Octet System Data Acquisition User Guide

Release 7.1

ForteBio, Inc.
1360 Willow Road, Suite 201
Menlo Park, CA 94025
888.OCTET-QK
650.322.1360
www.fortebio.com

fortéBIO®
A Division of Pall Life Sciences

Copyright 2011© ForteBio, Inc. All rights reserved

Table of Contents

CHAPTER 1:

Welcome

Welcome to the *ForteBio Octet System Data Acquisition User Guide*. This guide explains how to:

- Operate the Octet instrument.
- Set up and run quantitation and kinetics experiments on the Octet instrument.
- Maintain the Octet instrument.
- Use the optional Octet system 21 CFR Part 11 Compliance Validation module.

ABOUT THE OCTET SYSTEM

The Octet system enables real-time quantitation or kinetic characterization of biomolecular interactions. A system includes the Octet instrument with the following components:

- Computer
- Hardware
- Software Modules—Data Acquisition and Data Analysis (see Table 1-1)

For more details on the Data Analysis software, see the *Data Analysis User Guide*.

Table 1-1: *Octet System Functions*

Octet Software	*Functions*
Data Acquisition 	• Define a quantitation or kinetic experiment and save the experiment for future use. • Define custom assays. • Run the experiment and acquire binding data. • View and save binding data to a user-specified location.
Data Analysis 	• Analyze binding data and view analysis results. • Export or copy analysis results. • Generate a report of quantitation or kinetic results in table and graph formats.

For information on preparing samples for quantitation or kinetics experiments, please see the appropriate ForteBio Octet Biosensor product instructions.

WHAT'S NEW IN THE OCTET SYSTEM DATA ACQUISITION SOFTWARE, RELEASE 7.1

The following features are new for the Octet Pro Data Acquisition software, Release 7.1:

1. Multiple instruments can co-exist on the same computer.
2. The .fmf file is saved in the experiment folder as read-only.
3. Added the Sample Plate and Sensor Tray **Print** button on the **Plate Definition** tab and the plate map.

   The associated table information prints after you click **Print** (Figure 1-1).

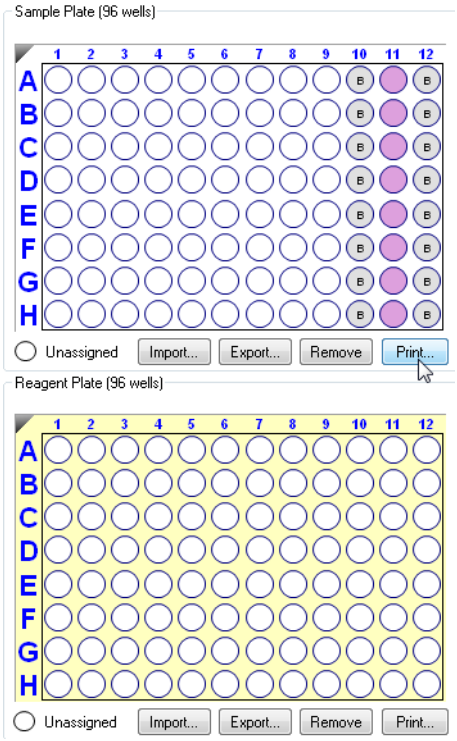


Figure 1-1: *Sample Plate and Sensor Tray Print Button*

4. Added a new **Regeneration** step that is similar to regeneration in Quantitation.
    a. On the **Plate Definition** tab, assign wells as **Regeneration** or **Neutralization** (Figure 1-2).

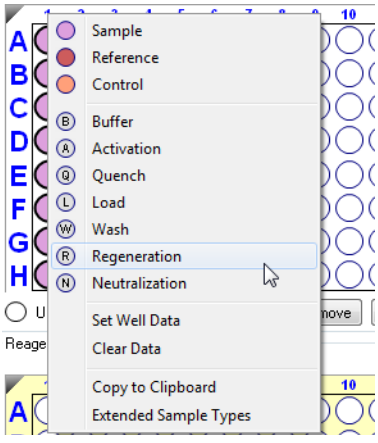


Figure 1-2: *Regeneration Step*

    b. Click **Add** (Figure 1-3) to display the Add Step Definition dialog box (Figure 1-4).

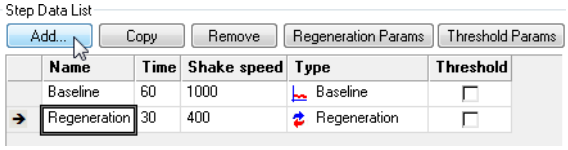


Figure 1-3: *Regeneration Step—Step Data List—Add Button*

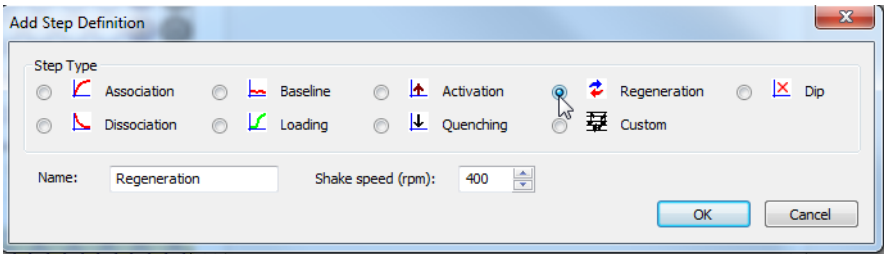


Figure 1-4: *Add Step Definition Dialog Box*

c. Select the **Regeneration** radio button and click **OK**.

d. Click **Regeneration Params** (Figure 1-5).

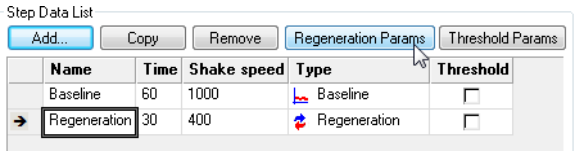


Figure 1-5: *Regeneration Step—Step Data List—Regeneration Params Button*

The **Regeneration Parameters** dialog box (Figure 1-6) displays, where you can edit Regeneration parameters, as necessary.

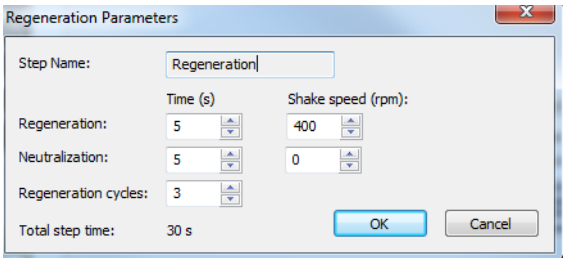


Figure 1-6: *Regeneration Parameters Dialog Box*

5. Added a new feature to align a Kinetic experiment at a given time.

   a. Right-click the Kinetic experiment running chart and select **Align at time...** (Figure 1-7).

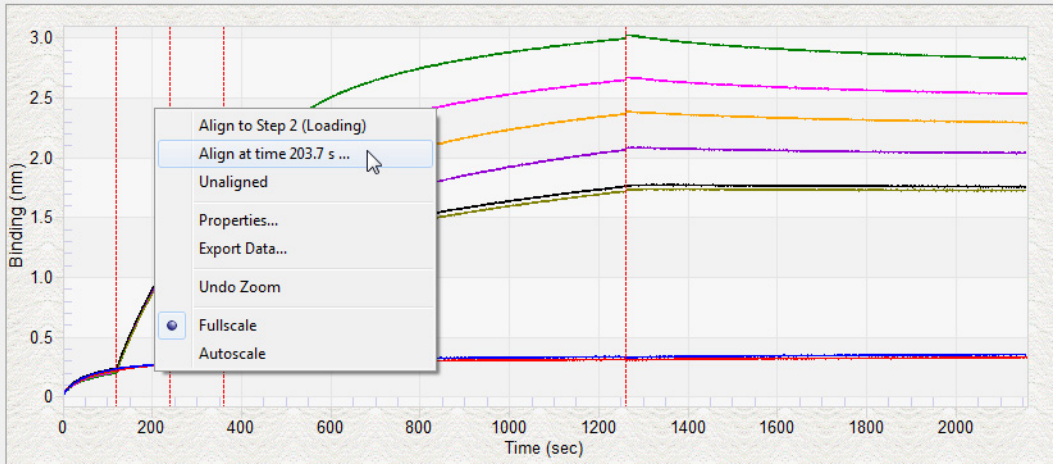


Figure 1-7: *Kinetic Experiment Running Chart—Align at Specified Time*

The Align at Time dialog box displays (Figure 1-8).

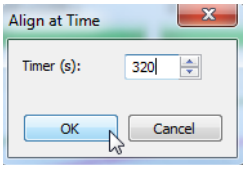


Figure 1-8: *Align at Time Dialog Box*

b. Specify the time point you want to align to and click **OK**.

The running chart aligns to the time point you specify.

6. Made changes to only permit samples in the sample plate.
7. Added printing for the **Assay Definition** tab via **File** > **Print & File** > **Print Preview**.

WHAT'S NEW IN THE OCTET SYSTEM DATA ACQUISITION SOFTWARE, RELEASE 7.0

Table 1-2 describes new features available in the Octet System Data Acquisition software, Release 7.0.

Table 1-2: *Octet System Data Acquisition Software—New Features for Release 7.0*

New Feature	*Description*
User-defined default start-up temperature	Allows you to define the default start-up temperature for all experiments. To access the Temperature field, click **File** > **Options**. ***NOTE:*** *To change the default setting, you must restart the Octet System Data Acquisition software after entering the new value.*
Post-condition biosensors	Post-condition biosensors after Basic Quantitation with Regeneration or Advanced Quantitation experiments, allowing re-racked tips to be stored in a regenerated state.
Sample plate temperature recorded in log file	The sample plate temperature is recorded in the Instrument Status window at the beginning of the experiment, as well as when each set of sensors is picked up by the manifold.
Enhanced legend options in the Runtime Binding Chart	The biosensor legend displayed in the Runtime Binding Chart provides four options for enhanced monitoring: Sensor Location, Sample ID, Sensor Information, and Concentration/Dilution.
Multiple Runtime Binding Charts	During data acquisition, multiple Runtime Binding Charts may be opened, allowing the comparison of different channel settings.

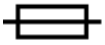
CONVENTIONS AND SYMBOLS USED IN THIS GUIDE

NOTE: *A note presents pertinent details on a topic.For example, general information about tips or alternate options.*

IMPORTANT: *An important message for instances where the assay or procedure will not work if not properly followed.*

WARNING: *A warning informs the user that specific actions could cause irreversible consequences or damage.*

Table 9: *Octet Instrument Labels*

Symbol	*Definition*
	Electrical hazard
	Heat/hot
	Fuse

FORTEBIO TECHNICAL SUPPORT

You can contact ForteBio technical support at any of the locations listed in Table 10.

Table 10: *ForteBio Technical Support*

Main Office	*European Office*	*Asia Office*
ForteBio, Inc. 1360 Willow Road, Suite 201 Menlo Park, CA 94025 USA Tel: +1-650-322-1360 Fax: +1-650-322-1370 E-mail: info@fortebio.com	ForteBio, UK, Ltd. 83 Victoria Street, Suite 407 London, SW1H 0HW UK Tel: +44-(0)20-31784425 Fax: +44-(0)20-31787070 E-mail: info@fortebio.co.uk	ForteBio (Aria Biotechnology Co. Ltd.) 917 Halley Road, Bldg. 4 Zhangjiang High Tech Park Shanghai, China 201203 Tel: +86-21-51320387 E-mail: info@fortebio.com

CHAPTER 2:

Octet System Specifications

OCTET RED96 SYSTEM SPECIFICATIONS

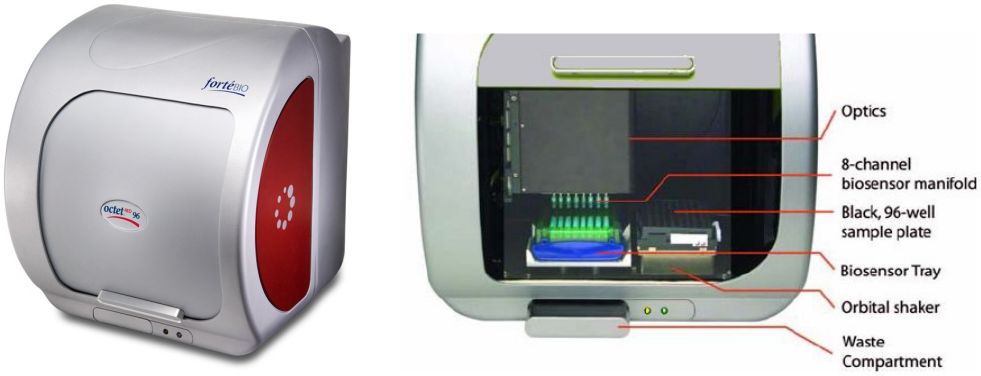


Figure 2-1: *Octet RED96 Instrument—Door Closed (Left) or Open (Right)*

Table 2-1: *Octet RED96 System Specifications*

Item	*Description*
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: -20 to 70 °C • Optimum Operating Temperature: 22 ± 4 °C • Safe Operating Temperature: 15 to 30 °C • Humidity: Non-condensing, 10 to 80% Relative Humidity • Indoor Use Only • Operating Altitude: 0 to 2,000 meters
Compliance	CE, CSA

Table 2-1: *Octet RED96 System Specifications (Continued)*

Item	*Description*
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening of proteins, peptides, and other biomolecules • Small molecule and fragments screening and kinetic analysis • Recommended analyte molecular weight of 150 Da or higher
Sampling Format	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate • Single sample plate capacity
Sampling Volume	180–220 µL/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tip tray, green color
Optics and Mechanics	• 8-channel biosensor manifold • Optical interferometer • Eight spectrometers (one dedicated spectrometer per biosensor)
Throughput	• Up to 8 biosensors in parallel, maximum of 96 tests unattended • One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Temperature Range	(Ambient + 4 °C)–40 °C, 1 °C increments
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)
Weight	63 lb (28.6 kg)
Electrical Requirements	• Mains: AC 100–240 V, 5.0–2.0 A, 50/60 Hz, single phase • Power consumption: 120 W (240 W peak)

OCTET RED384 SYSTEM SPECIFICATIONS

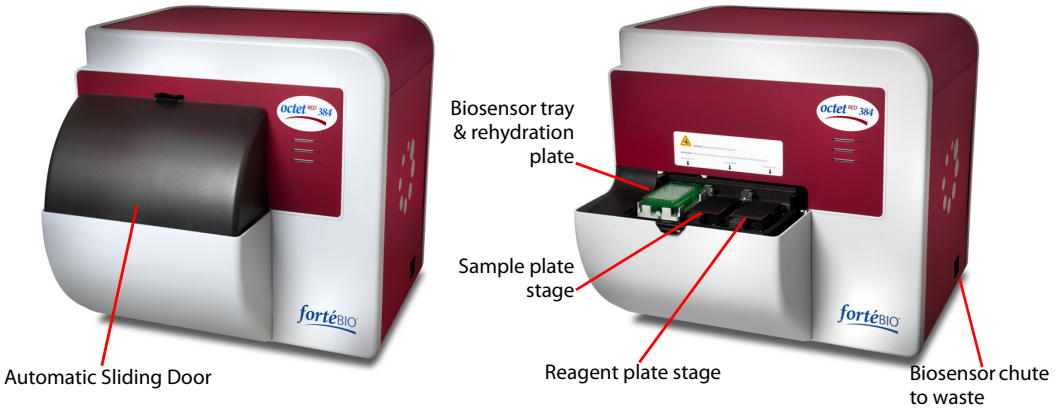


Figure 2-2: *Octet RED384 Instrument—Door Closed (Left) or Open (Right)*

Table 2-2: *Octet RED384 System Specifications*

Item	*Description*
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: -20 to 70 °C • Optimum Operating Temperature: 22 ± 4 °C • Safe Operating Temperature: 15 to 30 °C • Humidity: Non-condensing, 10 to 80% Relative Humidity • Indoor Use Only • Operating Altitude: 0 to 2,000 meters
Compliance	CE, CSA
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening • Small molecule kinetic analysis

Table 2-2: *Octet RED384 System Specifications (Continued)*

Item	*Description*

Sampling Format	• Required plates:
	  • 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate
	  • 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209)
	  • 384-well black, tilted-bottom polypropylene (ForteBio, #18-5076 or #18-5080), SBS standard microplate
	• Two plate stations
	• Test volume:
	  • 180–300 µL in a 96-well plate, non-destructive and recoverable
	  • 80–130 µL in a 384-well plate, non-destructive and recoverable
	  • 40–100 µL in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable

Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tip tray, green color
Automation	• Up to 16 biosensors in parallel • Ability to integrate the Octet instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
Optics and Mechanics	• 16-channel biosensor manifold • Optical interferometer • Sample plate platform temperature range: from 4 °C above ambient to 40 °C • 16 spectrometers (one dedicated spectrometer per biosensor)
Throughput	• Up to 16 biosensors in parallel, maximum of 384 tests unattended • Two microplates, either 96- or 384-well at once. Only one plate can be used for samples. The second plate is used for reagents.

Table 2-2: *Octet RED384 System Specifications (Continued)*

Item	*Description*
Orbital Flow Capacity	Static or 100–1,500 rpm
Dimensions	30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D)
Weight	150 lb (68 kg)
Electrical Requirements	• Mains: AC 100–240 V, 5.0–2.0 A, 50/60 Hz, single phase • Power consumption: 195 W (240 W peak)

Table 2-3: *Sensor Offset and Well Volumes for Octet RED384 and Octet QK384*

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

OCTET QK^{e} SYSTEM SPECIFICATIONS

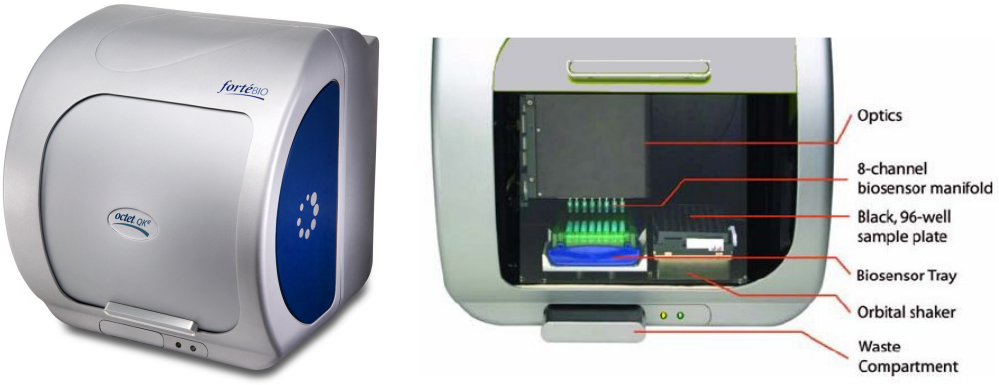


Figure 2-3: *Octet QK^{e} Instrument—Door Closed (Left) or Open (Right)*

Table 2-4: *Octet QK^{e} System Specifications*

Item	*Description*
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: -20 to 70 °C • Optimum Operating Temperature: 22 ± 4 °C • Safe Operating Temperature: 15 to 30 °C • Humidity: Non-condensing, 10 to 80% Relative Humidity • Indoor Use Only • Operating Altitude: 0 to 2,000 meters
Compliance	CE, CSA

Table 2-4: *Octet QK^e System Specifications (Continued)*

Item	*Description*
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening of proteins, peptides and other biomolecules • Biosensor re-racking • Recommended analyte molecular weight of 5,000 Da or higher
Sampling Format	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209), SBS standard microplate • Single sample plate capacity
Sample Volume	180–220 µL/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tip tray, green color
Optics and Mechanics	• 8-channel biosensor manifold • Optical interferometer • One spectrometer (shared by eight biosensors)
Throughput	• Up to eight biosensors in parallel, maximum of 96 tests unattended • One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Temperature Range	(Ambient + 4 °C)–40 °C, 1 °C increments
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)
Weight	54 lb (24.5 kg)
Electrical Requirements	• Mains: AC 100–240 V, 5.0–2.0 A, 50/60 Hz, single phase • Power consumption: 120 W (240 W peak)

OCTET QK SYSTEM SPECIFICATIONS

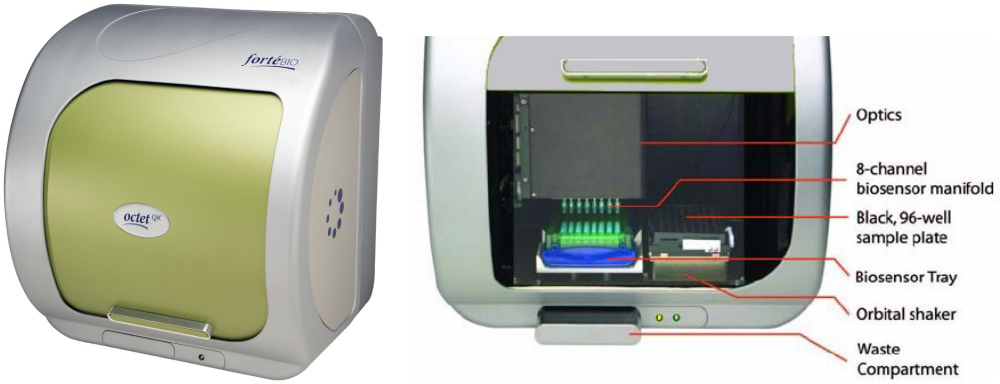


Figure 2-4: *Octet QK Instrument—Door Closed (Left) or Open (Right)*

Table 2-5: *Octet QK System Specifications*

Item	*Description*
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: -20 to 70 °C • Optimum Operating Temperature: 22 ± 4 °C • Safe Operating Temperature: 15 to 30 °C • Humidity: Non-condensing, 10 to 80% Relative Humidity • Indoor Use Only • Operating Altitude: 0 to 2,000 meters
Compliance	CE, CSA
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening of proteins, peptides, and other biomolecules • Recommended analyte molecular weight of 10,000 Da or higher

Table 2-5: *Octet QK System Specifications (Continued)*

Item	*Description*
Sampling Format	• Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209), SBS standard microplate • Single sample plate capacity
Sample Volume	180–220 µL/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration
Biosensor Tray Type	8 x 12 format 96-biosensor tip tray, green color
Optics and Mechanics	• 8-channel biosensor manifold • Optical interferometer • One spectrometer (shared by eight biosensors)
Throughput	• Up to 8 biosensors in parallel, maximum of 96 tests unattended • One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Temperature Range	(Ambient + 4 °C)–40 °C, 1 °C increments
Dimensions	18.6″ H x 17″ W x 20.8″ D (47 cm H x 43 cm W x 53 cm D)
Weight	50 lb (23 kg)
Electrical Requirements	• Mains: AC 100–240 V, 5.0–2.0 A, 50/60 Hz, single phase • Power consumption: 120 W (240 W peak)

OCTET QK384 SYSTEM SPECIFICATIONS

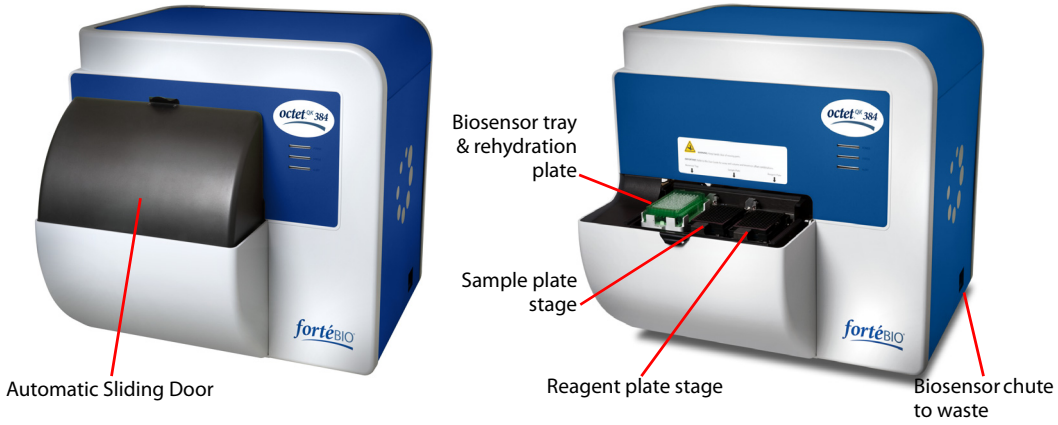


Figure 2-5: *Octet QK384 Instrument—Door Closed (Left) or Open (Right)*

Table 2-6: *Octet QK384 System Specifications*

Item	*Description*
Equipment Classifications	• Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	• Storage Temperature: -20 to 70 °C • Optimum Operating Temperature: 22 ± 4 °C • Safe Operating Temperature: 15 to 30 °C • Humidity: Non-condensing, 10 to 80% Relative Humidity • Indoor Use Only • Operating Altitude: 0 to 2,000 meters
Compliance	CE, CSA
Capabilities	• Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening

Table 2-6: *Octet QK384 System Specifications (Continued)*

Item	*Description*

Sampling Format	• Required plates:
	  • 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate
	  • 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209)
	  • 384-well black, tilted-bottom polypropylene microplate (ForteBio, #18-5076 or #18-5080), SBS standard microplate
	• Two plate stations
	• Test volume:
	  • 180–300 µL in a 96-well plate, non-destructive and recoverable
	  • 80–130 µL in a 384-well plate, non-destructive and recoverable
	  • 40–100 µL in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable

Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tip tray, green color
Automation	• Up to 16 biosensors in parallel • Ability to integrate the Octet instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
Optics and Mechanics	• 16-channel biosensor manifold • Optical interferometer • Sample plate platform temperature range: From 4 °C above ambient to 40 °C • 2 spectrometers (one dedicated spectrometer per eight biosensors)
Throughput	• Up to 16 biosensors in parallel, maximum of 384 tests unattended • Two microplates, either 96- or 384-well at once. Only one plate can be used for samples. The second plate is used for reagents.

Table 2-6: Octet QK384 System Specifications (Continued)

Item	Description
Orbital Flow Capacity	Static or 100–1,500 rpm
Dimensions	30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D)
Weight	150 lb (68 kg)
Electrical Requirements	• Mains: AC 100–240 V, 5.0–2.0 A, 50/60 Hz, single phase • Power consumption: 195 W (240 W peak)

Table 2-7: Sensor Offset and Well Volumes for Octet RED384 and Octet QK384

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

CHAPTER 3:

Getting Started

STARTING THE OCTET SYSTEM AND DATA ACQUISITION SOFTWARE

NOTE: *The installation shall be performed by ForteBio, Inc. personnel only.*

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

NOTE: *Do not position the Octet instrument such that it is difficult to disconnect the power.*

For information about how to connect the Octet instrument to the computer, please refer to the insert sheet that is provided with the Octet instrument.

To start the system and software:

1. Turn on the computer.
2. Turn the Octet instrument on using the power switch located on the external electrical box.

NOTE: *The instrument requires a minimum of one-hour warm-up time. It is recommended that you leave the instrument on for a minimum of eight hours prior to use.*

3. Launch the Octet System Data Acquisition software by double-clicking on the Data Acquisition desktop icon.

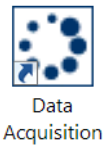


Figure 3-1: *Desktop Icon*

NOTE: *When using the CFR 11 version of the Octet System Data Acquisition software, users are required to log in and start a user session before the software will launch. Please refer to "Starting a User Session" on page 56 for more information.*

SOFTWARE OVERVIEW

Launching the application displays the Octet System Data Acquisition software **Main Screen**. Screen components along with the default windows displayed are shown in Figure 3-2.

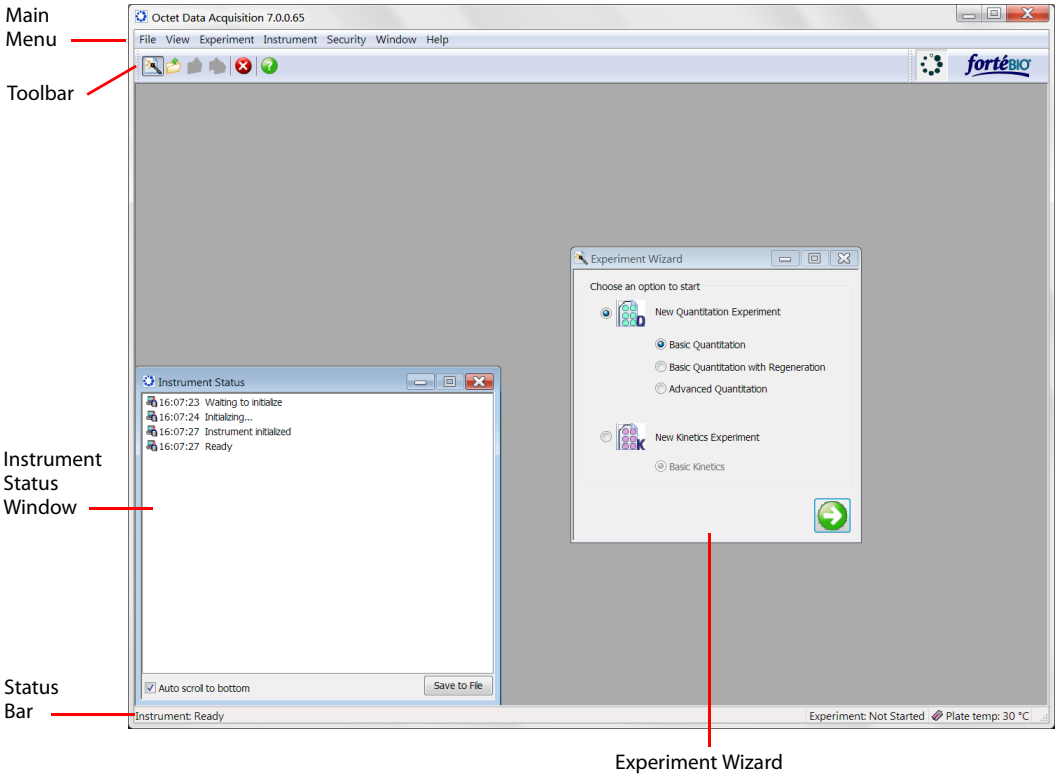


Figure 3-2: *Main Screen*

Main Menu and Toolbar

The Main Menu and Toolbar are located in the upper left corner of the **Main Screen** (Figure 3-3). Menu options and toolbar buttons are described in this section.

