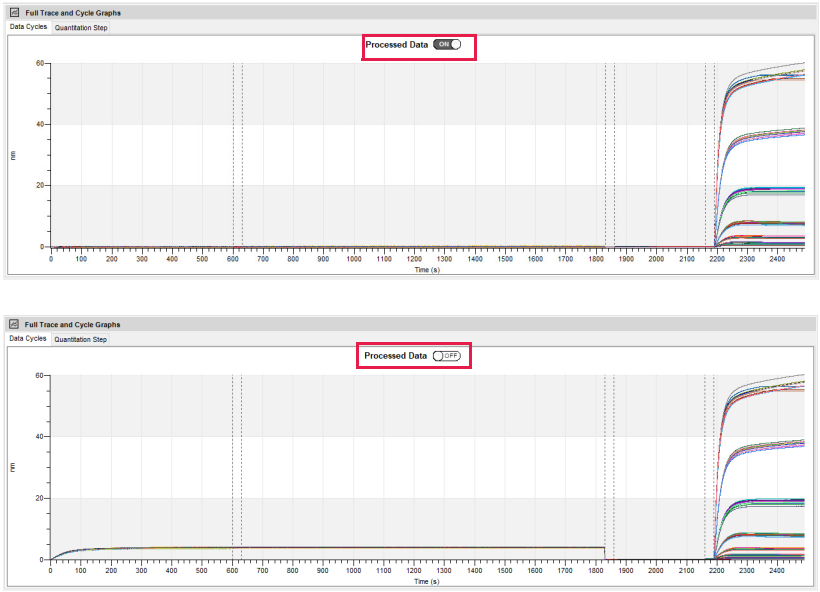


Figure 9-3: Processed Data ON (Top) and OFF (Bottom)

Reference Subtraction

To subtract any non-specific binding of your sample/analyte to the biosensor (no capture or ligand molecule) or the matrix components to the capture molecule or biosensor (no analyte) in your experiment, click the **Reference Subtraction for Quantitation** tab in the Reference Subtractions and Data Corrections window (Figure 9-4).

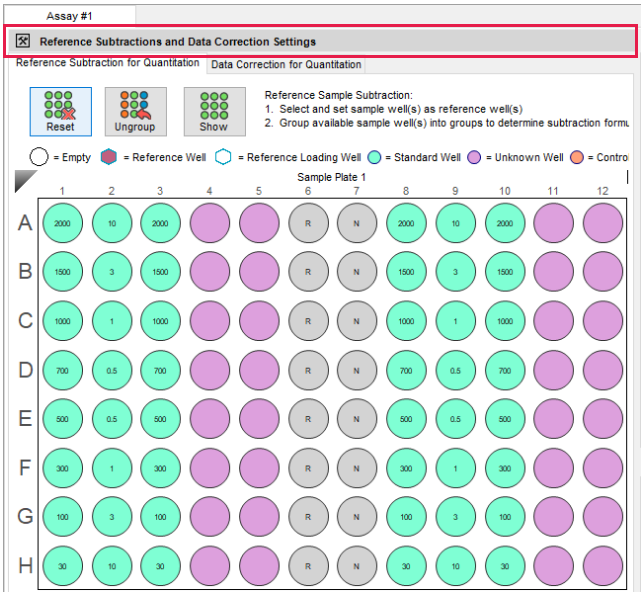


Figure 9-4: Reference Subtraction for Quantitation Tab

Viewing Data

To view data for different assays in the experiment or combined data set, click the **Assay** tab above the Reference Subtractions and Data Corrections window (Figure 9-5).

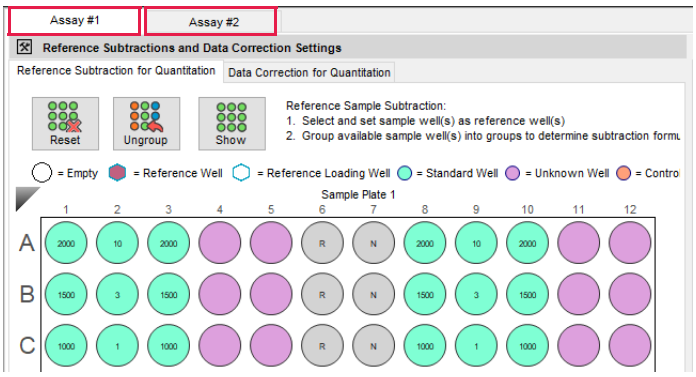


Figure 9-5: Assay Tabs

Click 

(**Show**) to display both the biosensor tray and the reagent plate (Figure 9-6). The default view shows only the reagent plate.

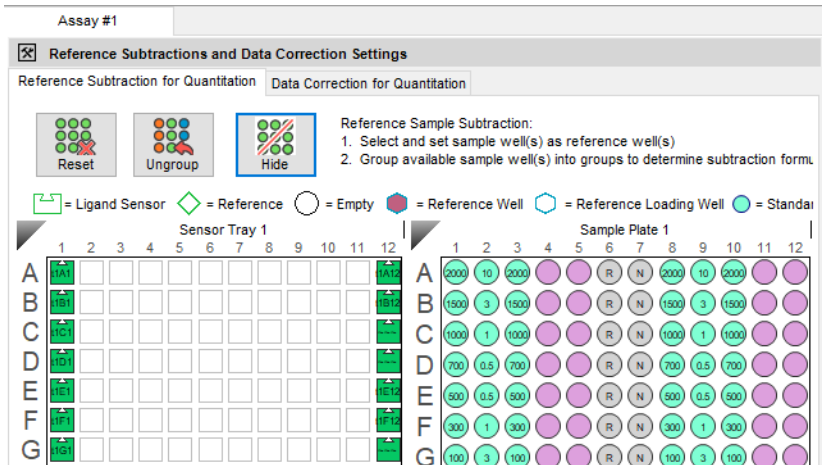


Figure 9-6: Biosensor Tray Displays After Show Selected

Click 

(**Hide**) to hide the biosensor tray.

If you used more than one biosensor tray or reagent plate in the experiment or combined dataset, use the scrollbars to move between the plates (Figure 9-7).

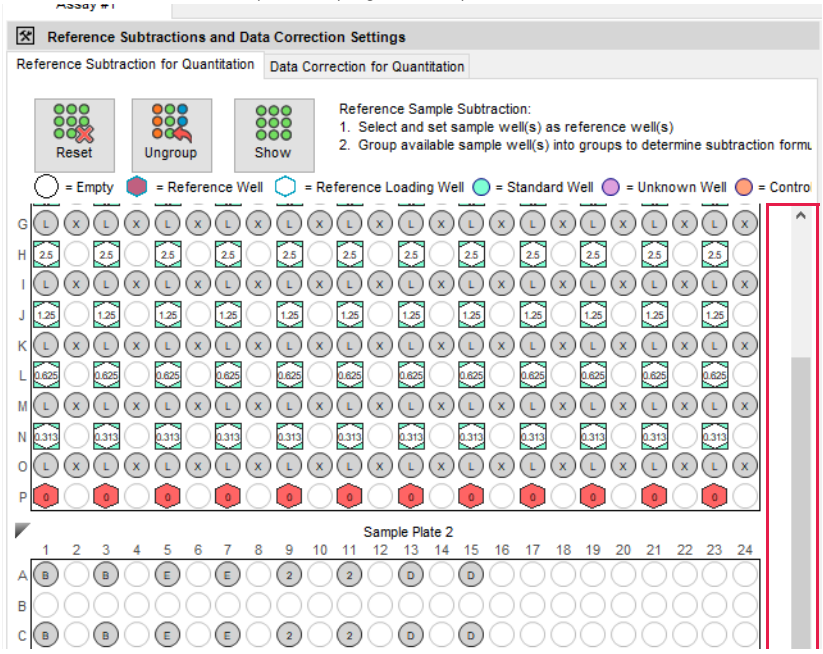


Figure 9-7: Scrolling Between Reagent Plates.

- Biosensors used in the experiment are green in the tray map and are set to ligand sensors by default, all other wells display empty.
- Biosensors are identified by their tray number and well number. For example t1A1 is the biosensor in well A1 on tray 1, t2H1 is the biosensor in well H1 on tray 2 (Figure 9-6).
- Selecting columns, rows or wells in the biosensor tray map also highlights the associated wells in the sample plate map and vice-versa (Figure 9-8).

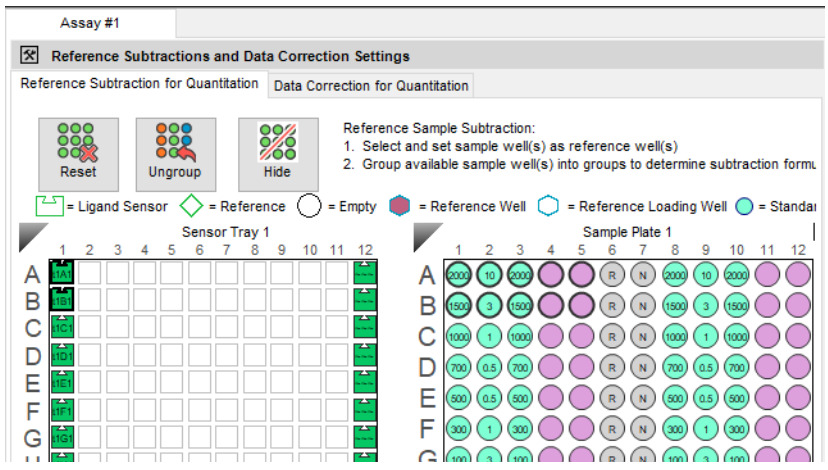


Figure 9-8: Biosensors and Associate Sample Wells Highlighted

- Analyte concentrations appear in the standard wells on the sample plate (Figure 9-9).

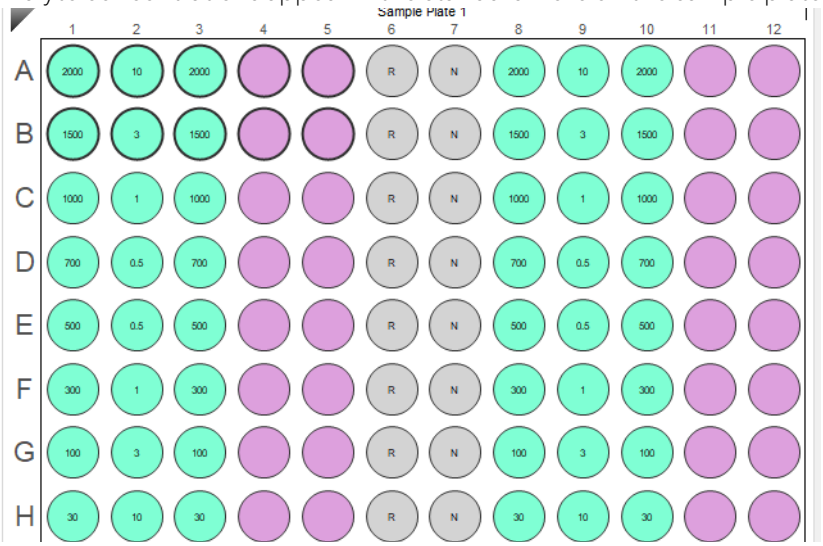


Figure 9-9: Reagent Plate Showing Concentrations for Standard Wells

Clicking on rows, columns or specific wells in a biosensor tray or sample plate displays the data for those biosensors in the Quantitation Cycle and Step Graphs window, and highlights the selected data in the Data Trace and Quantitation Step tables (Figure 9-10).

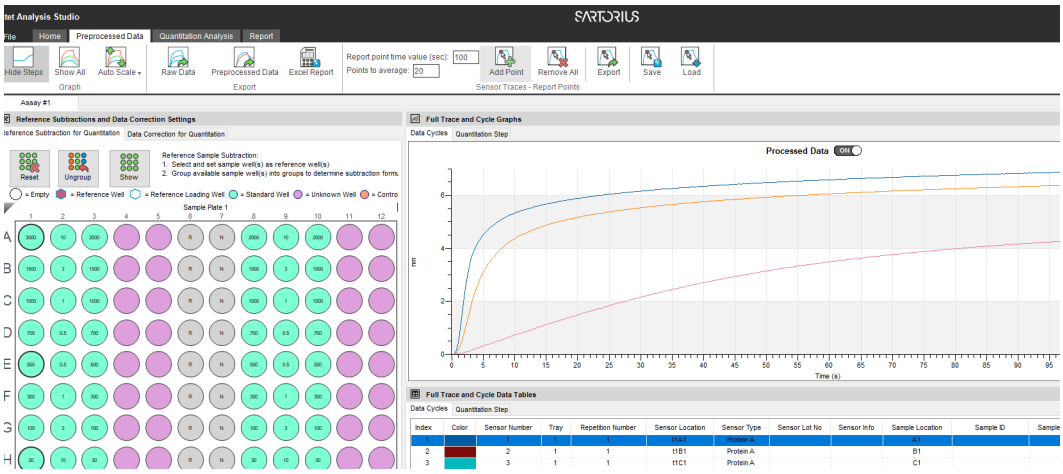


Figure 9-10: Well Selection Data Display

The Data Cycles tab shows the raw or reference-corrected data for all steps of the experiment including sample association step (1-step direct assay) or multiple steps for 2- or 3-step quantitation assays such as capture, secondary antibody, buffer, substrate and enzyme steps (Figure 9-11).

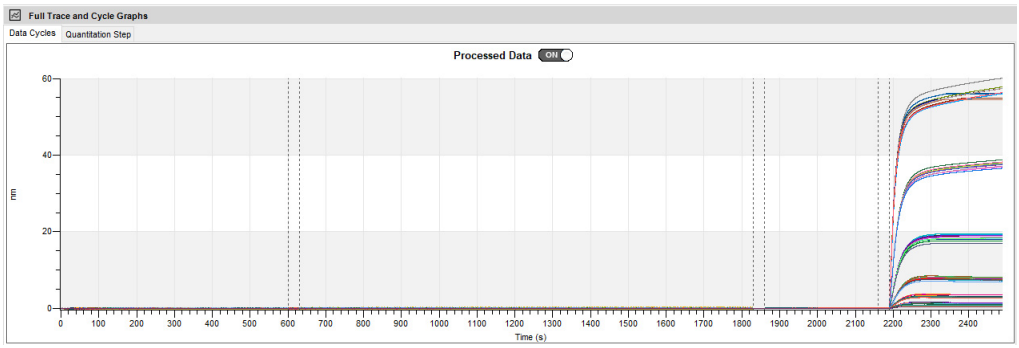


Figure 9-11: Data Cycles Tab

The Quantitation Step tab shows the sample step that was quantitated (Figure 9-12). If you're doing a direct quantitation assay, the same data is displayed in the Quantitation Step graph.

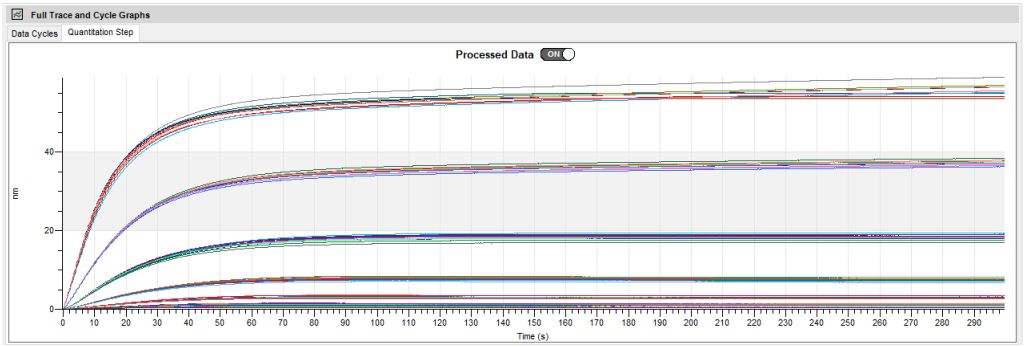


Figure 9-12: Quantitation Steps Tab

When corrected data is displayed, the Processed Data toggle at the top of the graph is ON. To view uncorrected data, click the button to toggle it OFF (Figure 9-13).

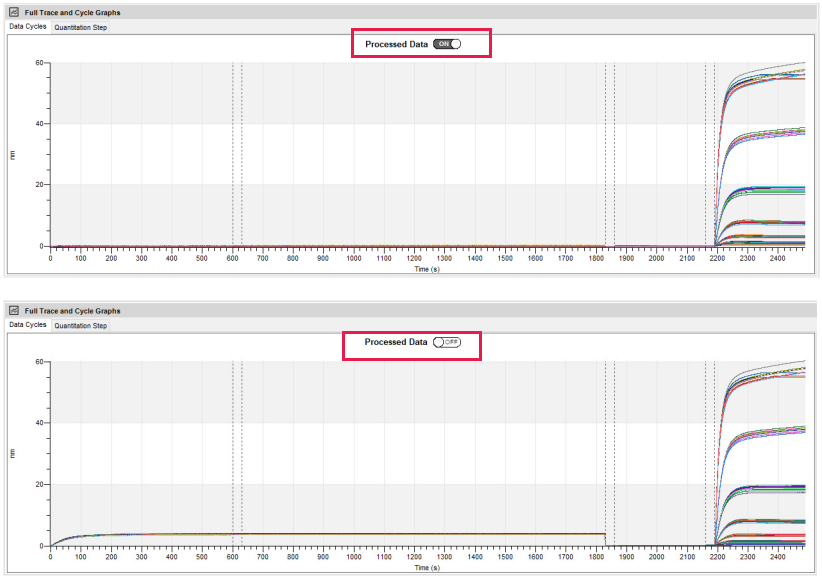


Figure 9-13: Processed Data ON (Top) and OFF (Bottom)

The Data Cycles table shows biosensor-specific information (Figure 9-14). Subtraction formulas display in the table after reference subtraction is performed.

Full Trace and Cycle Data Tables

Data Cycles | Quantitation Step

Index	Color	Sensor Number	Tray	Repetition Number	Sensor Location	Sensor Type	Sensor Lot No	Sensor Info	Sample Location	Sample ID	Sample Info	Concentration(ng/ml)	Replicate C
1		1	1	1	t1A1	Anti-Human IgG Fc			B1			20	
2		2	1	1	t1B1	Anti-Human IgG Fc			D1			10	
3		3	1	1	t1C1	Anti-Human IgG Fc			F1	5		5	
4		4	1	1	t1D1	Anti-Human IgG Fc			H1	2.5		2.5	
5		5	1	1	t1E1	Anti-Human IgG Fc			J1	1.25		1.25	
6		6	1	1	t1F1	Anti-Human IgG Fc			L1	0.625		0.625	
7		7	1	1	t1G1	Anti-Human IgG Fc			N1	0.313		0.313	
8		8	1	1	t1H1	Anti-Human IgG Fc			P1	0		0	
9		9	1	1	t1A2	Anti-Human IgG Fc			B3	20		20	

Figure 9-14: Data Cycles Table

The Quantitation Step table shows only the sample step information such as sample location, sample ID and sample concentration (Figure 9-15).

Full Trace and Cycle Data Tables

Data Cycles | Quantitation Step

Index	Color	Tray	Sensor Location	Sensor Type	Sensor Lot No	Sensor Info	Plate	Sample Location	Sample ID	Sample Info	Concentration(ng/ml)	Dilution F
1		1	t1A1	Anti-Human IgG Fc			1	B1			20	
2		1	t1B1	Anti-Human IgG Fc			1	D1			10	
3		1	t1C1	Anti-Human IgG Fc			1	F1	5		5	
4		1	t1D1	Anti-Human IgG Fc			1	H1	2.5		2.5	
5		1	t1E1	Anti-Human IgG Fc			1	J1	1.25		1.25	
6		1	t1F1	Anti-Human IgG Fc			1	L1	0.625		0.625	
7		1	t1G1	Anti-Human IgG Fc			1	N1	0.313		0.313	
8		1	t1H1	Anti-Human IgG Fc			1	P1	0		0	
9		1	t1A2	Anti-Human IgG Fc			1	B3	20		20	

Figure 9-15: Quantitation Step Table

- To view a single row or column of biosensor data, click the number above the column or the letter next to the row in the biosensor tray or sample plate (Figure 9-16).

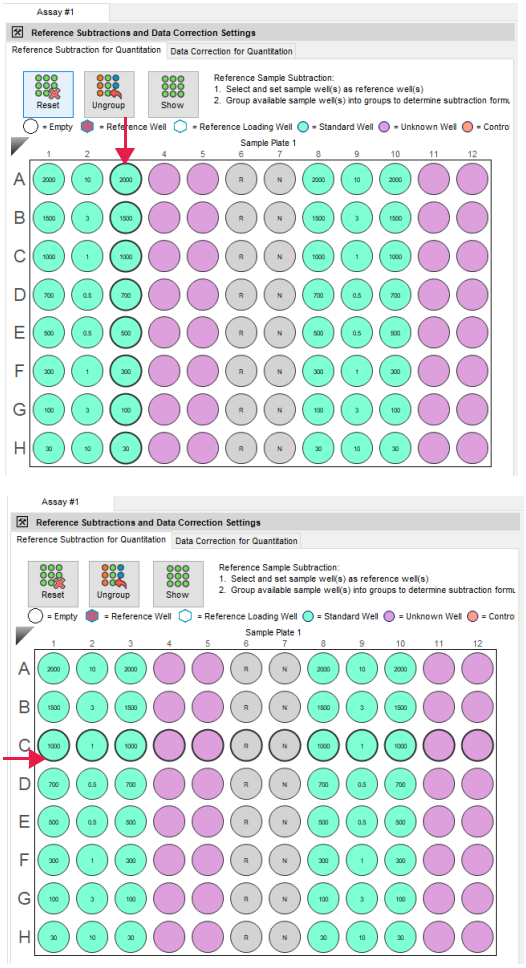


Figure 9-16: Selecting a Single Row or Column

- To view data for multiple sequential rows or columns, click the letter of the first row or the number of the first column you want to view in the biosensor tray or sample plate, then hold the mouse and drag it across all the other row/columns you want to view (Figure 9-17).

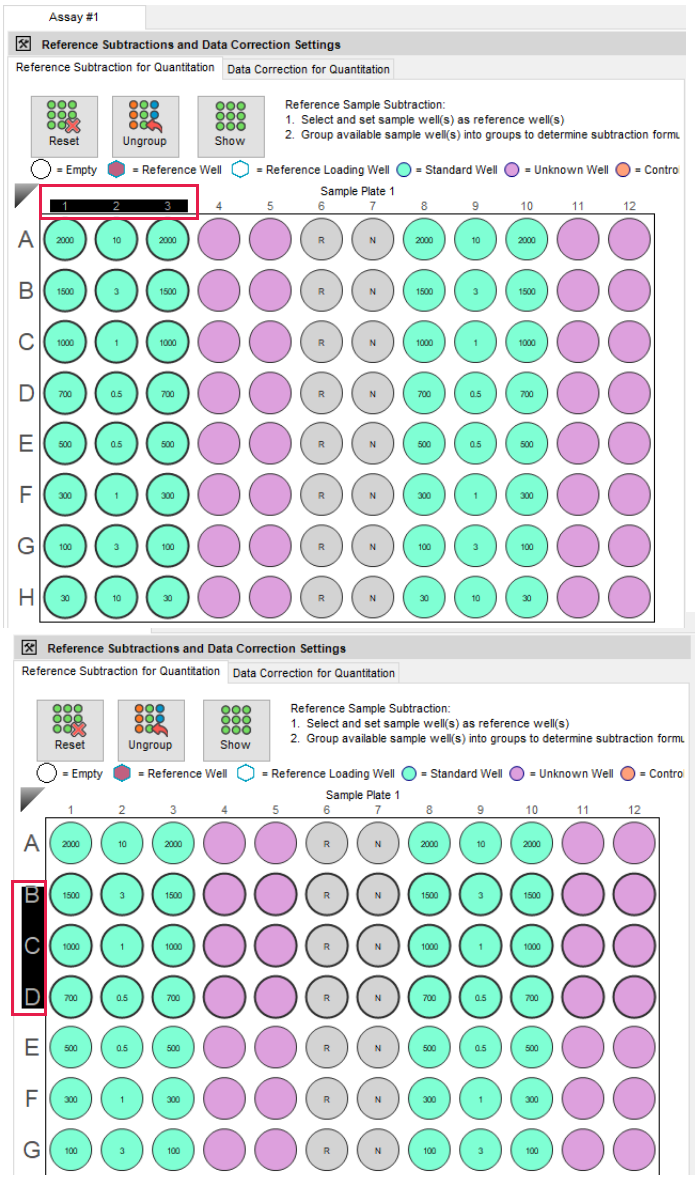


Figure 9-17: Selecting Multiple, Sequential Rows or Columns

- To view data for multiple, non-sequential rows or columns, click the number above the first column or letter next to the first row you want to view in the biosensor tray or sample plate, hold **Ctrl** and select the other rows/columns you want to view.