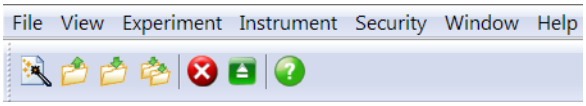


Figure 3-3: *Main Menu and Toolbar*

NOTE: *The* ***Security*** *menu is only available in the 21 CFR Part 11 version of the Octet System Data Acquisition software.*

File Menu

The **File** menu (Figure 3-4) allows users to open and save method files, view experiments, print files and set system and software options.

A method file (.fmf) contains sample plate configuration, sample plate table information, sensor assignments and assay step information that allow the Octet instrument and software to run an experiment. When the run is complete, the data in the experiment folder can then be reviewed.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

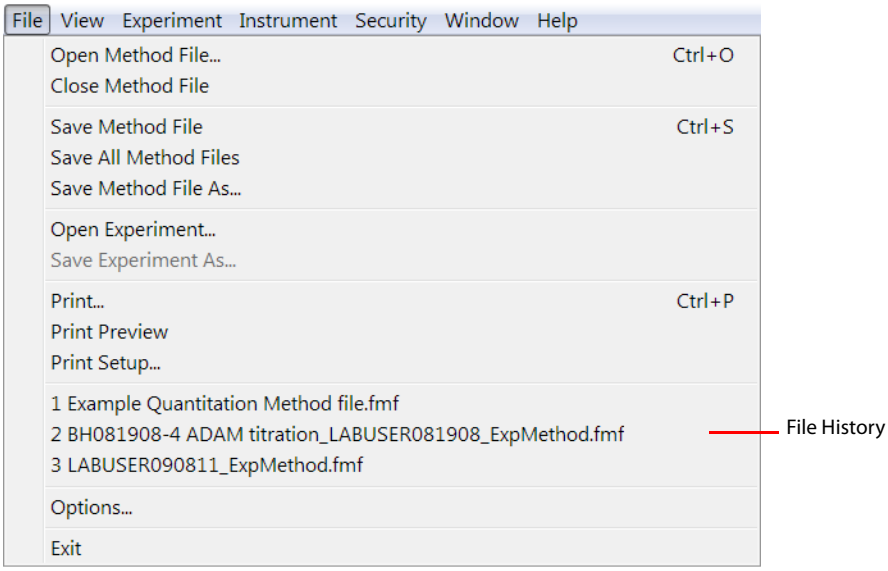


Figure 3-4: *File Menu*

Table 3-1: *File Menu Commands*

Menu Command	*Toolbar Button*	*Function*
Open Method File		Opens an experiment method file (.fmf).
Close Method File	N/A	Closes the active experiment method file but does not save changes.
Save Method File		Saves the active experiment method file (.fmf).
Save All Method Files		Saves all open method files (.fmf).
Save Method File As	N/A	Allows the active experiment method file to be saved as a new file without overwriting the original method file.
Open Experiment	N/A	Opens an experiment folder.
Save Experiment	N/A	Saves the active experiment.
Print	N/A	Opens the **Print** dialog box to print a file.
Print Preview	N/A	Opens a print preview window of a method file.
Print Setup	N/A	Opens the **Print Setup** dialog box to print a file.

Table 3-1: File Menu Commands (Continued)

Menu Command	Toolbar Button	Function
File History	N/A	Displays a list of previously opened files.
Options	N/A	Opens the Options dialog box. Please refer to "Octet System Data Acquisition Options" on page 40 for more information on changing system and software options.
Exit	N/A	Closes the application after prompting users to save any changes.

View Menu

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

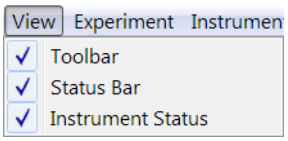


Figure 3-5: View Menu

Table 3-2: View Menu Commands

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

Experiment Menu

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

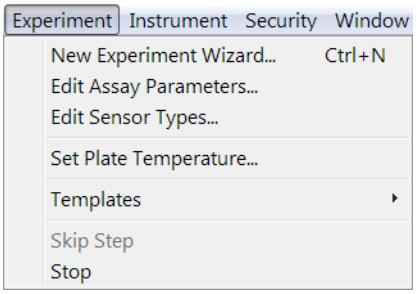


Figure 3-6: *Experiment Menu*

Table 3-3: *Experiment Menu Commands*

Menu Command	*Toolbar Button*	*Function*
New Experiment Wizard		Opens the **Experiment Wizard**.
Edit Assay Parameters	N/A	Opens the **Edit Assay Parameters** dialog box to define a new assay, edit an existing assay, or remove an assay from the quantitation application. See "Managing Assay Parameter Settings" on page 167 for more information.
Edit Sensor Types	N/A	Opens the **Sensor Types** dialog box to view current biosensor types, add new biosensor types and remove biosensor types. See "Managing Biosensor Types" on page 48 for more information.
Set Plate Temperature	N/A	Opens the **Temperature Setting** dialog box that displays the current sample plate temperature and allows users to change the current temperature setting of the instrument. See "Setting the Plate Temperature" on page 43 for more information. To set the default temperature, see "Defining a New Default Sample Plate Temperature" on page 44.
Templates	N/A	Allows users to select from a set of predefined ForteBio quantitation or kinetics method templates.
Skip Step	N/A	Skips the step in the method that is currently executing (kinetics experiments only).

Table 3-3: Experiment Menu Commands (Continued)

Menu Command	Toolbar Button	Function
Stop		Stops the experiment. Data from the active biosensor is not saved, but all data prior to the active biosensor will be available.

Instrument Menu

The **Instrument** menu provides direct control of Octet instrument functions.

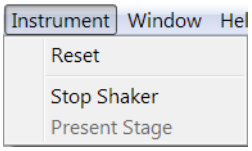


Figure 3-7: Instrument Menu

Table 3-4: Instrument Menu Commands

Menu Command	Toolbar Button	Function
Reset	N/A	Resets the instrument and the log in the **Instrument Status window**.
Stop Shaker	N/A	Stops the sample plate shaker.
Present Stage		Presents the instrument stage that houses the biosensor tray, sample and reagent plates (Octet RED384 and Octet QK384 only).

Security Menu

The **Security** menu is only available in the 21 CFR Part 11 version of the Data Acquisition software. For complete details on menu options, please refer to “Compliance Features” on page 59.

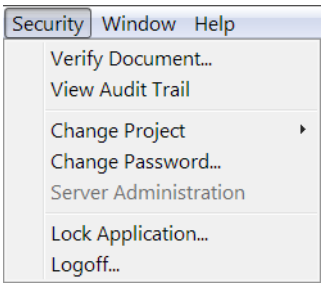


Figure 3-8: *Security Menu*

Window Menu

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

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

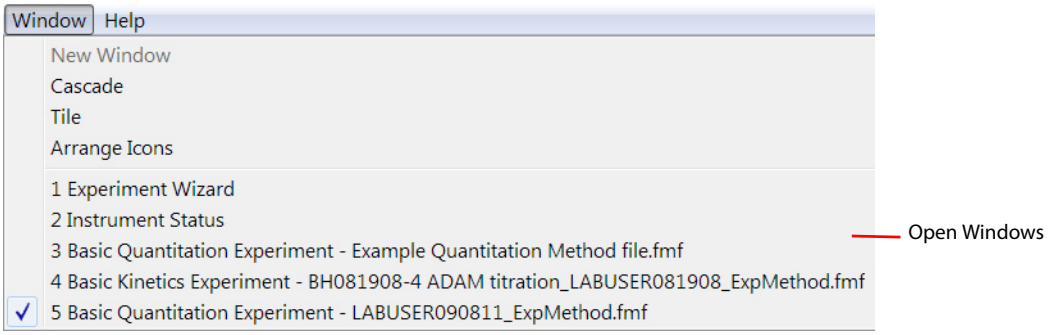


Figure 3-9: *Window Menu*

Table 3-5: *Window Menu Commands*

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

Help Menu

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

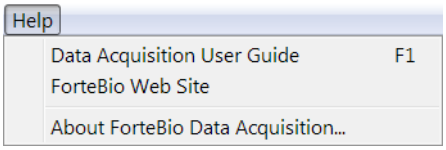


Figure 3-10: *Help Menu*

Table 3-6: *Help Menu Commands*

Menu Command	*Toolbar Button*	*Function*
Data Acquisition User Guide	N/A	Opens the online *Data Acquisition Software User Guide*.
ForteBio Web Site	N/A	Opens a web browser and displays the ForteBio web page (www.forte-bio.com).
About ForteBio Data Acquisition		Displays software, user and instrument information.

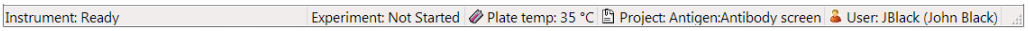
NOTE: *Clicking on the ForteBio logo in the upper right corner of the* ***Main Screen*** *also displays the About ForteBio Data Acquisition window.*

Status Bar

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

Figure 3-11: *Status Bar*

In the 21 CFR Part 11 version of the Data Acquisition software, the **Status Bar** will also display the User and Project name entered at login.



Instrument Status Window

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

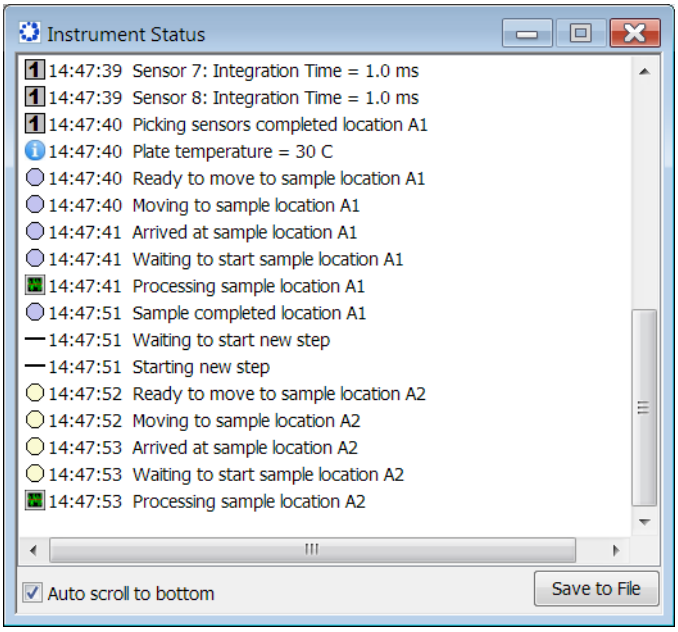


Figure 3-12: *Instrument Status Window*

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

NOTES:

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

The instrument log automatically resets when the Octet System Data Acquisition software application is closed.

Experiment Wizard

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

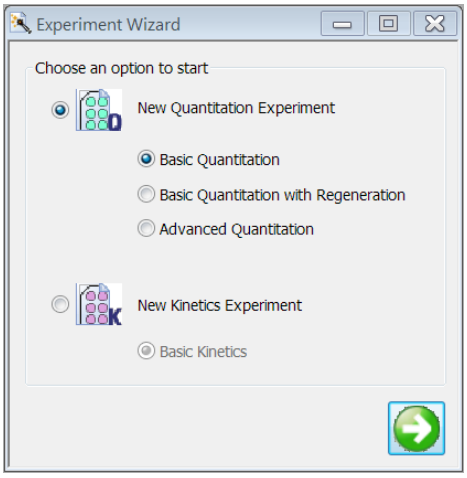


Figure 3-13: *Experiment Wizard*

OCTET SYSTEM DATA ACQUISITION OPTIONS

Acquisition options allow users to set system and data preferences for quantitation and kinetic data acquisition. To view user options (Figure 3-14), click **File** > **Options** from the **Main Menu**.

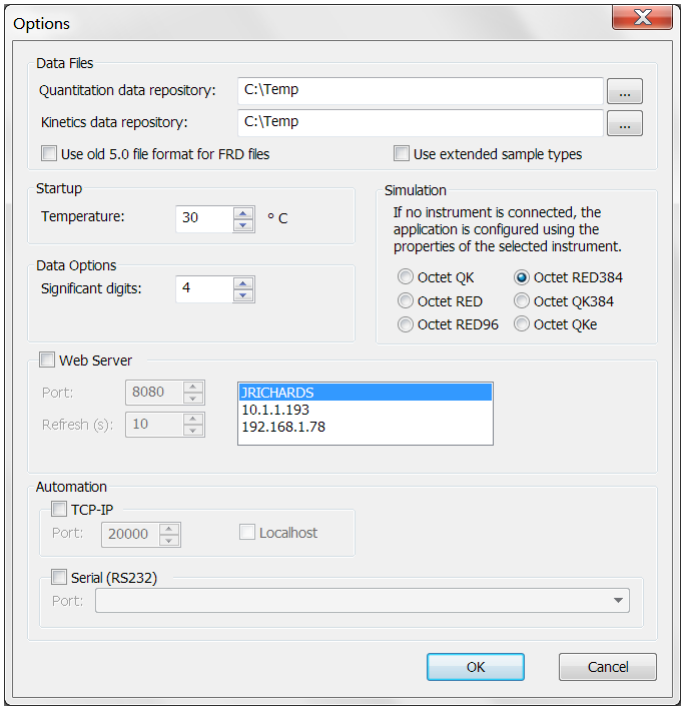


Figure 3-14: *Options Dialog Box*

Table 3-7: *User Options*

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

Table 3-7: User Options (Continued)

Item	Description
Use old 5.0 file format for FRD files	Select this option to save data in the earlier Octet RED software 5.0 format. ***NOTE:*** *Saving data in the old file format produces larger files and may result in slower data analysis.*
Use extended sample types	Select this option to extend the sample types available in the right-click menu of the **Sample Plate Map** and **Sample Plate Table** to include negative and positive controls.
Startup	
Temperature	User-defined default startup plate temperature. This temperature is used as the default setting for all experiments. ***NOTE:*** *To change the default setting, the software must be restarted after entering the new value. This changes the startup plate temperature only, not the current plate temperature.*
Data Options	
Significant digits	Specifies the number of significant digits for the values of Molecular Weight, Concentration and Dilution used during data analysis. ***NOTE:*** *Six decimal places are recommended for the Protein A assay.*

Table 3-7: User Options (Continued)

Item	Description
Simulation	If the workstation is not connected to an instrument, this option enables users to create and save an experiment to a method file (.fmf) using the properties of the selected instrument type.
Web Server	Selecting this option enables remote monitoring of the experiment using a web browser. See "Monitoring Experiments Remotely" on page 45 for more information.
Automation	Allows users to select the appropriate connection for automation interfaces used with OctetRED384 and OctetQK384 systems only. For more information, please refer to Appendix A, Using Octet384 Systems with an Automation Interface on page 345.

SETTING THE PLATE TEMPERATURE

The settable plate temperature can range from ambient plus 4 °C to a high of 40 °C. A factory-set default plate temperature of 30 °C is used as a system startup plate temperature and the experiment default temperature. This default value can be customized by the user. In addition, the plate temperature setting can be changed for individual experiments when needed. The current plate temperature displays in the **Status bar** at the bottom of the **Main Screen**.

Changing the Plate Temperature for Individual Experiments

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

1. From the **Main Menu**, click **Experiment** > **Set Plate Temperature**.
2. Click the **Set temperature to** field (Figure 3-15) to the desired value or enter the preferred temperature and click **OK**.

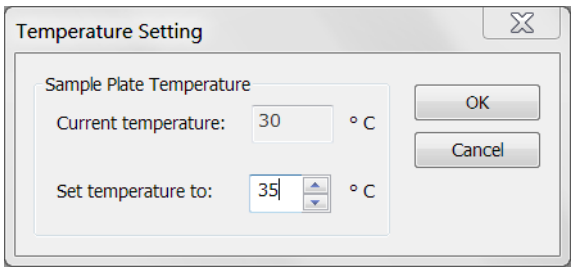


Figure 3-15: Temperature Setting

3. Allow sufficient time for the sample plate to equilibrate to the new temperature before beginning an experiment (approximately 5 minutes for a plate at room temperature or 15 minutes for a plate at ambient + 4 °C).

NOTE: *If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the* ***Options*** *dialog box when the software is relaunched.*

Defining a New Default Sample Plate Temperature

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

1. From the **Main Menu**, click **File** > **Options**.
2. In the **Options** dialog box (Figure 3-16), select a new temperature in the **Startup** box and click **OK**. The plate temperature will then adjust to the new value, and this setting will be used as the new default startup temperature whenever the software is launched.

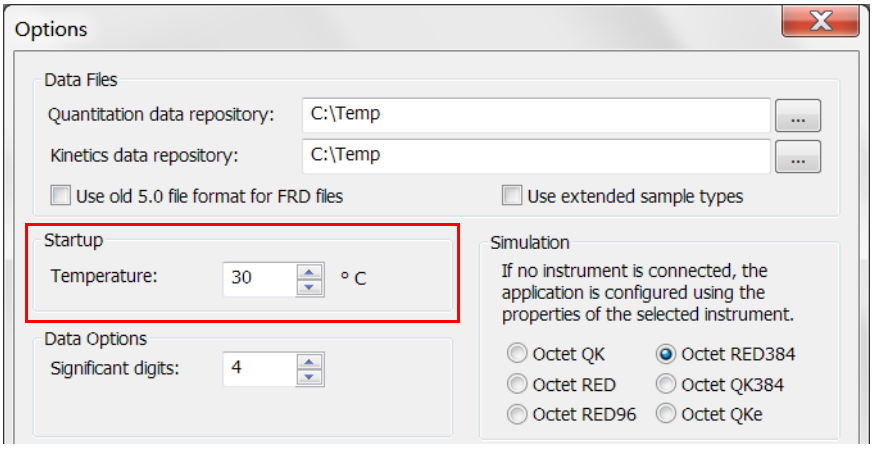


Figure 3-16: *Setting the Default Startup Temperature in the Options Dialog Box*

3. Allow sufficient time for the sample plate to equilibrate to the new temperature before beginning an experiment (approximately 5 minutes for a plate at room temperature or 15 minutes for a plate at ambient + 4 °C).

IMPORTANT: *To save the new default temperature value, you must restart the software.*

MONITORING EXPERIMENTS REMOTELY

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

1. From the **Main Menu**, click **File** > **Options**.
2. In the **Options** dialog box (Figure 3-18), select the **Web Server** check box. Adjust the **Port** and **Refresh** settings and change the **Connect as** IP address if needed. The default **Refresh** rate of 10 will refresh the experiment view in the web browser every 10 seconds. Click **OK**.

NOTE: *ForteBio recommends using the **Port** and **Connect as** (IP address) settings shown as default in the **Web Server** box, as they are unique to your particular Octet system.*

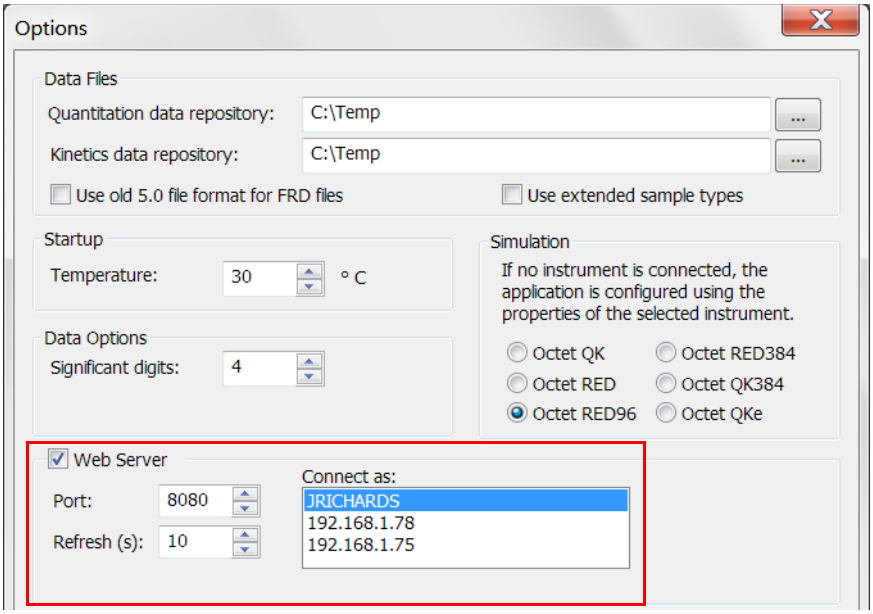


Figure 3-17: *Selecting the Web Server in the Options Dialog Box*

3. Click **File** > **Options** to access the **Options** dialog box again. A **Web Server URL** will now be listed under the **Connect as** box (Figure 3-18). Note this URL as it will be needed to access the experiment remotely.

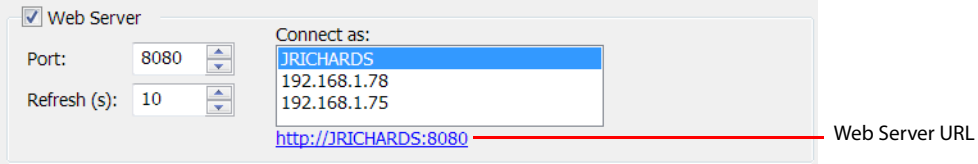


Figure 3-18: *Web Server URL*

4. Start the experiment in the Octet System Data Acquisition software as you normally would.
5. Open a web browser on a remote computer or device that is on the same network as the Octet system.

NOTE: *The remote computer or device must be on the same network as the Octet system, or connected to the network the instrument is on via VPN.*

---

6. Enter the **Web Server URL** in the browser window or click the **Web Server URL** link in the **Options** dialog box. The experiment in progress will display (Figure 3-19).

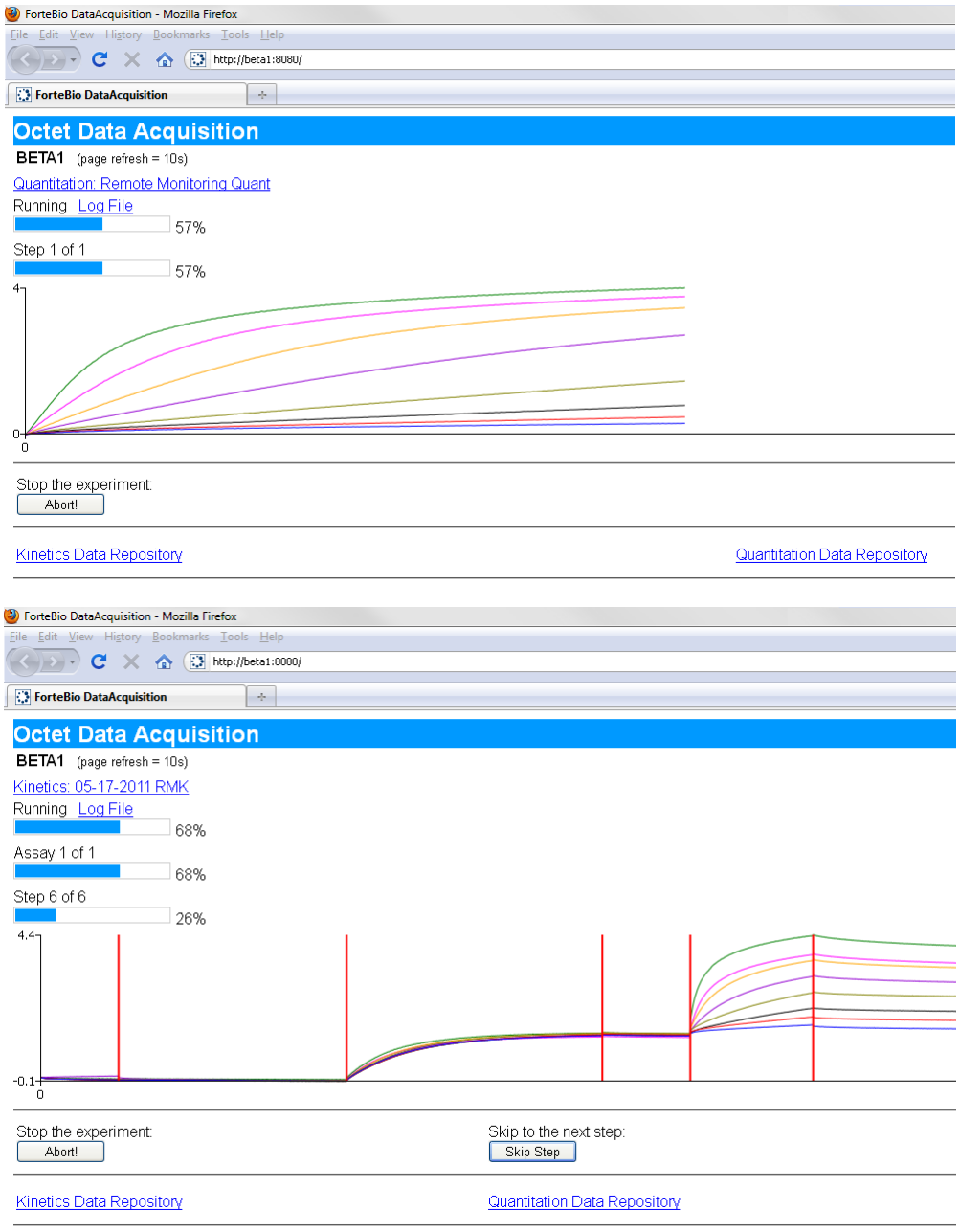


Figure 3-19: *View of Quantitation Experiment (top) and Kinetics Experiment (bottom) via Web Browser*

In the browser window, you can:

- Click the experiment name to view experiment details.
- Click **Log File** to display a log of current instrument activity.
- Click **Kinetics Data Repository** or **Quantitation Data Repository** to open and view previously run experiments.

MANAGING BIOSENSOR TYPES

The Octet System Data Acquisition software includes a factory set list of the types of biosensors available for quantitation or kinetic analysis. The available biosensor types display in the **Sensor Assignment** tab. Users can add custom biosensors as needed.

Viewing Available Biosensor Types

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

The **Sensor Types** dialog box will display (Figure 3-20).

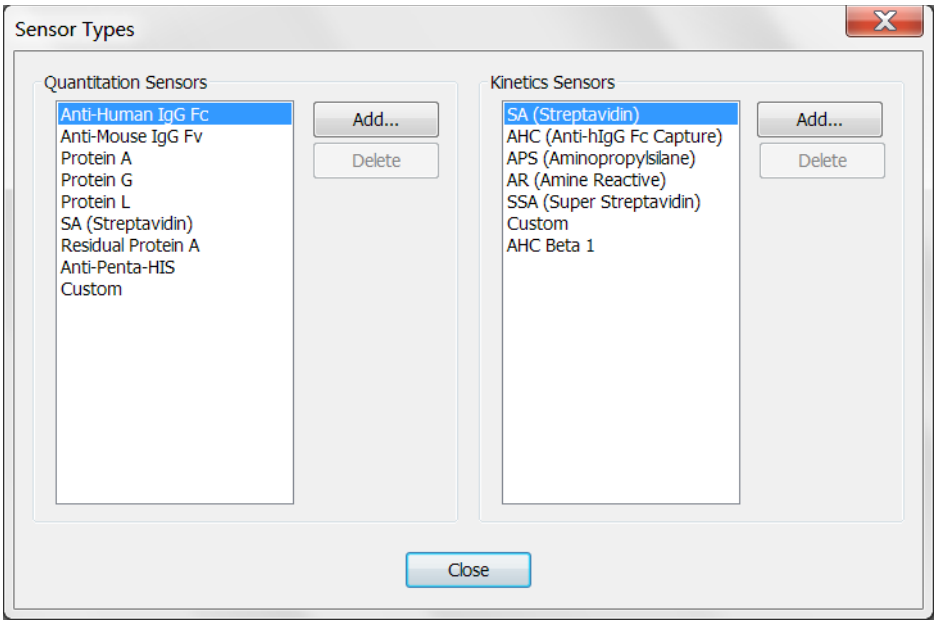


Figure 3-20: *Sensor Types Dialog Box*

Adding a Biosensor Type

To add a biosensor type:

1. From the **Main Menu**, click **Experiment** > **Edit Sensor Types**.
2. In the **Sensor Types** dialog box (Figure 3-21), click **Add** next to the **Quantitation Sensors** or **Kinetic Sensors** box (depending on the type of biosensor that will be added).
3. In the **Add Sensor** dialog box, enter a name for the biosensor type and click **OK**.

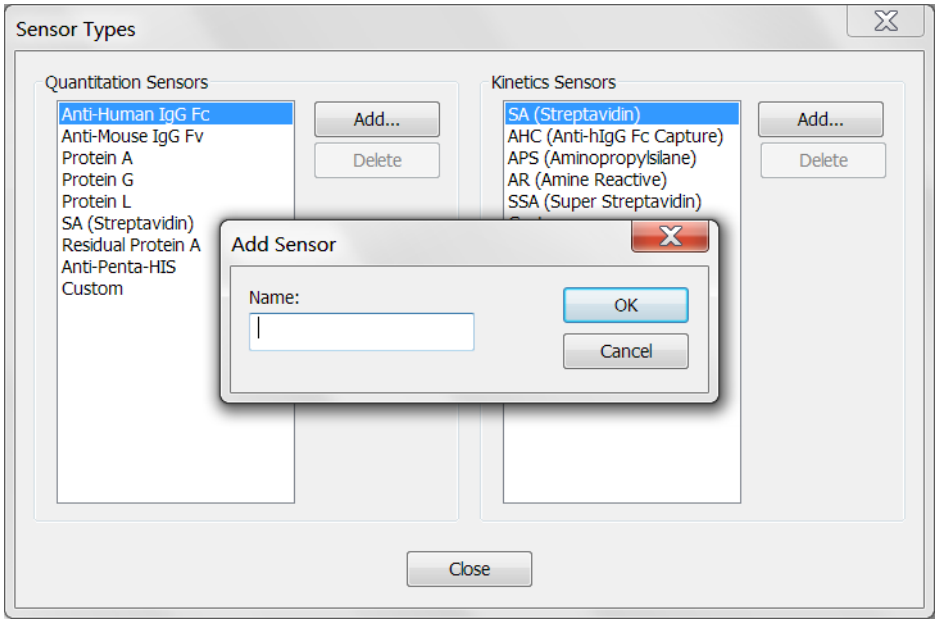


Figure 3-21: *Adding a Biosensor Type*

Removing a Biosensor Type

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

Factory-loaded biosensor types cannot be deleted. Only the biosensor types that users add to the system can be deleted.

CHAPTER 4:

21 CFR Part 11 Compliance

21 CFR PART 11 SOFTWARE

The Data Acquisition and Data Analysis software for Octet systems is available in an optional 21 CFR Part 11 version that enables users in GMP and GLP laboratories to comply with 21 CFR Part 11 regulations. This version of the software includes features such as user account management, audit trails and electronic signatures. In addition, the 21 CFR Part 11 version utilizes the ForteBio GxP Server module to manage the information recorded during user sessions.

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

FORTEBIO GXP SERVER MODULE

When the Data Acquisition or Data Analysis 7.0 21 CFR Part 11 software is launched, users are prompted to log on to the ForteBio GxP Server module. This initiates a user session where all system, software and user events are recorded. During user sessions, the GxP Server module manages and stores this recorded information. User sessions are closed when the user logs out or a set period of inactivity is reached. A new user session is initiated each time a user accesses the software.

SELECTING A SERVER LOCATION

NOTES:

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

Once the GxP Server module host location is selected, this location will be used as the default selection for the user account. It does not need to be reselected each time a new user session is initiated.

Users must select the host location of the GxP Server module during the login process. The GxP server can be run on the local host computer where the Data Acquisition or Data Analysis software is installed or from a network location.

To select a server location:

1. Launch the Data Acquisition or Data Analysis software by double-clicking on the desktop icon:

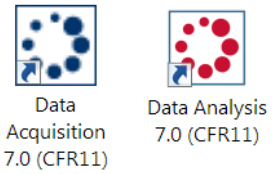


Figure 4-1: *Login Box*

The **Login** dialog box will display:

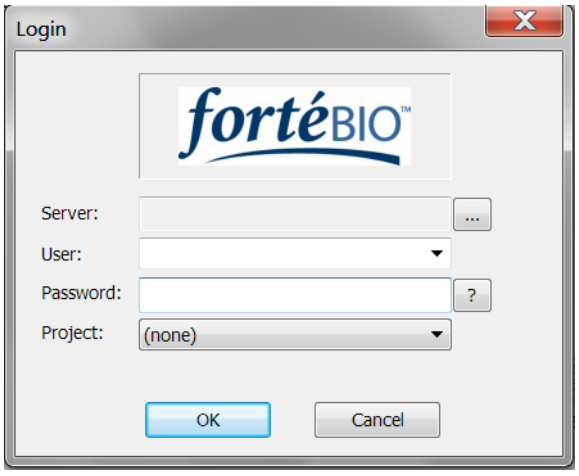


Figure 4-2: *Login Dialog Box*

2. Select a **Server** location by clicking on **...** (**Browse**).

   The **Authentication Server** dialog box will display:

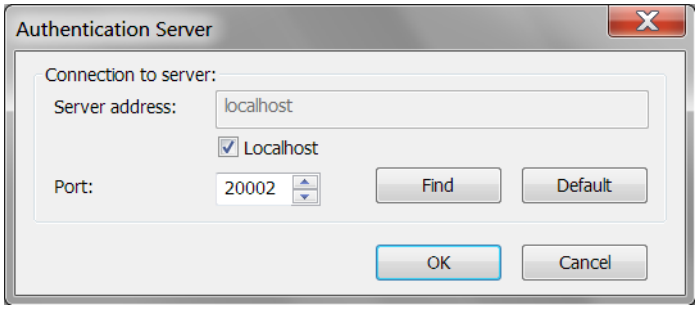


Figure 4-3: *Authentication Server Dialog Box*

Click **Default** to recall the default server settings of localhost and Port 2002.

- ***Local host*—**If the local computer is to be used as the GxP Server module host, select the **Localhost** check box. Change the Port number if needed.
- ***Remote host on same subnet*—**If the GxP Server module is hosted on the same subnet, deselect the **Localhost** check box and click **Find**. A list of potential GxP Server module addresses will be listed. Choose the desired location from the list and click **OK**.

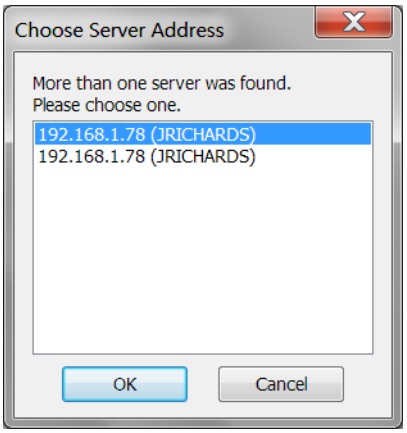


Figure 4-4: *GxP Server Address Search Results*

- ***Remote host on another subnet*—**If the GxP Server module is hosted on a different subnet, deselect the **Localhost** check box. Enter the IP address of the computer hosting the GxP Server module.

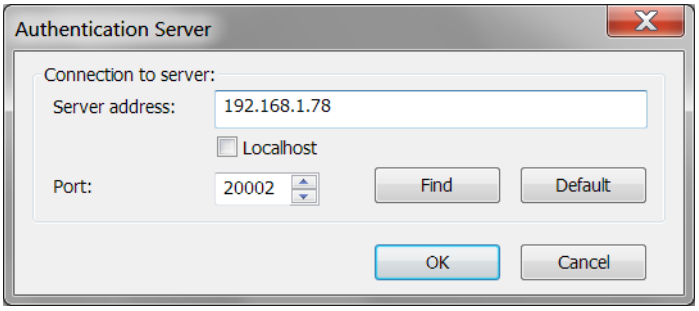


Figure 4-5: *Manual Entry of Remote Host Address*

When the GxP Server module host location has been selected or entered, click **OK** to save changes and exit the **Authentication Server** dialog box. The GxP Server module location will now be listed as the **Server** in the **Login** box.

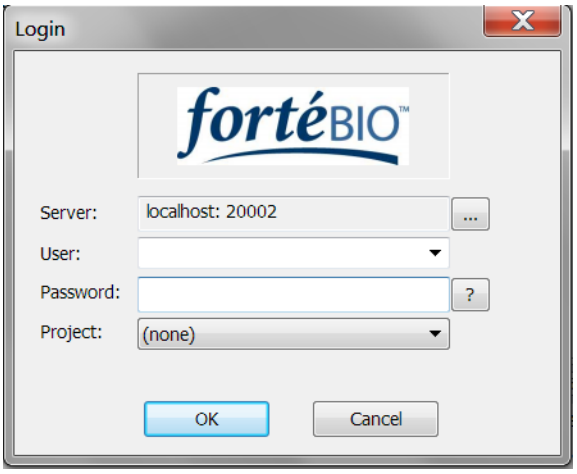


Figure 4-6: *Login Dialog Box—Displaying GxP Server Location*

STARTING A USER SESSION

NOTE: *Before starting your first user session, please contact your administrator to determine the GxP Server module host location that should be used.*

To start a user session:

1. Launch the Data Acquisition or Data Analysis software by double-clicking on the desktop icon:

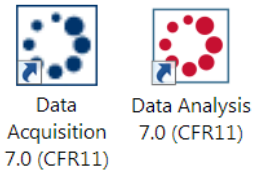


Figure 4-7: *Data Acquisition and Data Analysis Desktop Icons*

The **Login** dialog box will display:

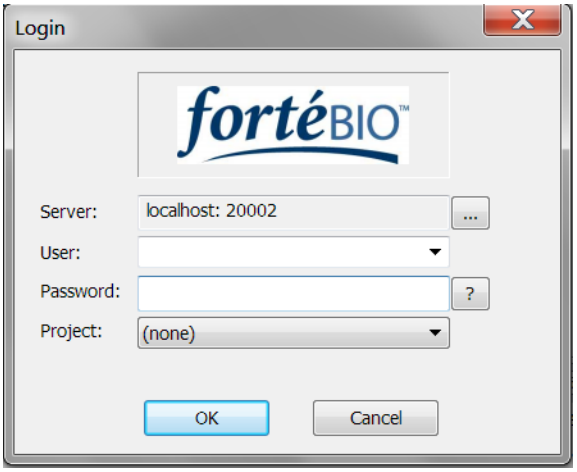


Figure 4-8: *Login Dialog Box*

2. Confirm that the **Server** location is correct. If not, please see "Selecting a Server Location" on page 52.
3. From the **User** drop-down list, select your login name.

NOTE: *To start an administrator session, select* ***Administrator*** *in the* ***User*** *drop down list.*

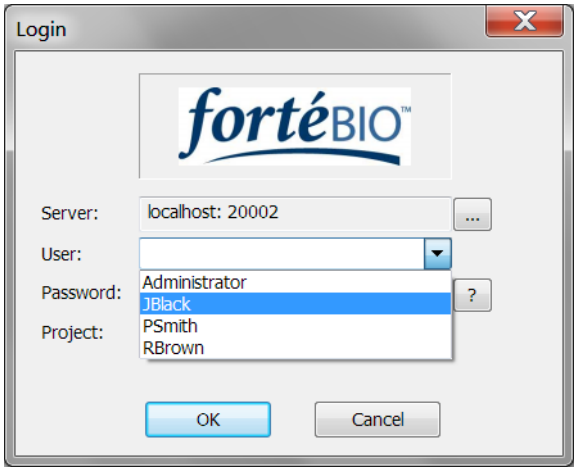


Figure 4-9: *Username Selection*

4. Enter your password in the **Password** text box. Click **?** for a password reminder if needed.

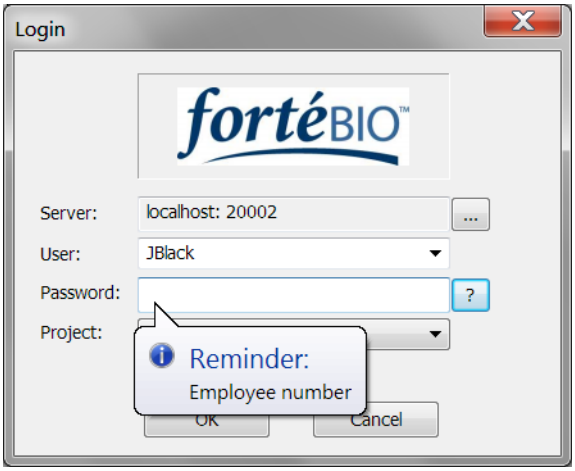


Figure 4-10: *Password Reminder*

5. Select a project from the **Project** drop-down list, if required.

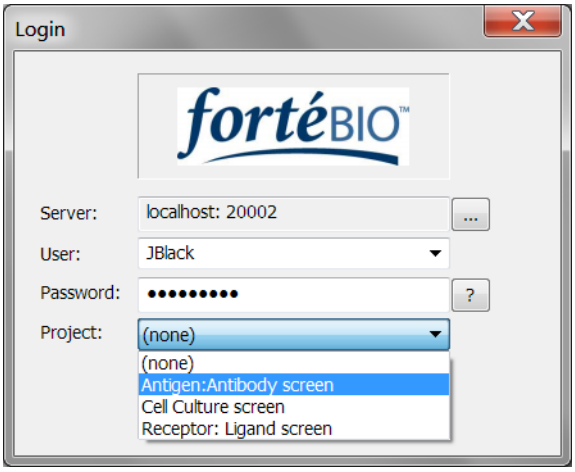


Figure 4-11: *Project Selection*

6. Click **OK.**

   The Data Acquisition or Data Analysis software will now launch and start the user session. During the session, the user account and project selected at login display in the software status bar:

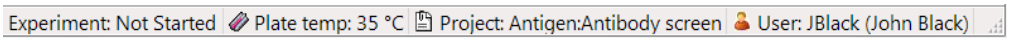


Figure 4-12: *Status Bar*

NOTES:

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

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

COMPLIANCE FEATURES

The 21 CFR Part 11-compliant features provided in the 21 CFR Part 11 versions of the Data Acquisition and Data Analysis software can be accessed by clicking the **Security** menu from the software's **Main Menu**:

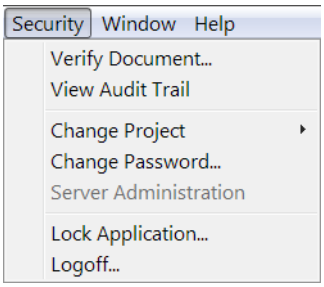


Figure 4-13: *Security Menu*

NOTES:

*The **Server Administration** menu option in the **Security** menu can be accessed only if you have administrator or review privileges.*

***Security** menu options in the Data Acquisition and Data Analysis software applications are identical.*

Experiment and Method File Compliance

When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11-compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software cannot be opened, and a message indicating this will be presented.

Verifying Digital Signatures

The electronic signature of method (.fmf) and data (.frd) files can be verified to ensure they were generated using 21 CFR Part 11 compliant software.

To verify digital signatures:

1. Click **Security** > **Verify Document**.

   The **Verify Digital Signature** dialog box will display:

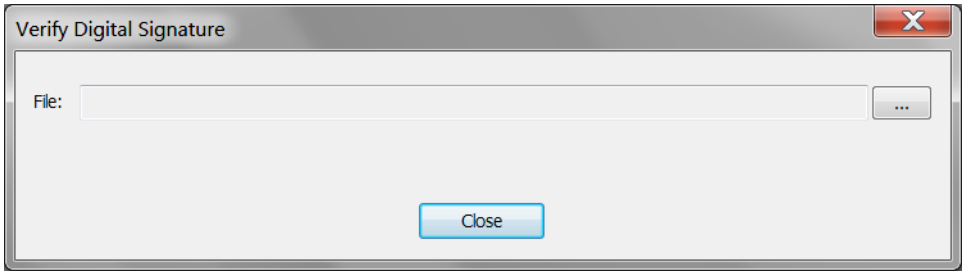


Figure 4-14: *Verify Digital Signature Dialog Box*

2. Click **...** to browse for the desired .fmf or .frd file.

NOTE: *When verifying digital signatures, both method (.fmf) and data (.frd) files can be selected in the Data Acquisition and Data Analysis software.*

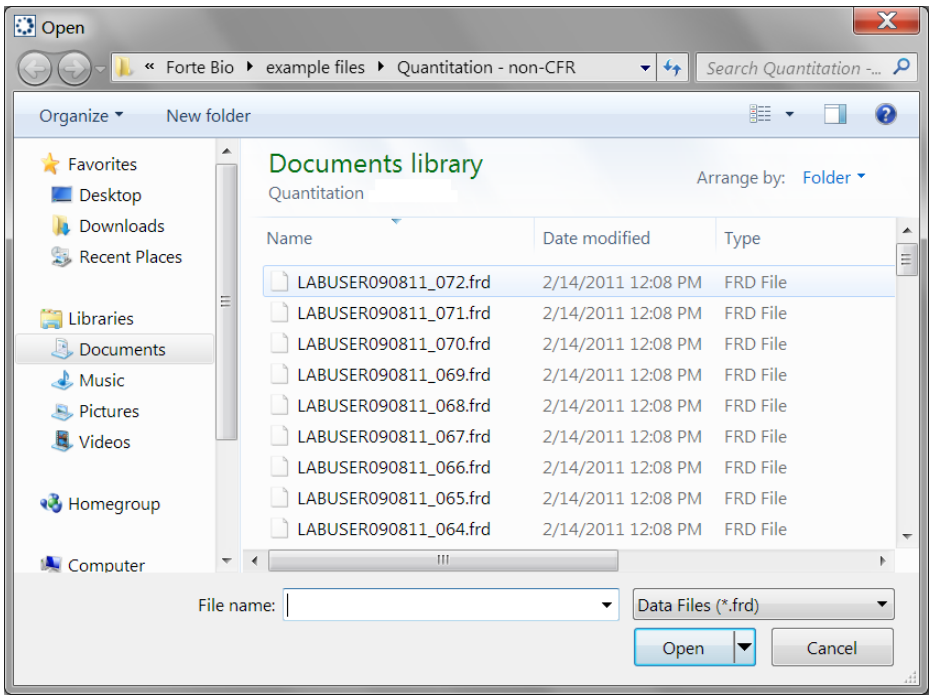


Figure 4-15: *File Selection*

To change the file type available for selection, click on the file type box and select a different format:

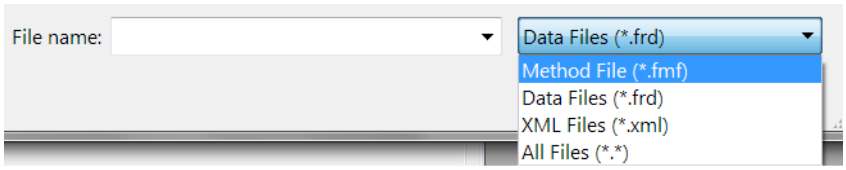


Figure 4-16: *File Type Selection*

3. Select the desired file and click **OK**.

   A message will display in the **Verify Digital Signature** dialog box indicating file compliance status:

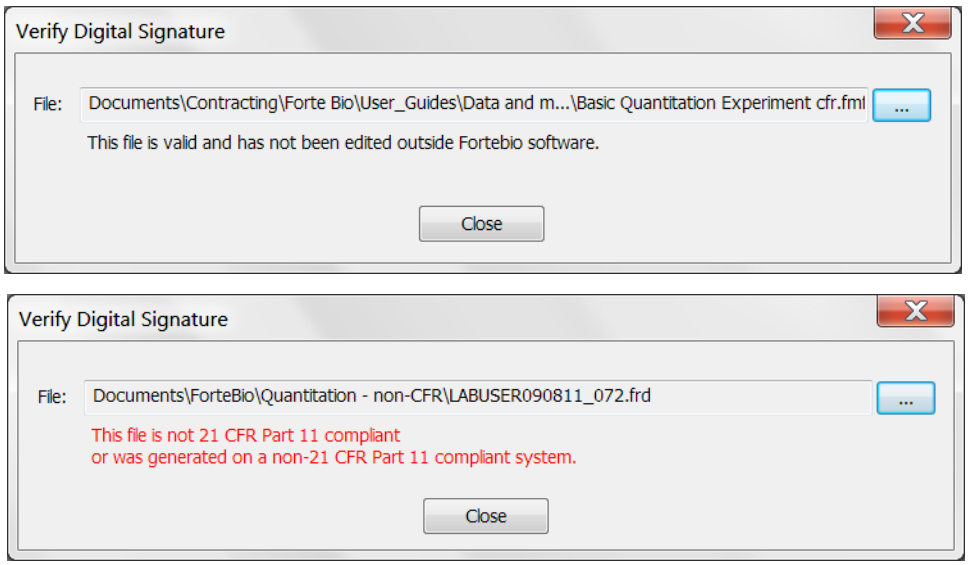
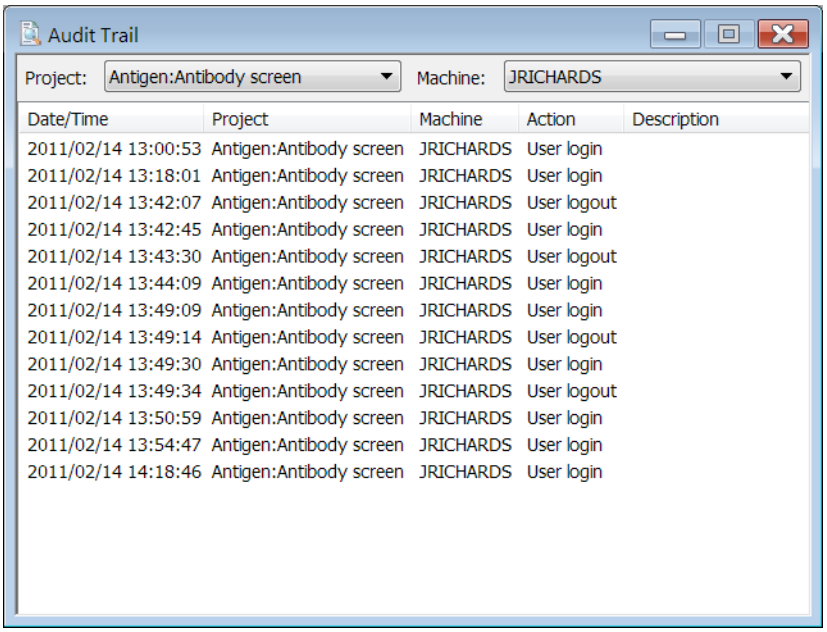


Figure 4-17: *File Compliant (top), File Not Compliant (bottom)*

Viewing the Audit Trail

The Audit Trail displays a historical log of user, system and software events recorded during user sessions. To view the Audit Trail, click **Security** > **View Audit Trail**.



Date/Time	Project	Machine	Action	Description
2011/02/14 13:00:53	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 13:18:01	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 13:42:07	Antigen:Antibody screen	JRICHARDS	User logout	
2011/02/14 13:42:45	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 13:43:30	Antigen:Antibody screen	JRICHARDS	User logout	
2011/02/14 13:44:09	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 13:49:09	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 13:49:14	Antigen:Antibody screen	JRICHARDS	User logout	
2011/02/14 13:49:30	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 13:49:34	Antigen:Antibody screen	JRICHARDS	User logout	
2011/02/14 13:50:59	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 13:54:47	Antigen:Antibody screen	JRICHARDS	User login	
2011/02/14 14:18:46	Antigen:Antibody screen	JRICHARDS	User login	

Figure 4-18: *Audit Trail*

NOTE: *Events shown in the Audit Trail are those associated with the user account that is currently logged in and active only.*

Events in the Audit Trail can be sorted by clicking on any of the column headers:

Date/Time	Project	Machine	Action	Description
2011/04/24 12:07:24	Antigen:Antibody screen	JRICHARDS	User login	
2011/04/13 22:33:48	Antigen:Antibody screen	JRICHARDS	User logout	
2011/04/13 21:52:57	Antigen:Antibody screen	JRICHARDS	User login	
2011/04/13 21:14:37		JRICHARDS	User logout	
2011/04/13 21:12:48		JRICHARDS	User login	
2011/04/05 13:54:51	Antigen:Antibody screen	JRICHARDS	User logout	
2011/04/05 10:16:25	Antigen:Antibody screen	JRICHARDS	User login	
2011/04/05 10:16:17	Antigen:Antibody screen	JRICHARDS	User logout	
2011/04/05 10:15:54	Antigen:Antibody screen	JRICHARDS	User login	
2011/04/05 10:15:45	Antigen:Antibody screen	JRICHARDS	User logout	
2011/04/05 10:13:41	Antigen:Antibody screen	JRICHARDS	User login	
2011/04/05 10:13:32	Antigen:Antibody screen	JRICHARDS	User logout	
2011/04/05 10:02:13	Antigen:Antibody screen	JRICHARDS	User login	
2011/04/04 12:59:34		JRICHARDS	User logout	

Figure 4-19: *Audit Trail Events Sorted by Date/Time*

By default, the events initially displayed in the Audit Trail will be those associated with the project selected at login and the machine (computer) currently being used. To view events for a specific project or computer, click on the **Project** or **Machine** drop-down list and select an entry:

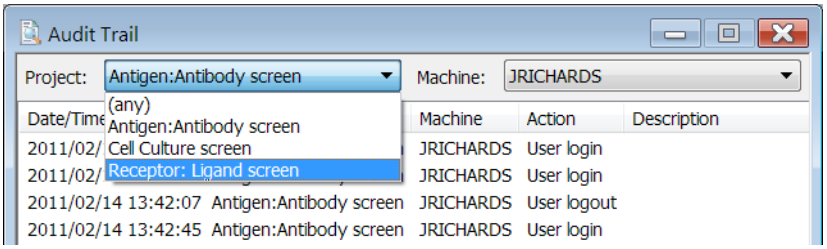


Figure 4-20: *Selecting a Project in the Audit Trail*

NOTE: *Selections can be made in either one or both of the **Project** or **Machine** drop-down lists.*

The list with then only display events for the entries selected:

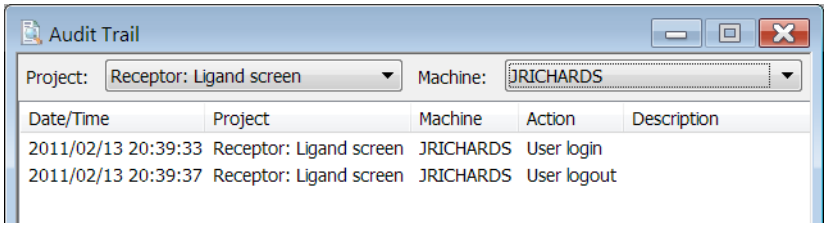


Figure 4-21: *Project-Based Audit Trail Events*

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

- **(any)**—Displays all project and/or machine events for the user account
- **(none)**—Displays all project or machine events not associated with a specific project (**Project** list only)

Changing Projects During a User Session

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

To change projects during a user session:

1. Click **Security** > **Change Projects**.

   A list of projects assigned to your user account will be shown with the active project highlighted:

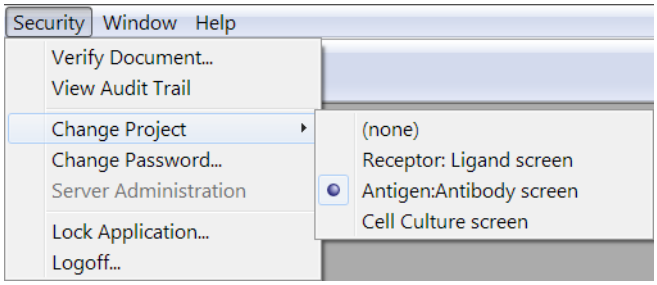


Figure 4-22: *Changing Projects*

2. Select the desired project from the list.

   The selected project will now become the active project for the user session.

Changing the User Password

To change the user password:

1. Initiate a new user session with your existing password.
2. When the software launches, click **Security** > **Change Password**=.

   The **Change Password** dialog box will display:

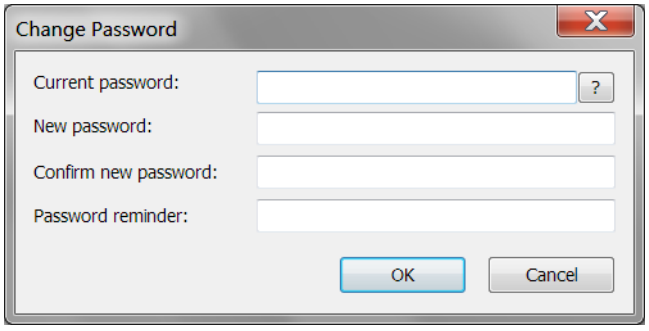


Figure 4-23: *Change Password Dialog Box*

3. Enter the **Current password** for your user account. Click **?** for a password reminder.
4. Enter the **New Password** and **Password reminder** (optional).
5. Click **OK** to save changes and exit.

Locking the Application

The Data Acquisition or Data Analysis software can be locked during a user session to prevent another user from interrupting a session or experiment. When the application is locked, any experiments started will continue to run.

To lock the application:

1. Click **Security** > **Lock Application**.

   The software will be placed in locked mode immediately and the **Application Locked** dialog box will display:

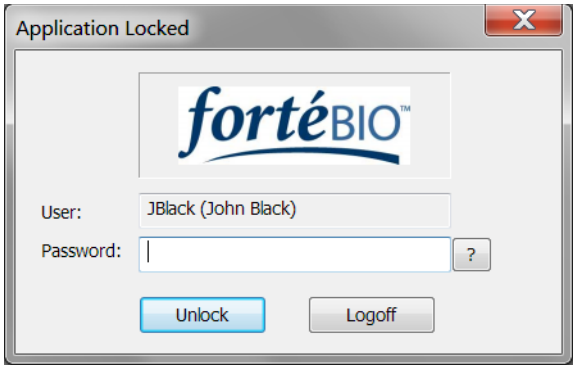


Figure 4-24: *Application Locked Dialog Box*

2. The application will remain locked until it is unlocked or the active user logs off.
   - ***Unlock***—To resume the user session, enter your password and click **Unlock**.
   - ***Log off***—To discontinue the user session, click **Logoff**.

Ending a User Session

To end a user session:

1. Click **Security** > **Logoff**.
2. In the displayed dialog box, click **OK**.

CHAPTER 5:

Quantitation Experiments: Octet RED96, QK^e and QK

INTRODUCTION

A quantitation experiment enables you to determine analyte concentration within a sample using a reference set of standards. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 5-1) to set up and analyze a quantitation experiment.

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

Software	*Step*	*See*
Data Acquisition 	1. Select a quantitation experiment in the **Experiment wizard** or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 69
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 70
	3. Confirm or edit the assay settings.	"Managing Assay Parameter Settings" on page 91
	4. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 96
	5. Run the experiment.	"Running a Quantitation Experiment" on page 116
Data Analysis 	6. Analyze the binding data. 7. Generate a report.	*Octet System Data Analysis Software User Guide*

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

STARTING A QUANTITATION EXPERIMENT

NOTE: *Before starting an experiment, check the plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the* ***Options*** *dialog box when the software is relaunched.*

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

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see, "Managing Experiment Method Files" on page 128.
- On the menu bar, click **Experiment** > **Templates** > **Quantitation**.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Starting an Experiment Using the Experiment Wizard

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

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

Table 5-2: *Quantitation Experiment Selection*

Quantitation Experiment	*Description*
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regeneration	A standard quantitation assay that enables regeneration of biosensors.