- To view data for multiple sequential wells, use your mouse to draw a box around the wells in the biosensor tray or sample plate (Figure 9-18).

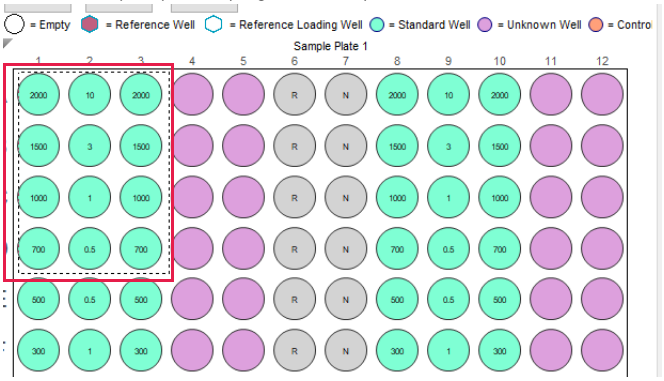


Figure 9-18: Selecting Multiple, Sequential Wells

- To view data for multiple, non-sequential wells, click the first well you want to view, hold **Ctrl** and select the others you want to view.

Setting Reference Wells and Changing Well Types

Well types can be changed if they were not designated correctly in Octet® BLI Discovery software. In the sample plate, select the well(s), right-click, and select the new well type from the menu. You can change the well type to Standard, Control, Unknown, or Reference (Figure 9-19).

- For direct quantitation or 1-step assays, set the well with zero analyte or sample as **Reference**.

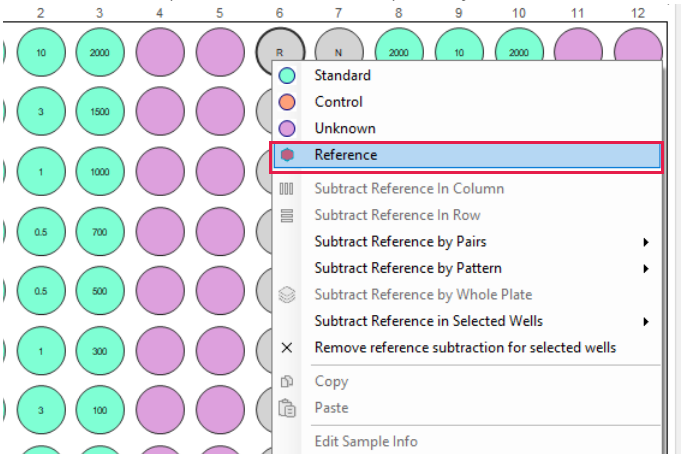


Figure 9-19: Change Well Type Options

- For multi-step quantitation assays, two types of references can be chosen:
  - A well containing no analyte or sample should be set as a **Reference**.
  - A well that contains no load, capture or ligand should be designated as a **Reference Load Well** (Figure 9-20).

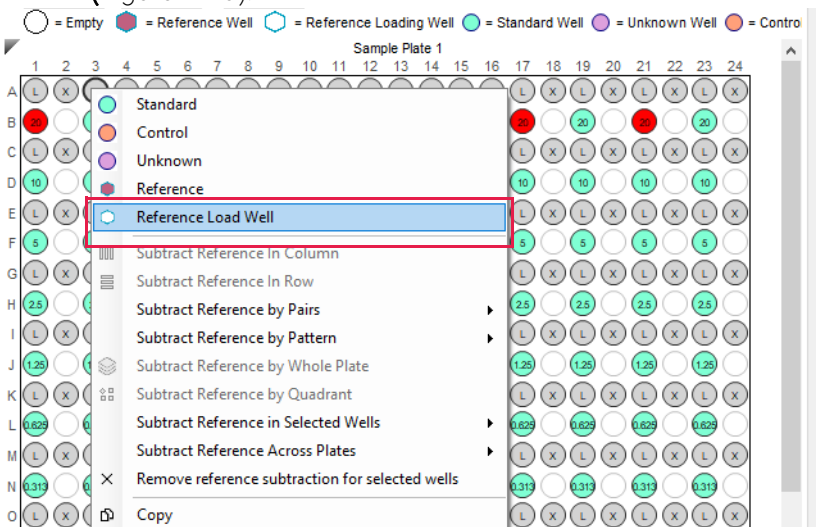


Figure 9-20: Change Well Type Options with Reference Load Well

Subtracting Reference or Non-Specific Binding

To subtract reference or non-specific binding:

1. Make sure all your reference wells have been assigned.
2. **To group reference wells for subtraction -** right click anywhere on the sample plate and select **Subtract Reference.** Select one of the following (Figure 9-21):

NOTICE: *For referencing similar to the All option in the Octet® BLI Analysis software, select the entire plate and choose* **Subtract Reference in Selected Wells**. *See page 204.*

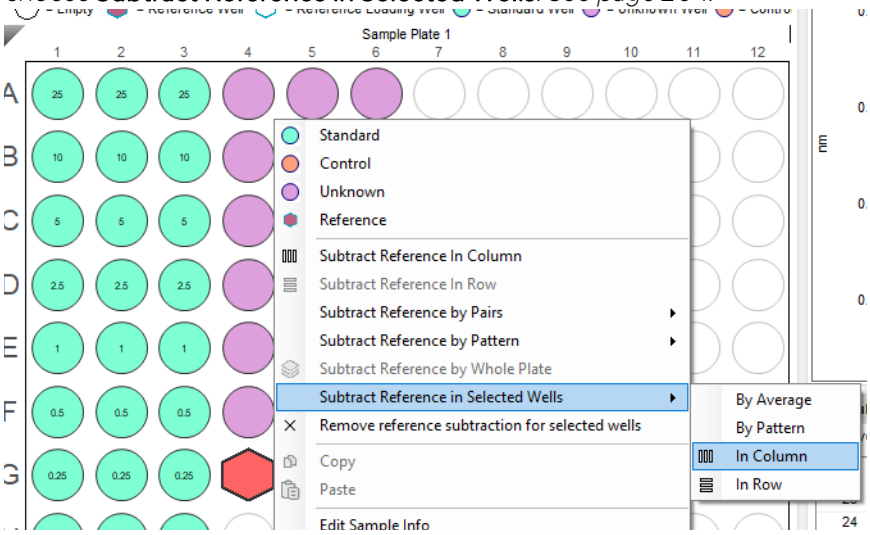


Figure 9-21: Subtract Reference Options

After making a selection, the software performs the subtraction and display the reference well subtraction formula in the Data Cycles table.

- **In Column** - If a column includes both non-reference and reference wells, the software computes the average reference curve for the column and subtracts this curve from the non-reference wells in the same column (Figure 9-22).

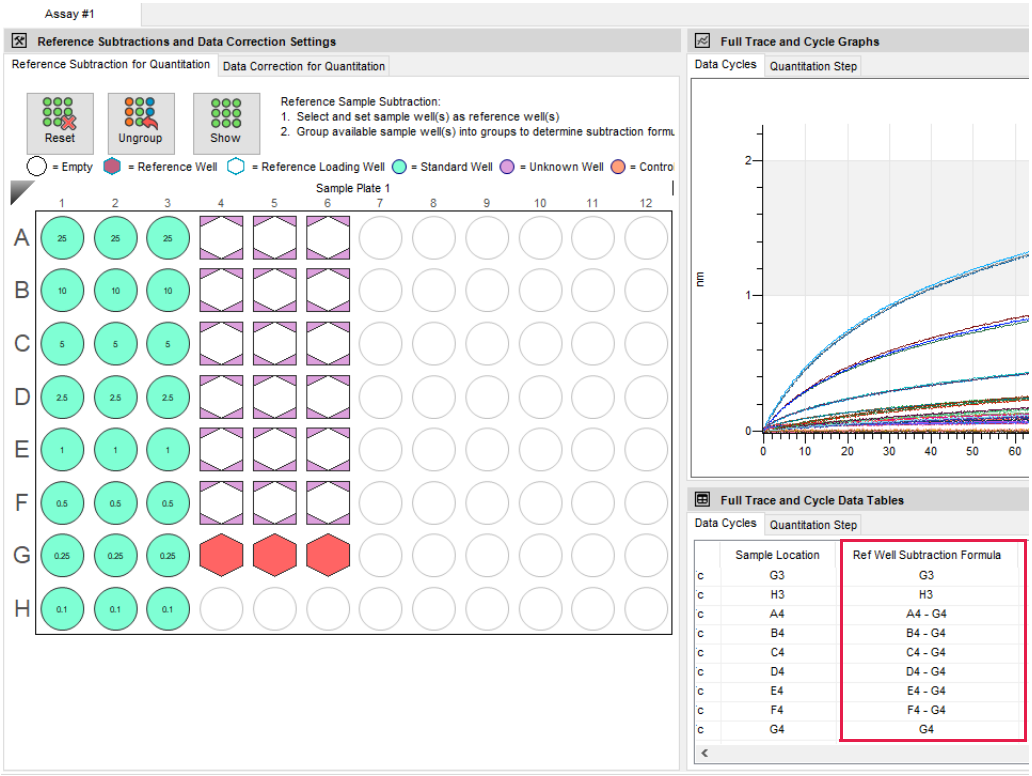


Figure 9-22: Subtract Reference In Column Plate View and Data Cycles Table Subtraction Formula

- **In Row** - If a row includes both non-reference and reference wells, the software computes the average reference curve for the row and subtracts this curve from the non-reference wells in the same row. (Figure 9-23).

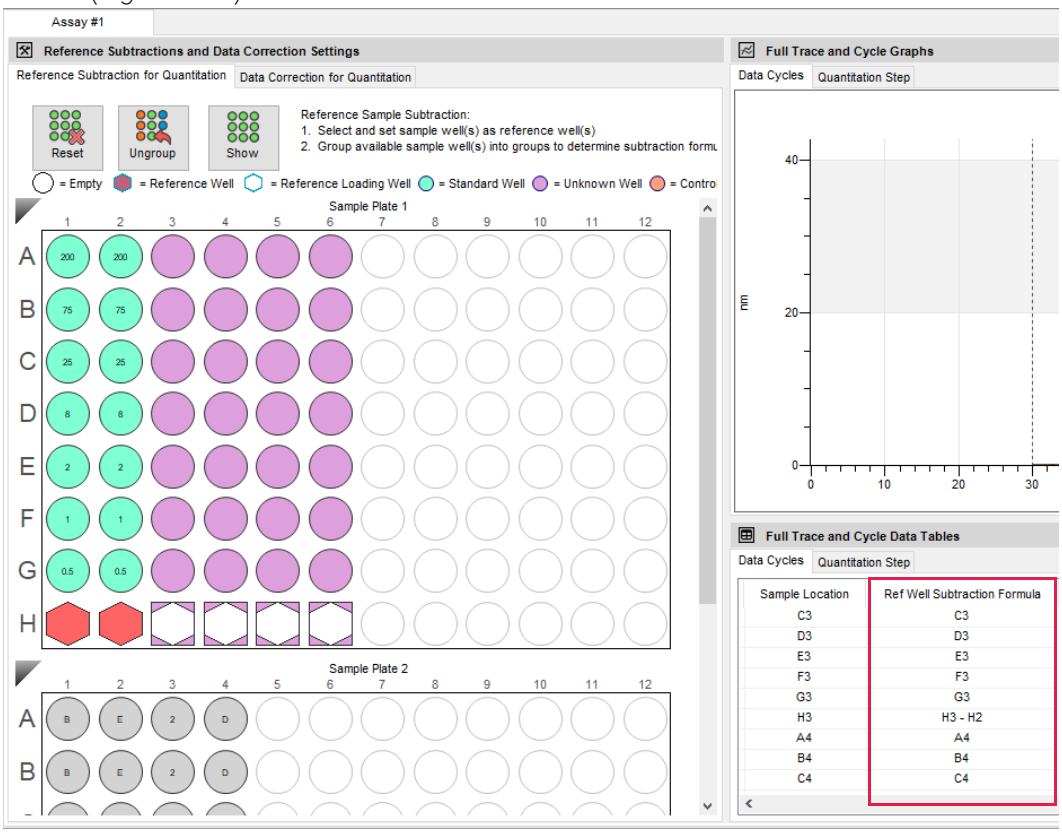


Figure 9-23: Subtract Reference In Row Plate View and Data Cycles Table Subtraction Formula

- **By Pairs Left** - Groups reference and sample/load wells in pairs and assumes that the reference wells are to the right of the sample/load wells. For example, if your sample/load wells are in column 1 and 3, and the reference wells are in column 2 and 4, this subtracts A2 from A1, A4 from A3, and so on.
- **By Pairs Right** - This function groups sensors similarly to By Pairs Left but it assumes that the reference wells (A1) are to the left of the sample/load wells (A2) so A1 subtracts from A2, A3 from A4 and so on.
- **By Pattern Right** - Groups reference and sample/load wells in a pattern and assumes that the reference wells are to the left of the sample/load wells. This option is typically used when you're running all reference wells first and then all the sample/load wells. For example, if your sample/load wells are in columns 3 and 4, and the reference wells are in columns 1 and 2, this subtracts A1 from A3, A2 from A4, and so on.
- **By Pattern Left** - Groups reference and sample/load wells in a pattern and assumes that the reference wells are to the right of the sample/load wells. This option is typically used when you're running all the sample/load wells first and then all the reference wells. For example, if your sample/load wells are in columns 1 and 2 and reference wells are in columns 3 and 4, this subtracts A3 from A1, A4 from A2, and so on.
- **By Sensor Plate** - Use this option if you've run multiple trays of sample/load wells with one entire tray of reference wells during an experiment or mega-experiment. Reference and sample/load wells are grouped so that the reference well A1 in tray 1 is subtracted from all A1 sample/load wells in other plates.

- **By Quadrant** - This option is for 384 well plates only. The four sample wells that are accessed by the same sensor are treated as a quadrant. Any of the four wells can be the reference well and is subtracted from the other three wells. This option is applied to the entire plate. For example, if well B1 is a reference well, it will be subtracted from A1, A2 and B2.
- **By Whole Plate** - Use this option if you've run multiple plates of sample/loaded wells with one entire plate of reference wells during an experiment or mega-experiment. After assigning the reference wells in the reference plate, right-click the other sample plate, choose the By Whole Plate option, and then choose the reference plate. More than one plate can be a reference plate. For example, plate 1 could be subtracted from plate 2, and plate 3 could be subtracted from plate 4. Reference and sample/load wells are grouped by corresponding positions on the plate. For example, reference well A1 is subtracted from sample well A1; B1 from B1, B2 from B2, etc.

NOTICE: *When using referencing by Whole Plate, the reference well subtraction formula displays the well names with a "p" prefix and the plate number. For example, p2A3 indicates well A3 on plate number 2.*

If multiple plates and sensor trays were overlaid to create a mega-experiment where all experiments were run in an identical manner, set the reference wells and grouping on one plate, then copy and paste the configuration to the other plate using the right-click menu.

- **Subtract Reference Across Plates** - Use this option if your workspace contains multiple sample plates. The selected wells in each plate will be grouped together so that the reference well is subtracted from the other selected sample wells. If more than one reference well is included in the selection, their responses will be averaged before subtracting from the sample wells.
- **Copy and Paste** - If multiple plates and sensor trays were overlaid to create a mega-experiment where all experiments were run in an identical manner, set the reference wells and grouping on one plate, then copy and paste the configuration to the other plate using the right-click menu.

To subtract specific reference(s) from specific non-reference well(s) - select the reference(s) and the non-reference(s). Your selection must include at least one reference well and one non-reference well. Right-click and select **Subtract Reference in Selected Wells > By Average** (Figure 9-24).

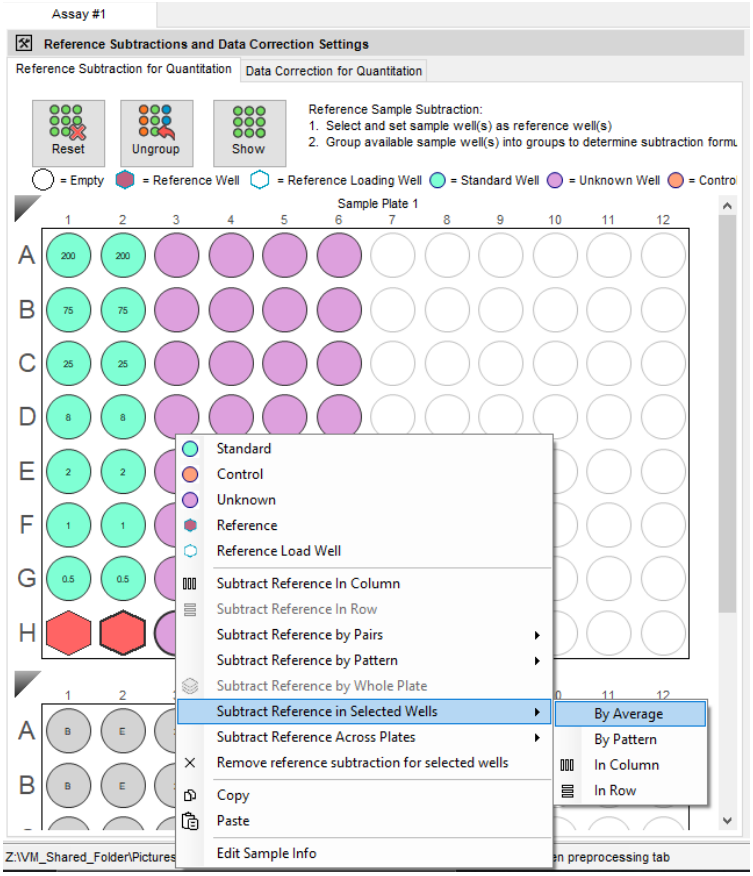


Figure 9-24: Subtracting Specific Wells

The software computes the average reference curve for the reference wells selected and subtracts this curve from the non-reference wells selected, and displays the reference well subtraction formula in the Data Cycles table.

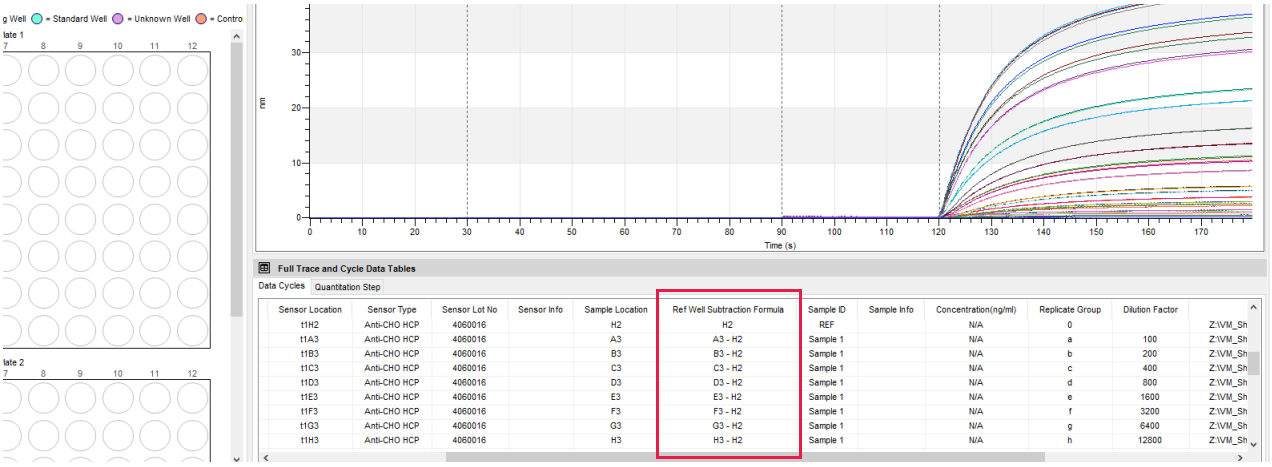


Figure 9-25: Subtract Selected Wells Plate View and Data Cycles Table Subtraction Formula

The corrected data displays in the Data Cycles and Quantitation Step graph tabs (Figure 9-26).

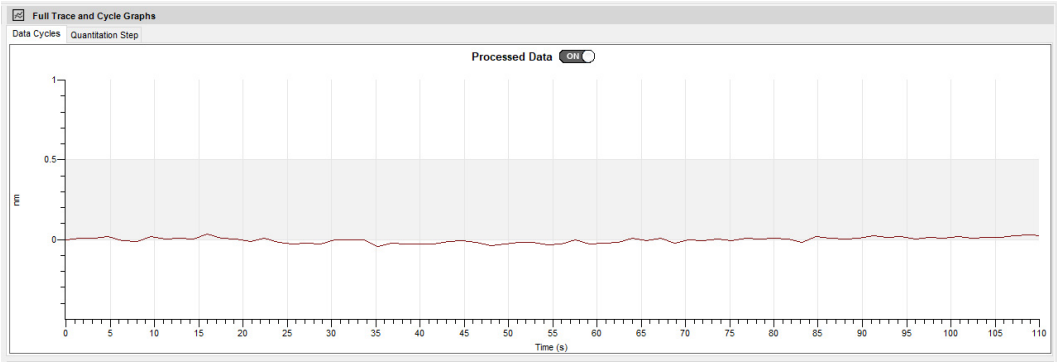


Figure 9-26: Corrected Data

When corrected data is displayed, the Processed Data toggle at the top of the graph is ON. To view uncorrected data, click the button to toggle it OFF (Figure 9-27).

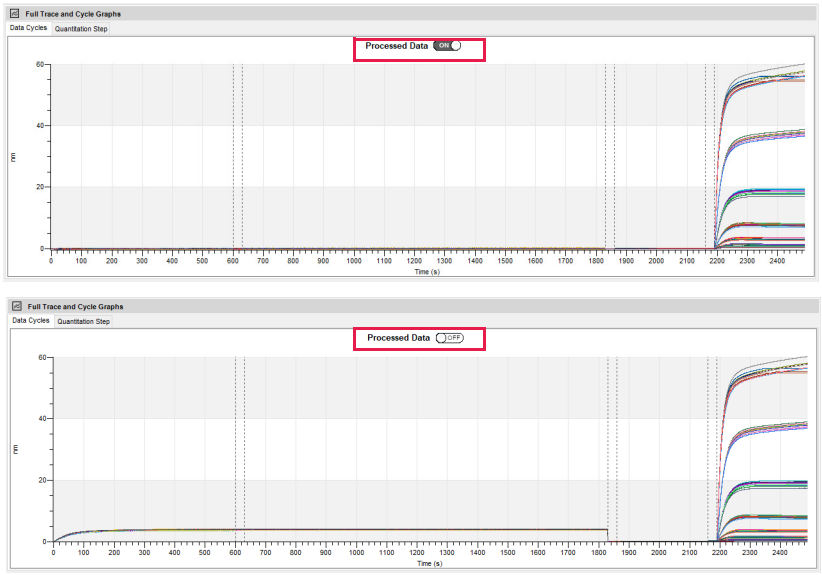


Figure 9-27: Processed Data ON (Top) and OFF (Bottom)

- Click 

(**Ungroup**) to remove all reference groupings. You can also right-click the reagent plate and select **Remove Reference Subtraction** > **Remove All Reference Subtraction**.
- To ungroup specific wells, select those wells then right click and select **Remove Reference Subtraction** > **Remove Selected Reference Subtraction Only**.
- Click 

(**Reset**) to clear all reference and group settings for all wells.

3. If there's more than one assay in your experiment or combined dataset, click the next **Assay** tab and repeat the prior steps as needed.

Editing Sample Information

You can edit sample information in the Reference Subtraction for Quantitation tab.

1. Select the well you want to edit in the reagent plate.
2. Right-click the well and select **Edit Sample Info** (Figure 9-28).