Table 5-2: *Quantitation Experiment Selection*

Quantitation Experiment	*Description*
Advanced Quantitation	A standard two- or three-step quantitation assay that enables signal amplification for higher detection sensitivity.

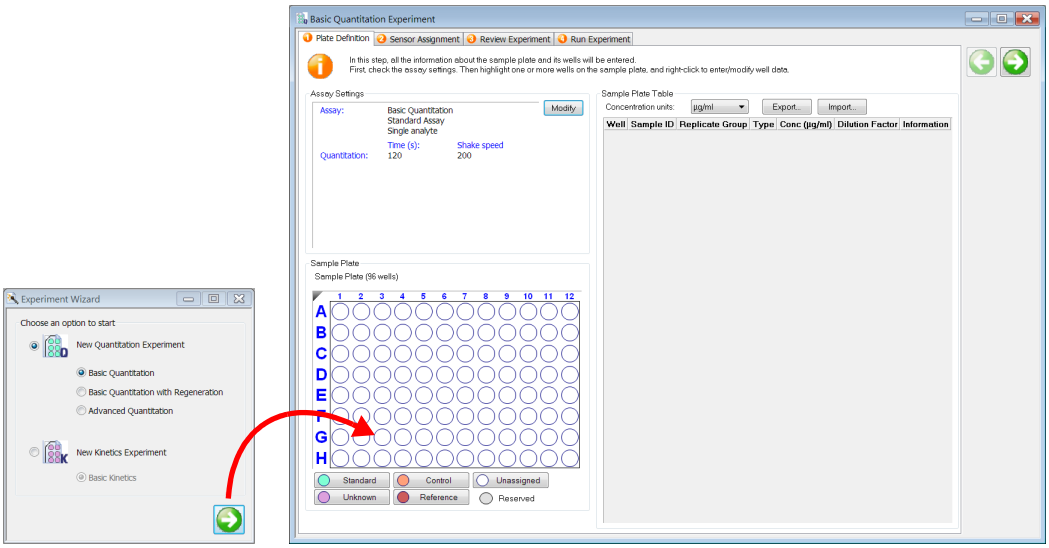


Figure 5-1: *Selecting an Experiment Type in the Experiment Wizard (for Octet RED96)*

4. Click the  arrow.

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

DEFINING THE SAMPLE PLATE

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

Table 5-3: *Defining a Sample Plate*

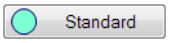
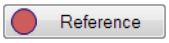
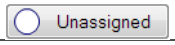
Step	*See Page*
1. Designate the samples.	70
2. Annotate the samples (optional).	82
3. Save the sample plate definition (optional).	88

Designating Samples

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

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

Table 5-4: *Types of Sample Wells*

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

Reserved Wells

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

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

Table 5-5: *Reserved Well Requirements*

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

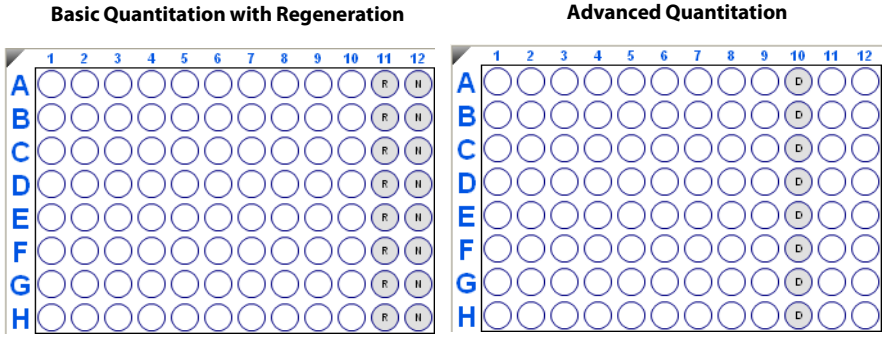


Figure 5-2: *Default Locations for Reserved Wells in a 96-Well Sample Plate Map*

Selecting Wells in the Sample Plate Map

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

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

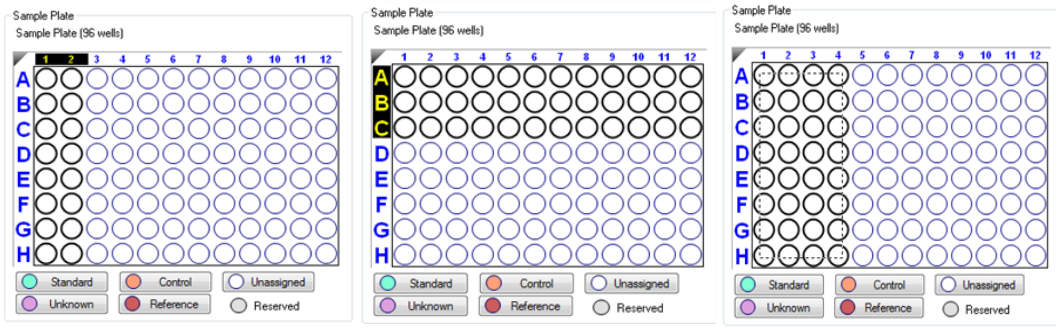


Figure 5-3: *Selecting Wells in the Sample Plate Map*

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

Designating Standards

To designate standards:

1. In the **Sample Plate Map**, select the wells to define as standards.
2. Click the **Standard** button below the **Sample Plate Map** (see Figure 5-3), or right-click and select **Standard**.

   The standards are marked in the plate map and the **Sample Plate Table** is updated.
3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

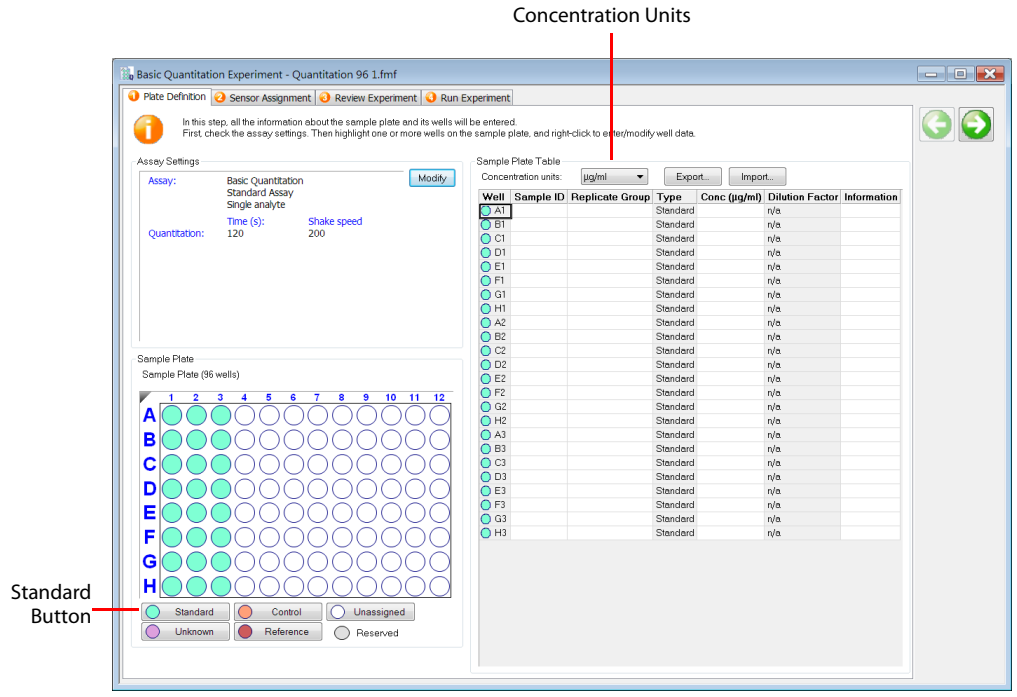


Figure 5-4: *Plate Definition Window—Designating Standards*

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

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

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

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

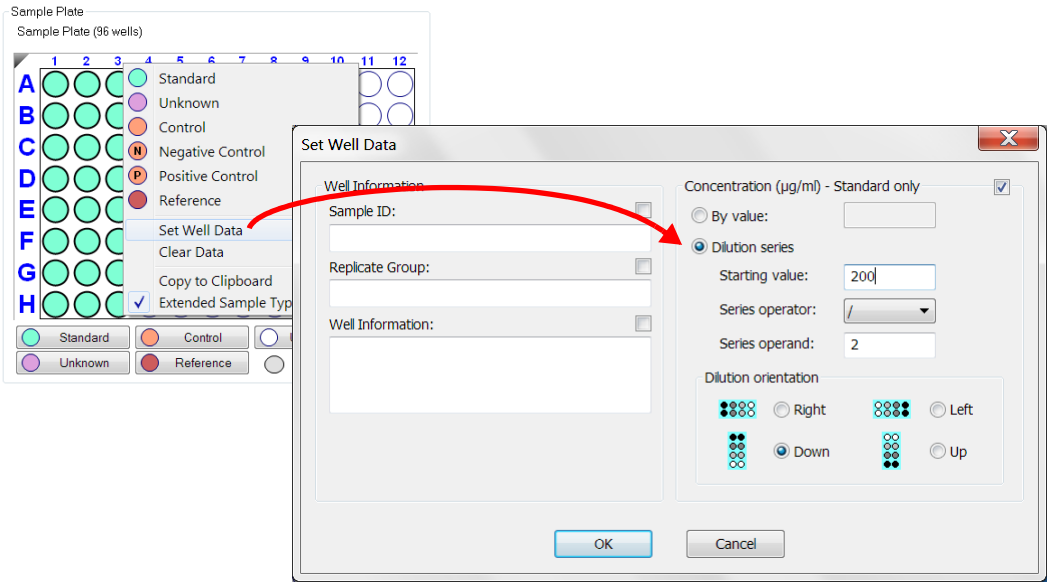


Figure 5-5: Sample Plate Map—Setting a Dilution Series

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

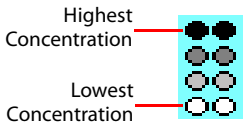


Figure 5-6: Concentration Representation in Dilution Series

4. Click **OK**.

   The **Sample Plate Table** will display the standard concentrations entered.

Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

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

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

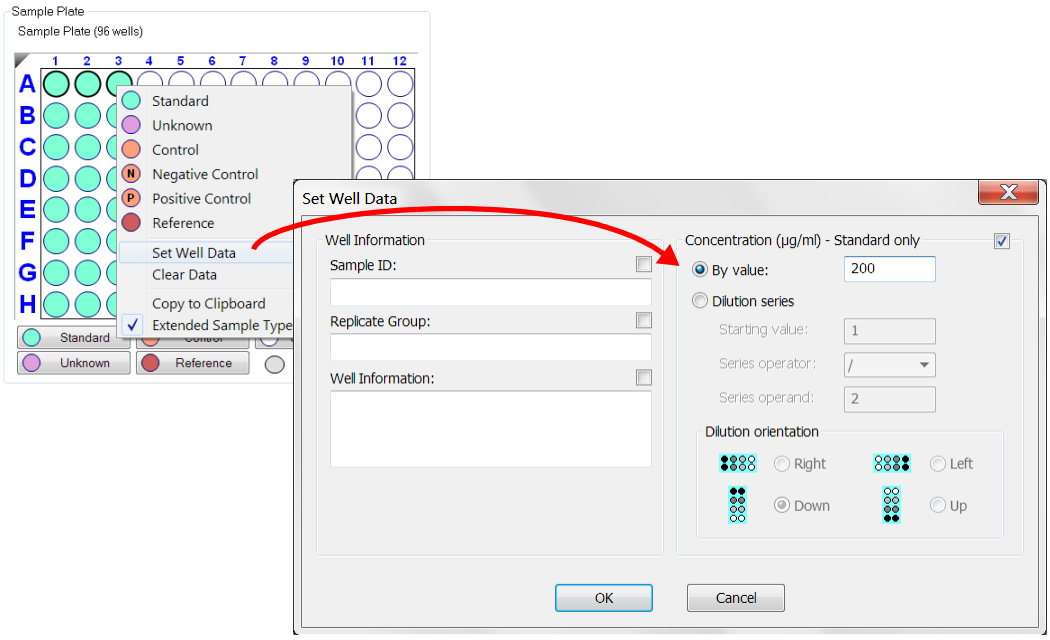


Figure 5-7: *Sample Plate Map—Assigning a Standard Concentration*

2. Select the **By value** option and enter the starting concentration value.
3. Click **OK**. The **Sample Plate Table** will display the standard concentrations entered.

Editing an Individual Standard Concentration

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

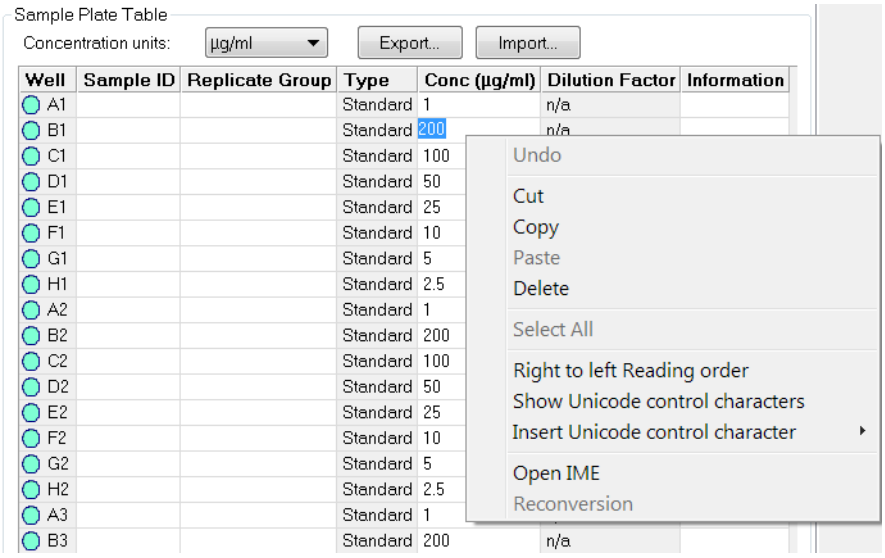


Figure 5-8: *Sample Plate Table—Shortcut Menu of Edit Commands*

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

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

Designating Unknowns

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

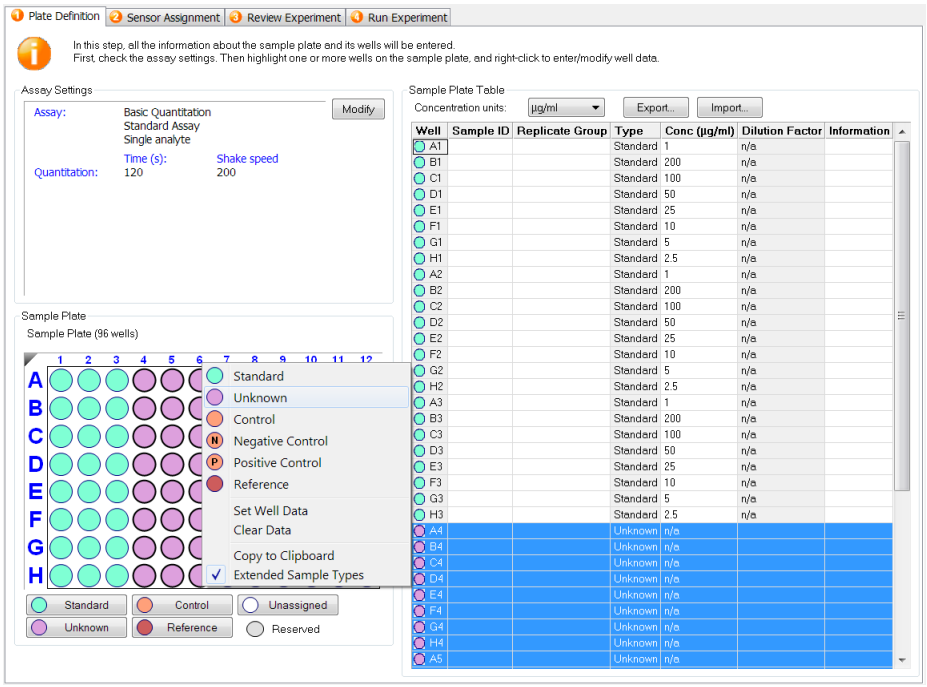


Figure 5-9: *Plate Definition Window—Designate Unknown Wells*

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

Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

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

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

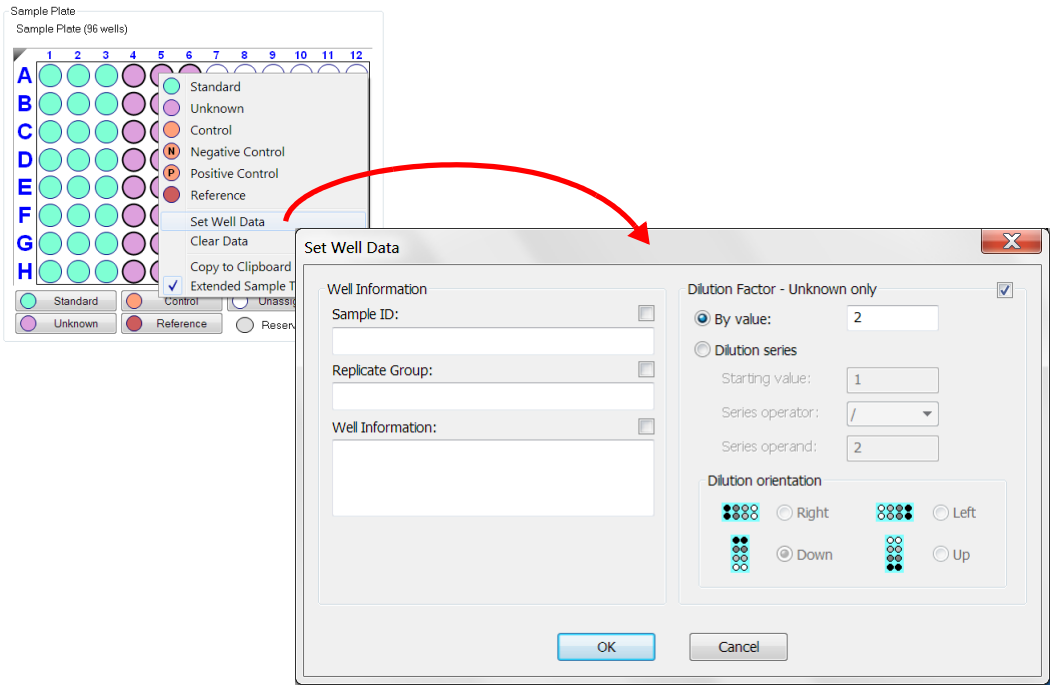


Figure 5-10: *Sample Plate Map—Setting a Dilution Factor or a Serial Dilution*

To assign a dilution factor to selected wells:

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

To assign a serial dilution to selected wells:

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

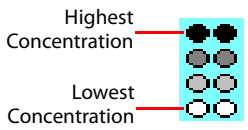


Figure 5-11: *Concentration Representation in Dilution Series*

4. Click **OK**.

   The **Sample Plate Table** will display the dilution factors entered.

Editing a Dilution Factor in the Sample Plate Table

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

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

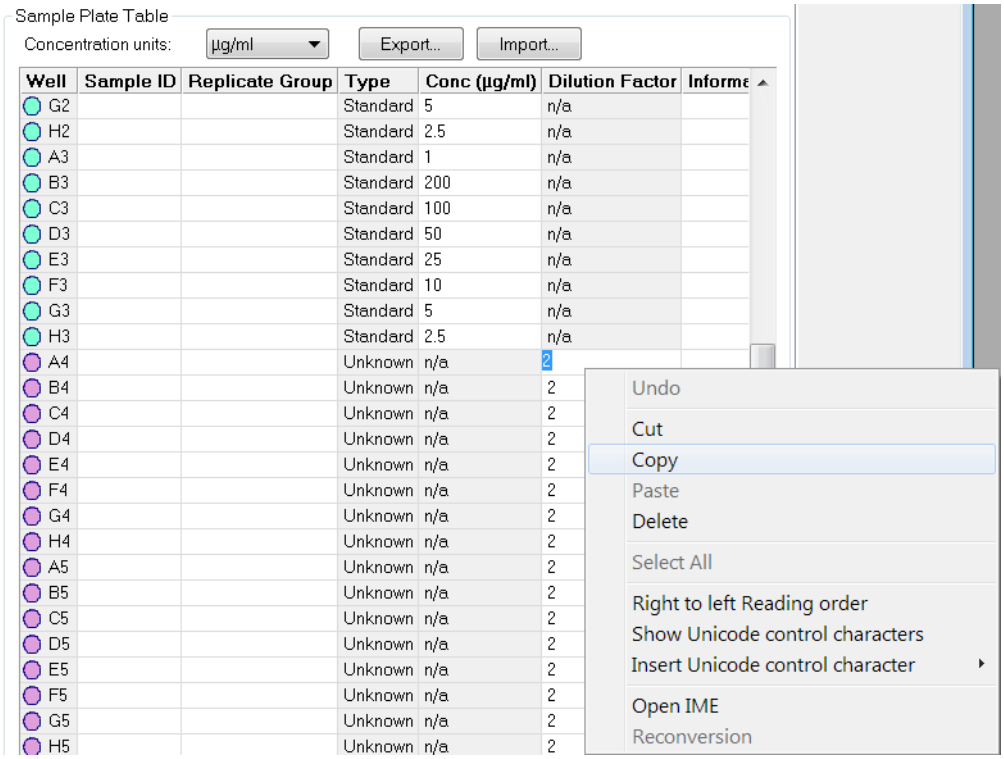


Figure 5-12: *Sample Plate Table—Shortcut Menu of Edit Commands*

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

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

Designating Controls or Reference Wells

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

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

The wells are marked in the **Sample Plate Map** and the **Sample Plate Table** is updated (see Figure 5-12).

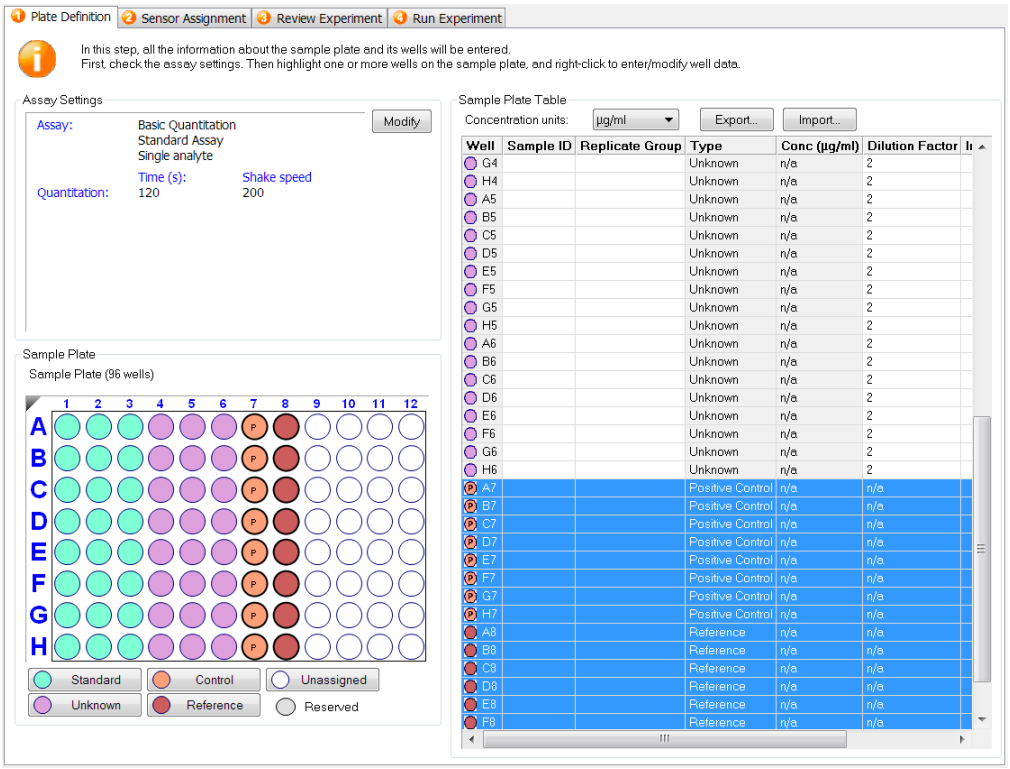


Figure 5-13: *Designate Controls or Reference Wells*

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

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

Annotating Samples

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

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

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

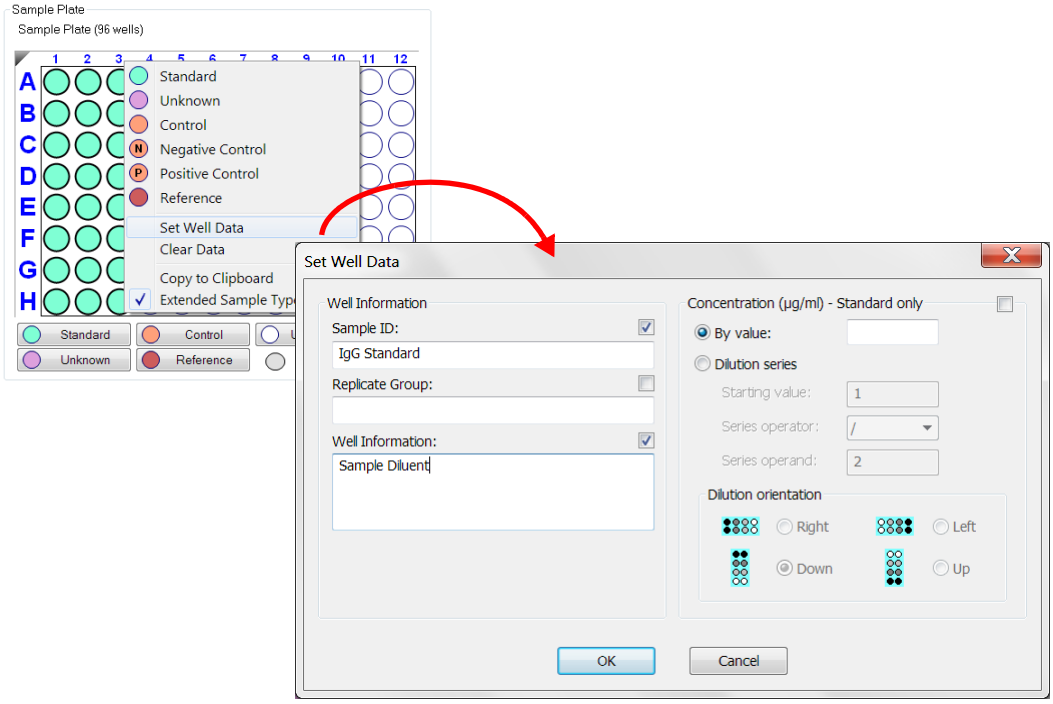


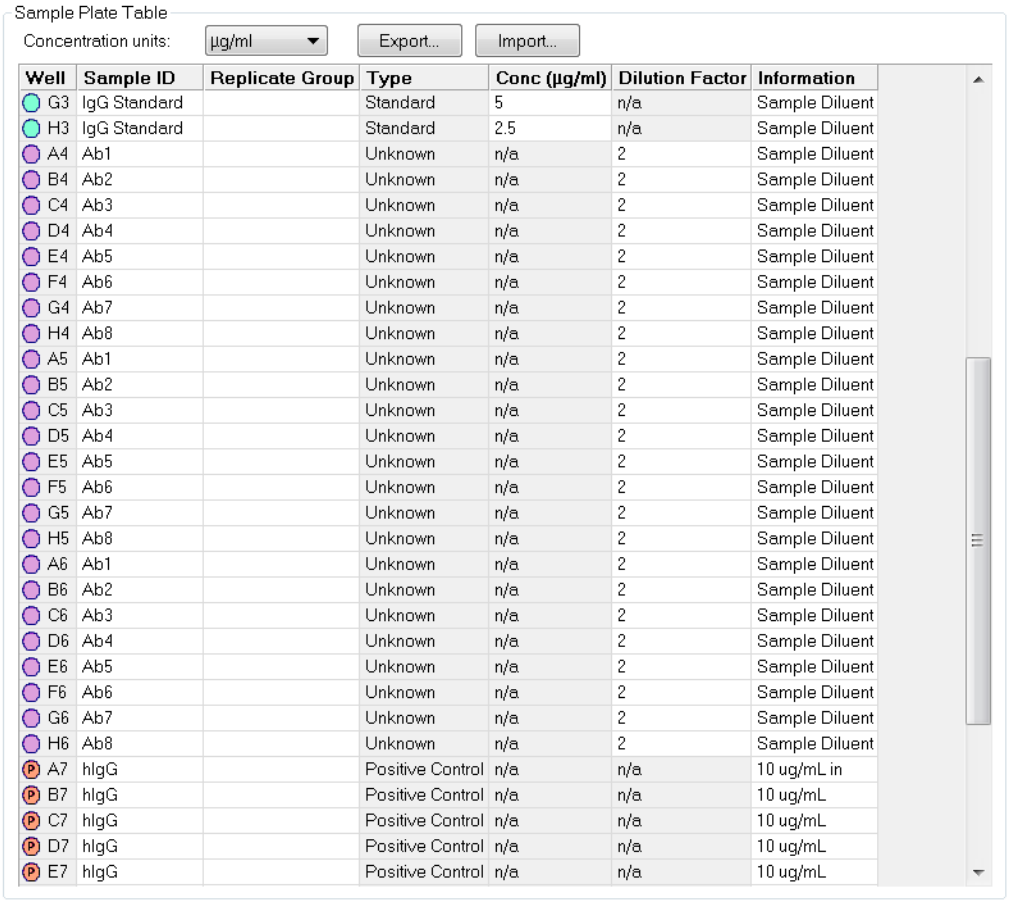
Figure 5-14: *Adding Sample Annotations from the Sample Plate Map*

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

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

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



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
G3	IgG Standard		Standard	5	n/a	Sample Diluent
H3	IgG Standard		Standard	2.5	n/a	Sample Diluent
A4	Ab1		Unknown	n/a	2	Sample Diluent
B4	Ab2		Unknown	n/a	2	Sample Diluent
C4	Ab3		Unknown	n/a	2	Sample Diluent
D4	Ab4		Unknown	n/a	2	Sample Diluent
E4	Ab5		Unknown	n/a	2	Sample Diluent
F4	Ab6		Unknown	n/a	2	Sample Diluent
G4	Ab7		Unknown	n/a	2	Sample Diluent
H4	Ab8		Unknown	n/a	2	Sample Diluent
A5	Ab1		Unknown	n/a	2	Sample Diluent
B5	Ab2		Unknown	n/a	2	Sample Diluent
C5	Ab3		Unknown	n/a	2	Sample Diluent
D5	Ab4		Unknown	n/a	2	Sample Diluent
E5	Ab5		Unknown	n/a	2	Sample Diluent
F5	Ab6		Unknown	n/a	2	Sample Diluent
G5	Ab7		Unknown	n/a	2	Sample Diluent
H5	Ab8		Unknown	n/a	2	Sample Diluent
A6	Ab1		Unknown	n/a	2	Sample Diluent
B6	Ab2		Unknown	n/a	2	Sample Diluent
C6	Ab3		Unknown	n/a	2	Sample Diluent
D6	Ab4		Unknown	n/a	2	Sample Diluent
E6	Ab5		Unknown	n/a	2	Sample Diluent
F6	Ab6		Unknown	n/a	2	Sample Diluent
G6	Ab7		Unknown	n/a	2	Sample Diluent
H6	Ab8		Unknown	n/a	2	Sample Diluent
A7	hIgG		Positive Control	n/a	n/a	10 ug/mL in
B7	hIgG		Positive Control	n/a	n/a	10 ug/mL
C7	hIgG		Positive Control	n/a	n/a	10 ug/mL
D7	hIgG		Positive Control	n/a	n/a	10 ug/mL
E7	hIgG		Positive Control	n/a	n/a	10 ug/mL

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

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

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

Replicate Groups

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

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

Figure 5-16: *Replicate Group Result Table Statistics*

NOTE: *Replicate Group information can also be entered in the Results table in the Octet System Data Analysis software.*

Assigning Replicate Groups in the Sample Plate Map

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

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

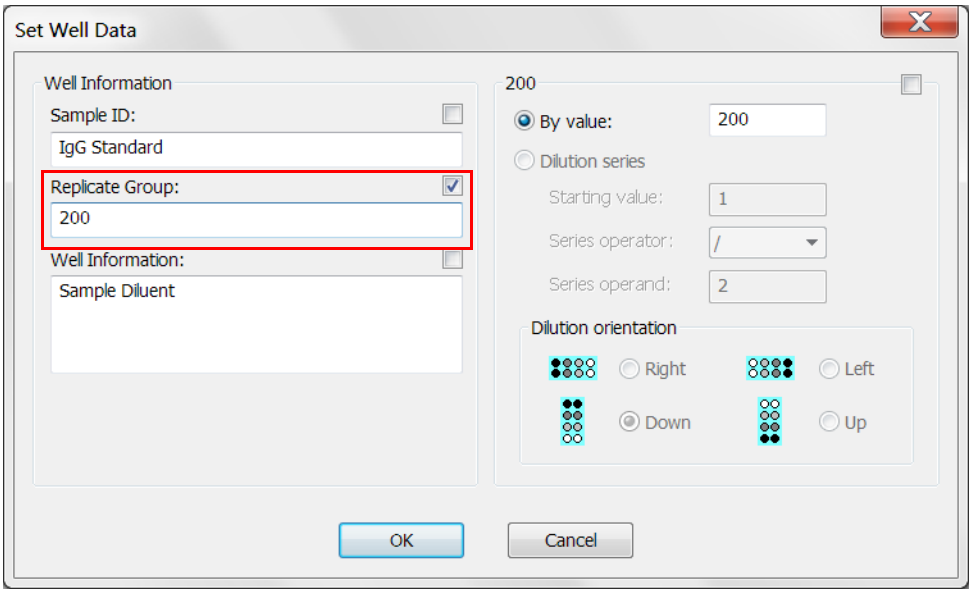


Figure 5-17: *Add Replicate Group from the Sample Plate Map*

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

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

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

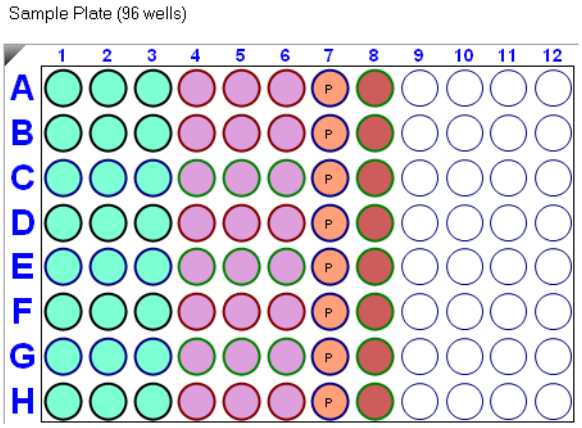


Figure 5-18: *Replicate Groups Displayed in Sample Plate Map*

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

Sample Plate Table

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

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG Standard	2.5	Standard	2.5	n/a
H1	IgG Standard	1	Standard	1	n/a
A2	IgG Standard	200	Standard	200	n/a
B2	IgG Standard	100	Standard	100	n/a
C2	IgG Standard	50	Standard	50	n/a
D2	IgG Standard	25	Standard	25	n/a
E2	IgG Standard	10	Standard	10	n/a
F2	IgG Standard	5	Standard	5	n/a
G2	IgG Standard	2.5	Standard	2.5	n/a
H2	IgG Standard	1	Standard	1	n/a
A3	IgG Standard	200	Standard	200	n/a
B3	IgG Standard	100	Standard	100	n/a
C3	IgG Standard	50	Standard	50	n/a
D3	IgG Standard	25	Standard	25	n/a
E3	IgG Standard	10	Standard	10	n/a
F3	IgG Standard	5	Standard	5	n/a
G3	IgG Standard	2.5	Standard	2.5	n/a
H3	IgG Standard	1	Standard	1	n/a
A4	Ab1	Ab1	Unknown	n/a	2
B4	Ab2	Ab2	Unknown	n/a	2
C4	Ab3	Ab3	Unknown	n/a	2
D4	Ab4	Ab4	Unknown	n/a	2
E4	Ab5	Ab5	Unknown	n/a	2
F4	Ab6	Ab6	Unknown	n/a	2
G4	Ab7	Ab7	Unknown	n/a	2

Figure 5-19: *Replicate Groups in Sample Plate Table*

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

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

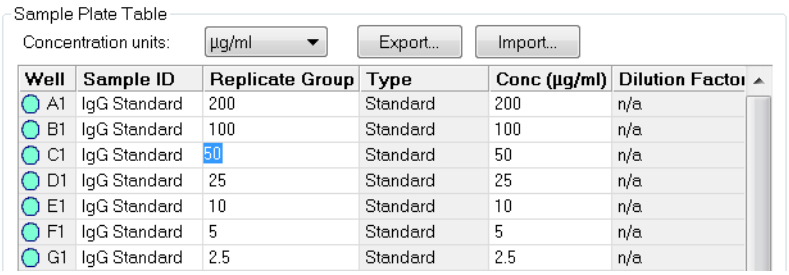


Figure 5-20: *Add Replicate Group from the Sample Plate Table*

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

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

---

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

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

MANAGING SAMPLE PLATE DEFINITIONS

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

Exporting a Plate Definition

To export a plate definition:

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

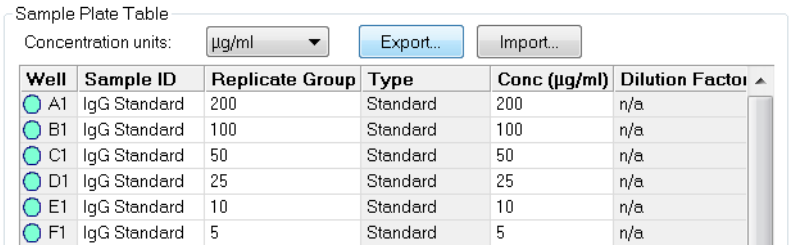


Figure 5-21: *Export Button in Sample Plate Table*

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

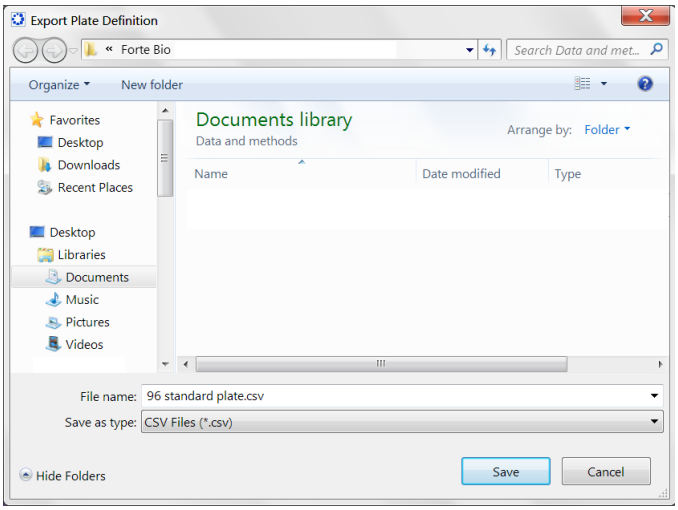


Figure 5-22: *Export Plate Definition Window*

Importing a Plate Definition

To import a plate definition:

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

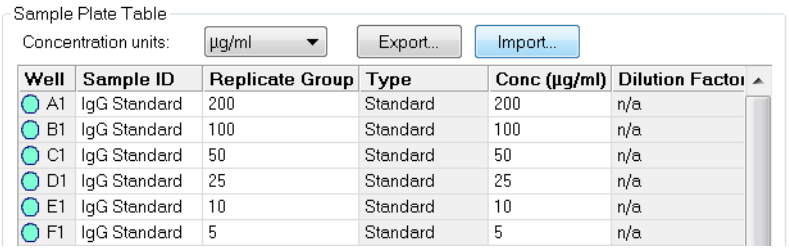


Figure 5-23: *Import Button in Sample Plate Table*

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

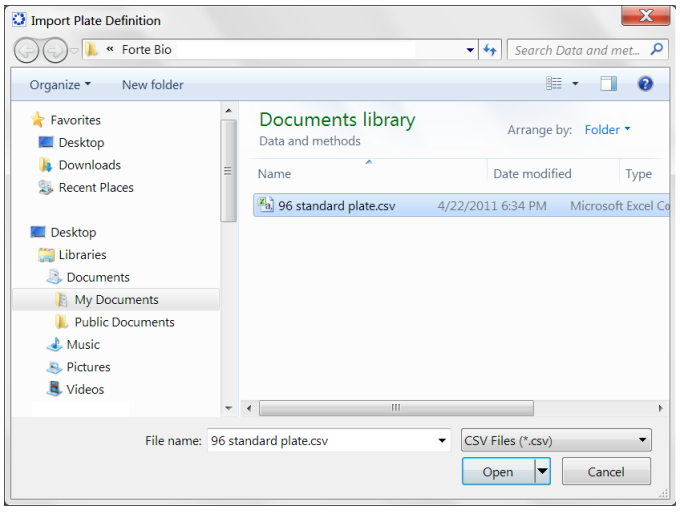


Figure 5-24: *Import Plate Definition Window*

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

	A	B	C	D	E	F	G
1	PlateWells	96					
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information
3	A1	IgG Standard	200	Standard	200		Sample Diluent
4	B1	IgG Standard	100	Standard	100		Sample Diluent
5	C1	IgG Standard	50	Standard	50		Sample Diluent
6	D1	IgG Standard	25	Standard	25		Sample Diluent
7	E1	IgG Standard	10	Standard	10		Sample Diluent
8	F1	IgG Standard	5	Standard	5		Sample Diluent
9	G1	IgG Standard	2.5	Standard	2.5		Sample Diluent
10	H1	IgG Standard	1	Standard	1		Sample Diluent
11	A2	IgG Standard	200	Standard	200		Sample Diluent

96 standard plate

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

MANAGING ASSAY PARAMETER SETTINGS

Modifying Assay Parameter Settings

You can modify the assay parameter settings during sample plate definition. However, the changes are only applied to the current experiment. To save modified parameter settings, you must define a new assay. For details on creating a new assay, see "Custom Quantitation Assays" on page 129.

Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box will display (Figure 5-26). The settings available are experiment-dependent.

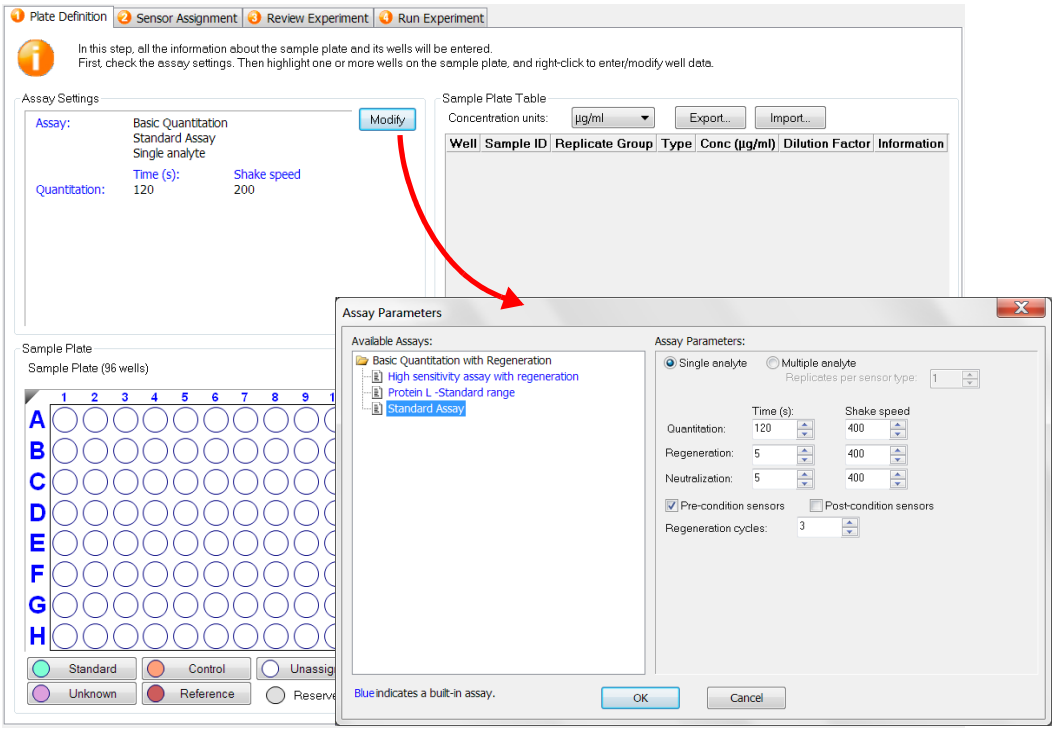


Figure 5-26: *Modifying Assay Parameters*

Basic Quantitation Assay Parameters

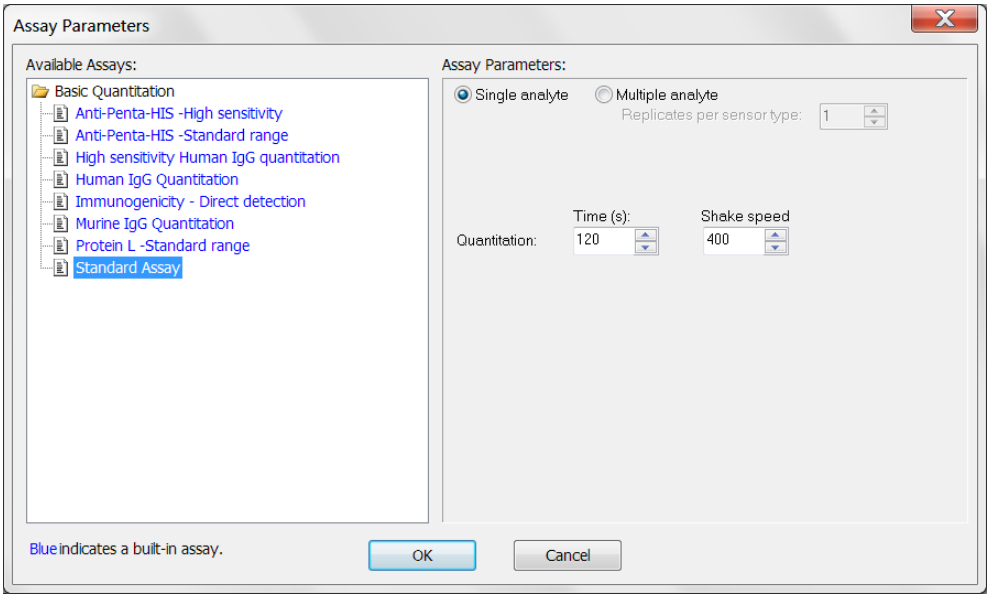


Figure 5-27: *Assay Parameters—Basic Quantitation Assay*

Table 5-6: *Basic Quantitation Assay Parameters*

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

Basic Quantitation with Regeneration Assay Parameters

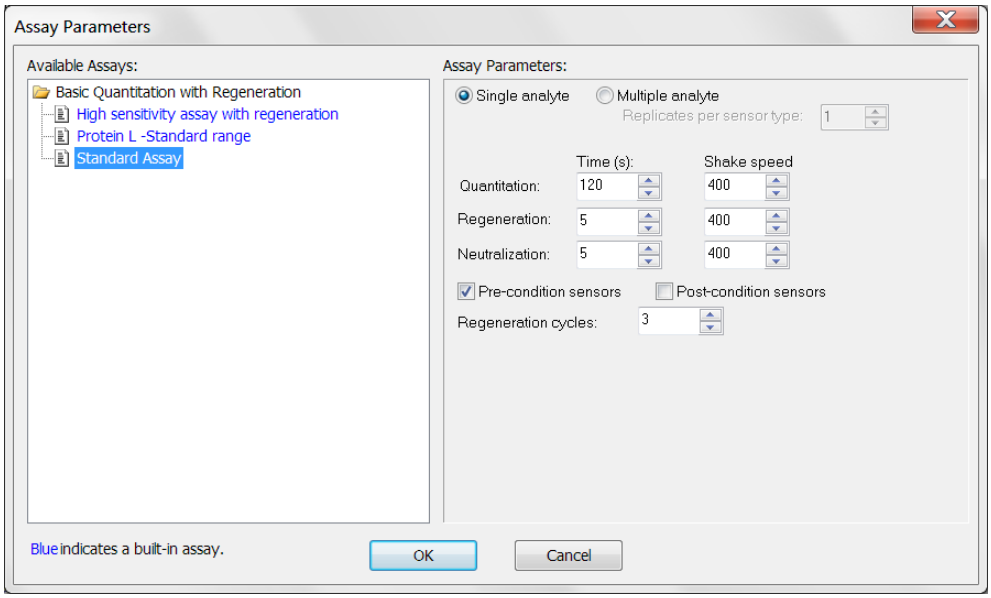


Figure 5-28: *Assay Parameters—Basic Quantitation with Regeneration*

Table 5-7: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	*Description*
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.

Table 5-7: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	Description
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

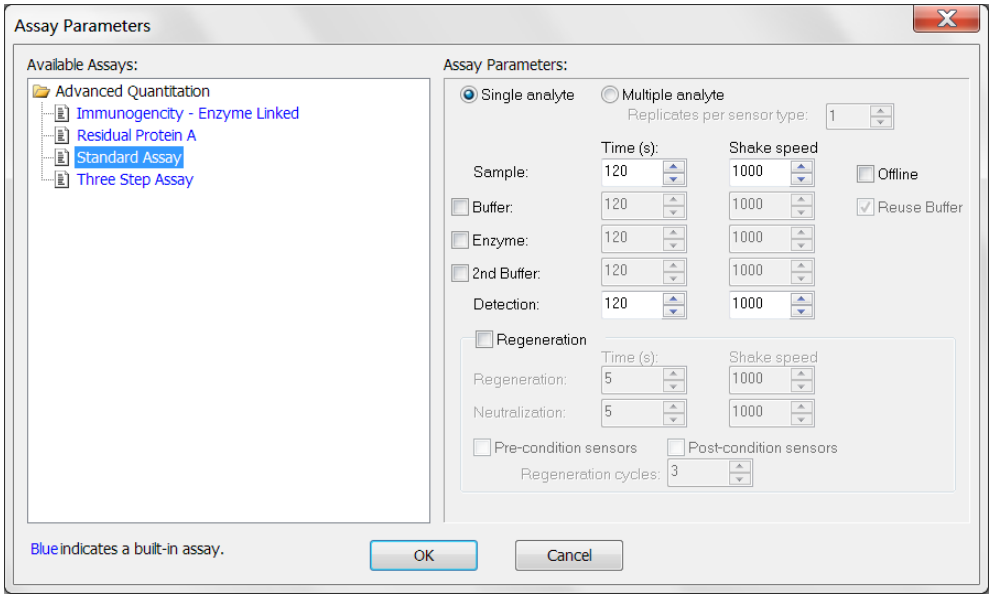


Figure 5-29: *Assay Parameters—Advanced Quantitation*

Table 5-8: *Advanced Quantitation Assay Parameters*

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.

Table 5-8: *Advanced Quantitation Assay Parameters (Continued)*

Parameter	*Description*
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample platform orbital shaking speed (rotations per minute).
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample platform orbital shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample platform orbital shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay. ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.

Table 5-8: *Advanced Quantitation Assay Parameters (Continued)*

Parameter	*Description*
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse. ***NOTE:*** *In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.*

ASSIGNING BIOSENSORS TO SAMPLES

After the sample plate is defined, biosensors must be assigned to the samples.

Biosensor Assignment in Single-Analyte Experiments

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

NOTE: *For single analyte experiments, the* ***Single Analyte*** *option must be selected in the* ***Assay Parameters*** *dialog box. For more information, please see "Managing Assay Parameter Settings" on page 91.*

Click the **Sensor Assignment** tab, or click the arrow to access the Sensor Assignment window (see Figure 5-30).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default.

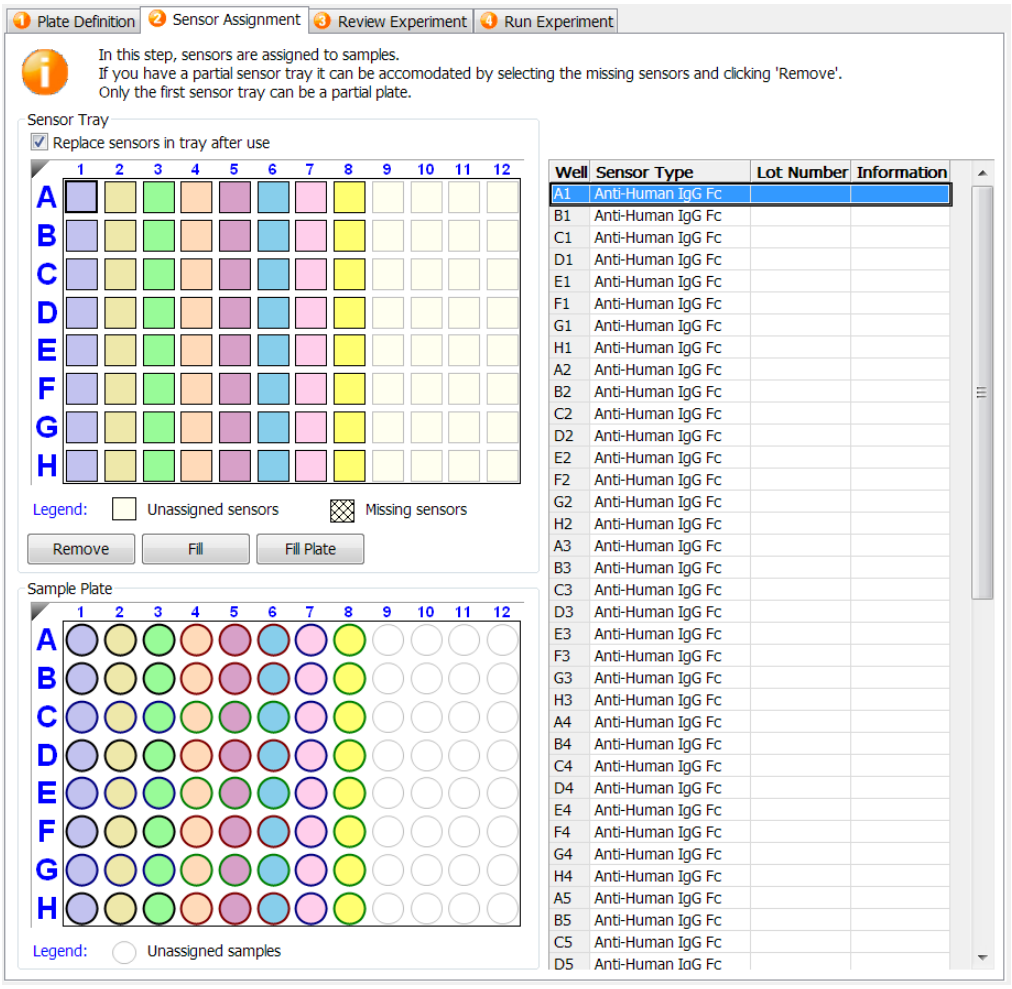


Figure 5-30: *Sensor Assignment Window for Basic Quantitation without Regeneration*

1. Assign biosensors in one of two ways:

   - Select a column(s) in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (see Figure 5-30 left).
   - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 5-30 right).

   All wells in the **Sensor Type** column will automatically populate with the biosensor type selected.

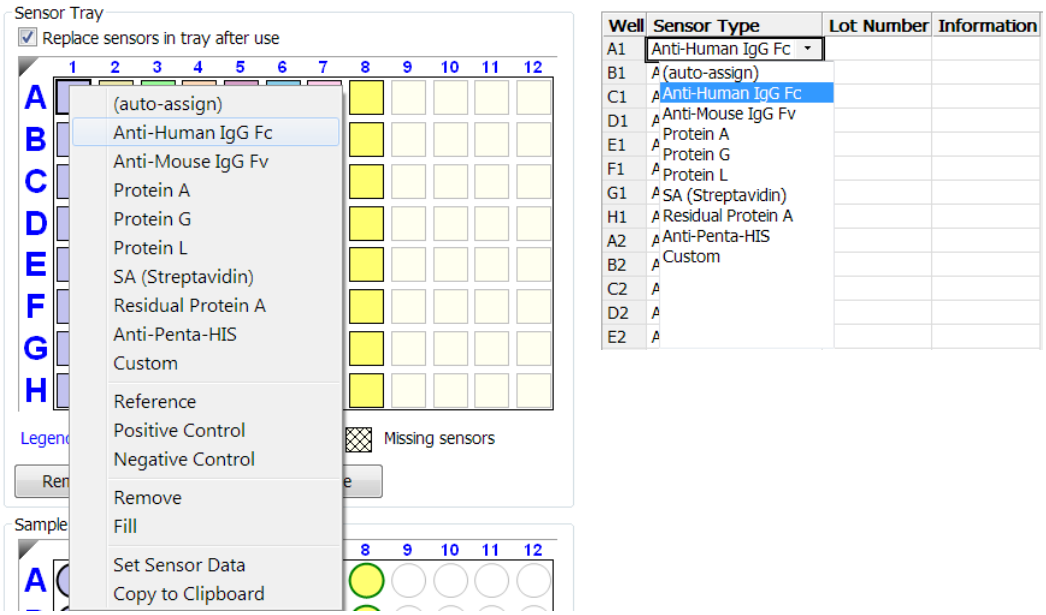


Figure 5-31: *Changing Biosensor Types in the Sensor Tray Map (left) and Sensor Type Column (right)*

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

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

---

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

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

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

5. Optional for the Octet RED96 instrument only: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 5-32).

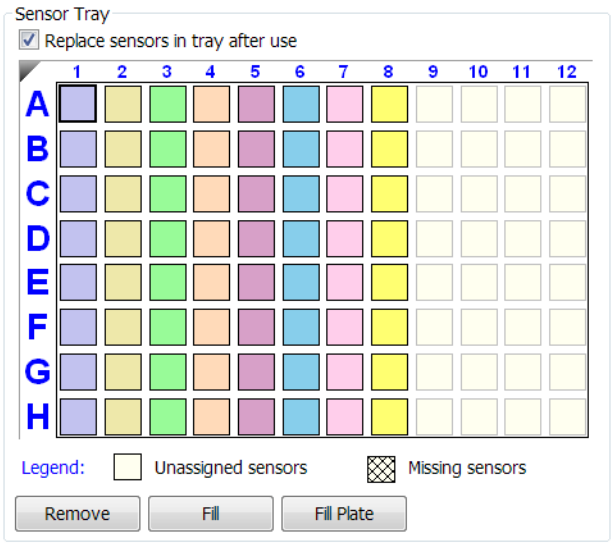


Figure 5-32: *Replace Sensors in Tray After Use Check Box*

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

Biosensor Assignment in Multiple Analyte Experiments

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

NOTE: *For multiple analyte experiments, the* ***Multiple Analyte*** *option must be selected in the* ***Assay Parameters*** *dialog box. For more information, please see "Managing Assay Parameter Settings" on page 91.*

Click the **Sensor Assignment** tab, or click the arrow to access the Sensor Assignment window (see Figure 5-30).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 5-30, **one** replicate had been previously selected with the **Multiple Analyte** assay parameter option.