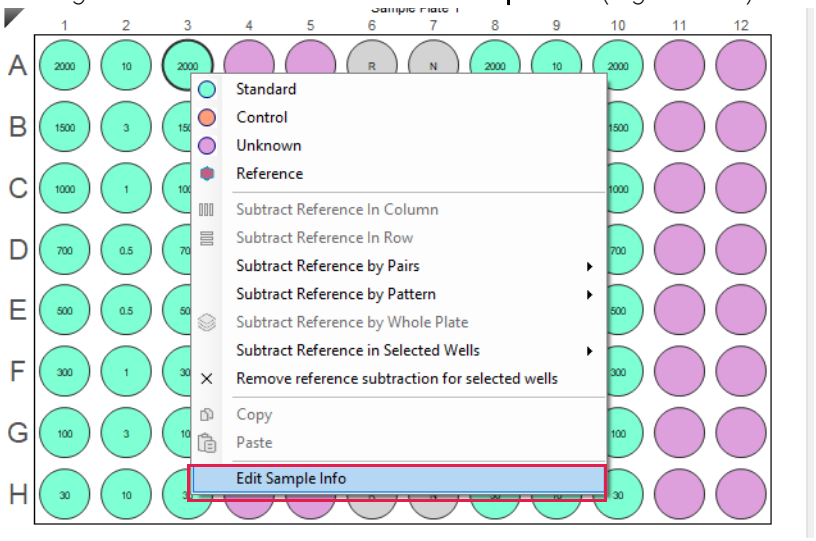


Figure 9-28: Selecting Edit Sample Info

3. Update the information in the Edit Sample Info window, then click **OK**.
   You can also double click in the cells in the Dilution Factor, Sample ID and Concentration columns in the Data Cycles table to change this information.

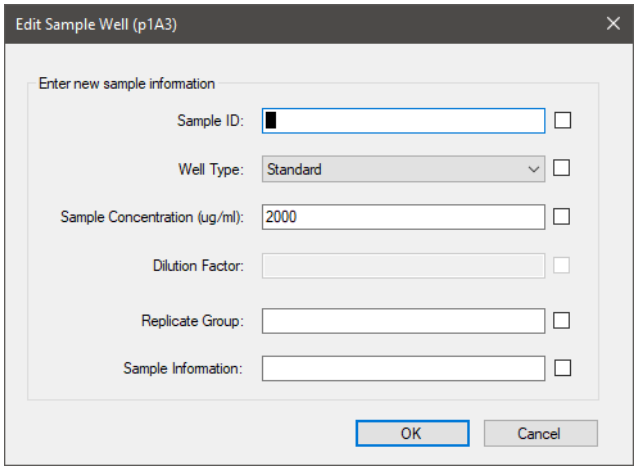


Figure 9-29: Edit Sample Info Window.

Data Correction

The Data Correction tab lets you flip data or align the step X-axis prior the applying the curve fitting (Figure 9-30).

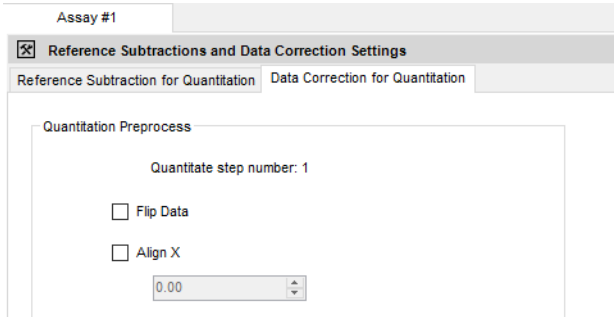


Figure 9-30: Data Correction Tab

Align X

Use this option if you need to remove artifacts at the beginning of the binding curves.

1. Select **Align X**.
2. Enter a time point (in seconds) or use the up/down arrows to assign a time point to begin alignment (Figure 9-31).

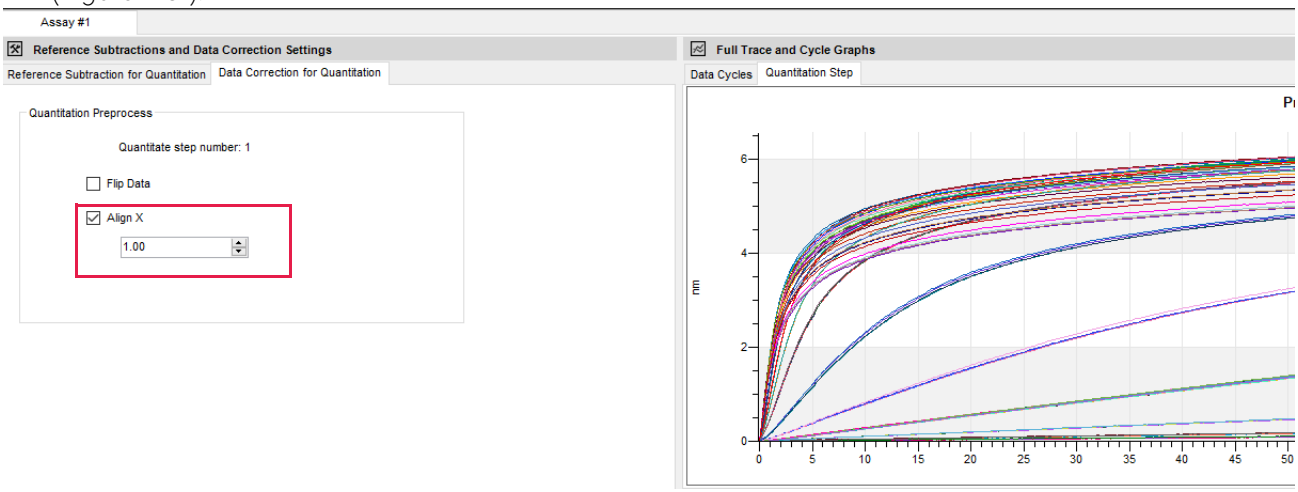


Figure 9-31: Align X

If there is more than one assay in your experiment or combined dataset, click the next **Assay** tab and apply the Align X option as needed.

Flip Data

The Flip Data function inverts binding curves from positive to negative or from negative to positive (Figure 9-32). Select this option when the observed nm shift goes negative due to presence of large analytes such as phage, cells, and lipoparticles on the biosensor surface.

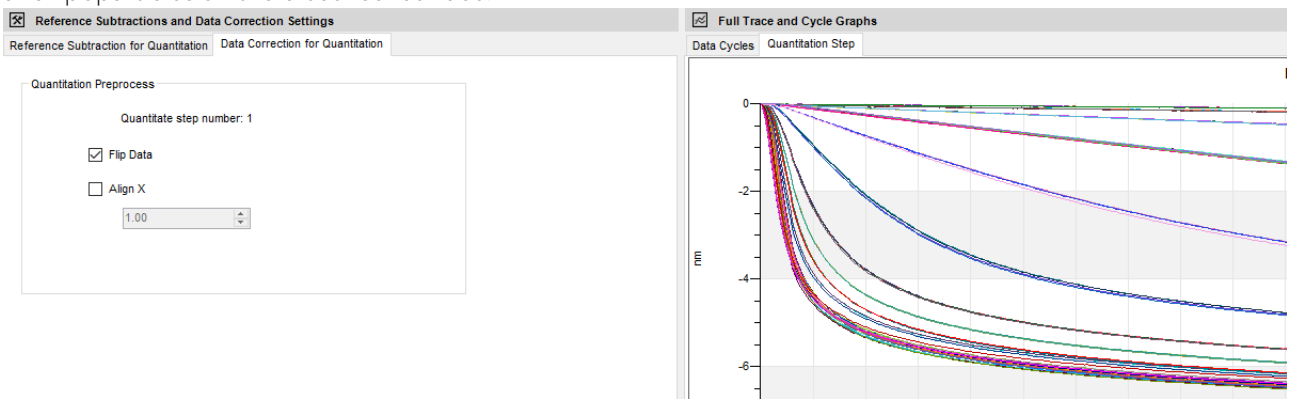


Figure 9-32: Flip Data

If there is more than one assay in your experiment or combined dataset, click the next **Assay** tab and apply the Flip Data option as needed.

Changing the Graph View

- To make the sensorgram traces thicker, right-click the graph and select **Thicken Line**:

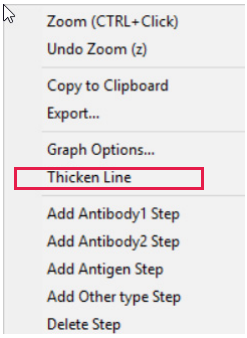


Figure 9-33: Selecting Thicken Line

Graph Options

To change the graph display options, right-click the graph and select **Graph Options**. The Graph Options window displays (Figure 9-34).

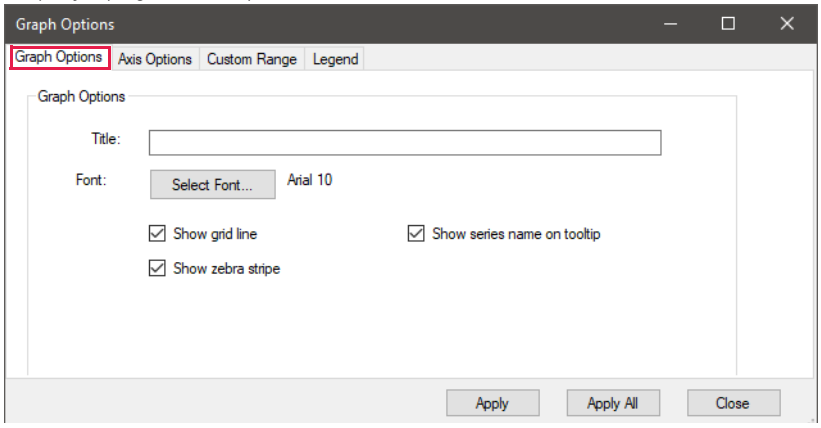


Figure 9-34: Graph Options Window

- **Title** - Adding text in the Title box displays that text above the graph (Figure 9-35).

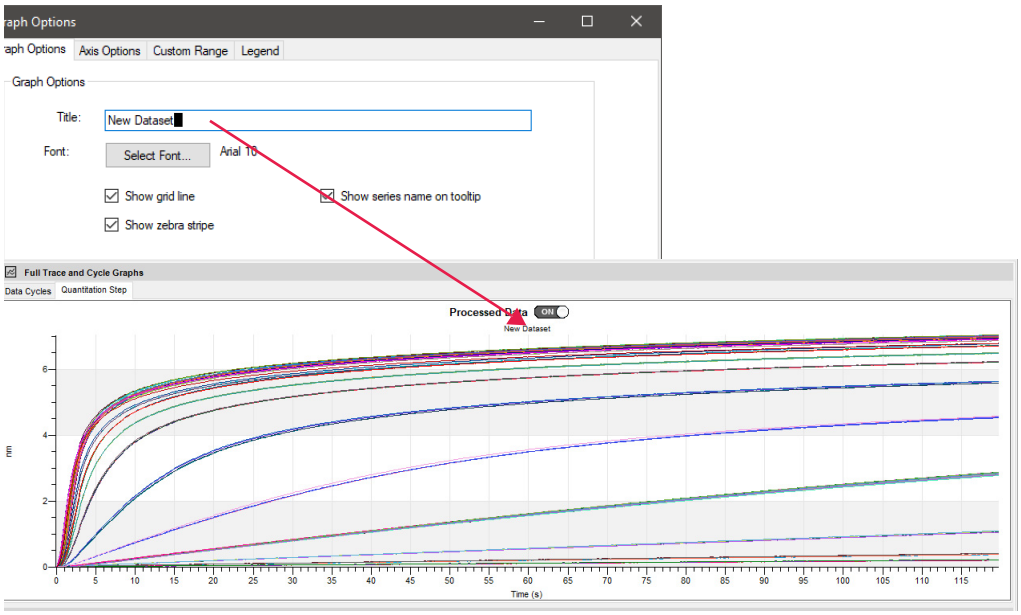


Figure 9-35: Adding a Graph Title

- **Font Size** - Lets you select the font size of the Title in pixels.
- **Show Grid Line** - Checking or deselecting this box shows or hides the grid lines on the graph.

- **Show Zebra Stripe** - Checking or deselecting this box shows or hides (Figure 9-36) the alternating white and grey horizontal rows on the graph.

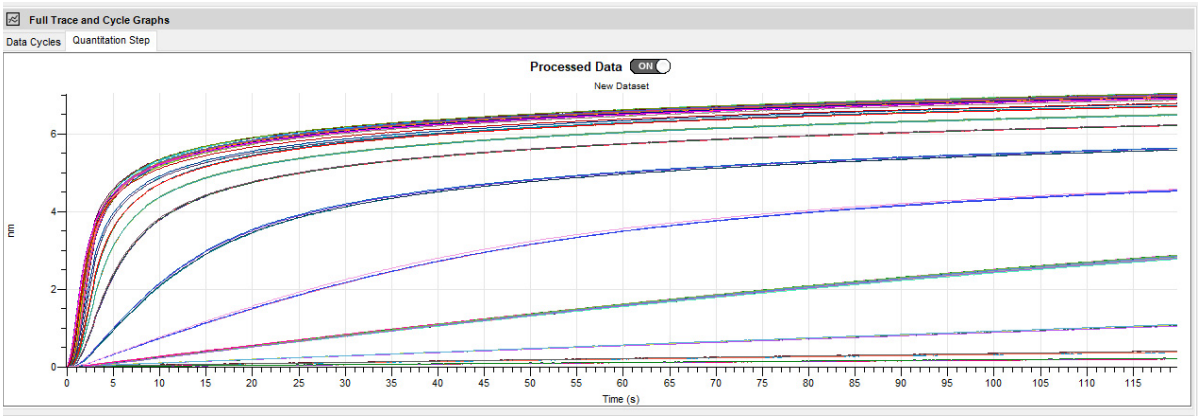


Figure 9-36: Show Zebra Stripe Deselected

- **Show Series Name on Tooltip** - Checking or deselecting this box shows (Figure 9-37) or hides the tooltip when you hover over a trace on the graph.

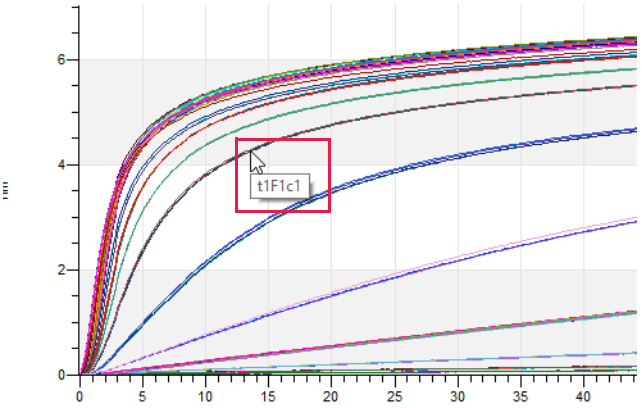


Figure 9-37: Show Series Name on Tooltip Selected

Axis Options

To change the graph's X and Y axis options, right-click the graph and select **Graph Options**, then select the **Axis Options** tab (Figure 9-38).

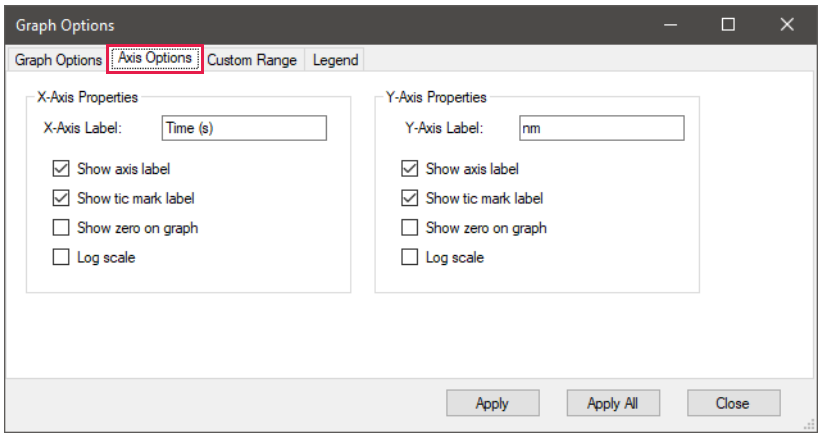


Figure 9-38: Axis Options Tab

- **Axis Label** - Changing text in the Axis Label boxes updates the X and Y axis labels on the graph.
- **Show Axis Label** - Checking or deselecting this box shows or hides the X and Y axis labels on the graph.
- **Show Tic Mark Label** - Checking or deselecting this box shows or hides the X and Y axes tic mark values on the graph.
- **Show Zero on Graph** - Shows the zero on the y-scale in the graph if the signals are too high and zero is not shown by default.
- **Log Scale** - Checking or deselecting this box changes the X and Y scale from linear to log scale (Figure 9-39).

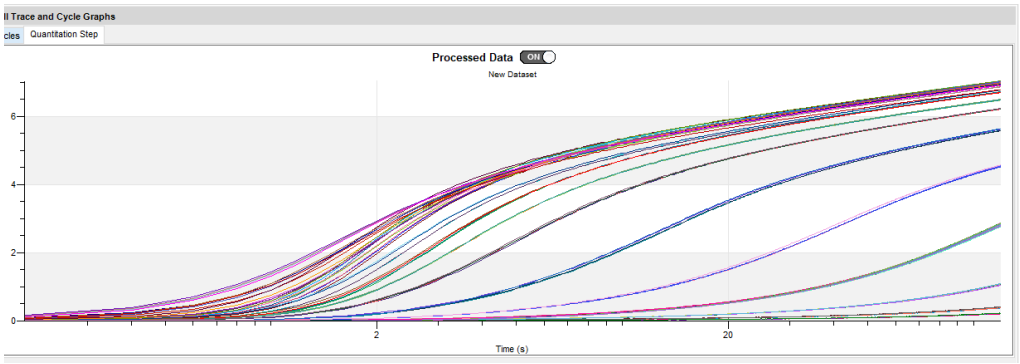


Figure 9-39: X-axis Log Scale Selected

Changing the Graph Axis Range

To change the graph's X and Y axis range, right-click the graph and select **Graph Options**, then select the **Custom Range** tab (Figure 9-40).

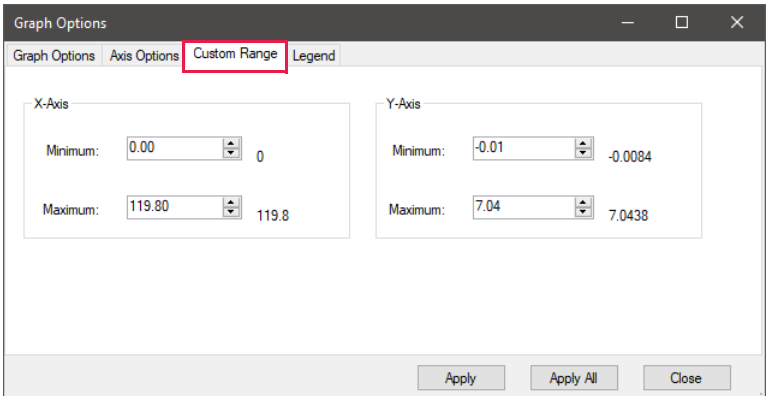


Figure 9-40: Custom Range Tab

Enter your own Minimum and Maximum values for the X and Y axes. After making your selections, click **Apply** to apply the change just to the graph you selected, or if you are viewing individual graphs, select **Apply All** to apply the change to all graphs.

Adding Fit Graph Legends

To show data legends on the graphs, right-click the graph and select **Graph Options**, then select the **Legend** tab (Figure 9-40).

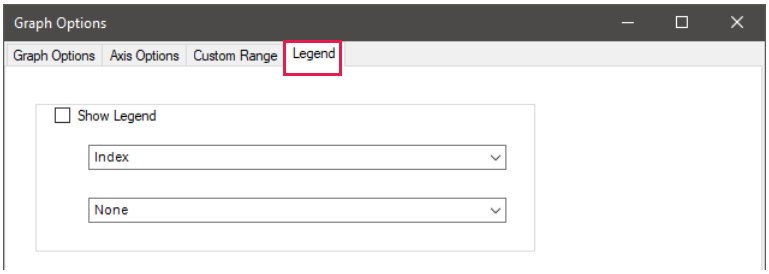


Figure 9-41: Legend Tab

Select up to two legend options from the drop down menus, then select Show Legends (Figure 9-42).

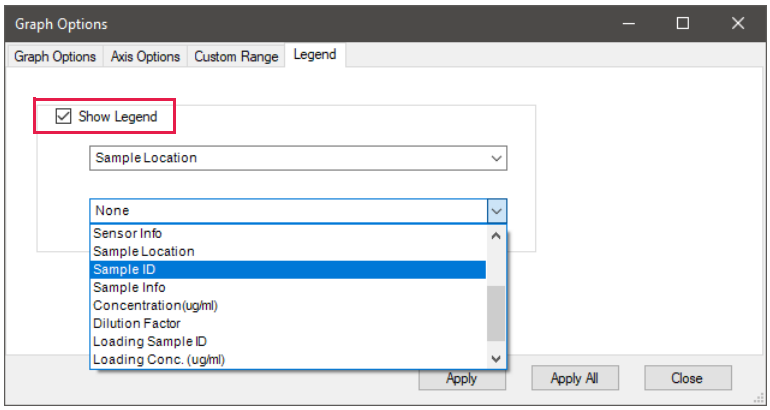


Figure 9-42: Selecting Legend Options

After making your selections, click **Apply** to apply the change. The legend option(s) selected display at the bottom of the graph (Figure 9-43).

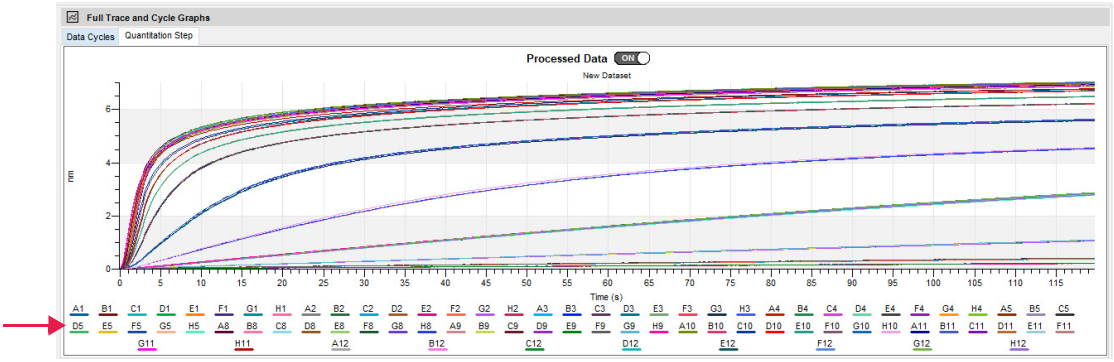


Figure 9-43: Legend Displayed

Export Options

To export the graphs, right-click the graph and select **Export**. The Export window displays (Figure 9-44).

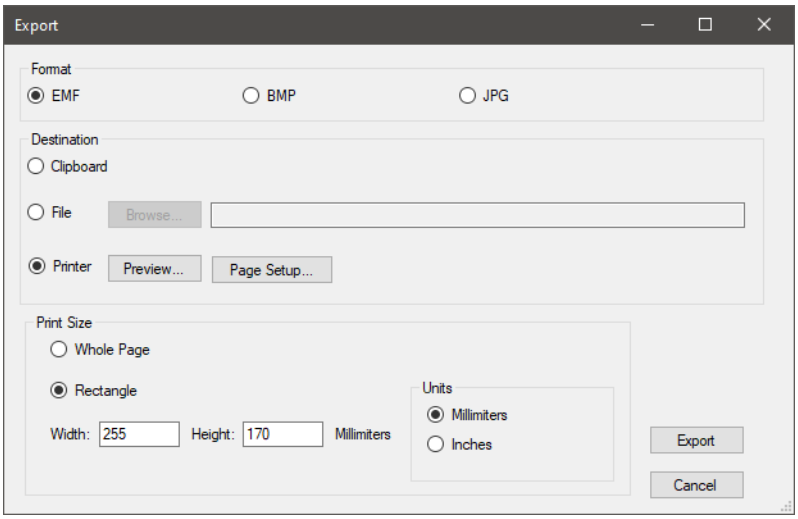


Figure 9-44: Export Window

Chose a format (EMF, BMP or JPG), an export destination (Clipboard, save to File, or Printer), set print size parameters if needed then click **Export**.

Changing the Table View

You can sort data in the Data Traces and Quantitation Step tables by clicking the column header (Figure 9-45). When a column header is blue, it indicates the table data is currently sorted by that parameter (Figure 9-45).

- Clicking a header for a column that contains numbers or letters sort the data alpha-numerically lowest to highest or A-Z. Clicking the header again resorts the data highest to lowest or Z-A.
- Clicking **Tray** sorts the data by tray first to last starting at 1, clicking **Tray** again resorts the data by trays last to first.
- Clicking **Sensor Number** sorts the data by sensor number in sensor trays 1-4 starting with A1 and sensor tray 1. Clicking on **Sensor Number** again sorts the data by sensor number in sensor trays 4-1, starting with A1 and sensor tray 4.