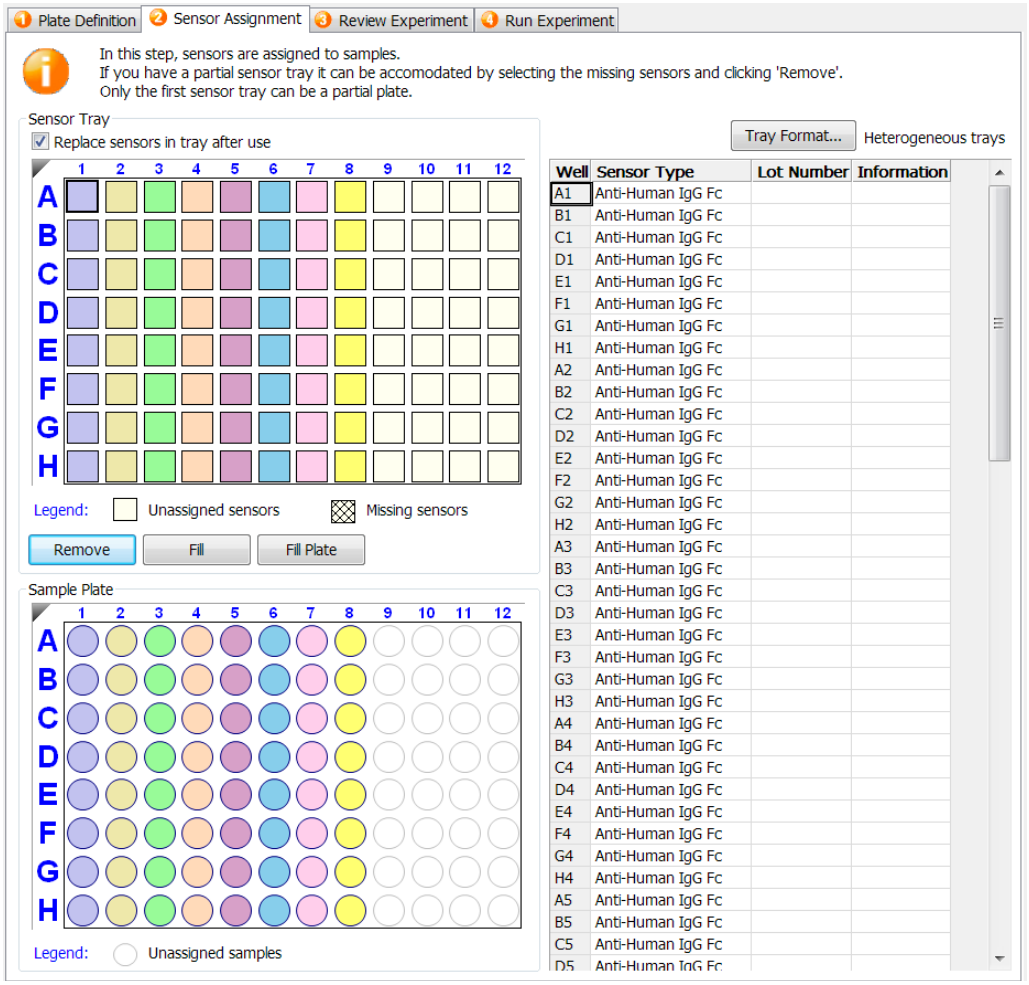


Figure 5-33: *Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option*

There are two ways to assign biosensors:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (see Figure 5-34 left).
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 5-34 right).

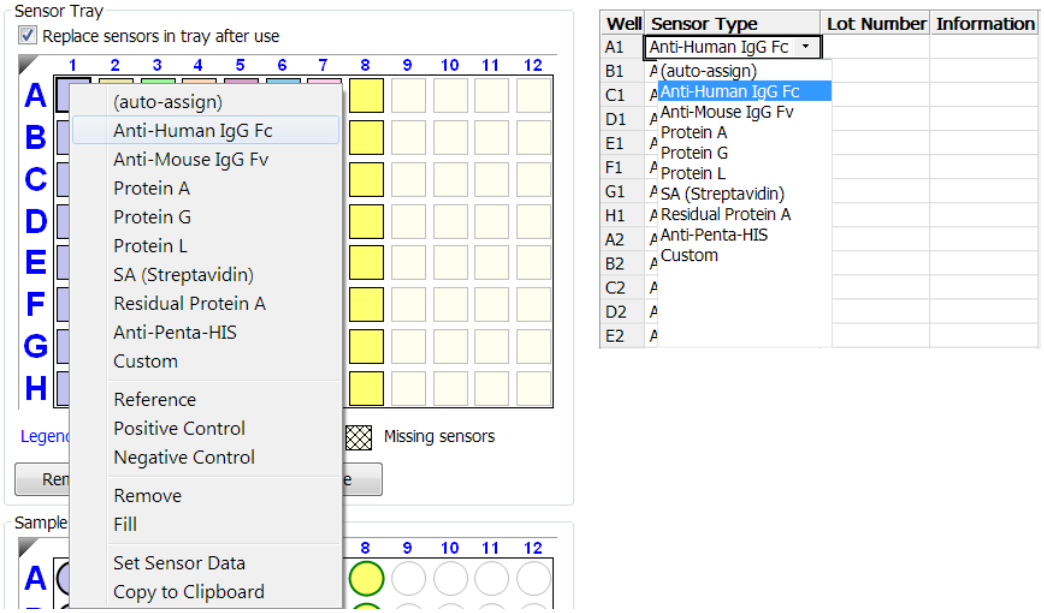


Figure 5-34: *Changing Biosensor Types in the Sensor Tray Map (left) and Sensor Type Column (right)*

Biosensor Assignment Using Heterogeneous Biosensor Trays

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

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

---

1. If Heterogeneous Trays is not displayed next to the **Tray Format** button, click the button.

   The **Tray Format** dialog box displays (see Figure 5-35).

2. Select **Heterogeneous** and click **OK**.

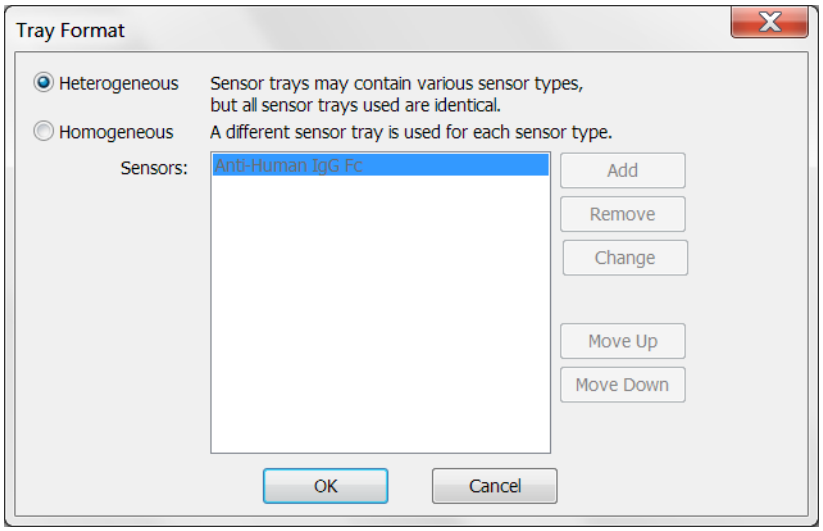


Figure 5-35: *Tray Format Dialog Box*

The Tray 1 **Sensor Tray Map** will be displayed by default.

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to be used (see Figure 5-36).

   The **Sensor Type** column will update accordingly.

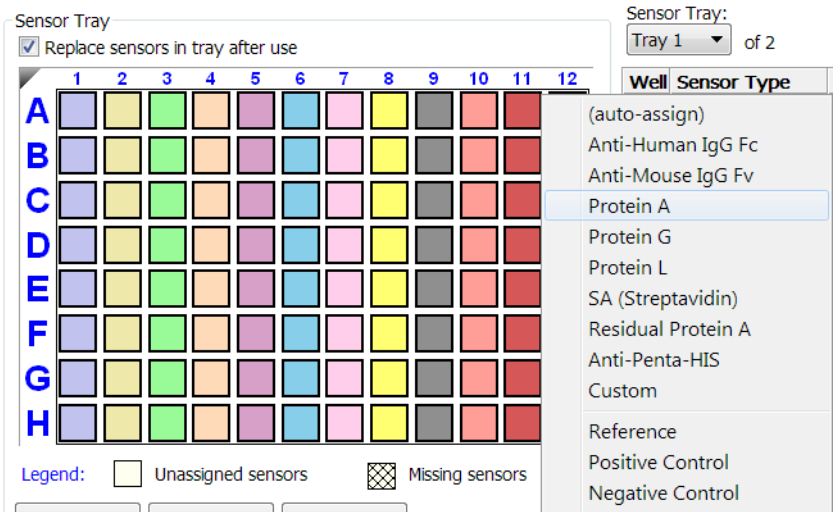


Figure 5-36: *Populating the Sensor Tray Map with First Biosensor Type*

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

   The **Sensor Type** column will update accordingly.

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

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

   In the example shown in Figure 5-38, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays will be needed for the experiment.

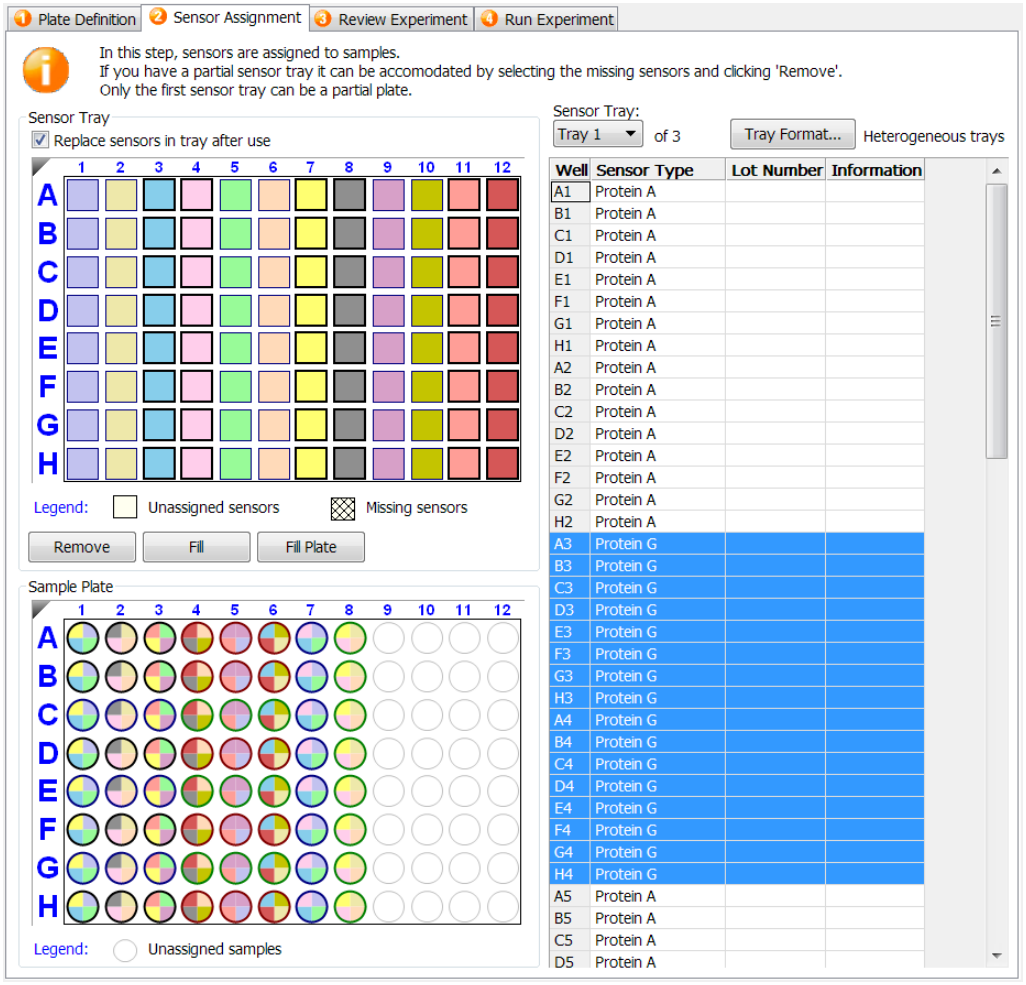


Figure 5-38: *Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types*

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

   The **Sensor Tray Map** and table for the tray selected will be shown and biosensor assignments can be changed as needed (see Figure 5-39).

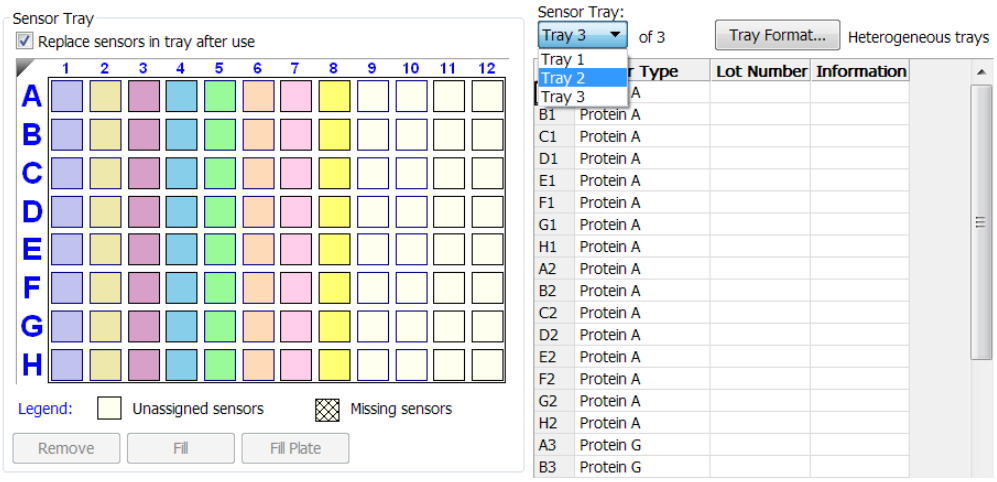


Figure 5-39: *Tray Selection*

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

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

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

---

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

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

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

---

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 5-40).

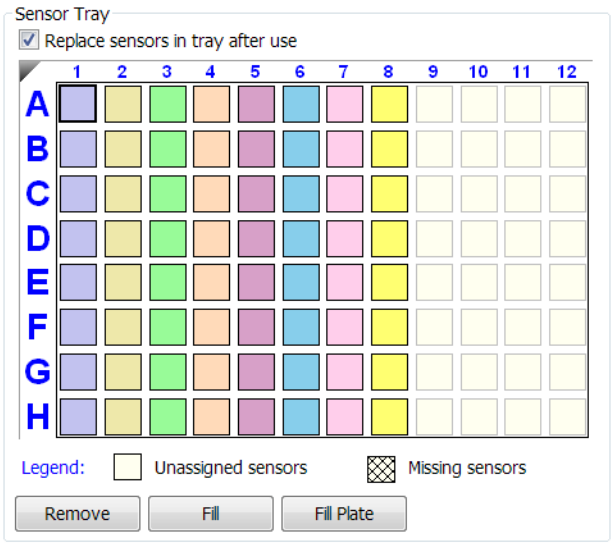


Figure 5-40: *Replace Sensors in Tray After Use Check Box*

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

Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.

NOTE: *Using the* ***Homogeneous*** *option will necessitate switching trays during the experiment.*

1. Click **Tray Format**.

   The **Tray Format** dialog box displays (see Figure 5-41) and the **Sensors** box will be populated with the default biosensor type.

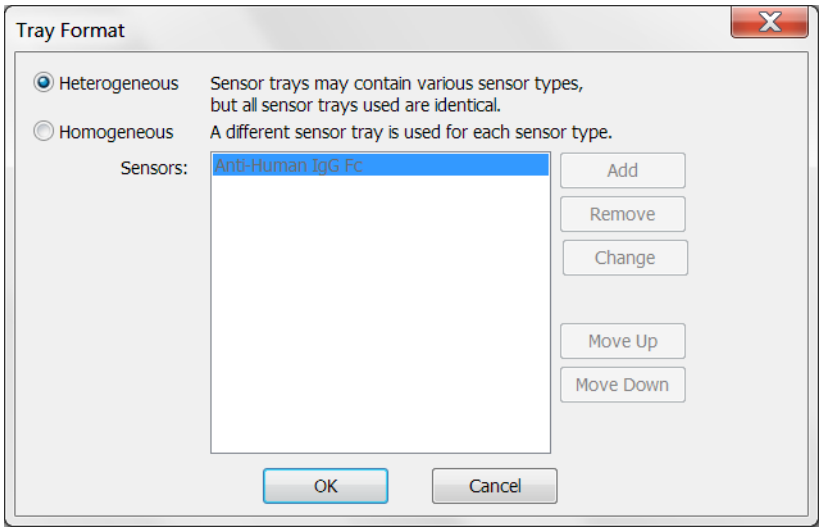


Figure 5-41: *Tray Format Dialog Box*

2. Select **Homogeneous**. Click **Add** to select the first biosensor type (see Figure 5-42).

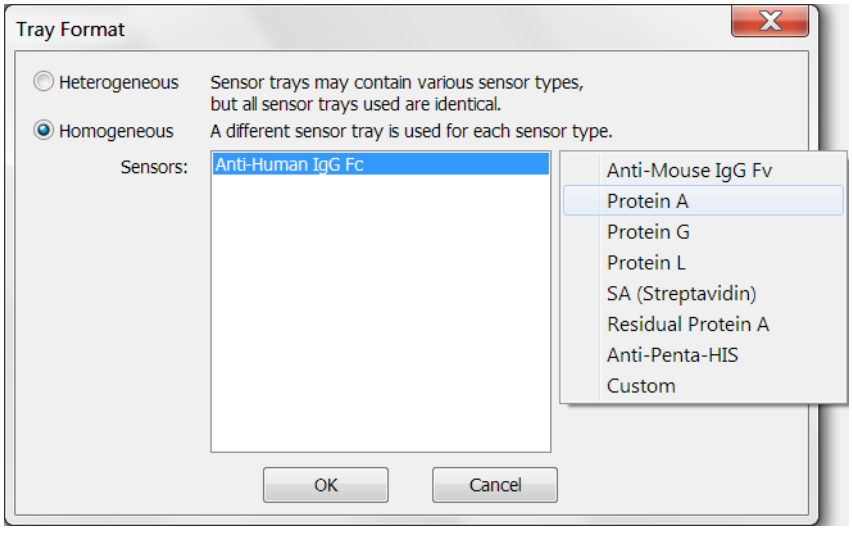


Figure 5-42: *Selecting a Biosensor Type in the Tray Format Dialog Box*

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

The order of biosensor types listed in the **Sensor** box will be used as the default tray assignment (see Figure 5-43).

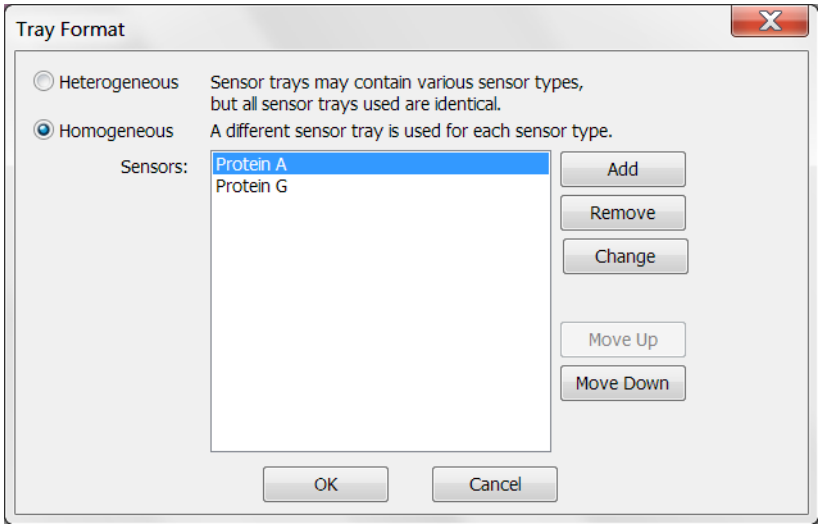


Figure 5-43: *Biosensor Types List Order in Sensor Box*

5. Click **OK**.

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

   In the example shown in Figure 5-44, Protein A and Protein G biosensor types will be used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) will be needed for the experiment. The Tray 1 **Sensor Tray Map** will be displayed by default.

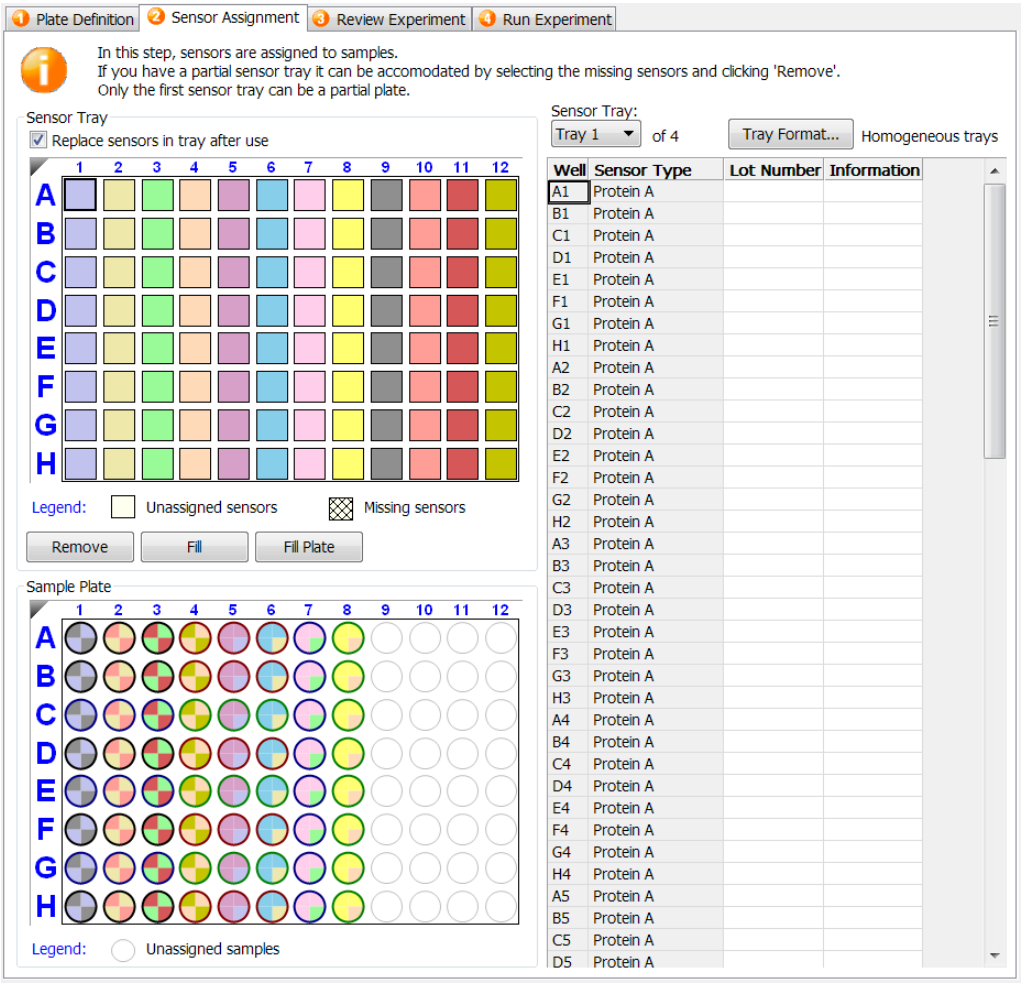


Figure 5-44: *Biosensor Assignment using Homogeneous Trays and Two Biosensor Types*

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

   The **Sensor Tray Map** and table for the tray selected will be shown (see Figure 5-39).

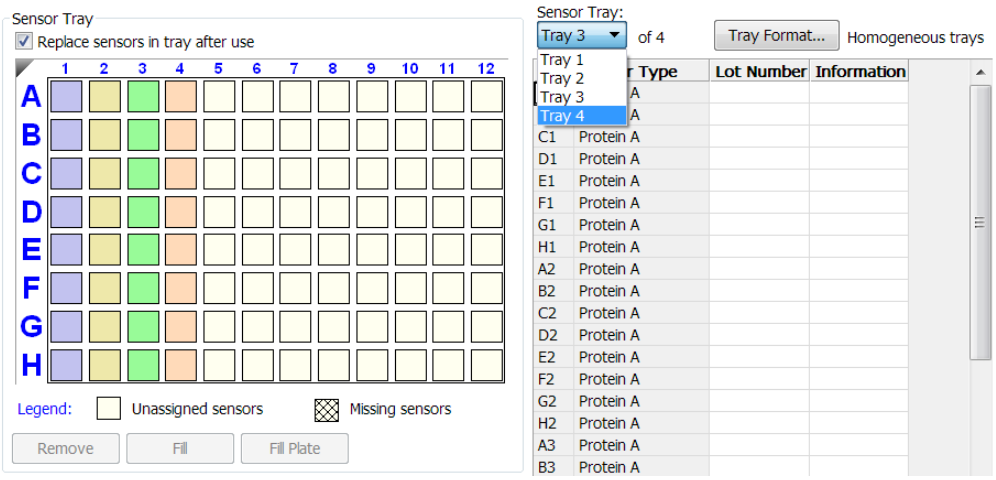


Figure 5-45: *Tray Selection*

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

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

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

---

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

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

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

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

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

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 5-46).

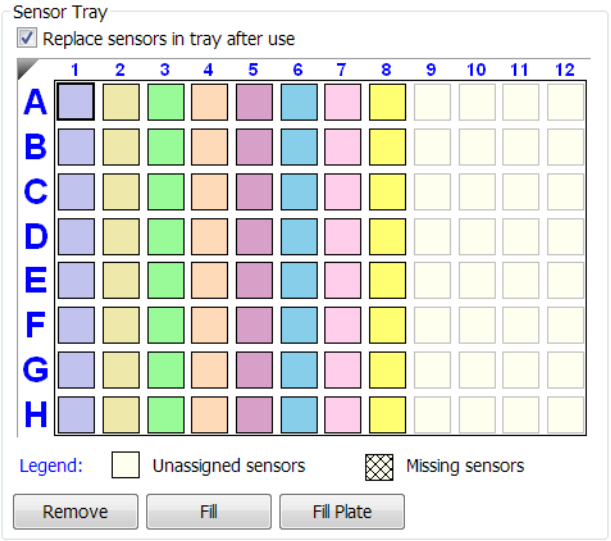


Figure 5-46: *Replace Sensors in Tray After Use Check Box*

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

Biosensor Regeneration

For Basic Quantitation with Regeneration experiments only, the **Sensor Assignment** tab includes the **Regenerations** parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The specified number of regeneration cycles determines the minimum number of cycles required for each column of sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by each column of sensors is represented by a pie chart (Figure 5-47).

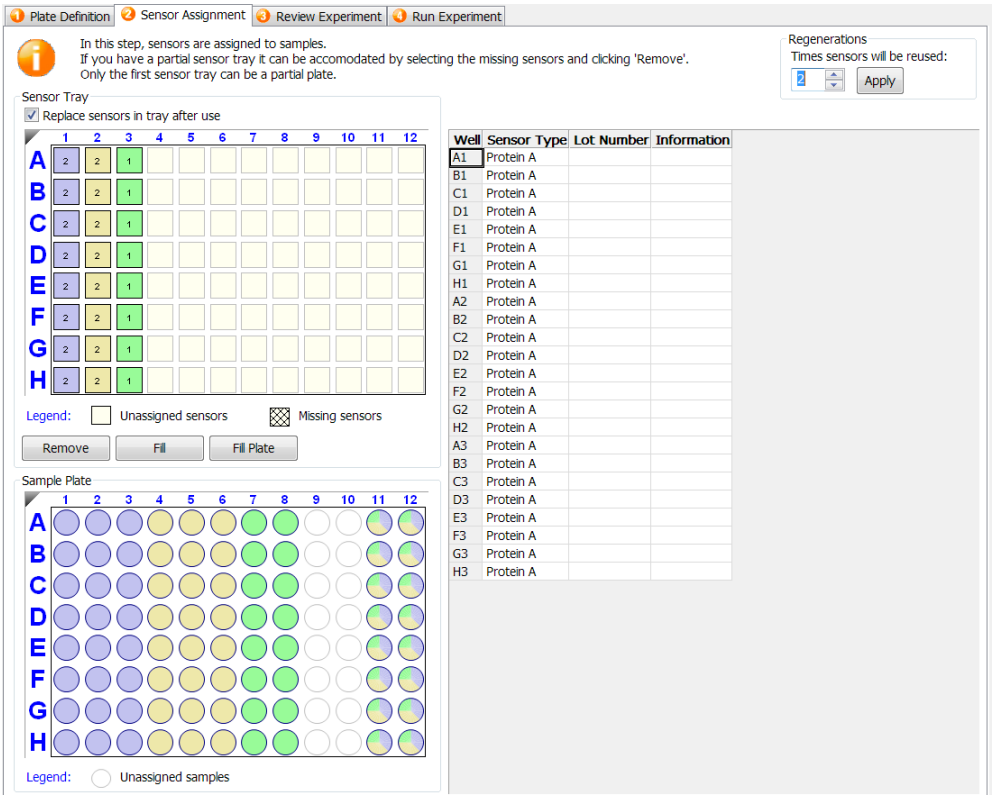


Figure 5-47: *Fractional Use of Regeneration and Neutralization Wells*

Using Partial Biosensor Trays

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

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

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

2. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 5-48). In the example shown, Tray 1 is a partial tray that does not contain enough biosensors for the assay. To designate a second tray, select Tray 2 from the **Sensor Tray** drop-down list (Figure 5-48 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 5-48 bottom).