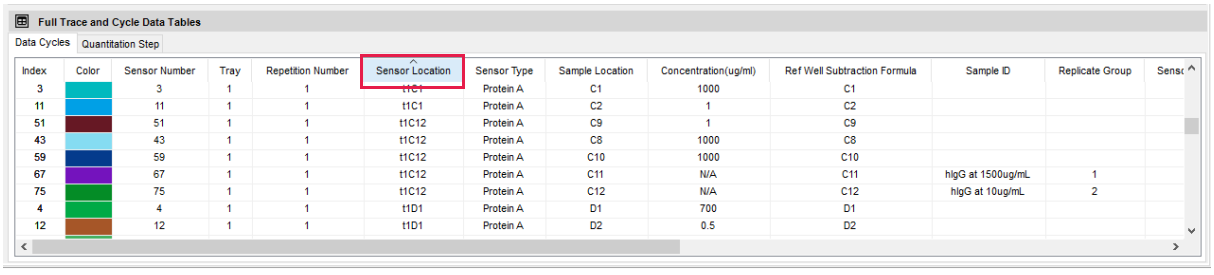


Figure 9-45: Sorting Data Table Columns

To resize the table columns, roll the cursor over the border between the column headers until the resizing cursor displays (Figure 9-46).

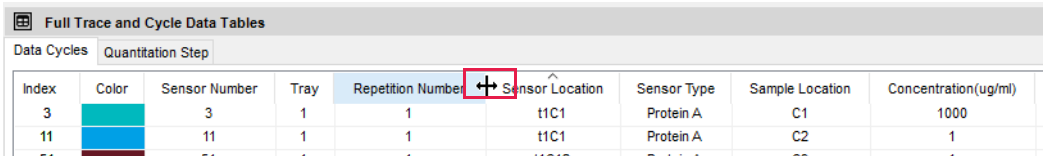


Figure 9-46: Column Resize Cursor

Then click and drag the column to resize (Figure 9-47).

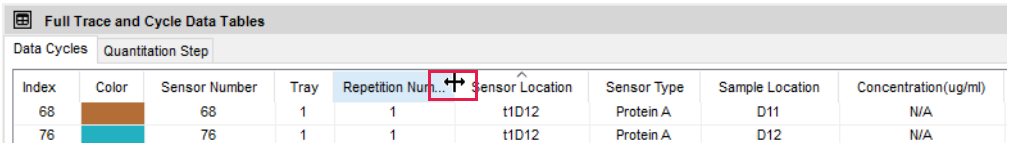


Figure 9-47: Resized Column

Exporting Datasets

You can use the Export options menu at the top of the Preprocess Data screen to export your datasets (Figure 9-48).

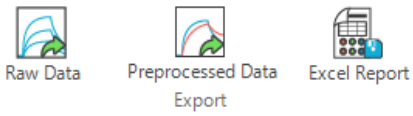


Figure 9-48: Export Options.

- Click (**Raw Data**) to export the raw, uncorrected data for your experiment or combined data set in .csv format.
- Click (**Preprocessed Data**) to export the processed (corrected) data for your experiment or combined data set in .csv format.
- Click (**Excel Report**) to export the processed (corrected) data for your experiment or combined data set in .xlsx format. Select which experimental components to export, use the default report name or click **...** to specify another name/location, then click **Export** (Figure 9-49).

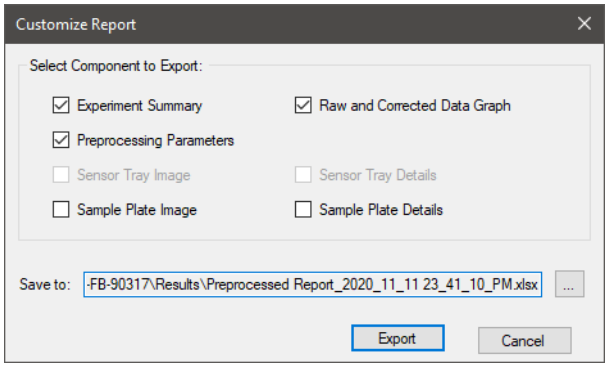


Figure 9-49: Excel Report Export Options

Chapter 10:

Quantitation Analysis

Quantitative Analysis

Octet® systems can be used to accurately determine the concentration of antibodies and other proteins in purified samples and complex matrices. Titer determination of human IgG and other recombinant proteins using Octet® systems have been critical to the selection of cell lines for development and optimization as they enable rapid turn-around time of results, thereby improving process efficiencies and productivity that cannot be achieved with existing methods such as HPLC and ELISA. Octet® systems are also being used in process development labs to quantitate sub ng/mL process contaminants such as Host Cell Proteins and residual Protein A.

Quantitation Analysis Screen Overview

The Quantitation Analysis Screen lets you determine sample concentration using a reference set of standards. Once you've made your reference subtraction and data corrections in the Preprocess Data screen, click the **Quantitation Analysis** tab to start your analysis. For information on how to pre-process your data before proceeding to quantitation, please refer to "Preprocessing Quantitation Datasets" on page 189. The software automatically analyzes the data using default settings, but you'll be able to modify analysis parameters as needed (Figure 10-1).

NOTICE:

See "Resizing, Hiding and Closing Windows" on page 11. for information on working with the software windows.

See "Resizing, Hiding and Closing Windows" on page 11. for more information on the displaying graphs and display options.

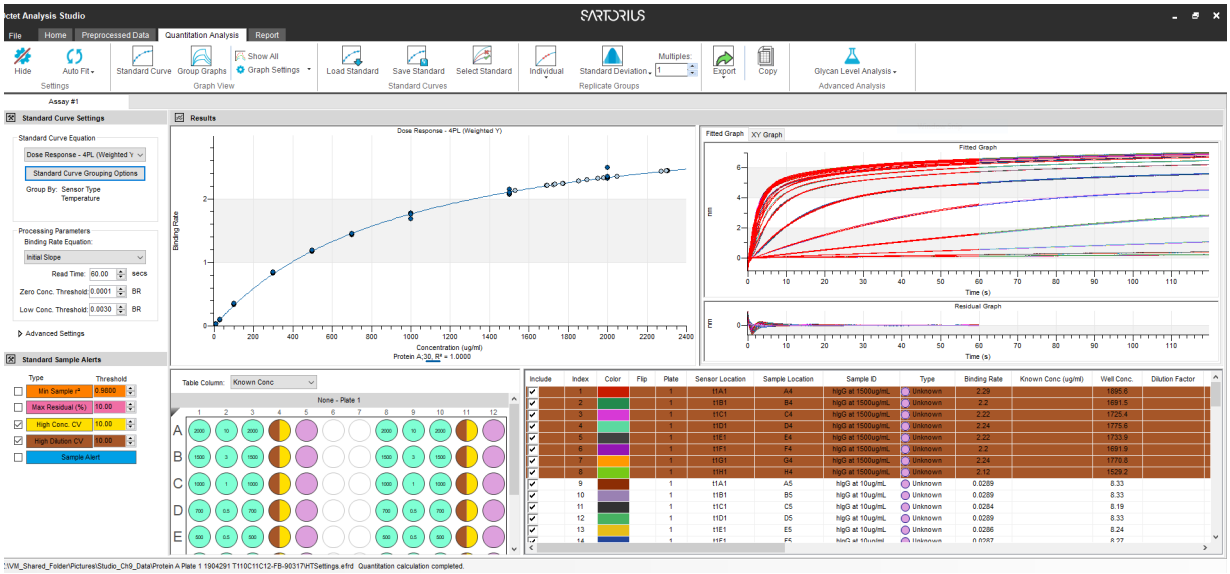


Figure 10-1: Quantitation Analysis Screen

To view or analyze data for different assays in the experiment, click an **Assay** tab. (Figure 10-2).

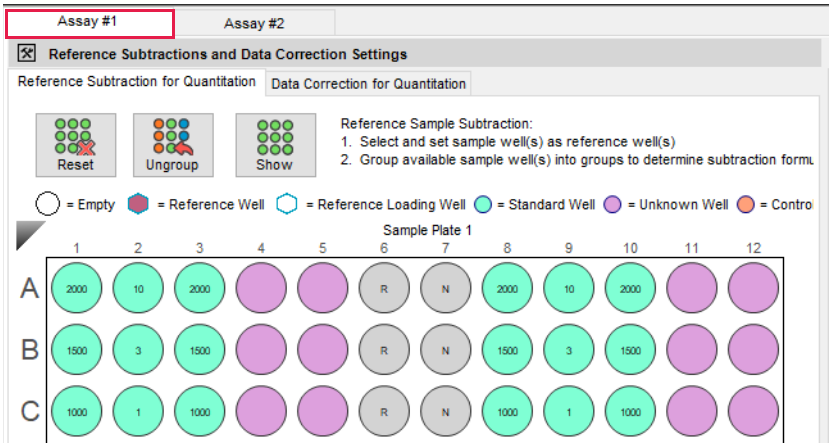


Figure 10-2: Assay Tabs

Viewing Settings

You can view quantitative analysis and other settings for your experiment in the Standard Curve Settings and Standard Sample Alert windows (Figure 10-3). Step-by-step details for performing quantitative analysis on your data can be found in "Analyzing Your Quantitation Data" on page 229.

- Click (**Settings**) to hide the panel.
- Click (**Settings**) to show the panel again.

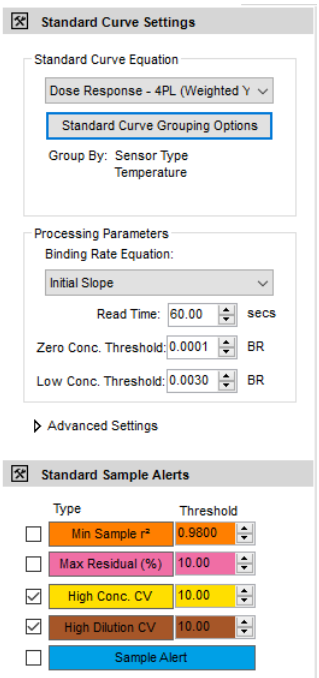


Figure 10-3: Quantitation Analysis Settings Panel

Viewing Data

To view the standard curve for your data, click the first icon in the Graph View section of the icon bar and select **Standard Curve** (Figure 10-4).

Figure 10-4: Selecting the Standard Curve View

Standard Curve and X-Y graphs (Figure 10-5).

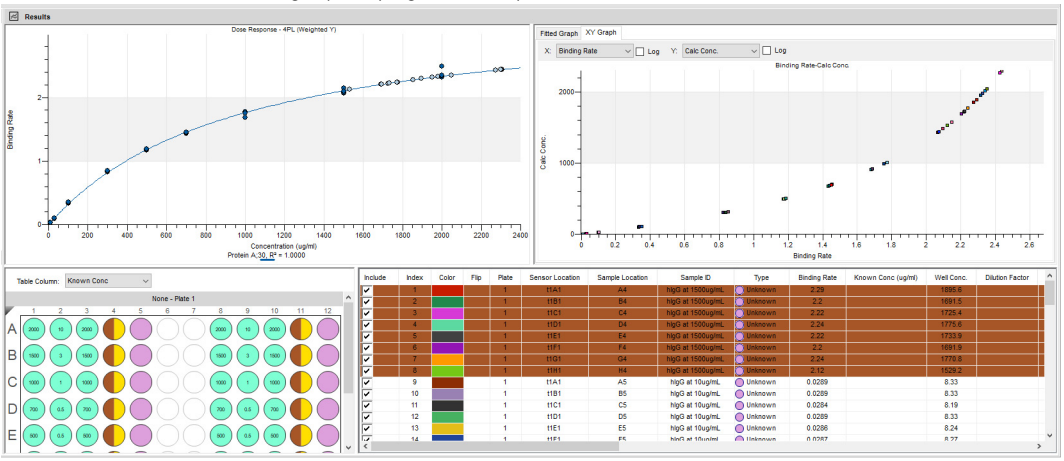


Figure 10-5: Standard Curve and X-Y Plots

Standard Curve Graph

The Standard Curve graph (Figure 10-6) lets you view the calculated standard curve of standards run in your experiment. The curve displayed depends on the option selected in the Standard Curve Settings window.

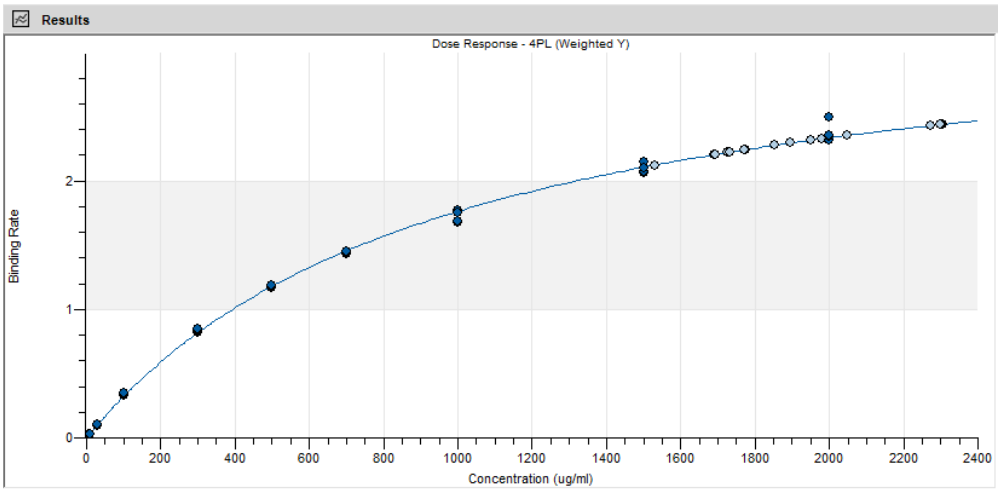


Figure 10-6: Standard Curve Graph—X Axis = Concentration, Y Axis = Binding Rate

Fitted Graph

The Fitted Graph shows the measured binding curves from the Quantitation step, and overlays the fitted binding model as a red trace. The residual graph shows the deviations of the measurements from the fitted model. (Figure 10-7) The Show Curve Fit option in the main ribbon toggles the display of the binding model traces.

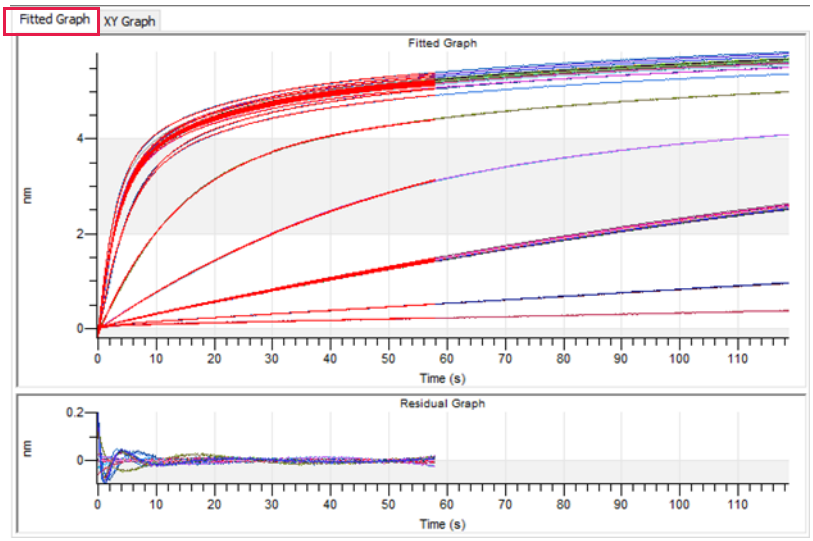


Figure 10-7: Fitted Graph Dialog

X-Y Graph

The X-Y graph is a scatter plot of analysis results based on x and y variables you can choose from (Figure 10-8).

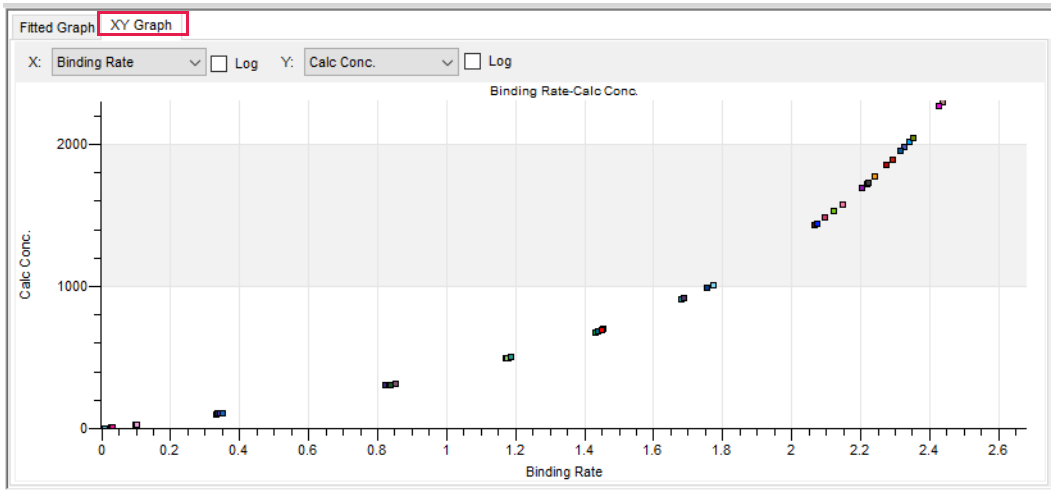


Figure 10-8: X-Y Graph

- **Changing the x/y variables** - You can choose which variables to plot by selecting the drop down menu next to either the x or y axis and selecting an option (Figure 10-9).

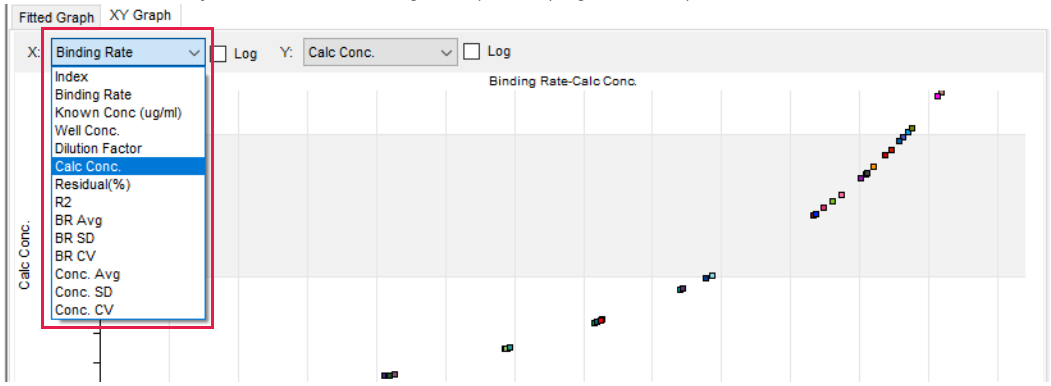


Figure 10-9: Variable Selection for X and Y Axis

- **Changing the scales** - A linear scale is displayed by default. Select one or both of the **Log** checkboxes to display either axes in a logarithmic scale.

Changing the Data View

- To view data for a single sample plate well, click a well in the plate map or row in the results table (Figure 10-5). The well you selected highlights in green on the Standard Curve, and only the well selected displays in the X-Y graph.

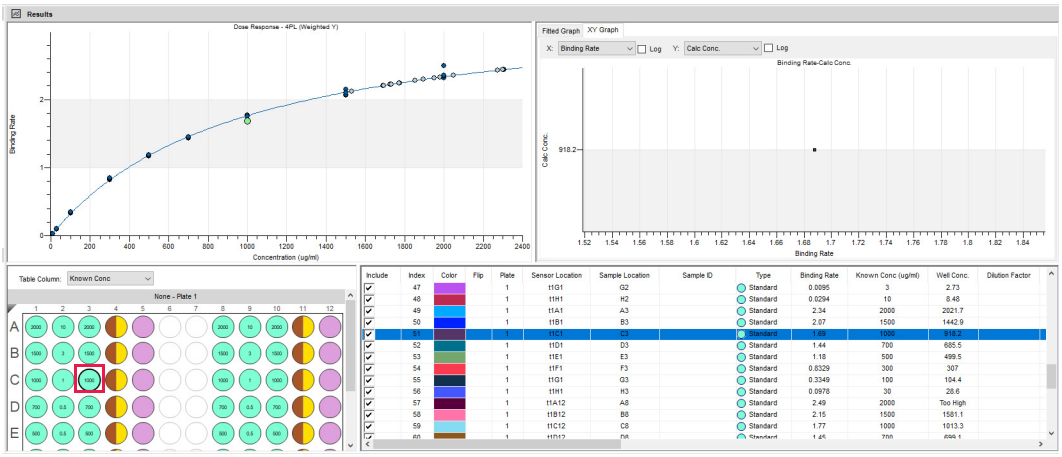
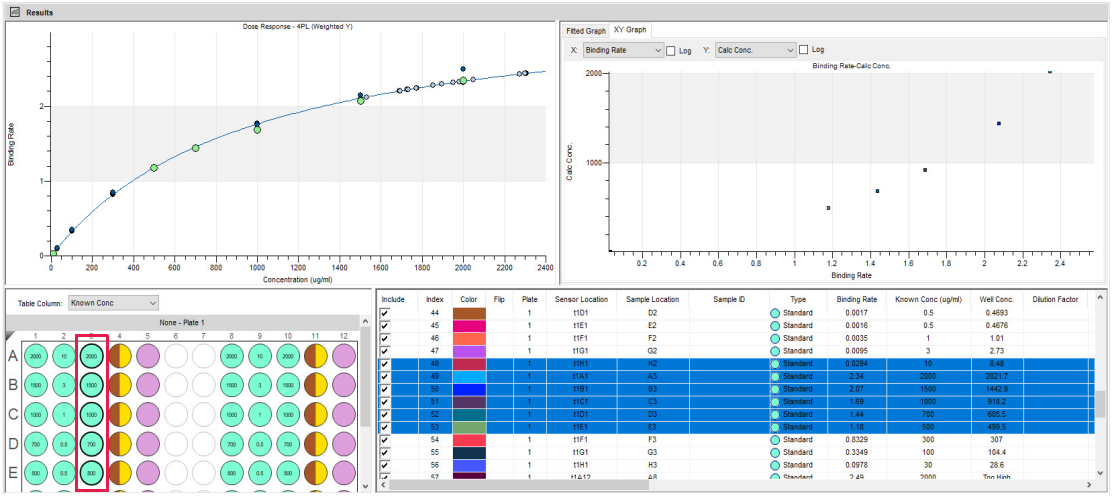


Figure 10-10: Viewing a Single Well

- To view data for multiple sample plate wells, press and hold **Ctrl**, then select the wells in the sample plate or rows in the table you want to view (Figure •). The wells you selected highlights in green on the Standard Curve, and only the wells selected display in the X-Y graph.



- Viewing Multiple Wells
- Click  (**Show All**) to view data for all sample plate wells.
- To view graphs organized into groups according to sample attributes or results category, click the first icon in the Graph View section of the icon bar and select **Group Graphs** (Figure 10-11). This is a useful feature when you're working with large data sets.
- To view graphs grouped by specific options, click **Graph Settings** in the Fit Graph View section (Figure 10-11).

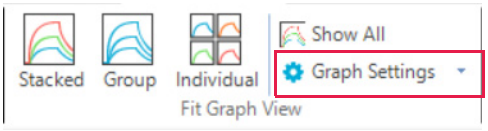


Figure 10-11: Selecting the Group View

Open the **Graph Setting** menu and select **Group Graph options**.

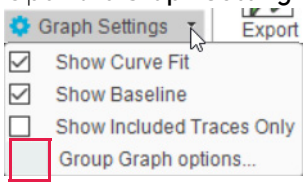


Figure 10-12: Graph Settings - Group Graph options

The **Group View Options** screen appears (Figure 10-13).

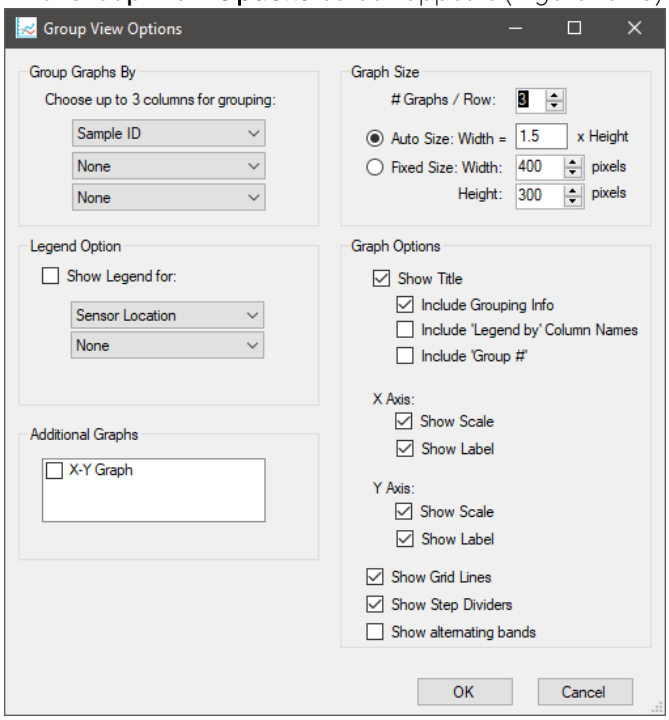


Figure 10-13: Group View Options

- **Group Graphs By** - Lets you select up to three categories for grouping the data across three independent parameters.
- **Legend Option** - Check the **Show Legend for:** box then select up to two categories to include in the graph legends.
- **Additional Graphs** - Lets you select other graphs to display with the analyzed (fitted) data.
- **Graph Size** - Options for the number of graphs to display per row and the graph size.
- **Graph Options** - Lets you choose options for graph labels and other graph display features.

After you've made your selections, click **OK**.

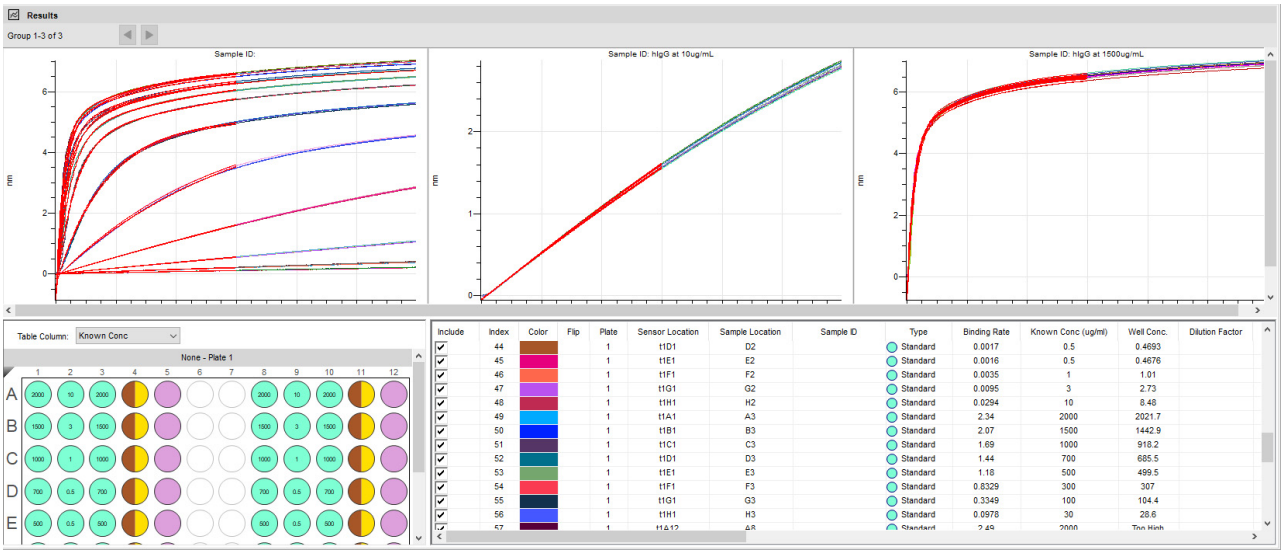


Figure 10-14: Grouped View

Viewing Analysis Results Table Data

Results for your quantitation analysis are shown in the Analysis Results table (Figure 10-15). Each row displays the results for one well on the sample plate. Sensor and Sample Well properties are entered during assay setup in the Octet® BLI Discovery software, but can be edited on the Preprocess Data tab.

Include	Index	Color	Flip	Plate	Sensor Location	Sample Location	Sample ID	Type	Binding Rate	Known Conc (ug/ml)	Well Conc.	Dilution Factor
☑	44			1	t1D1	D2		Standard	0.0017	0.5	0.4693	
☑	45			1	t1E1	E2		Standard	0.0016	0.5	0.4676	
☑	46			1	t1F1	F2		Standard	0.0035	1	1.01	
☑	47			1	t1G1	G2		Standard	0.0095	3	2.73	
☑	48			1	t1H1	H2		Standard	0.0294	10	8.48	
☑	49			1	t1A1	A3		Standard	2.34	2000	2021.7	
☑	50			1	t1B1	B3		Standard	2.07	1500	1442.9	
☑	51			1	t1C1	C3		Standard	1.69	1000	918.2	
☑	52			1	t1D1	D3		Standard	1.44	700	685.5	
☑	53			1	t1E1	E3		Standard	1.18	500	499.5	
☑	54			1	t1F1	F3		Standard	0.8329	300	307	
☑	55			1	t1G1	G3		Standard	0.3349	100	104.4	
☑	56			1	t1H1	H3		Standard	0.0978	30	28.6	

Figure 10-15: Analysis Results Table

- **Include** - When this box is checked, the data is included in the analysis. If this box is blank, the data is excluded from the analysis.
- **Index** - This is the number assigned to each data point during data analysis.
- **Color** - The color corresponding to the binding curve.
- **Flip** - An **X** in this column indicates the data has been flipped. This is done in the Data Corrections tab in the Preprocess Data screen.
- **Plate** - This is a unique number assigned to individual sample plates. If a standard curve was loaded from another experiment, the plate column displays the file name used for the standard curve.
- **Sensor Location** - Location of the biosensor in the sensor tray.

- **Sample Location** - Location of the well in the sample plate.
- **Sample ID** - The sample ID of the well
- **Type** - The well designation (standard, control, unknown or reference).
- **Binding Rate** - The rate of sample binding to the biosensor calculated by the software using the binding rate equation specified.
- **Known Conc.** - The user-specified standard concentration. Applicable to the Standard and Control well types only.
- **Well concentration** - The concentration of the analyte determined from the standard curve. The well concentration is multiplied by the dilution factor to determine the calculated concentration.
- **Dilution factor** - The dilution factor used to prepare the sample. The dilution factor is multiplied by the well concentration to determine the calculated concentration.
- **Calc. Conc.** - The sample concentration calculated from the standard curve.
- **Residual (%)** - Residual = (Calculated concentration - Known concentration) / Known concentration. The residual is computed for any well that has a known concentration, typically standards and controls.
- **R2** - The R^2 of the curve fit used to determine the binding rate.
- **Sample Information** - Annotations about the sample well.
- **Sensor Type** - The biosensors used in the assay.
- **Sensor Info** - Custom information about the biosensor.
- **Replicate Group** - If the samples were set as replicate groups, then they indicate as replicate groups in the table (showing same alpha numeric letter for replicates) and BR AVG, BR SD, BR CV, Conc Avg, Conc SD and Conc CV populate.
- **BR AVG** - The average binding rate of the replicate group.
- **BR SD** - The standard deviation of the binding rate of the replicate group.
- **BR CV** - The coefficient of variance of the binding rate of the replicate group.
- **Conc. Avg** - The average concentration of the replicate group.
- **Conc. SD** - The standard deviation of the concentration of the replicate group.
- **Conc. CV** - The coefficient of variance of the concentration of the replicate group.
- **Calc. Dilution CV** - Calculates dilution linearity for same samples (same sample ID) assayed at multiple dilutions.
-
- **Lot Number** - The lot number of the biosensor tray used in the assay.
- **File location** - Location of the data file.

The following columns are only shown for assays that include a loading step, such as a Capture Antibody step or Loading step from a K to Q analysis.

- **Loading Conc.** - concentration of the loading well.
- **Loading Response** - Amount of binding shift that occurred during the loading step.
- **Loading Loc**. - Location of the loading well (if used in the assay).
- **Loading Sample ID** - sample ID of the loading well.

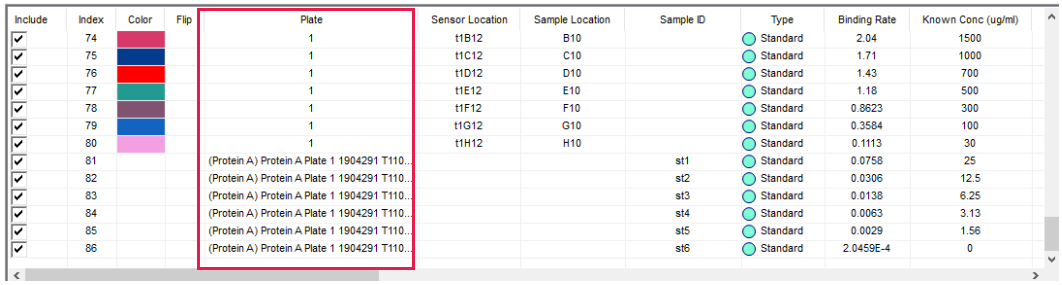
Working with Saved Standard Curve Files

Loading a Saved Standard Curve

If you have a previously saved standard curve you'd like to use, you can load it into the current data set for analysis.

1. Click  (**Load Standard**) in the Standard Curves section of the icon bar.
2. Select the standard curve file you'd like to load and click **Open**.

   The binding rates of the loaded standard curve display in the results table, and the plate column displays the standard curve file name (Figure 10-16).



Include	Index	Color	Flip	Plate	Sensor Location	Sample Location	Sample ID	Type	Binding Rate	Known Conc (ug/ml)
☑	74			1	t1B12	B10		Standard	2.04	1500
☑	75			1	t1C12	C10		Standard	1.71	1000
☑	76			1	t1D12	D10		Standard	1.43	700
☑	77			1	t1E12	E10		Standard	1.18	500
☑	78			1	t1F12	F10		Standard	0.8623	300
☑	79			1	t1G12	G10		Standard	0.3584	100
☑	80			1	t1H12	H10		Standard	0.1113	30
☑	81			(Protein A) Protein A Plate 1 1904291 T110...			st1	Standard	0.0758	25
☑	82			(Protein A) Protein A Plate 1 1904291 T110...			st2	Standard	0.0306	12.5
☑	83			(Protein A) Protein A Plate 1 1904291 T110...			st3	Standard	0.0138	6.25
☑	84			(Protein A) Protein A Plate 1 1904291 T110...			st4	Standard	0.0063	3.13
☑	85			(Protein A) Protein A Plate 1 1904291 T110...			st5	Standard	0.0029	1.56
☑	86			(Protein A) Protein A Plate 1 1904291 T110...			st6	Standard	2.0459E-4	0

Figure 10-16: Standard Curve Loaded

Selecting a Standard Curve

1. Click  (**Select Standard**) in the Standard Curves section of the icon bar.
2. Select the standard(s) you want to use and then click **OK** (Figure 10-18).

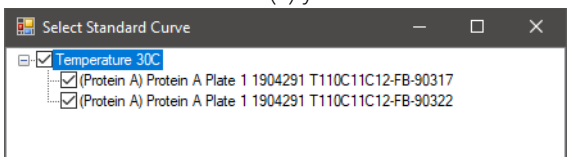


Figure 10-17: Selecting a Standard Curve

Saving a Standard Curve

1. Click  (**Save Standard**) in the Standard Curves section of the icon bar.

2. Select a standard to save and enter a file name (Figure 10-18).

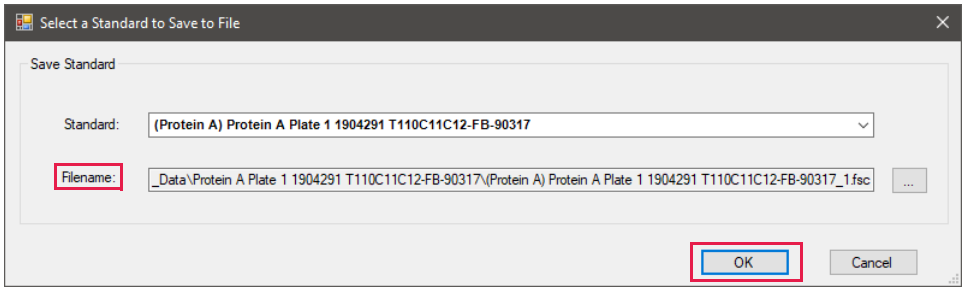


Figure 10-18: Saving a Standard

3. Click **OK**.

Analyzing Your Quantitation Data

You can select options for data analysis in the Standard Curve Settings and Standard Sample Alerts windows. Before you get started, first make sure you've made any reference subtractions and data corrections needed for your experiment in the Preprocess Data screen.

Step 1: Fit the Standard Curve

1. Select an equation in the Standard Curve Equation box (Figure 10-19).

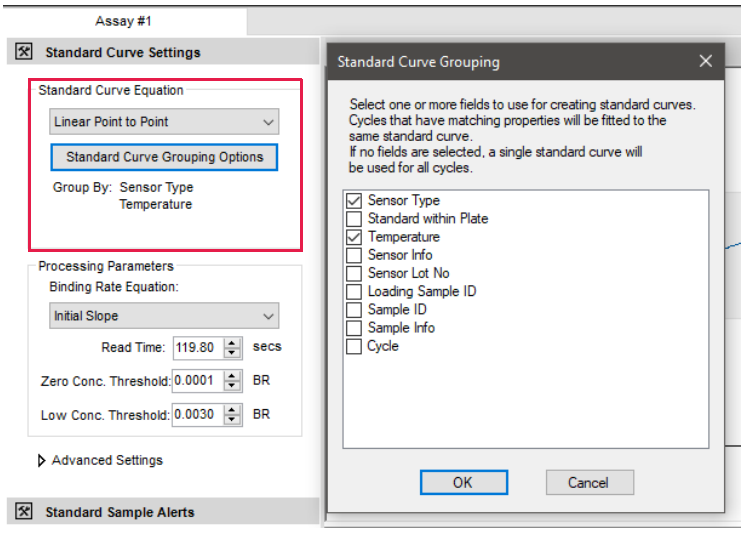


Figure 10-19: Selecting a Standard Curve Equation

- **Linear Point to Point** - The software connects the points of the standard curve with straight line segments. This option is often used to view the data trend before selecting a standard curve equation.
- **Dose Response-4PL (Unweighted)** - A symmetrical dose response curve. No points are weighted during the curve fitting.
- **Dose Response-4PL (Weighted Y)** - A symmetrical dose response curve with weighting applied as 1/Y (as Y increases, weighting decreases).
- **Dose Response-4PL (Weighted Y^2)** - A symmetrical dose response curve with weighting applied as $1/Y^2$.
- **Dose Response-5PL (Unweighted)** - A non-symmetrical dose response curve. No points are weighted during the curve fitting.
- **Dose Response-5PL (Weighted Y)** - A non-symmetrical dose response curve with weighting applied as 1/Y.
- **Dose Response-5PL (Weighted Y^2)** - A non-symmetrical dose response curve with weighting applied as $1/Y^2$.
- **Linear (Y = a*X + b)** - Linear regression fit that assumes that the overall relationship between concentration and binding rate is best described by a straight line.

You can display the fitting equation for the curve can on the Standard Curve graph by right-clicking on the graph and selecting **Show Fitting Equation** (Figure 10-20)

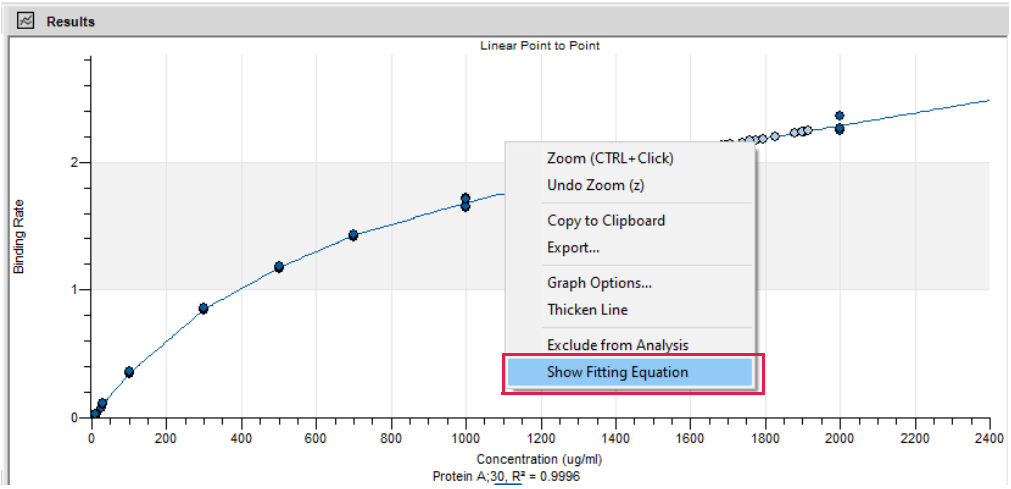


Figure 10-20: Show Fitting Equation on Graph

2. Select one or more fields in the Standard Curve Grouping Options dialog to use for creating standard curves. Cycles with matching properties are fitted to the same standard curve. If no fields are selected, a single standard curve will be used for all cycles.
   - **Standard Within Plate** - Select this option when multiple data sets are loaded and you want the binding rates and concentrations of the unknowns to be calculated from standards within each individual plate.
   - **Standard Curve by Temperature** - When you select this option, standard curves are generated based on temperature, and binding rates and concentrations are calculated according to the standards within the same temperature.
   - **Standard Curve by Sensor Type** - When you select this option, standard curves are generated based on biosensor type, and binding rates and concentrations are calculated according to the standards with the same biosensor type. This option is useful if different types of biosensors are being tested for the same samples to select which works best.
   - **Sensor Info** - Standard Curves are generated based on the custom Sensor Info filed.
   - **Sensor Lot No** - Standard Curve are generated based on the biosensor Lot Number.
   - **Loading Sample ID** - Standard Curve are generated based on loading sample ID (if loading step is used).
   - **Sample ID** - Standard Curve are generated based on Sample ID.
   - **Sample Info** - Standard Curve are generated based on Sample Info.
   - **Cycle** - Standard Curve are generated based on Cycle number.

Step 2: Set Processing Parameters

1. In the Processing Parameters box, select a binding rate equation (Figure 10-21). This is the curve-fitting equation that models the binding data.

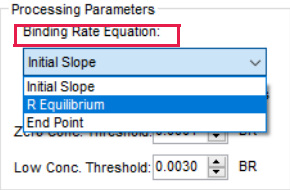


Figure 10-21: Selecting a Binding Rate Equation

- **Initial Slope** - Calculates the initial slope of the acquired quantitation data (nm/second). Choose this equation for a basic quantitation or basic quantitation with regeneration experiment.
- **R equilibrium** - This equation is recommended for an advanced quantitation experiment that includes amplification. This equation uses the calculated equilibrium, not the initial slope, to model the data.
- **End Point** - Calculates the average binding response for a segment of the binding curve.

2. Set the **Read Time** (Figure 10-22). This represents the duration of binding data to be included in the analysis. Type in a number or use the up/down arrows next to each cell.
3. Set the **Zero Conc. Threshold** (Figure 10-22). Binding rates that are less than the zero concentration threshold are considered zero. Type in a number or use the up/down arrows next to each cell.
4. Set the **Low Conc. Threshold** (Figure 10-22). Used with the Initial Slope Binding Rate Equation, the low concentration threshold acts as a filter for low binding responses. The binding curve is fitted with a linear regression. If the slope of the linear fit is less than the low concentration threshold, then the linear slope is reported as the binding rate in the results table. Changing this threshold can improve the precision of low concentration samples. Type in a number or use the up/down arrows next to the value.

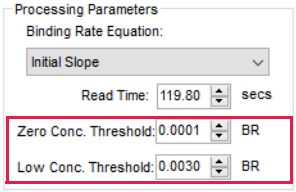


Figure 10-22: Setting Read Time, Zero Conc. Threshold and Low Conc. Threshold

Advanced Settings

- **Model Classifier** - When using the Initial Slope Binding Rate Equation, each binding curve is evaluated independently and fit with three binding models: Linear, Single Exponential and Double Exponential. The model classifier is used to determine which of the three models are appropriate for the binding curve.

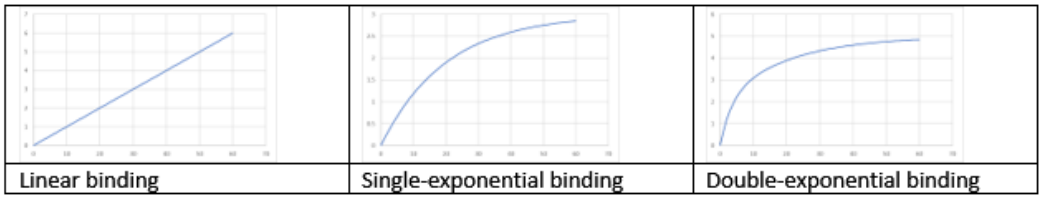


Figure 10-23: Binding Curve Examples

  - **Legacy** - The model with the greatest slope is selected, and a proprietary filter checks for the possibility of overfitting by the double exponential model. The Legacy classifier is the same classifier used by Octet® BLI Analysis software and previous versions of Octet® Analysis Studio software. This is the default option when analyzing a new experiment.
  - **Best Fit** - Chooses the model with the best fit to the binding curve, but taking into account the model complexity. The simpler model is selected for statistically equivalent fits.

NOTICE: *For most experiments, the Legacy and Best Fit classifiers report identical results. The Best Fit classifier is recommended when binding curves are in the transition region from a single-exponential profile to a double-exponential profile. Use the Fitted Graph display to confirm the appropriate setting for your data.*

  - **Single Exponential** - The classifier is constrained to only use a single exponential model for each binding curve.
- **DE Significance** - Used with the Best Fit classifier. When a double exponential model is selected as the best fit, this threshold removes exponential components that contribute less than the given binding shift to the overall response. Useful for removing binding rate bias that may be introduced from a non-specific binding event. Set the DE significance to zero to disable this filter. Figure 10-24 shows the effect of the DE Significance setting. A setting of 0.05 to 0.1 is usually sufficient to filter out matrix effects such as this. Use the Fitted Graph display to confirm the appropriate setting for your data.

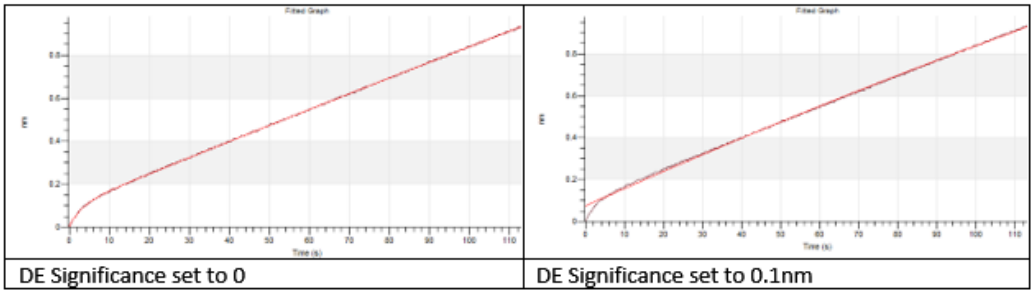


Figure 10-24: Effect of DE Significance

- **End point average** - Used with the End Point Binding Rate Equation, this parameter controls the number of data points to average when computing the binding rate.

Step 3: Setting Standard Sample Alerts (Optional)

In the Standard Sample Alerts window, select one or more sample alerts (Figure 10-25). Rows and wells that match criteria specified here are highlighted in corresponding colors in the results table and sample plate map.

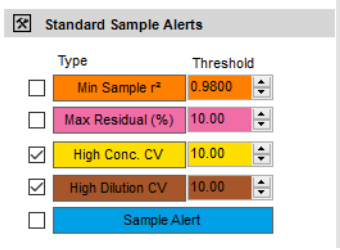


Figure 10-25: Selecting Standard Sample Alerts

To change the threshold for an alert, click in the **Threshold** cell and type in a number or use the up/down arrows to select a value.