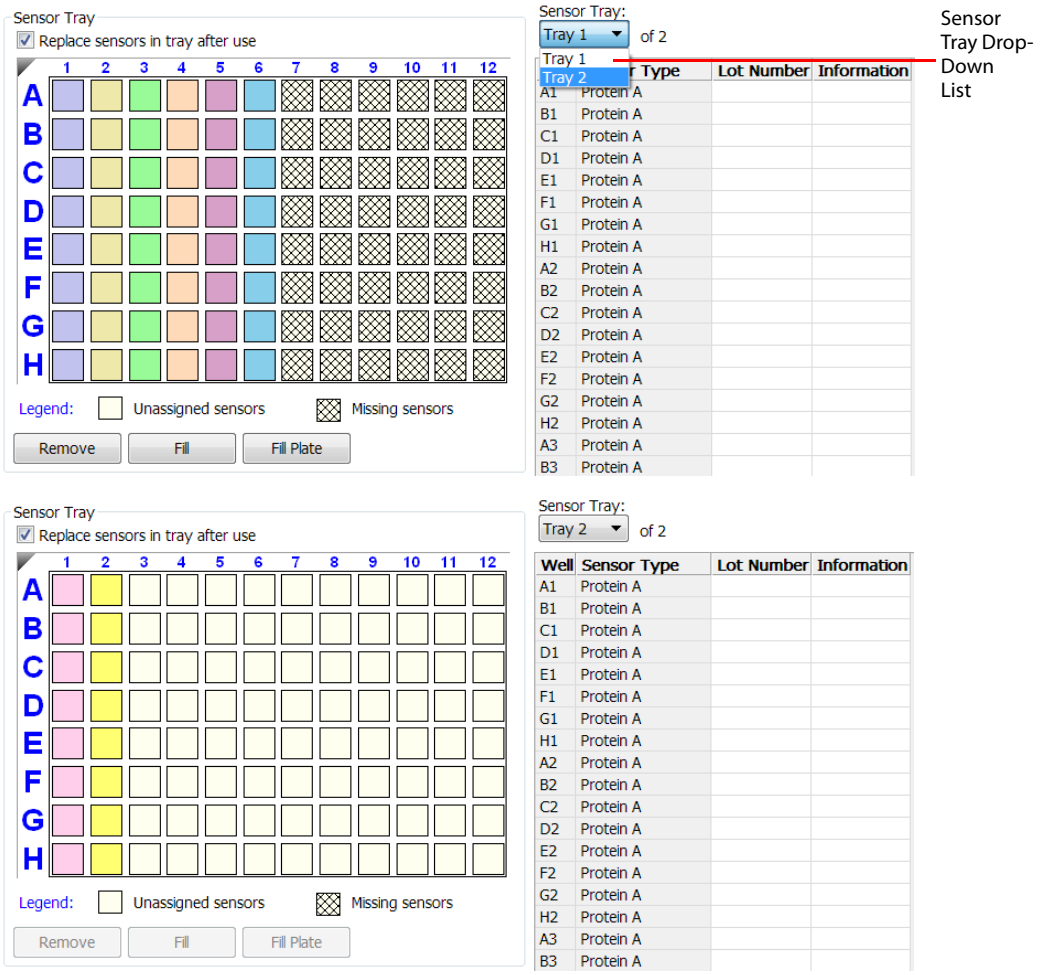


Figure 5-48: *Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray*

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

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

REVIEWING EXPERIMENTS

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

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

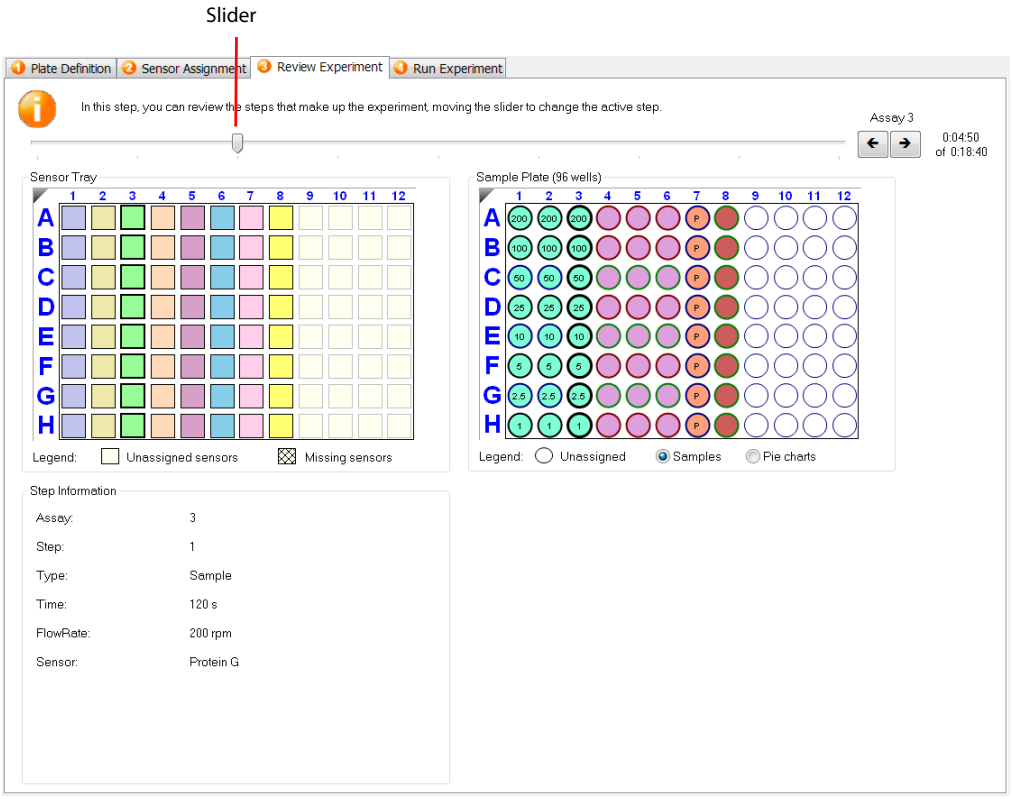


Figure 5-49: *Review Experiment Window*

SAVING EXPERIMENTS

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

To manually save an experiment method:

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

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

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available on the menu bar. To view templates click **Experiment** > **Templates** > **Quantitation** > **Experiment Name** (see Figure 5-50).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.

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

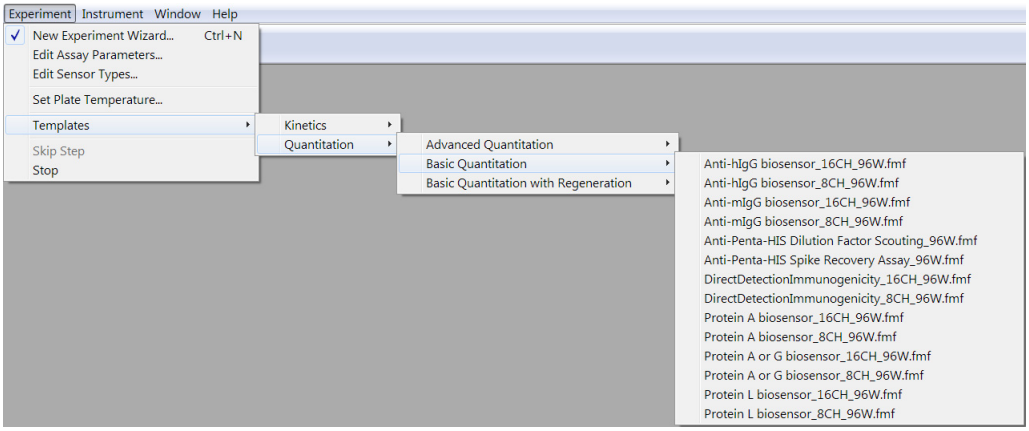


Figure 5-50: *Experiments in the Template Folder*

RUNNING A QUANTITATION EXPERIMENT

IMPORTANT: *Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare the biosensors, see the appropriate biosensor product insert.*

Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

1. Open the Octet instrument door (lift the handle up).
2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 5-51).
3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 5-51).

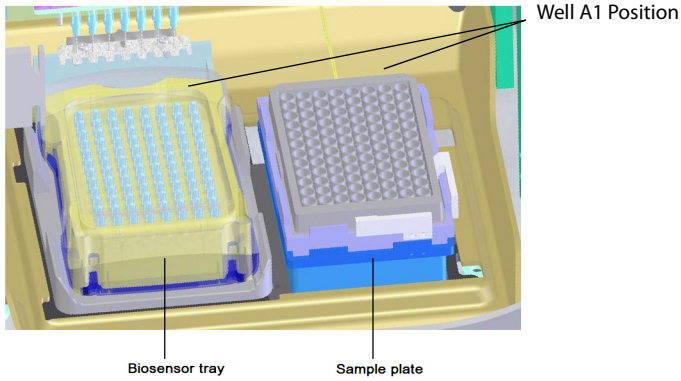


Figure 5-51: *Biosensor Stage (left) and Sample Stage (right)*

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

4. Close the Octet instrument door.
5. Allow the plate to equilibrate.

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

Starting an Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow to access the Run Experiment window (see Figure 5-52).

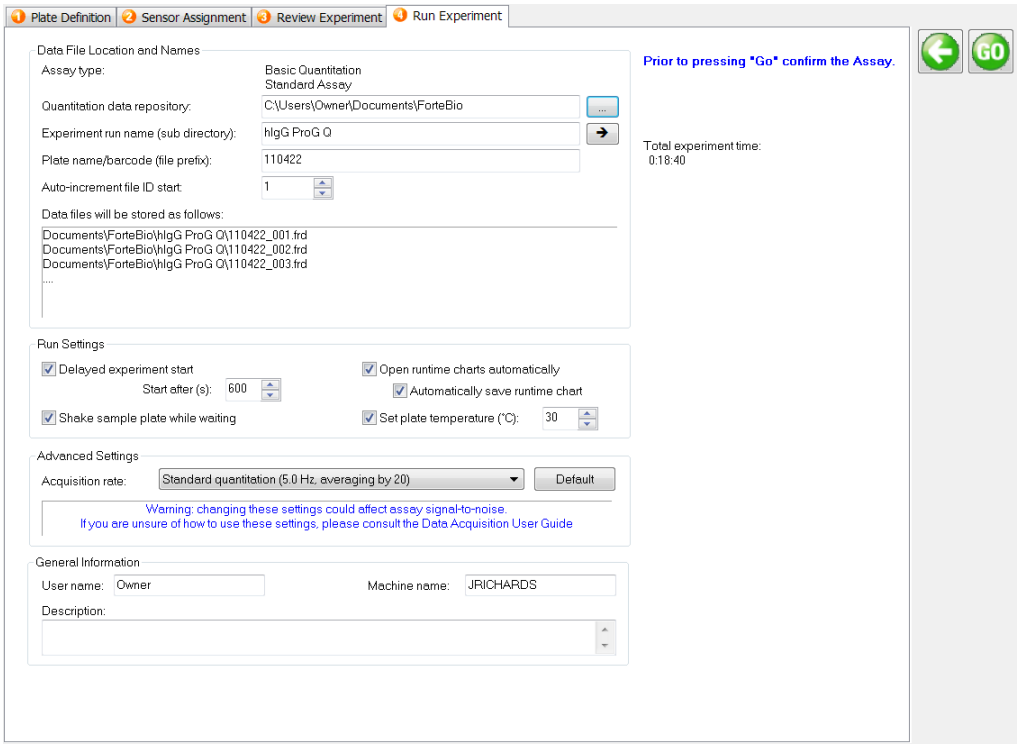


Figure 5-52: *Run Experiment Window—Octet RED96*

2. Confirm the defaults or enter new settings. See "Run Experiment Window Settings" on page 119 for more information on experimental settings.

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

---

3. To start the experiment, click GO.

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

   If you selected the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time and the experiment progress (see Figure 5-53).

NOTE: *For more details about the* ***Runtime Binding Chart****, see "Managing Runtime Binding Charts" on page 122.*

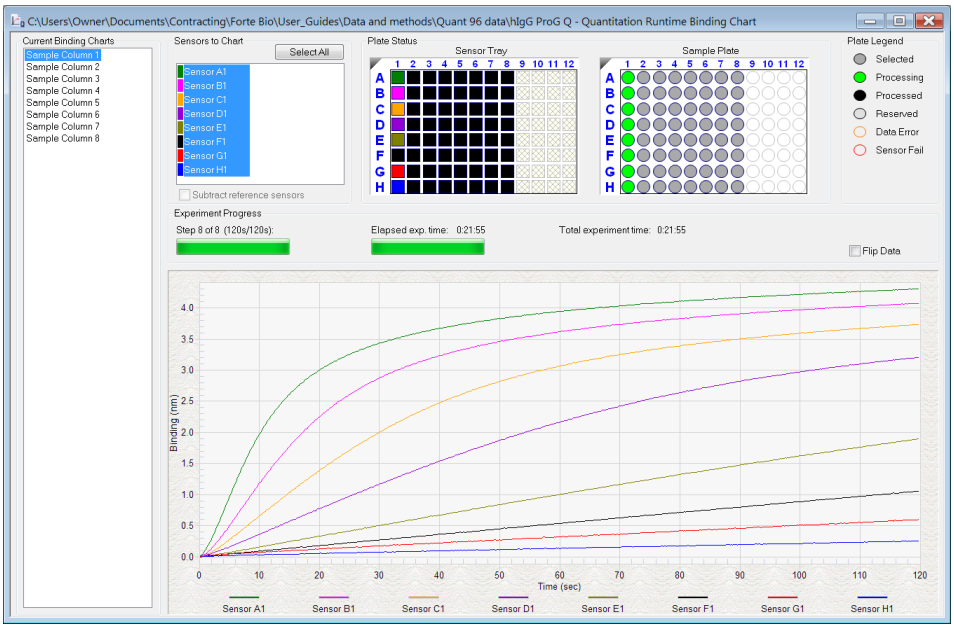


Figure 5-53: *Runtime Binding Chart*

4. Optional: Click **View** > **Instrument Status** to view the log file (see Figure 5-54).

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

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

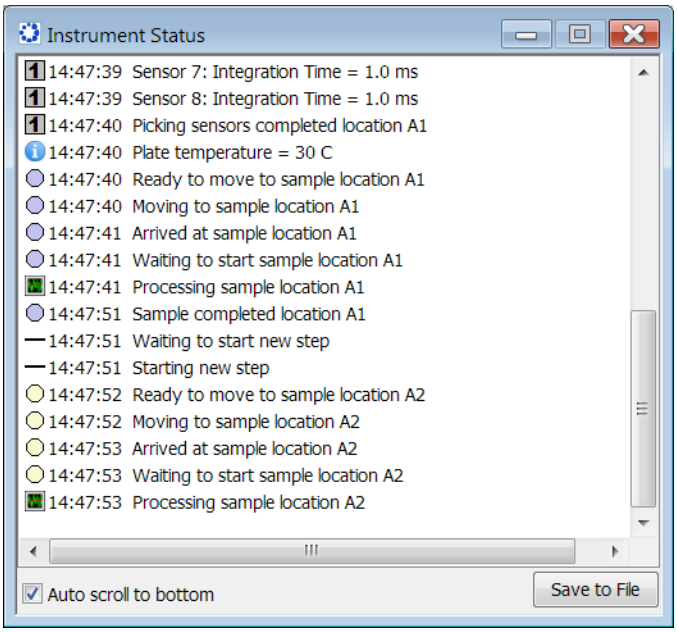


Figure 5-54: *Instrument Status Log*

Run Experiment Window Settings

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

Table 5-9: *Data File Location and Name*

Item	*Description*
Assay type	The name of the selected assay.
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click **Browse** to select another data location. ***NOTE:*** *It is recommended that you save the data to the local machine first, then transfer to a network drive.*
Experiment Run name (sub-directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software generates one data file for each biosensor.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.

Table 5-9: *Data File Location and Name (Continued)*

Item	*Description*
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

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

Table 5-10: *Run Settings*

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

Advanced settings are available for the Octet QK^e, Octet RED and Octet RED96 systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per second and is reported in Hertz (per second). A higher acquisition rate

generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

Table 5-11: *Advanced Settings for Octet QK^e, Octet RED and Octet RED96*

Item	*Description*
Acquisition rate, Octet QK^e	• High sensitivity quantitation (0.3 Hz, averaging by 40)—The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. • Standard quantitation (0.6 Hz, averaging by 5)—The average of five data frames is reported as one data point. One data point is reported every 1.6 seconds.
Acquisition rate, Octet RED and Octet RED96	• High sensitivity quantitation (2 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second. • Standard quantitation (5 Hz, averaging by 20)—The average of 20 data frames is reported as one data point. Five data points are reported per second.
Sensor offset (mm)—Octet QK^e only	Recommended sensor offset for quantitation—3 mm
Default	Sets acquisition rate and sensor offset to the defaults.

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

Table 5-12: *General Settings*

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

Stopping an Experiment

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

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

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

MANAGING RUNTIME BINDING CHARTS

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 5-55). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

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

To selectively display acquisition data for a particular acquisition step:

1. Click the corresponding **Sample Column** number.
2. Select a sub-set of sensors for a displayed column under **Sensors to Chart** box (see Figure 5-55).

WARNING: *Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.*

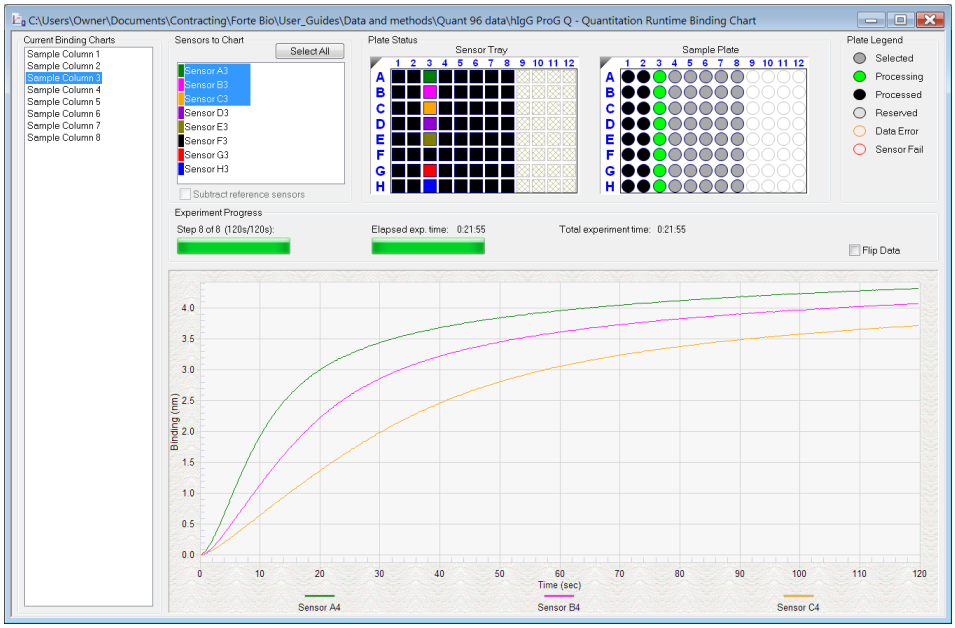


Figure 5-55: Runtime Binding Chart Window

Opening a Runtime Binding Chart

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

1. Click **File** > **Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data during acquisition in the chart by clicking the **Subtract reference sensors** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

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

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 5-57).

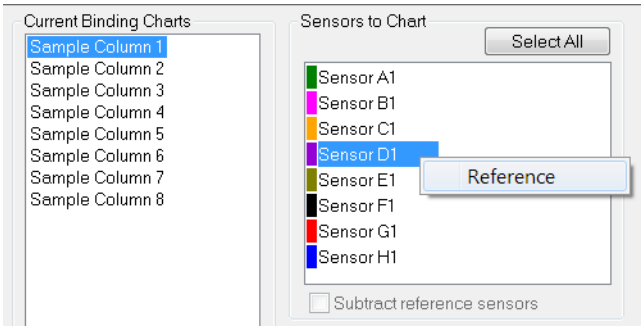


Figure 5-56: *Designating a Reference Biosensor in the Runtime Binding Chart*

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 5-57).

2. Click the **Subtract reference sensors** check box (see Figure 5-57).

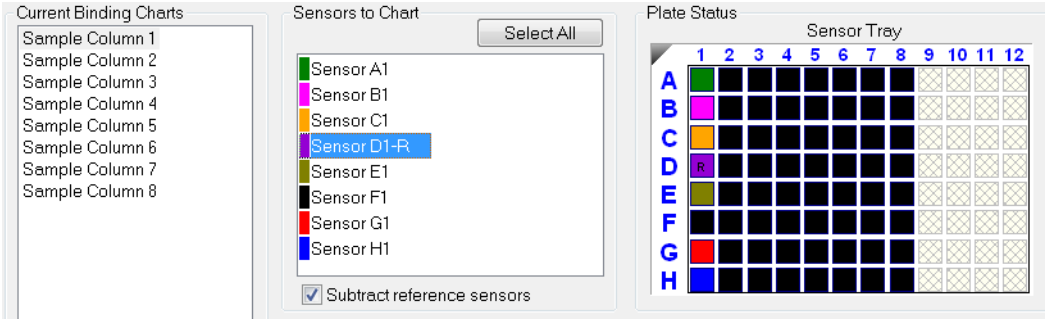


Figure 5-57: *Subtract Reference Sensors check box in the Runtime Binding Chart*

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

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 5-58). Uncheck the box to return to the default data display.

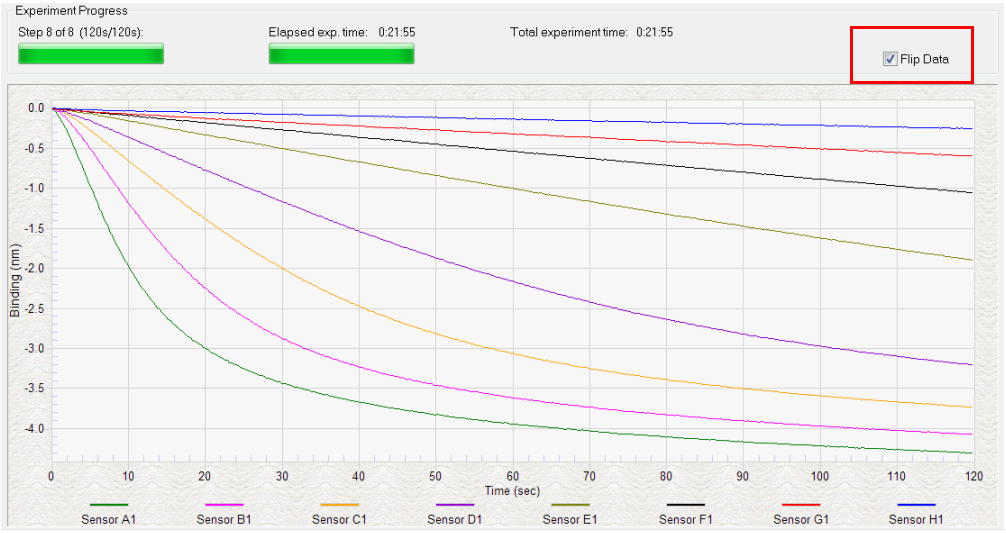


Figure 5-58: *Data Inverted Using Flip Data Function*

Magnifying the Runtime Binding Chart

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

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

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

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

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

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

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 5-59), select one of the following legends:
   - Sensor Location
   - Sample ID
   - Sensor Information
   - Concentration/Dilution

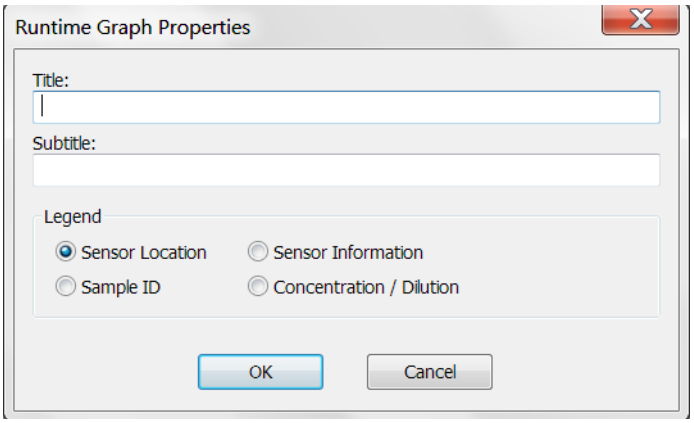


Figure 5-59: *Selecting a Runtime Binding Chart Legend*

---

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

---

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

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

Exporting or Printing the Runtime Binding Chart

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

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 5-60), select the export options and click **Export**.

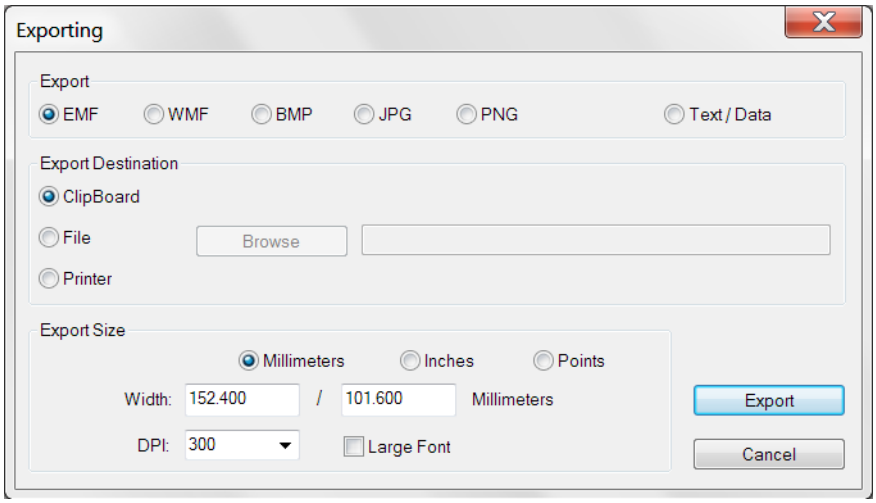


Figure 5-60: *Exporting Dialog Box*

Table 5-13: *Runtime Binding Chart Export Options*

Task	*Export*	*Option*	*Export Destination*	*Result*
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click **File** > **Browse** to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click **File** > **Browse** to select a folder and enter a file name.	Creates a graphic image.

Table 5-13: *Runtime Binding Chart Export Options (Continued)*

Task	*Export*	*Option*	*Export Destination*	*Result*
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it if necessary.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Table 5-14: *Managing Experiment Method Files*

Menu Bar Command/ Toolbar Button	*Description*
File > **Open Method File**	Enables you to select and open a method file (.fmf)
File > **Save Method File** or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > **Save Method File As**	Saves a method file to a new name so that the original file is not overwritten.

CUSTOM QUANTITATION ASSAYS

Defining a Custom Assay

To define a custom assay:

1. Click **Experiment** > **Edit Assay Parameters**.

   The **Edit Assay Parameters** dialog box appears; see Figure 5-61.

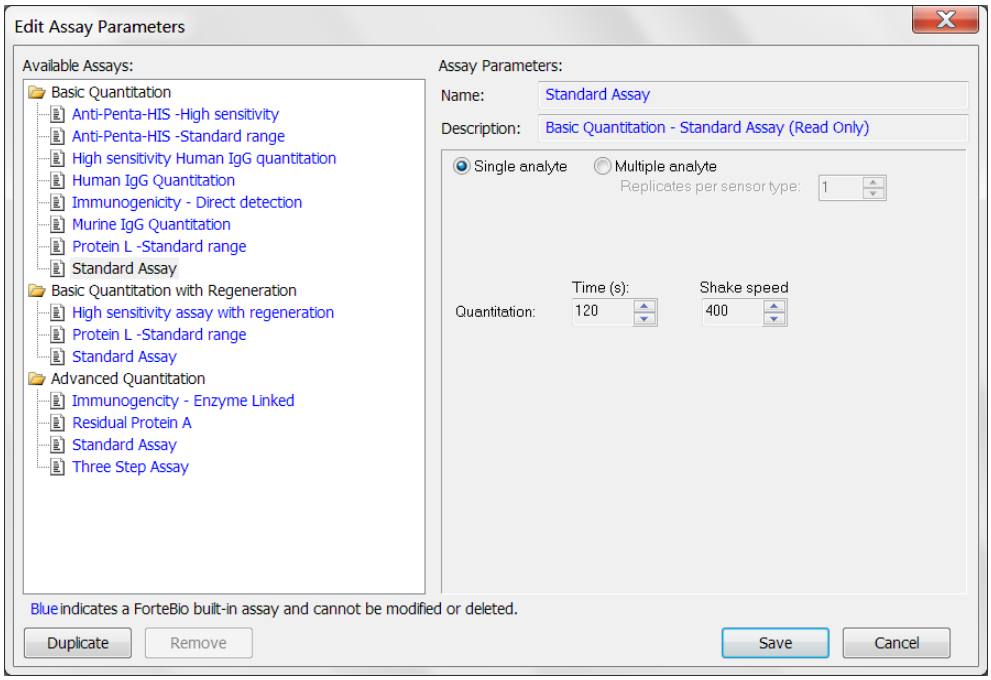


Figure 5-61: *Edit Assay Parameters Dialog Box*

2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
3. Click **Duplicate**.
4. In the **New Assay** dialog box (see Figure 5-62 top), enter an **Assay name**.
5. Optional: In the **Assay Description**, enter information about the assay.
6. Click **Save**.

   The new assay appears in the directory tree of available assays (see Figure 5-62 bottom).