- **Min Sample r^2** - The threshold r^2 value for a standard or unknown binding curve. If the r^2 value of a standard or unknown binding curve is less than the threshold value, the standard or unknown sample is highlighted in the results.
- **Max Residual (%)** - Specifies a threshold residual value for standards. If a calculated standard concentration deviates +10% or greater from the expected concentration, the standard is highlighted in the results.
- **High Conc. CV** - This option is useful to evaluate the precision within an assay. For the same concentration and replicate group of a sample, the percent coefficient of variation (% CV) is calculated. This will not yield an alert if the replicate group column is not populated in the method file or during analysis.
- **High Dilution CV** - This option is useful to evaluate dilution linearity of an assay. In order for the software to calculate dilution CV correctly, the sample ID should be the same and the dilution factor column must be populated in the method file or during analysis. The software calculates the unknown well concentration from the standard curve, then back-calculate the final concentration using the dilution factor. The % CV calculates by evaluating the variation between the back-calculated values of the final concentrations.
- **Sample Alert** - Specifies and highlights data that fit your specified criteria. Thresholds can be set for r^2, max residual, or both. When you select this option, a Sample Alert window displays (Figure 10-26). Set either a positive or negative threshold on the standard deviation of either the binding rate or the calculated well concentration.

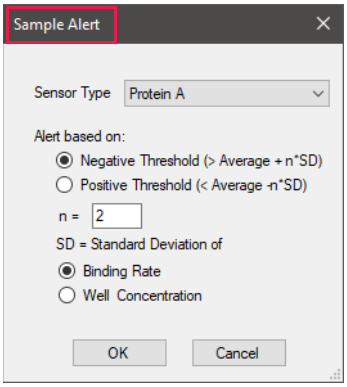


Figure 10-26: Sample Alert Settings

Step 4: Analyze the Next Assay

If your data includes more than one experiment or combined data set, repeat Steps 1-3 on the next Assay tab.

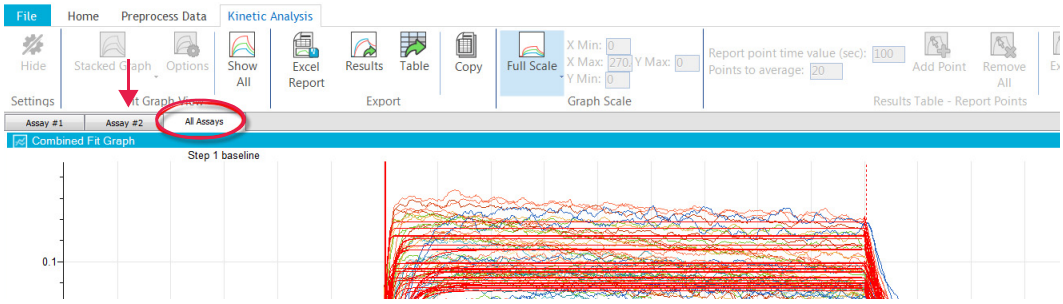


Figure 10-27: Assay Tabs

Excluding Data from the Analysis

To remove specific data from the analysis, deselect the box in the **Include** column for that row of data. The software updates the Analysis Results table automatically (Figure 10-28).

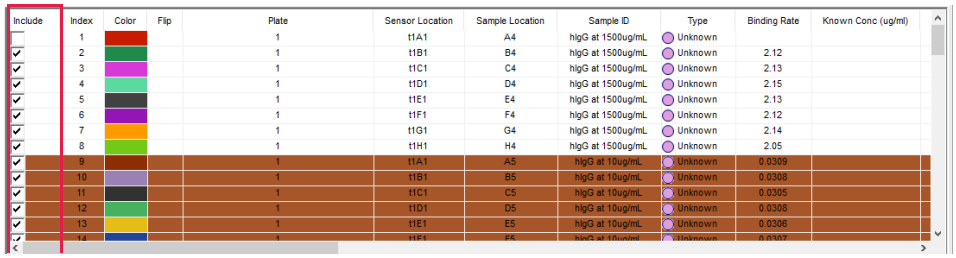


Figure 10-28: Excluding Data

You can also right-click a sample well or a data point in the Standard Curve and select **Exclude from Analysis**.

Replicate Groups

Turning Replicates On and Off

Click the first icon in the Replicates section of the menu bar (Figure 10-29).

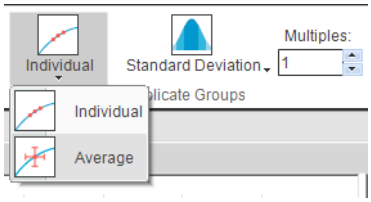


Figure 10-29: Selecting Replicates

To turn replicates on, click **Average** (Figure 10-30). Replicates displays in the Standard Curve graph.fif.

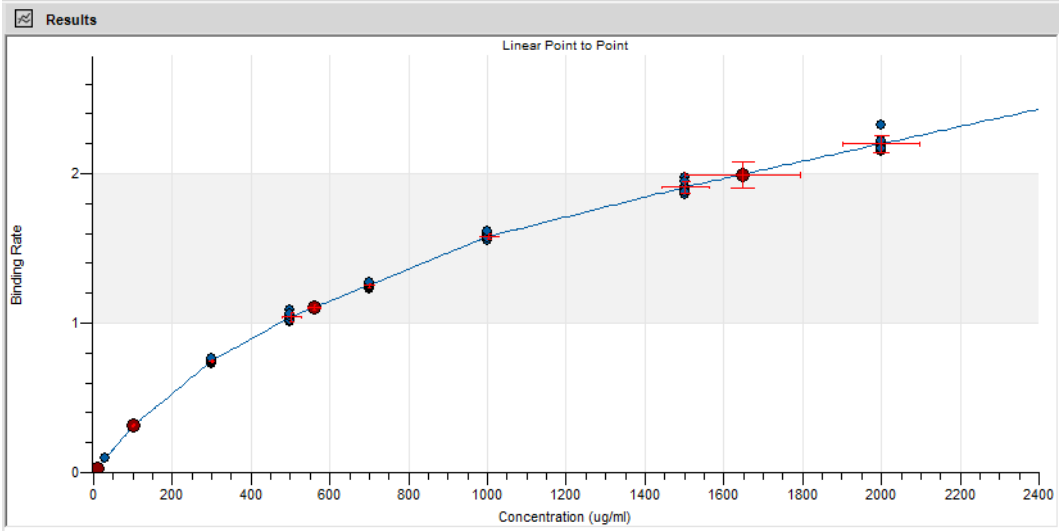


Figure 10-30: Replicates Displayed on Standard Curve Graph

To turn replicates off, click the first icon in the Replicates section of the menu bar and select **Individual**.

Displaying the Standard Deviation

Click the second icon in the Replicates section of the menu bar and select **Standard Deviation** (Figure 10-31).

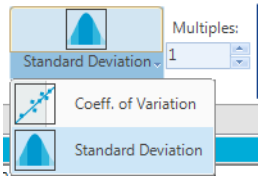


Figure 10-31: Selecting Standard Deviation

The standard deviation of the replicate groups displays on the Standard Curve graph (Figure 10-32).

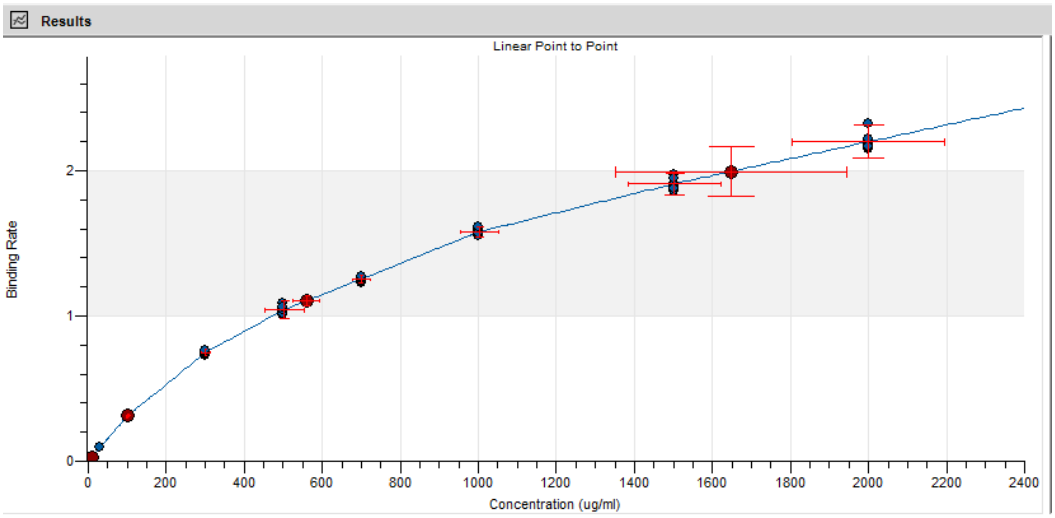


Figure 10-32: Standard Deviation Displayed on Standard Curve

Displaying the Coefficient of Variation

Click the second icon in the Replicates section of the menu bar and select **Coeff of Variation** (Figure 10-33). Multiples is a scaling factor that allows you to grow or shrink the plotted standard deviation.

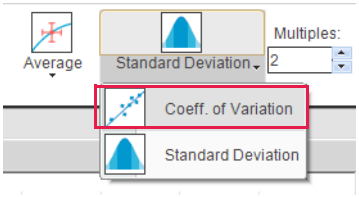


Figure 10-33: Selecting Coeff. of Variation

Multiples is a scaling factor that allows you to grow or shrink the plotted standard deviation. Multiples can be changed by typing in the cell or using the up/down arrows (Figure 10-34).

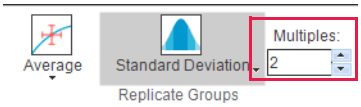


Figure 10-34: Setting Multiples

The coefficient of variation displayed here is the standard deviation of the binding rate of a replicate group divided by the average binding rate of the same group. The magnitude of this coefficient of variation is then plotted as a red vertical line centered on the average binding area of the associated replicate group.

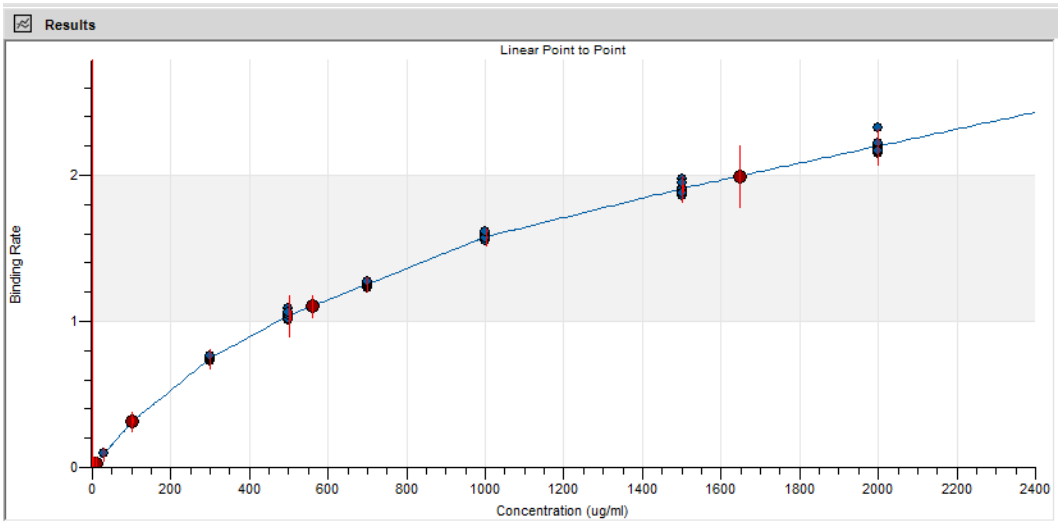


Figure 10-35: Coefficient of Variation Displayed on Standard Curve

Multiples can be changed by typing in the cell or using the up/down arrows (Figure 10-36). Multiples is a scaling factor that allows you to grow or shrink the plotted coefficient of variation.

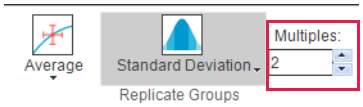


Figure 10-36: Multiples

Exporting Graphs

To export a Standard Curve or X-Y graph, right-click the graph and select **Export**. The Export window displays (Figure 10-37).

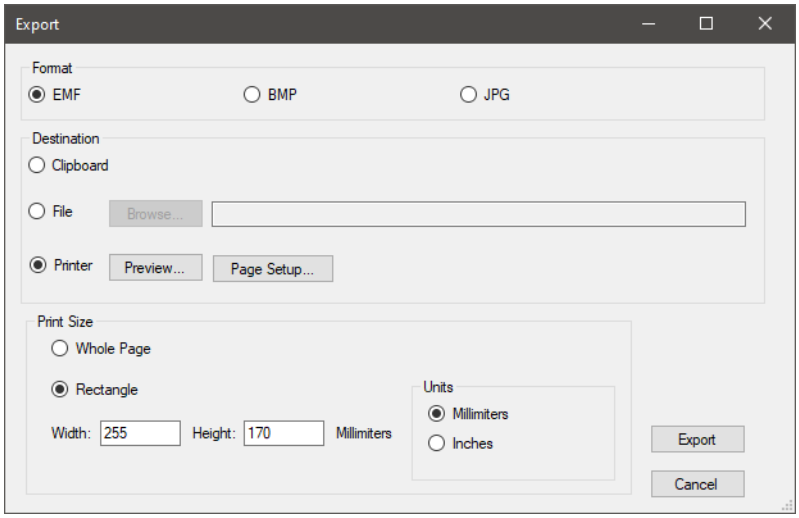


Figure 10-37: Export Window

Chose a format (EMF, BMP or JPG), an export destination (Clipboard, save to File, or Printer), set print size parameters if needed then click **Export**.

Analysis Results Table Options

The Analysis Results table has several options to let you change the display.

- To sort data in the table alphabetically or numerically, just click the column header.
- To view table display options, right-click a populated table row or column to see the menu (Figure 10-38).

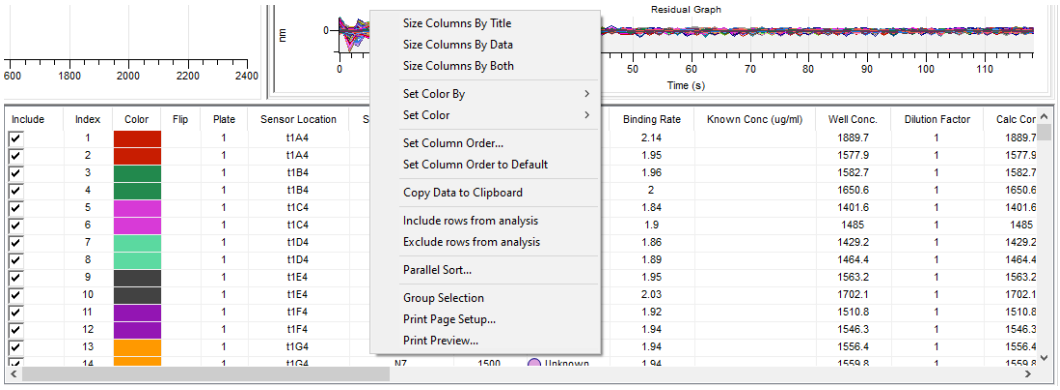


Figure 10-38: Analysis Results Table Menu

Sizing Columns

The following automatic column sizing options are available in the table's right-click menu:

- **Size Columns by Title** - Sets all column widths to fit the column titles.
- **Size Columns by Data** - Sets all column widths to fit the data.
- **Size Columns by Both** - Sets all column widths to best fit both the column titles and the data.

To manually resize the columns, roll the cursor over the border between the column headers until the resizing cursor displays (Figure 10-39). Then just click and drag the column to resize.

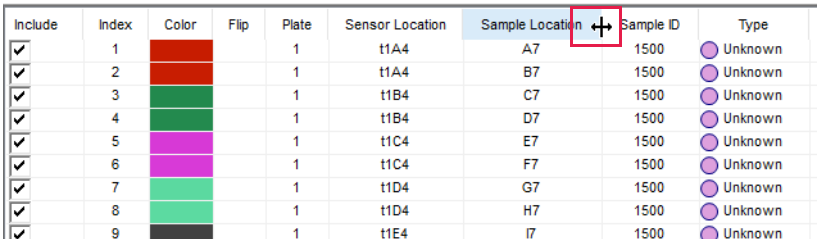


Figure 10-39: Column Resize Cursor

Color-Coding Data

You can assign specific colors to data as needed. Select one or more rows in the Analysis Results table, right-click and choose one of the following options:

- **Set Color By** - Lets you color-code data by a table column variable (Figure 10-40). Once you select a variable, the binding curve and table row colors automatically updates.

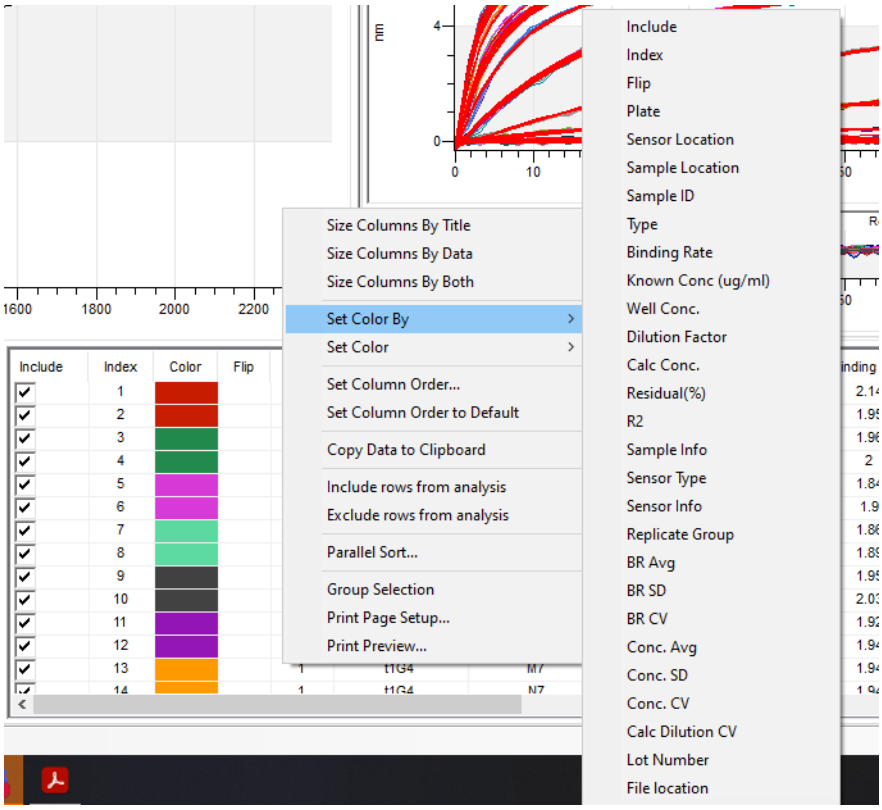


Figure 10-40: Set Color By

- **Set Color** - Opens the color palette so you can change the color for the table row(s) currently selected (Figure 10-41). Select a color from the palette or define a custom color then click **OK**. The binding curve and table row colors automatically updates.

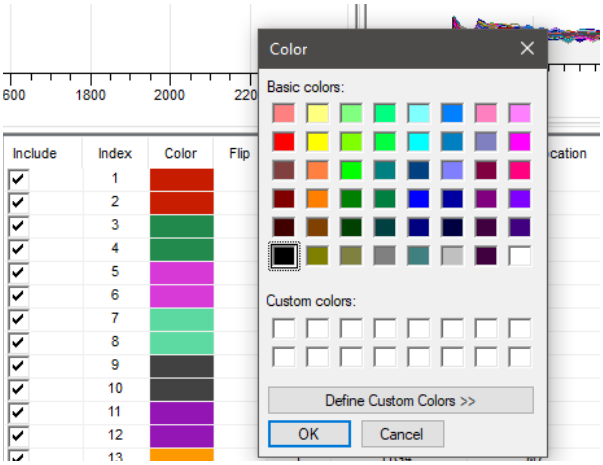


Figure 10-41: Set Color

Changing the Column Order

To change the order of columns shown in the Analysis Results table, right-click the table and select **Set Column Order**. The window that displays lets you shuffle columns (Figure 10-42).

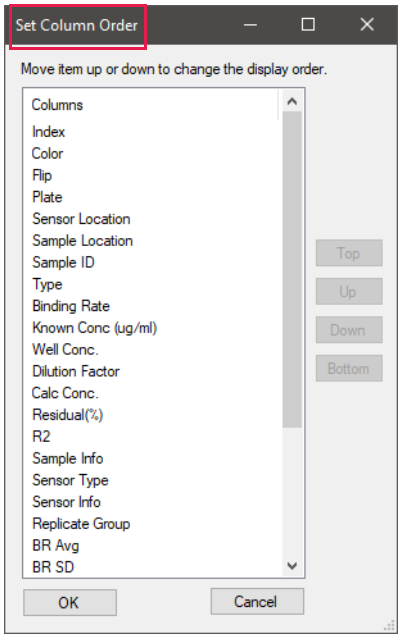


Figure 10-42: Set Column Order Window

Exporting Datasets

You can use the Export options menu at the top of the Quantitation Analysis screen to export your datasets (Figure 10-43).

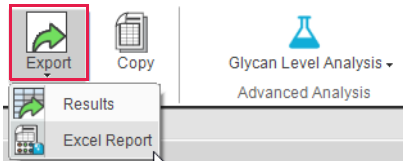


Figure 10-43: Export Options.

- Click (**Excel Report**) to export the processed (corrected) data for your experiment or combined data set in .xlsx format. Select which experimental components to export, use the default report name or click **...** to specify another name/location, then click **Export** (Figure 10-44).

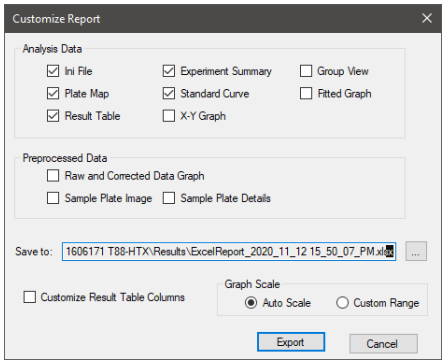


Figure 10-44: Excel Report Export Options

- Click (**Results**) to export the results in .csv format.
- Click (**Copy**) to copy the results in the Analysis Results table and paste into another program like Microsoft® Excel®.

Chapter 11:

Custom Reports

Custom Reporting

Use the Report screen to generate customized reports. Reports can contain any combination of data elements you choose from all analyses performed on the selected experiment including graphs, data tables, and experimental details from the Preprocess Data, Quantitation Analysis, Epitope Binning and/or Kinetic Analysis Screens. You can also save report templates to be auto-filled with data from subsequent experiments so that report formats can be standardized.

Launching the Report Screen

After completing a quantitation, kinetics or epitope binning analysis, click the **Report** tab at the top of the screen.

The Report screen displays (Figure 11-1):

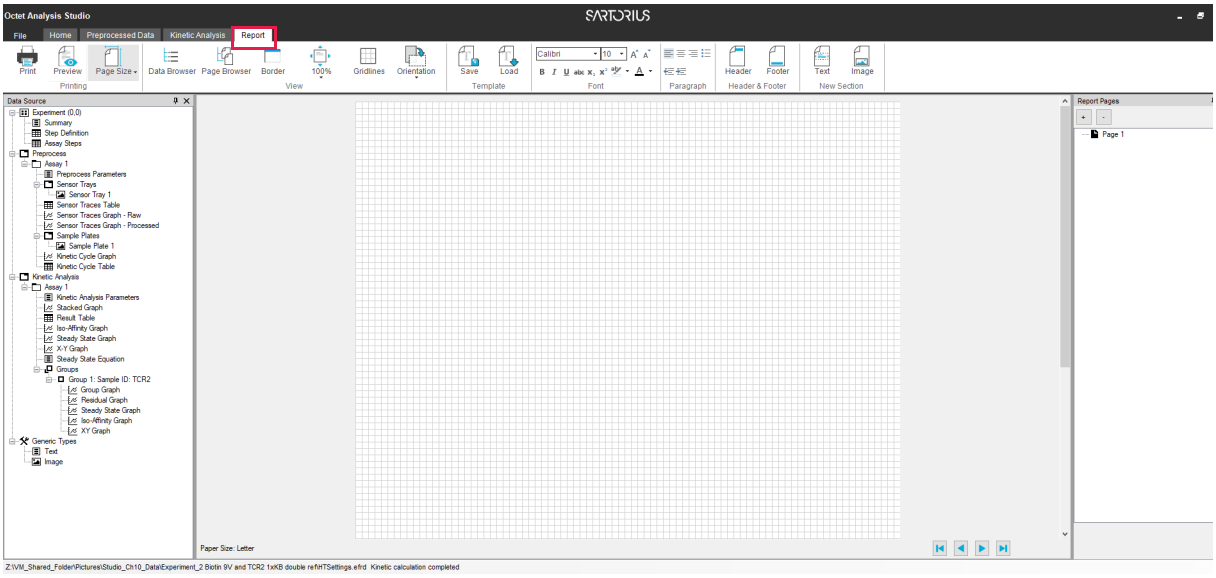


Figure 11-1: Report Screen

The tree in the Data Source window shows all the data elements you can choose from pre-processing or analyses performed on the current experiment. For information about customizing colums see “Adjusting Data Elements” on page 248.

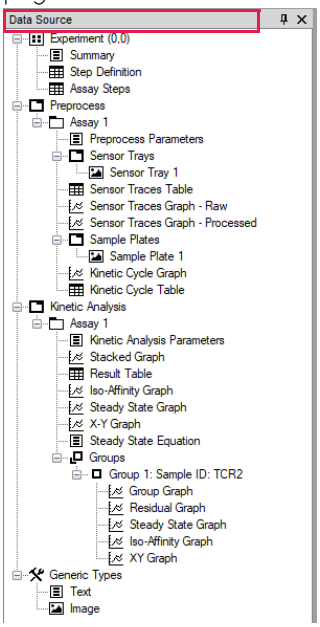


Figure 11-2: Data Source Window

You can drag data elements into the Report Grid to create the report (Figure 11-3).

Figure 11-3: Grid Plot

The tree in the Report Pages window shows the number of report pages and the data elements on each page.

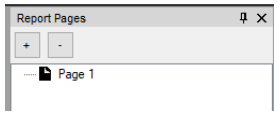


Figure 11-4: Report Pages Tree Window

Once a report is populated the tree shows which data elements are contained on each page, and you can add or subtract pages from the report (Figure 11-5).

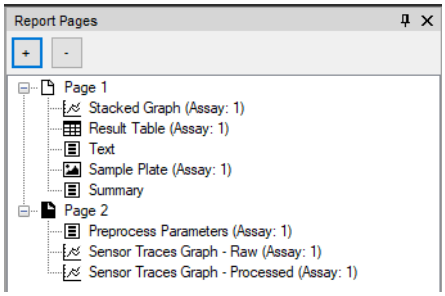


Figure 11-5: Report Pages Tree Window Showing Pages and Data Elements

Adding Data Elements to the Report

To add data elements to the report, drag the element from the Data Source tree anywhere into the Report Grid area. You can then can drag the data element wherever you'd like it to go. Depending on the nature of the analysis you have a choice of the following elements you can add:

- **Text** - Prefilled with information about the experiment or analysis
- **Table** - Tabular data, inputs and outputs of the analysis.
- **Graph** - Various spectra, lines and scatter plots, and histograms of the analysis.
- **Images** - Usually an image of the sensor trays and sample plates.

Two Generic Types data elements are included in the Data Source tree:

- **Text** - An empty text box. You can add text to annotate the report. The text font type, color, size and orientation can be customized in the Report Toolbar.
- **Image** - Lets you paste a saved image file (PNG, JPEG, or TIFF) into the report.

An example kinetic analysis report is shown in Figure 11-6.

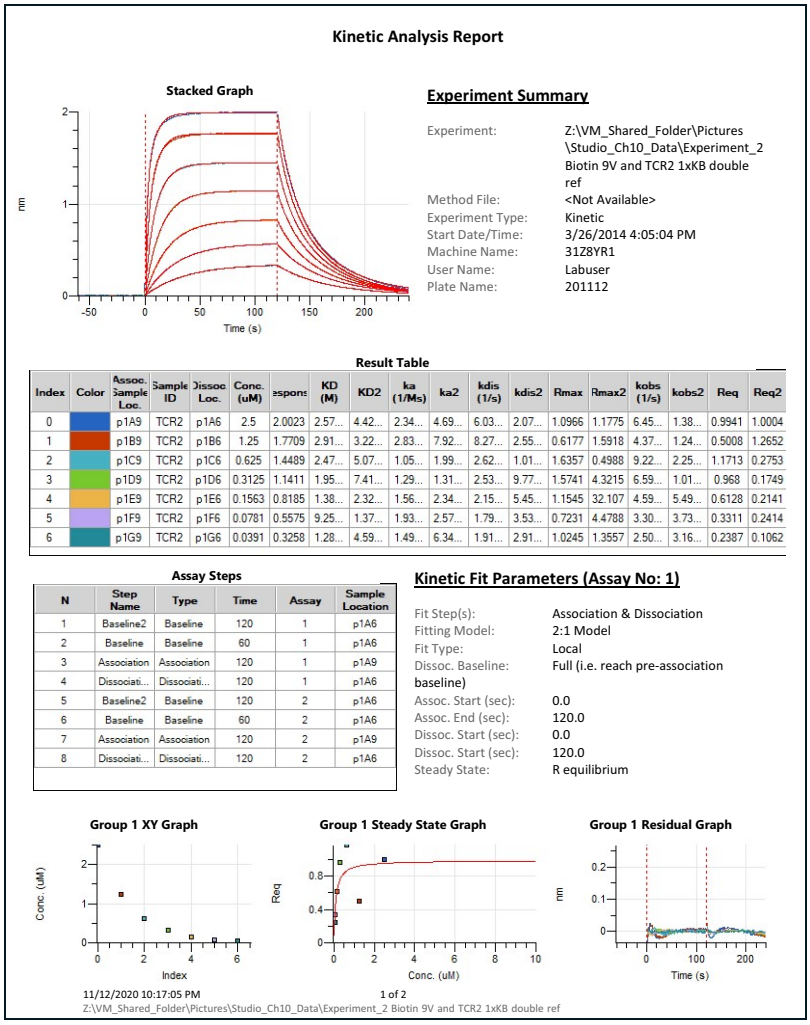


Figure 11-6: Example Analysis Report

Adjusting Data Elements

Once a data element has been added to the Report Grid, its size and appearance can be adjusted. All elements can be resized by dragging the corners and/or sides of the element. Right-clicking on the element displays a menu of adjustments that can be made. The menu is different depending on the type of element.

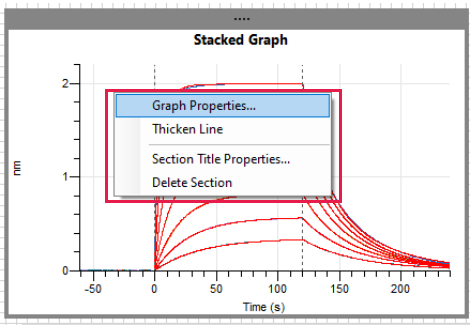


Figure 11-7: Graph Right-Click Menu

For images, the following option are displayed:

- **Section Title Properties** - Lets you set the title and font properties.
- **Delete Section** - Removes the images from the report.

For text boxes, the following options are displayed:

- **Cut**
- **Copy**
- **Paste**
- **Delete Section** - Removes the text box from the report.

Note that predefined text boxes cannot be edited so the Cut and Paste selections are disabled. If you wish to change or add to the predefined text, copy the text and paste it into a generic text box.

For graphs, the following options display:

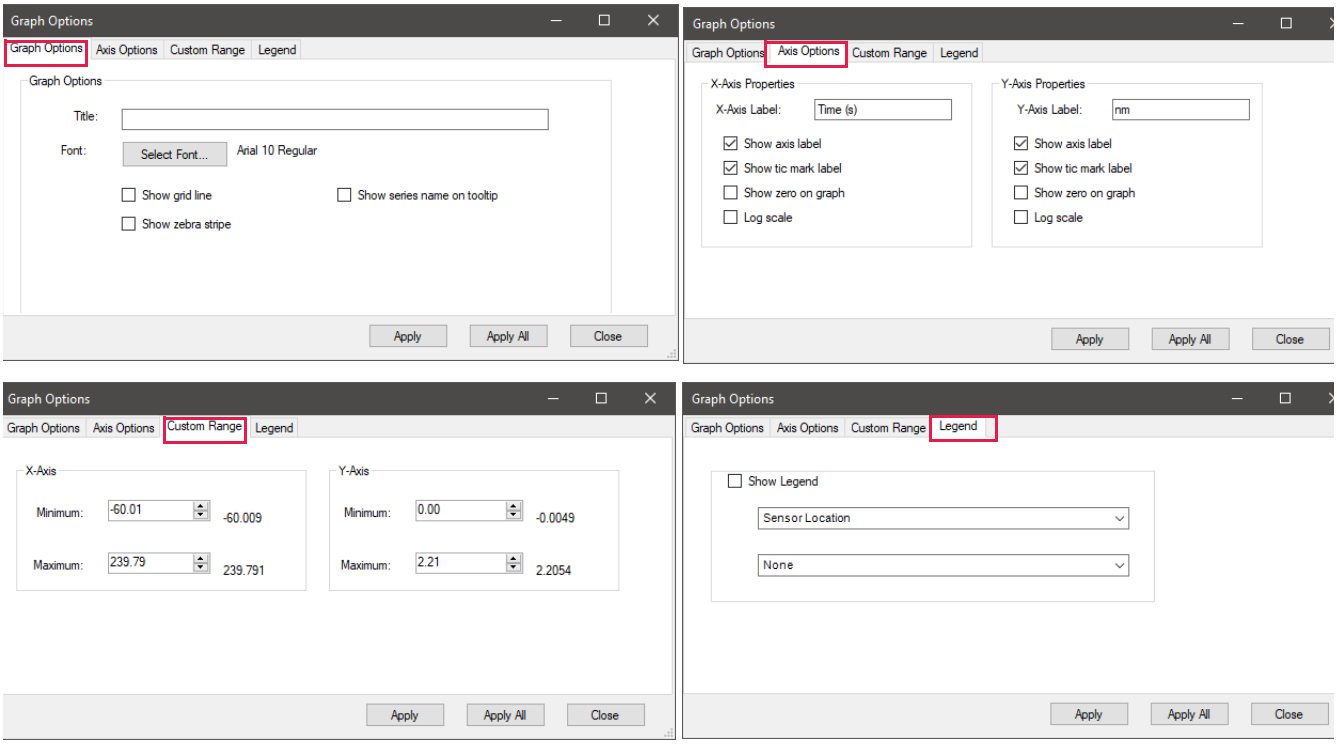


Figure 11-8: Graph Options

- **Graph Properties** - Lets you set the graph a title, set axis labels, and adjust the graph axes.
- **Thicken Line** - Lets you thicken the sensorgram lines for easier viewing.
- **Section Properties** - Lets you change the position and font of the element title, or remove it entirely.
- **Delete Section** - Removes the graph from the report.

For tables, the following options are displayed:

- **Size Columns by Title** - Sets the column width based on the title of the column.
- **Size Columns by Data** - Sets the column width based on the size of the data contained.
- **Size Columns by Both** - Uses both the title and data size to set the column width.
- **Size Columns to fit available Width** – Fits all selected columns into the available width.

- **Choose Columns and display order** - Lets you pick which parameters are included in the table from a list of options. Check/uncheck an item to show/hide it. Click an item name and then click the Top, Up, Down, or Bottom to change the order of the column. The top item will appear on the far-left side of the table (Figure 11-9).

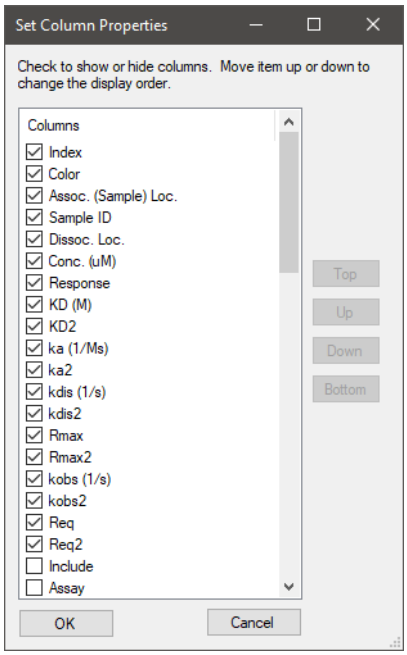


Figure 11-9: Set Column Properties Options

- **Table properties** – Select the font properties for the text in the table.

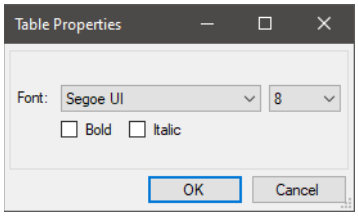


Figure 11-10: Set Table Font Properties

- **Section Title Properties** – Lets you change the position and font of the element title, or remove it entirely.

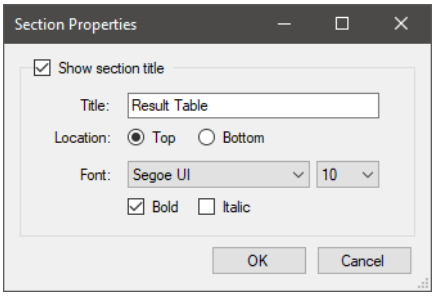


Figure 11-11: Set Section Title Properties

- **Delete Selection** - Removes the table from the report.

Other element types (text, images, plate maps, etc.) have simpler menus that allow for cutting and pasting text, and adjusting the element title.

NOTICE: *If any of the pre-processing or analyses parameters are modified in their respective screens, the data elements in the Report Screen updates accordingly. If you set the table column preferences, return to the analysis tab and change any analysis settings, you should reexamine the column settings to ensure the table is displaying the desired data and not displaying empty fields.*

Report Toolbar

The Report Toolbar has several options you can use to save and print the report, edit data elements or edit the overall report layout.

Figure 11-12: Report Toolbar

- **Print** - Lets you print the report to a printer or PDF.
- **Preview Printing** - Lets you view the report before you print it.
- **Page Size** - Lets you choose from a drop-down menu of different paper sizes to print the report on.
- **Orientation** - Lets you choose whether the report is displayed and printed in a landscape or portrait orientation. If you switch between orientations, the position of the data elements in the report remains fixed, so they may need to be moved or adjusted. You can set the page orientation for the entire report or for individual pages.
- **DataBrowser** - Restores the Data Source tree window, if it's been closed.
- **PageBrowser** - Displays the Report Pages tree window, if it's been closed.
- **Border View** - Lets you turn borders on or off around each data element.
- **Size** - Lets you toggle between a 100% zoomed-in view and a full-page, fit to window view of the report.
- **Gridlines** - Toggles gridlines on and off. Gridlines aren't included in the printed reports.
- **Save and Load (Template)** - Lets you save and load report template files (see "Saving and Loading Report Templates" on page 252).
- **Font and Paragraph** - Lets you adjust the selected text in text boxes **only**. Text in other data elements must be adjusted in the properties of those elements (see "Adjusting Data Elements" on page 248).
- **Header and Footer** - Let you generate custom headers and footers for your report. Headers are a text box that can be edited as desired. Footers are automatically generated with three optional outputs: experiment name (including the file path to the data), date and time, and page number.
- **Text and Image (New Section)** - Lets you generate a new text or image box for the report.

Saving and Loading Report Templates

You can save report templates that auto-fill with data generated in later experiments. This lets you easily standardize your report formats.

To create and use a template:

1. Generate a report in the format that you would like to save as a template.
2. Click **Save Template** in the Report Toolbar. Choose the location where you would like to save the template.
3. After a future experiment, click **Load Template** and select your template. The experimental data for the current experiment auto-fill into the appropriate data elements.

NOTICE:
Data elements may need to be adjusted after loading a template file due to possible differences in the size of the information contained (longer file path names, etc.).

Text manually entered in text boxes is not stored with the template and must be re-entered for each report.

Column properties are stored with tables, and must be adjusted if different columns are required than what is in the template report.

Chapter 12:

Compliance

Compliance Features

You can access the 21 CFR Part 11-compliant features provided in the 21 CFR Part 11 version of Octet® Analysis Studio software by clicking the **Security** tab (Figure 12-1).

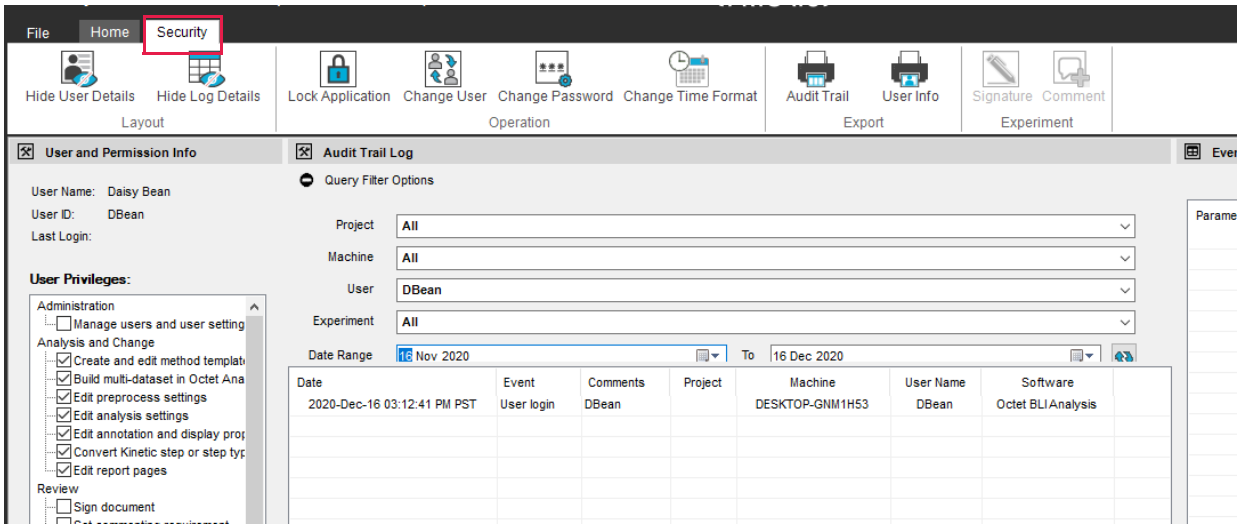


Figure 12-1: Security Screen

The Security tab lets you:

- Display User and Permission Info and Log Event Details unless they have been (optionally) hidden
- Display the Audit Trail
- Lock the application, change Users and change passwords
- Export the Audit Trail and User Info
- Review and Sign Experiments and add comments (for Users with the requisite privileges)

Viewing User Privileges

Make sure that User and Permission Info window is visible (Figure 12-2). If not, click **Show User Details** to open the window and see privileges assigned to the current User.

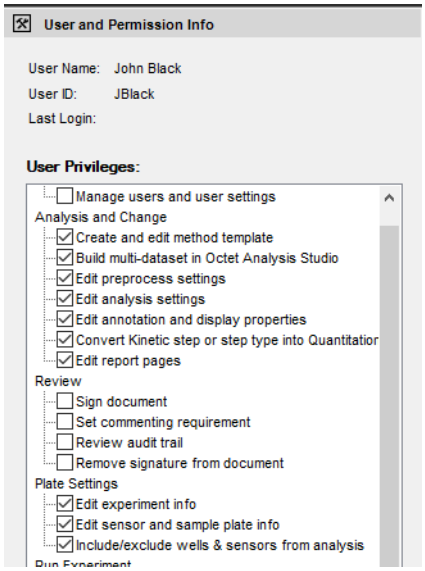


Figure 12-2: Users and Permission Info

Click any privilege to view all operations linked to that privilege. For example, the linked operations for Edit preprocess settings are shown in (Figure 12-3).

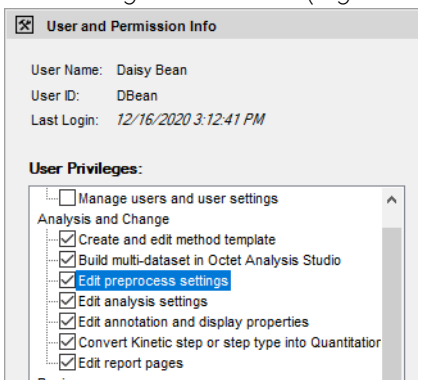


Figure 12-3: User and Permissions Info

Operations Linked to All Privileges

Table 12-1: Administration Operations Linked to Privileges

Privilege	Linked Operations in Octet® Analysis Studio
Administration	
Manage users and user settings	• User administrative options (add, remove, edit users) • View audit trail for any user

Table 12-2: Analysis and Change Operations Linked to Privileges (Sheet 1 of 2)

Privilege	Linked Operations in Octet® Analysis Studio
Analysis and Change	
Create and edit method template	• Modify method file settings
Build multi-dataset	• Build dataset - append experiment • Build dataset - overlay experiment • Build dataset - move experiment • Build dataset - delete experiment
Edit preprocess settings	• Modify reference sensor settings • Modify reference sample settings • Modify correction settings for kinetic analysis • Modify correction settings for quantitation analysis

Table 12-2: Analysis and Change Operations Linked to Privileges (Sheet 2 of 2)

Privilege	Linked Operations in Octet® Analysis Studio
Analysis and Change	
Edit analysis settings	• Modify kinetic steps to analyze • Modify kinetic binding model • Modify kinetic fitting parameters • Modify kinetic fitting type • Modify window of interest for kinetic analysis • Modify steady state analysis • Set kinetic group by color for fitting • Set quantitation binding rate settings • Set quantitation curve fit settings • Modify standard for quantitation analysis • Modify epitope binning (EB) cycle settings • Modify EB step settings • Modify EB matrix correction settings • Modify EB subtraction settings • Enable EB normalization settings • Modify EB clustering options
Edit annotation/display properties	• Modify color of kinetic cycles • Set column orders in kinetic result table • Modify color in quantitation analysis • Modify sample alerts for quantitation analysis • Set column orders in quantitation table • Modify EB color threshold settings • Modify trace color
Convert Kinetic step/step type into Quantitation	• Enable quantitation analysis of kinetic step(s)
Edit report pages	• Add report items to report page • Modify report page layout • Save report page template
(LEGACY) Change	• No linked operation

Table 12-3: Review Operations Linked to Privileges

Privilege	Linked Operations in Octet® Analysis Studio
Review	
Sign Document	• Sign document
Set commenting requirement	• Enable customized comment preferences
Review Audit Trail for any user	• View audit trail for any user
Remove signature from document	• Remove signature(s) from a document

Table 12-4: Plate Settings Operations Linked to Privileges

Privilege	Linked Operations in Octet® Analysis Studio
Plate Settings	
Edit experiment info	• Modify step type
Edit sensor and sample plate info	• Modify sample information • Change well location and well type • Modify sensor information
Include/exclude wells and sensors from analysis	• Exclude sensors from analysis • Exclude sample wells from analysis • Exclude data from quantitation analysis • Exclude sample from EB clustering • Exclude cycles from kinetic analysis
(LEGACY) Plate	• There are no linked operations.

Table 12-5: Run Experiment Operations Linked to Privileges (Sheet 1 of 2)

Privilege	Linked Operations in Octet® Analysis Studio
Run Experiment	
Run Experiment	• Run an experiment in Octet® BLI Discovery software • Load report page template
Import analysis settings template to new dataset	• Import analysis settings
Export data and Excel report	• Export data and Excel reports • Modify and export report points

Table 12-5: Run Experiment Operations Linked to Privileges (Sheet 2 of 2)

Privilege	Linked Operations in Octet®Analysis Studio
Run Experiment	
Choose repository directory when running an experiment	• Set the full repository directory when running an experiment

Viewing the Audit Trail

By default, the information in the Audit Trail window applies only to the current user. Administrators and users with the Review Audit Trail permission can view entries of all users. (Figure 12-4).

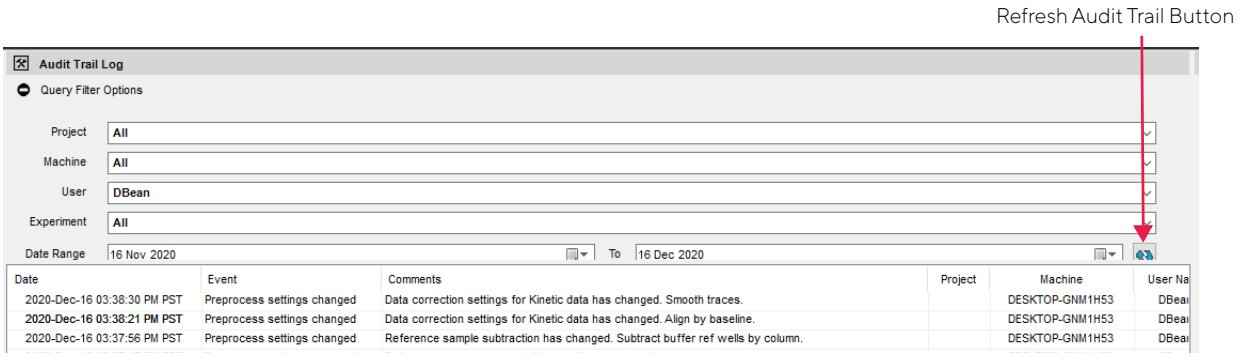


Figure 12-4: Audit Trail

- **Sorting the table** - Click a column header to sort either alphabetically or by time/date, and the other column data sort accordingly.
- **Filtering the table** - You can filter (restrict) the display to a particular Project, Machine, User, Experiment and Date Range. To do this, select your choices from the corresponding dropdown menus (Figure 12-5). Next, click the **Refresh Audit Trail** button (Figure 12-4) to update the view.

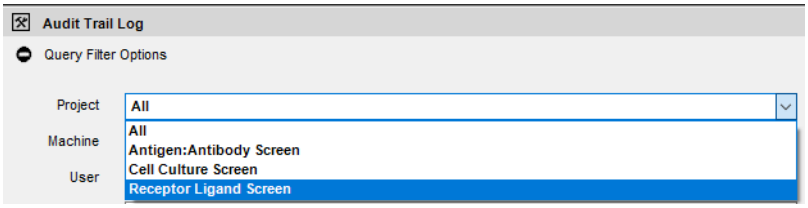


Figure 12-5: Filter Dropdown Menu

Viewing the Event Log Details

Settings changes are logged in the Event Log Details. To view the details of a particular event, click that event. For example, in Figure 12-6 the Kinetic analysis was changed from Association only to Both.

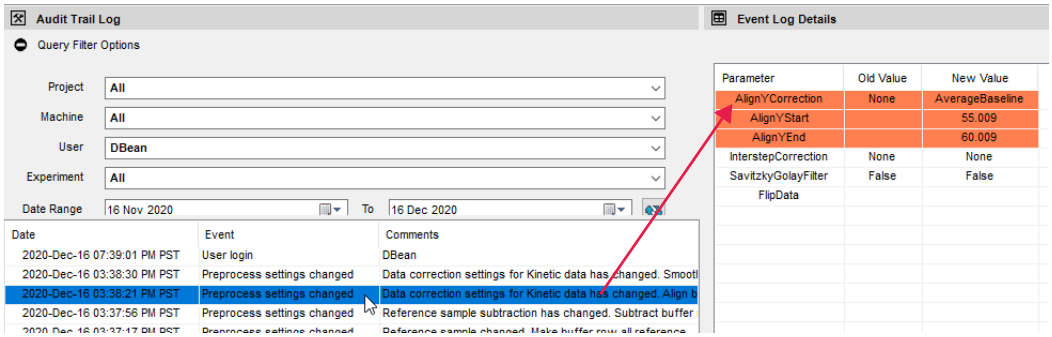


Figure 12-6: Viewing Event Details

Adding Comments to the Audit Trail

If a user has been assigned the Set commenting requirement privilege under Review, they have permission to turn commenting requirements on or off.

To enable or disable comment additions, go to **File** > **Preferences** in Octet® Analysis Studio software. In the Preferences dialog box, select or deselect the **Prompt user to enter customized comments** check box (Figure 12-7).

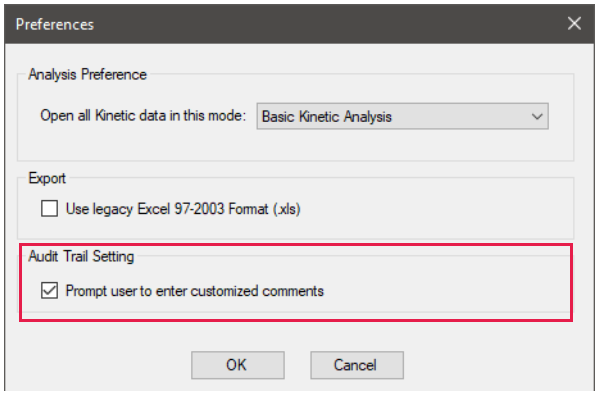


Figure 12-7: Preferences Dialog Box

If this option is selected, the software prompts users to add a custom comment if any analysis setting is changed. A default statement is displayed in the comment box, but this is editable by the user and should be modified prior to moving to the next step (Figure 12-8). The exact modifications show in the Differences box (old value vs. new value). All information is also logged in the Audit Trail.

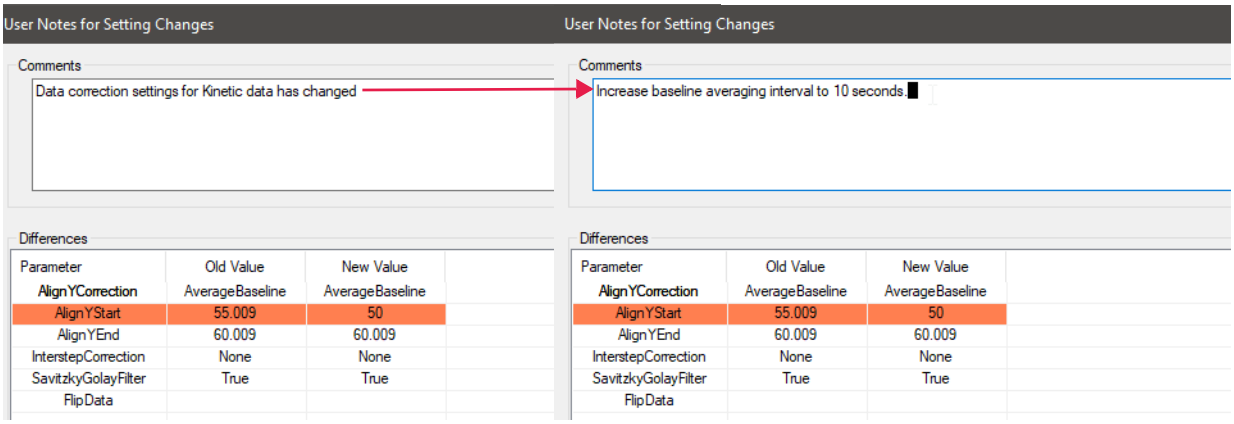


Figure 12-8: User Comments for Analysis Settings Changes

If this option is disabled, there is no prompt to enter a custom comment. The audit trail entry records a default comment. To add a comment directly in the audit trail, click **Comment** in the Experiment pane to bring up the Add Comment dialog (Figure 12-9).Type in your comment and click **OK**.

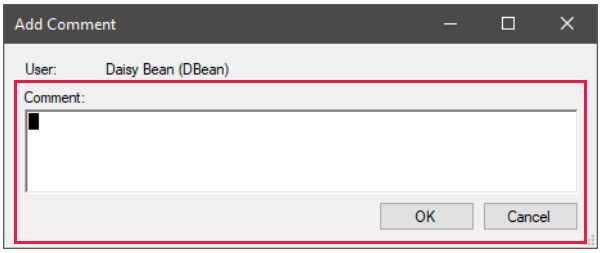


Figure 12-9: Adding a Comment

Locking the Application

Click **Lock Application** in the Operations section of the tab menu. The Application Locked dialog box (Figure 12-10) displays and remains until you unlock it or log off.

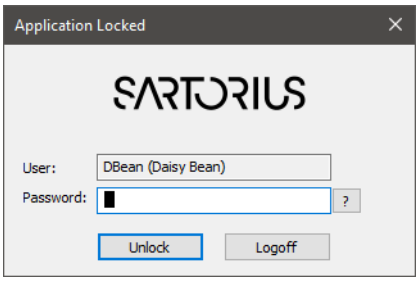


Figure 12-10: Application Locked Dialog Box

Changing the User

Click **Change User** in the Operations section of the tab menu. In the Login dialog box, select the new User from the menu, enter the password and click **Login (**Figure 12-11).

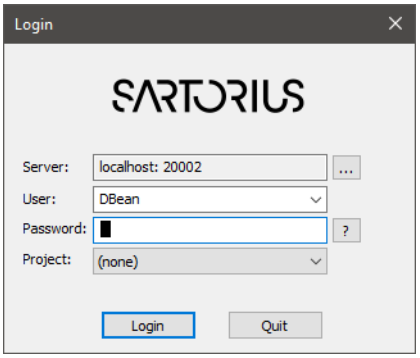


Figure 12-11: Login Dialog Box

Changing Your Password

Click **Change Password** in the Operations section of the tab menu. In the Change Password dialog box, enter your current password, then enter/re-enter the new one. Add a reminder (optional), then click **OK** (Figure 12-12).

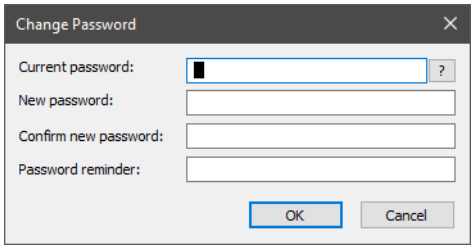


Figure 12-12: Change Password Dialog Box

Exporting the Audit Trail

Click **Audit Trail** in the Export section of the tab menu. Change the name and destination if needed and click **Save** (Figure 12-13).

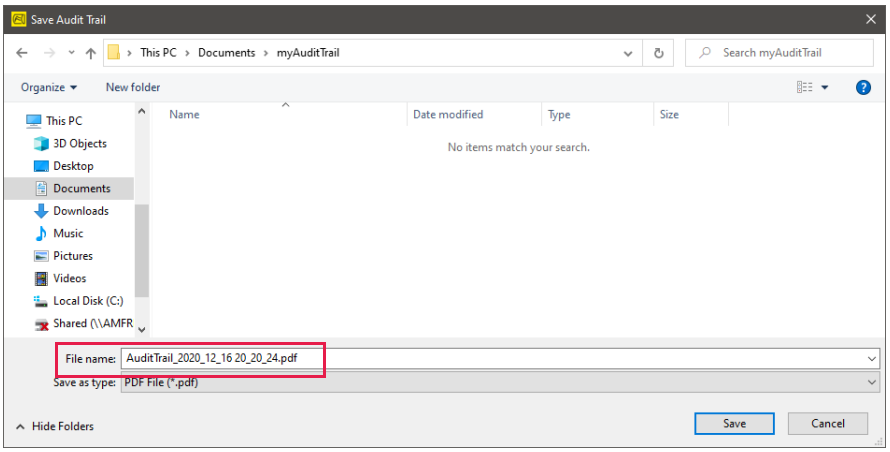


Figure 12-13: Saving the Exported Audit Trail

Exporting User Info

Click **User Info** in the Export section of the tab menu. Change the name and destination if needed and click **Save** (Figure 12-14).

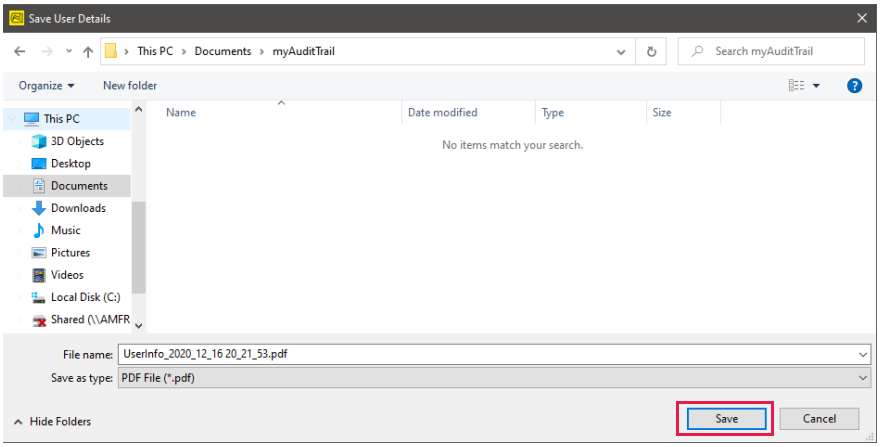


Figure 12-14: Saving User Details

Signing a Document

After a document is signed, it can't be edited. We recommend two different Reviewers sign documents.

To sign a document:

1. **First Reviewer** - Click **Signature** in the Experiment section of tab menu. The Sign Document dialog box displays (Figure 12-15).

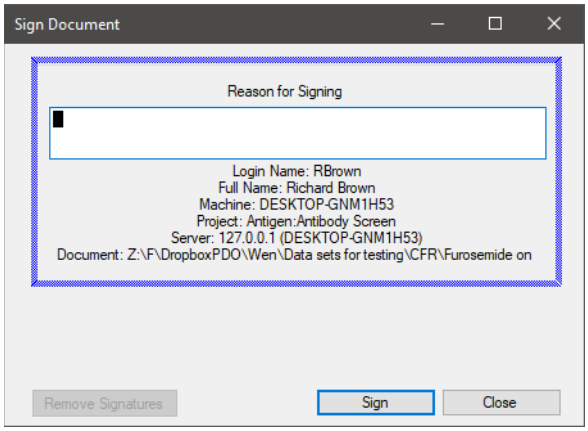


Figure 12-15: Sign Document Dialog Box

2. Enter your reason for signing and click **Sign**. The Re-enter Password dialog box displays (Figure 12-16).

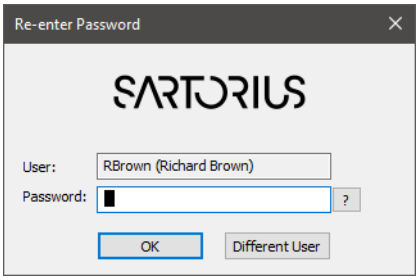


Figure 12-16: Re-enter Password Dialog Box

3. Enter your password and click **OK**. The Sign Document dialog box now shows Signature 1 of 1 (Figure 12-17).

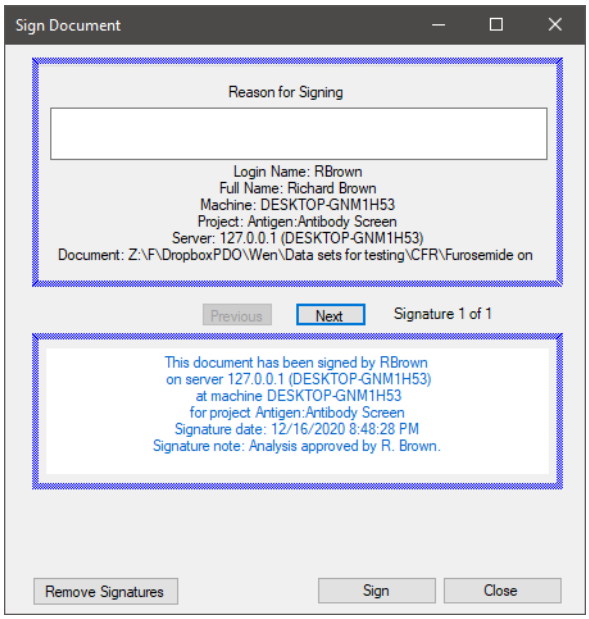


Figure 12-17: Sign Document Dialog After First Signature

4. **Second Reviewer** - Repeat Steps 1-3. The Sign Document dialog now indicates that the document is fully signed and shows Signature 2 of 2 (Figure 12-18).

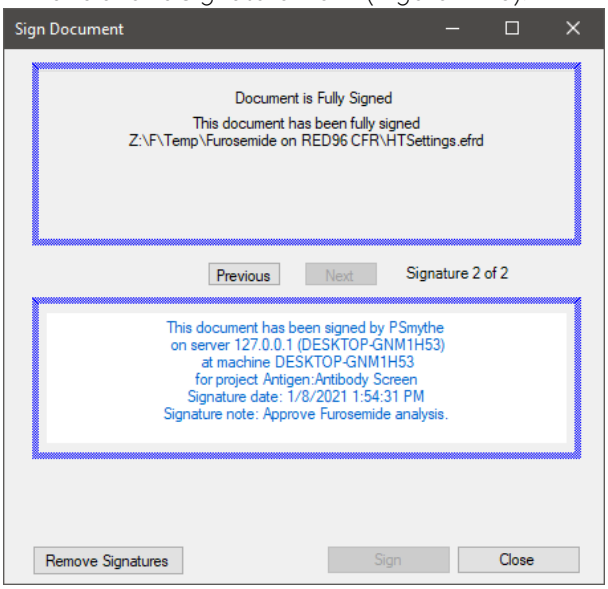


Figure 12-18: Sign Document Dialog After Second Signature

Removing a Signature

In case a change needs to be made after signing, users who have the Remove Signature privilege can remove signatures from a document. All signatures are removed from the document.

To remove signatures from a document:

1. Click **Experiment** > **Signature.** The Sign Document dialog displays (Figure 12-19).

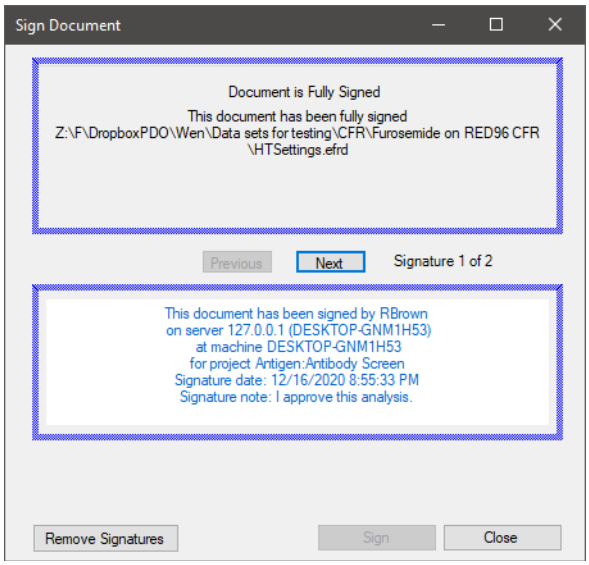


Figure 12-19: Sign Document Dialog

2. Click **Remove Signatures**. The Re-enter password dialog displays (Figure 12-20).

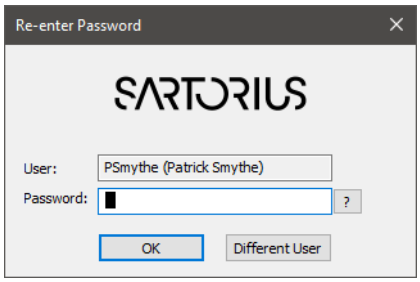


Figure 12-20: Re-enter Password Dialog Box

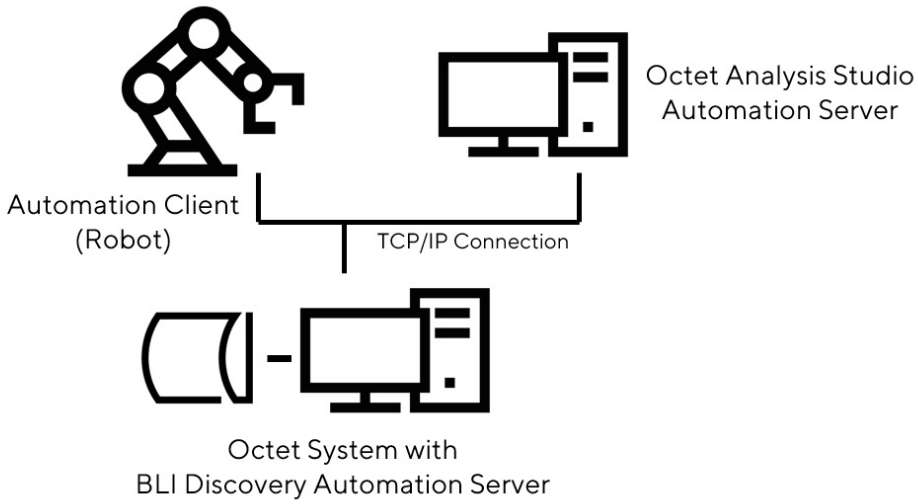
After entering the correct password, the signatures are removed, and the document can be edited again. The remove signature activity is recorded in the Audit Trail.

Appendix A:

Using an Automation Interface

Using a Robotic System

You can configure your Octet® System connected to a robotic system to acquire data and then send it to Octet® Analysis Studio software to analyze.



Creating a Successful Automation Client

Step 1: Establishing the Connection

Automation is supported via Serial Port (RS-232) or TCP/IP socket.

Before the Octet® software can be controlled using an automation interface, the correct automation options must be set. To do this, go to **File > Automation Connection Settings** and select the appropriate port in the Automation box.

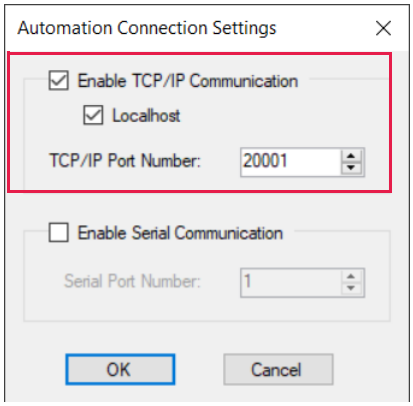


Figure A-1: Autmation Connection Settings

Step 2: Executing Commands

Each software package has its own set of commands that can be sent to execute an operation or analyze a dataset. Once each command is sent you can expected to receive a response:

- **OK** - The command is accepted and the operation is initiated.
- **Error** - The command syntax is wrong or there are some issues initiating the operation. The error should contain an explanation to what is wrong.

Step 3: Monitoring Status and Handling Errors

Once the command is sent and the response received is OK, the status changes to Busy until the operation finishes. The Automation Client should monitor the status until the command is complete, then the status changes from Busy to OK or Error.

Example source code for Automation Client to Octet® Analysis Studio is available upon request.

Automation Commands

Get Status Command

```
Command:
Status <none>
```

Get Version Command

```
Command:
Version <none>
```

Create Command

```
Command:
Create -d <path to experiment>
Create a new multi-experiment dataset starting with the given experiment.
```

Append Command

```
Command:
Append -d <path to experiment> -a <front/back>
Append an experiment to the currently open multi-experiment dataset (argument -a is optional. If omitted, the experiment will be appended to the back).
```

Overlay Command

```
Command:
Overlay -d <path to experiment> -n <segment number>
Overlay an experiment to the currently open multi-experiment dataset (segment number is optional, and should be an integer greater than or equal to 1. If not specified, segment 1 is used).
```

Save Command

Command:

Save -f <efrd filename>

Save the current multi-experiment dataset to the given filename.

Load a Multi-experiment efrd Data File

Command:

Load -f <efrd file path>

-f: load efrd file path which contains the data in segments and its settings.

-f <exp-path>: open an experiment folder in Octet Analysis Studio. If the file was previously analyzed with Octet Analysis Studio, any default settings for this experiment load.

Validate the Open efrd Data File

Command:

Validate <none>

Validate the currently open data file is valid for analysis

Preprocess Data File

Command:

Preprocess -s <efrd setting file> -x <export type> -p <output path>

-s: efrd setting file to overwrite the settings opened

-x: export types include: report, result, (processed data), raw

-p: output path: indicate output directory

Example: Preprocess -s "c:\test.efrd" -x "report" -p "c:\output"

Analyze Kinetic Data

Command:

Kinetic -s <efrd setting file> -x <export type> -p <output path>

-s: efrd setting file to overwrite the settings opened

-x: kinetic export types include: report, result, table

-p: output path: indicates output directory

Example: Kinetic -s "c:\test.efrd" -x "report" -p "c:\output"

Analyze Epitope Binning Data

Command:

EpitopeBinning -s <efrd setting file> -x <export type> -p <output path >

-s: efrd setting file to overwrite the settings opened

-x: epitope binning export types include: raw, result, matrix, report.

-p: output path: indicates output directory

Example: EpitopeBinning -s "c:\test.efrd" -x "report" -p "c:\output"

Analyze Quantitation Data

Command:

Quantitation -s <efrd setting file> -x <export type> -p <output path>

-s: efrd setting file to overwrite the settings opened

-x: Quantitation export types include: report, result, ambr

-p: output path: indicates output directory

Example: Quantitation -s "c:\test.efrd" -x "report" -p "c:\output\report.xls"

Analyze Dose Response Data

Command:

DoseResponse -s <efrd setting file> -x <export type> -p <output path>

-s: efrd setting file to overwrite the settings opened

-x: Dose Response export types include: report, result, table

-p: output path

Build an efrd File with These Commands

1. Create a new efrd file with this experiment

   Command:

   Create *-d <exp-path>*

2. Add an experiment by overlay it on a segment

   Command:

   Overlay *-d < exp-path> -o <1-n segment#> (1-based)*

   Add an experiment by append it to either the front or the back of the current dataset:

   Command:

   Append *-d < exp-path> -a <front/back>*

3. Save the currently opened efrd file to disk

   Command

   Save *-f <efrd-filename>*

   Example: Build a multi-experiment workspace by overlaying three experiments.

   Command1: Create -d <path to experiment 1>

   Command2: Overlay -d <path to experiment 2>

   Command3: Overlay -d <path to experiment 3>

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