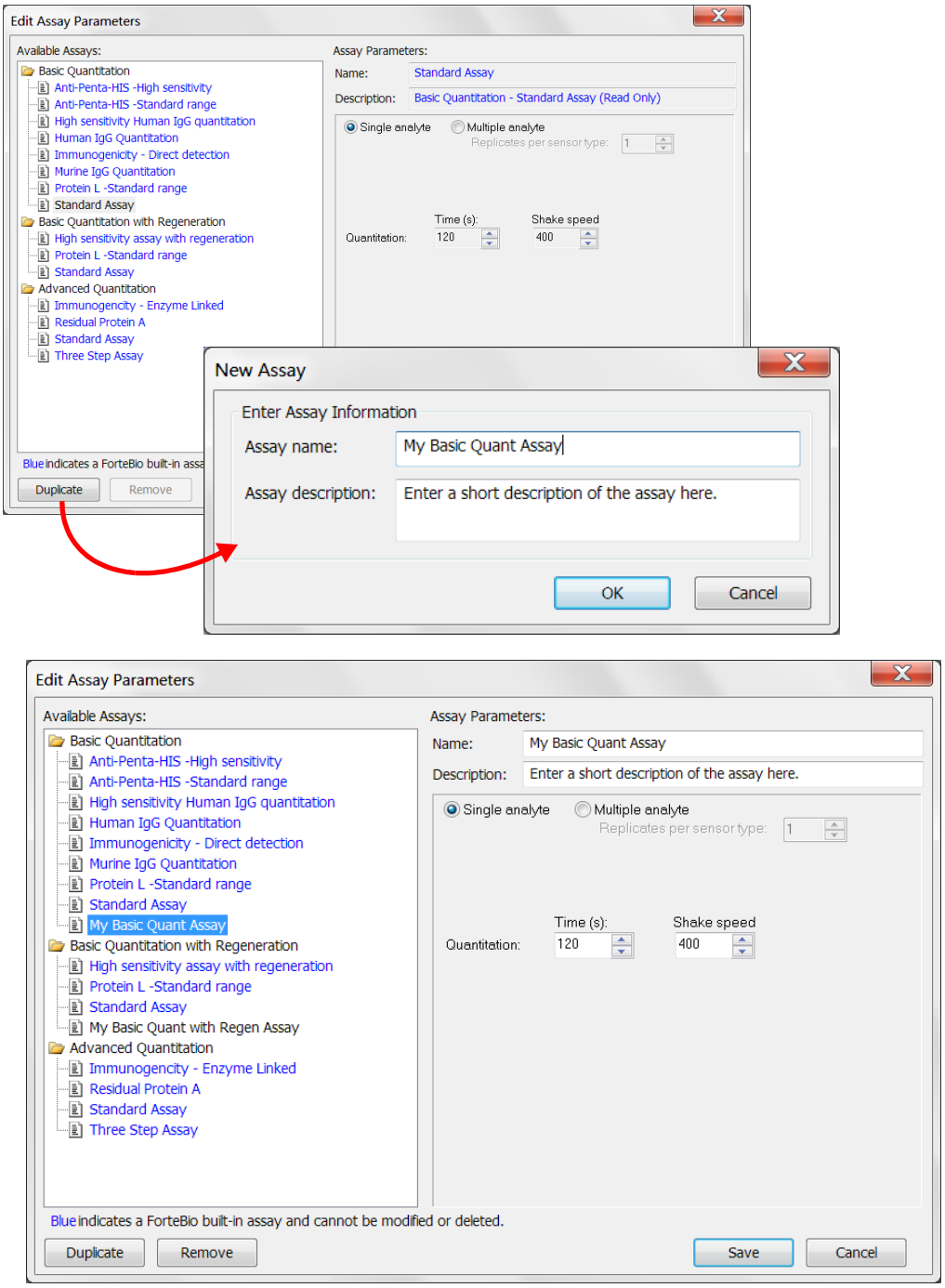


***Figure 5-62:** Defining a New Assay*

Editing Assay Parameters

To edit assay parameters:

1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 5-62 bottom).
2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
3. Click **Save** to accept the new parameter values. The new assay is added to the system.

NOTE: *Not all parameters are available for all of the assays.*

Basic Quantitation Assay Parameters

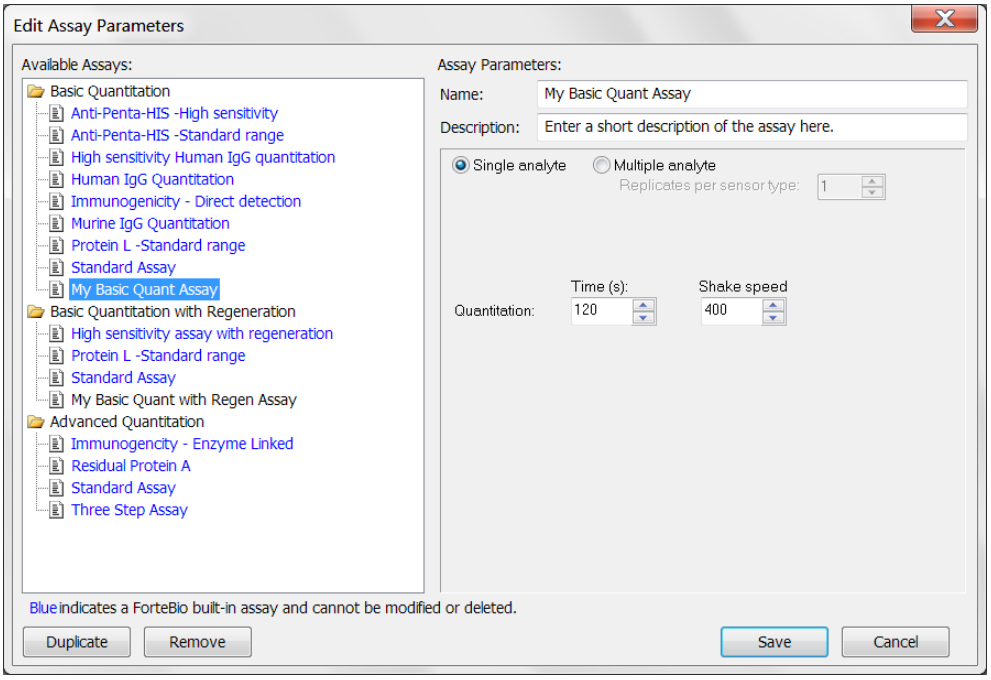


Figure 5-63: *Assay Parameters—Basic Quantitation Assay*

Table 5-15: *Basic Quantitation Assay Parameters*

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.

Table 5-15: *Basic Quantitation Assay Parameters (Continued)*

Parameter	*Description*
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample. ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample platform orbital shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

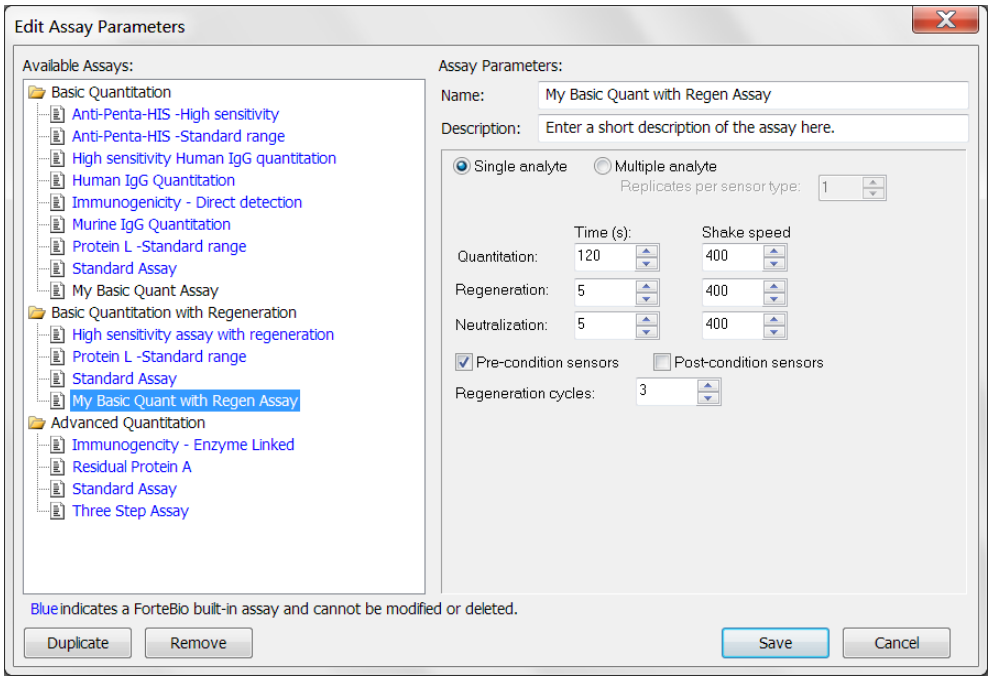


Figure 5-64: *Assay Parameters—Basic Quantitation with Regeneration*

Table 5-16: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	*Description*
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 5-16: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	*Description*
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

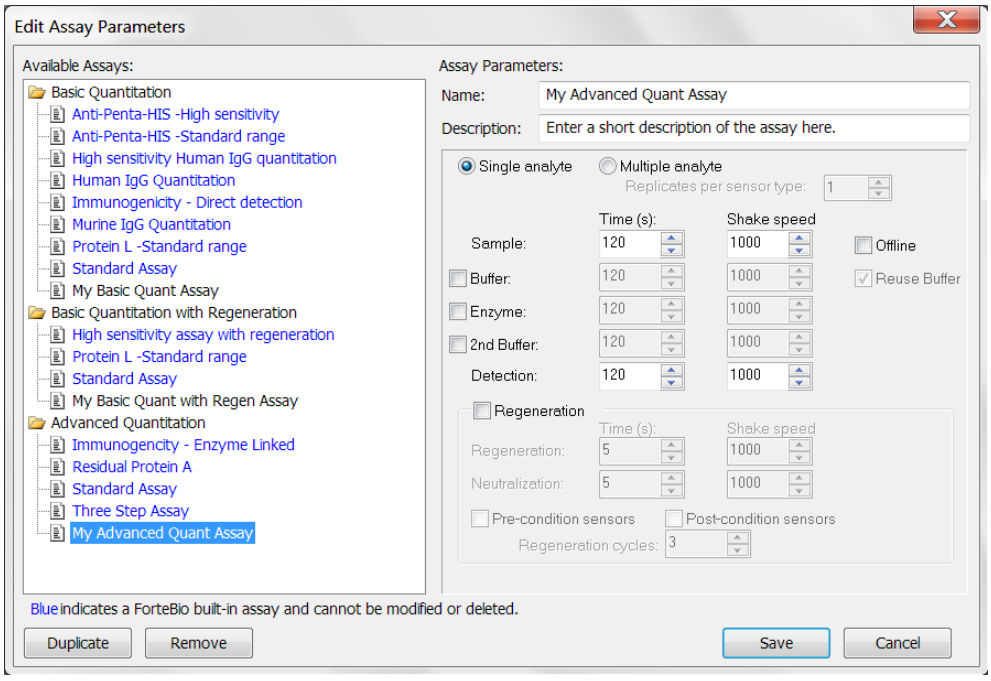


Figure 5-65: *Assay Parameters—Advanced Quantitation*

Table 5-17: *Advanced Quantitation Assay Parameters*

Parameter	*Description*
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample platform orbital shaking speed (rotations per minute).

Table 5-17: *Advanced Quantitation Assay Parameters*

Parameter	*Description*
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample platform orbital shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample platform orbital shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay. ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.

Table 5-17: Advanced Quantitation Assay Parameters

Parameter	Description
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse. ***NOTE:*** *In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.*

Selecting a Custom Assay

You can select a custom assay when you define a sample plate.

To select a custom assay:

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

   The **Edit Assay Parameters** dialog box displays (see Figure 5-66).

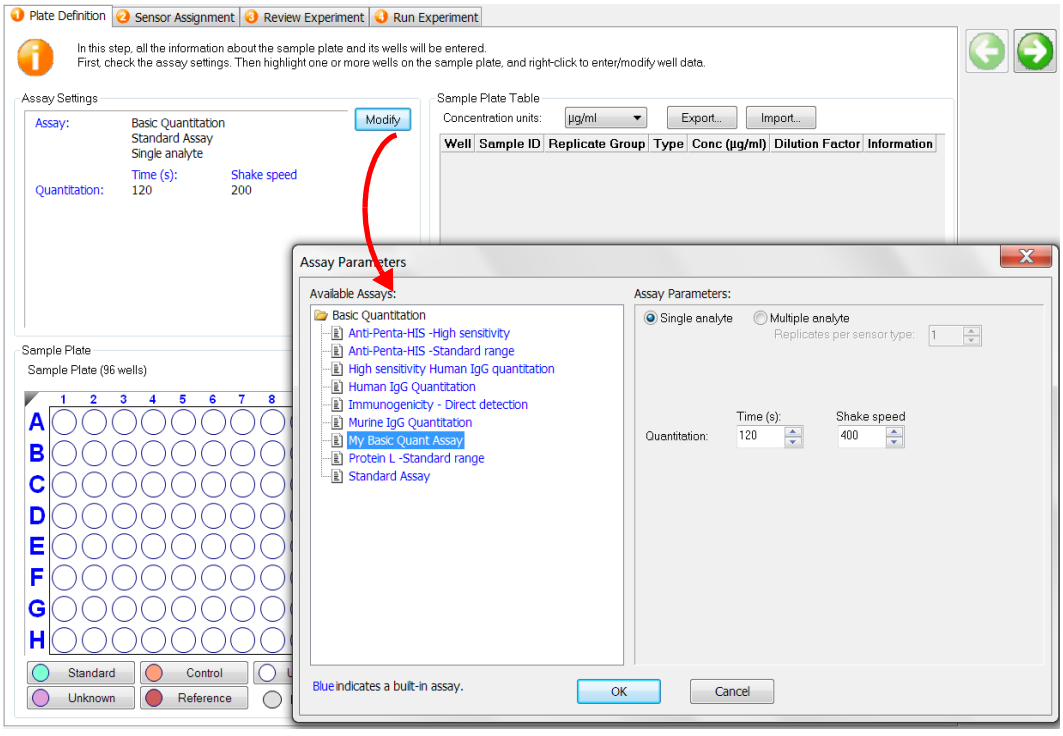


Figure 5-66: Selecting a Custom Assay

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

CHAPTER 6:

Quantitation Experiments: Octet RED384 and QK384

INTRODUCTION

A quantitation experiment enables you to determine analyte concentration within a sample using a reference set of standards. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 6-1) to set up and analyze a quantitation experiment.

Table 6-1: *Setting Up and Analyzing a Quantitative Experiment*

Software	*Step*	*See*
Data Acquisition 	1. Select a quantitation experiment in the **Experiment Wizard** or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 141
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 142
	3. Define a or import a reagent plate (optional) for a Basic Quantitation with Regeneration experiment or an Advanced Quantitation experiment).	"Working with a Reagent Plate" on page 165
	4. Confirm or edit the assay settings.	"Modifying Assay Parameter Settings" on page 167
	5. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 173
	6. Run the experiment.	"Running a Quantitation Experiment" on page 193
Data Analysis 	7. Analyze the binding data. 8. Generate a report.	*Octet System Data Analysis Software User Guide*

STARTING A QUANTITATION EXPERIMENT

NOTE: *Before starting an experiment, check the plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the* ***Options*** *dialog box when the software is relaunched.*

You can start a quantitation experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see "Managing Experiment Method Files" on page 205.
- On the menu bar, click **Experiment** > **Templates** > **Quantitation**.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Starting an Experiment Using the Experiment Wizard

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

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

Table 6-2: *Quantitation Experiment Selection*

Quantitation Experiment	*Description*
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regeneration	A standard quantitation assay that enables regeneration of biosensors.

Table 6-2: *Quantitation Experiment Selection*

Quantitation Experiment	*Description*
Advanced Quantitation	A standard two-or three-step quantitation assay that enables signal amplification for higher detection sensitivity.

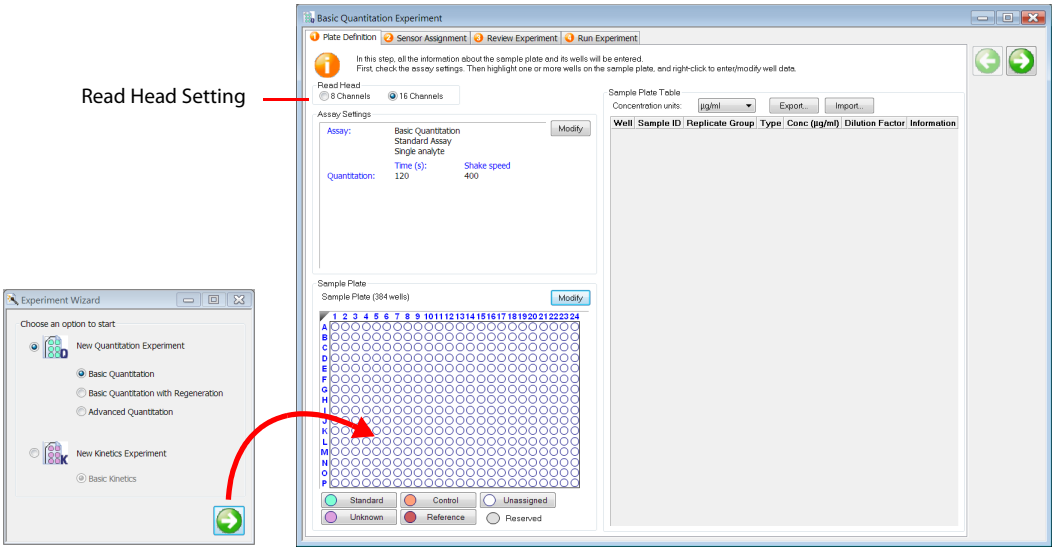


Figure 6-1: *Selecting an Experiment Type in the Experiment Wizard (for Octet RED384)*

4. Click the arrow.

   The **Experiment** window displays (Figure 6-1, right).

DEFINING THE SAMPLE PLATE

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

Table 6-3: *Defining a Sample Plate*

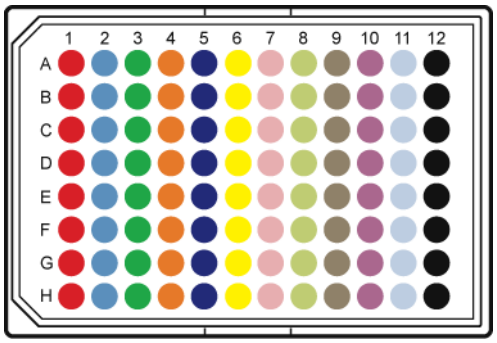
Step	*See Page*
1. Select the instrument read head configuration (8 or 16 channels).	143
2. Select the sample plate format (96 or 384 wells).	144
3. Designate the samples.	144
4. Annotate the samples (optional).	157
5. Save the sample plate definition (optional).	163

Read Head Configuration and Plate Layout

The Octet read head contains the collection optics. If the read head is set to 8 channels, one column of 8 biosensors interrogate 8 plate wells. If the read head is set to 16 channels, two columns of biosensors interrogate 16 wells (Figure 6-1).

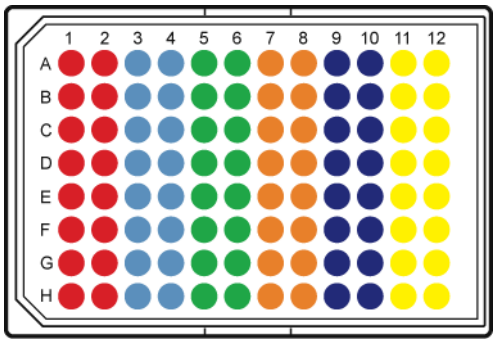
The read head configuration and the plate format (96 or 384 wells) determine the plate layout (Figure 6-2).

8 Channel Read Head



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

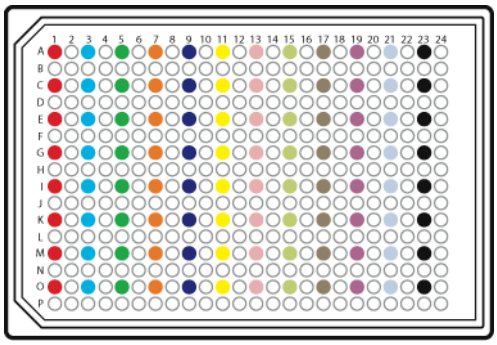
16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.

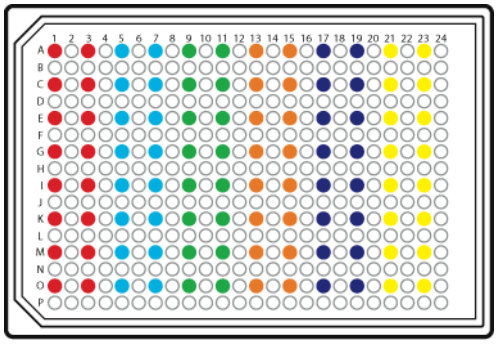
Figure 6-2: *Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head*

8 Channel Read Head



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

16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.

Figure 6-3: *Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head*

NOTE: *Keep the read head configuration in mind when laying out the sample plate. While reading a 384-well sample plate, both the 8 channel and 16 channel read heads can freely step through the plate by either moving left or right to step across columns or step one row up or down.*

Changing the Plate Format

To change the sample plate format:

1. Click the **Modify** button above the plate map.
2. In the **Modify Plates** box, select **96 Well** or **384 Well** format.

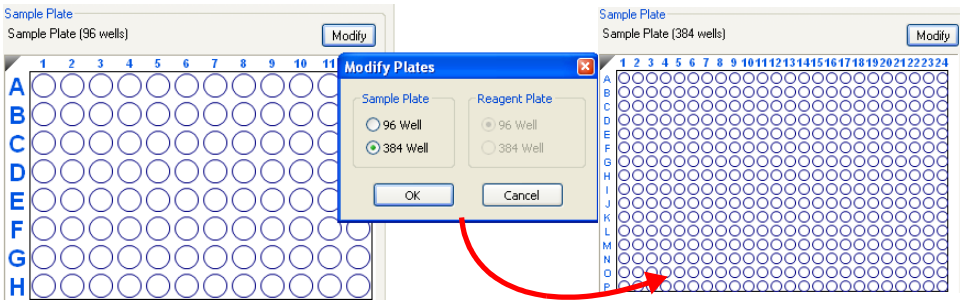


Figure 6-4: *Changing the Sample Plate Format*

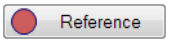
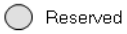
NOTE: *In Basic Quantitation with Regeneration and Advanced Quantitation experiments, a reagent plate format option is also available. Please refer to "Working with a Reagent Plate" on page 165 for more information.*

Designating Samples

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

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

Table 6-4: *Types of Sample Wells*

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

Reserved Wells

In a Basic Quantitation with Regeneration or an Advanced Quantitation experiment, the **Sample Plate Map** includes gray wells. These wells are reserved by the system and specify the location of particular sample types. The default location of the reserved wells depends on the sample plate format (96 or 384-wells) and the Octet instrument read head configuration (8 or 16 channels).

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

Table 6-5: *Reserved Well Requirements*

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

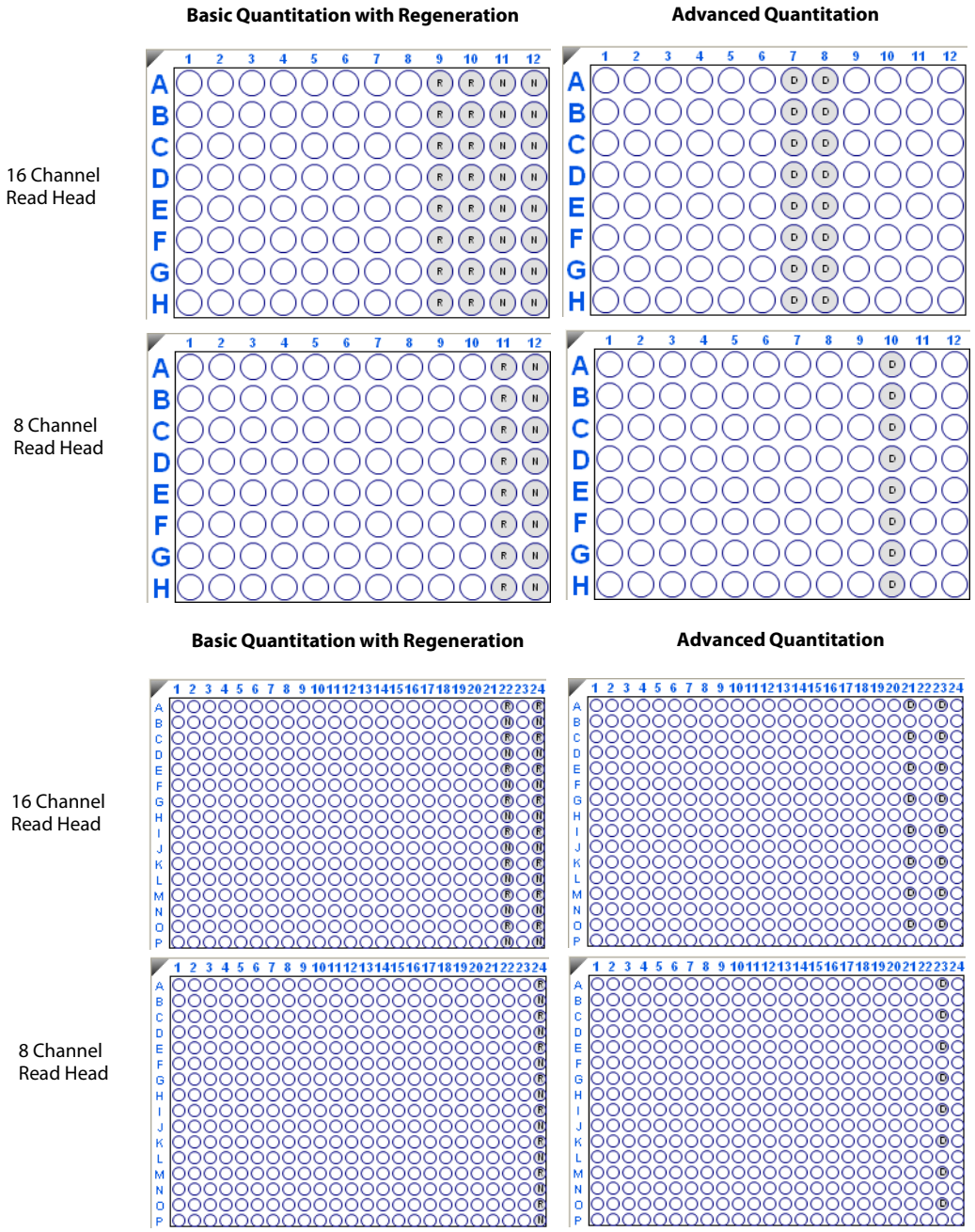


Figure 6-5: Default Locations for Reserved Wells in 96-well (top) and 384-well Sample Plate Maps (bottom)

Selecting Wells in the Sample Plate Map

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

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

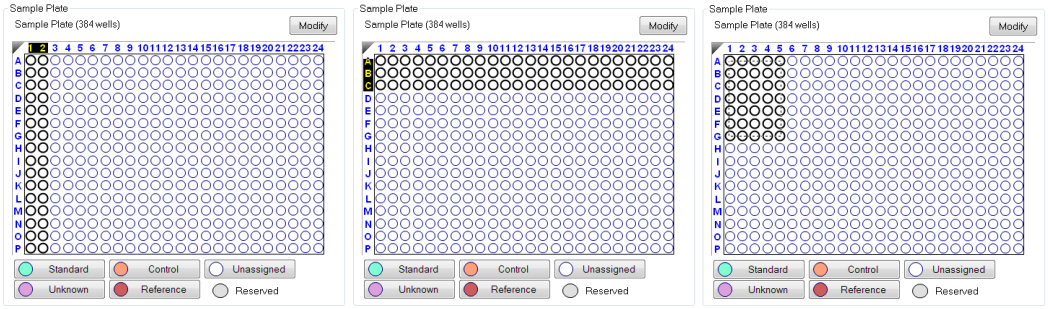


Figure 6-6: *Selecting Wells in the Sample Plate Map*

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

Designating Standards

To designate standards:

1. In the **Sample Plate Map**, select the wells to define as standards.
2. Click the **Standard** button below the **Sample Plate Map** (see Figure 6-7), or right-click and select **Standard**.

   The standards are marked in the plate map and the **Sample Plate Table** is updated.
3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

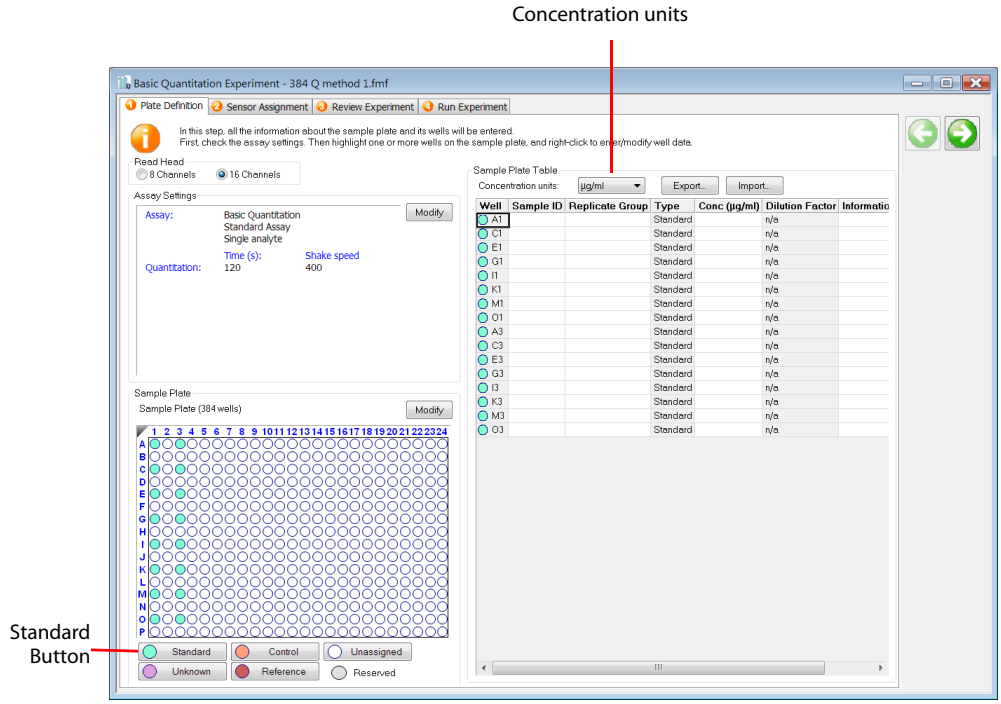


Figure 6-7: *Plate Definition Window—Designating Standards*

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

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

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

   The **Set Well Data** dialog box displays (see Figure 6-8).

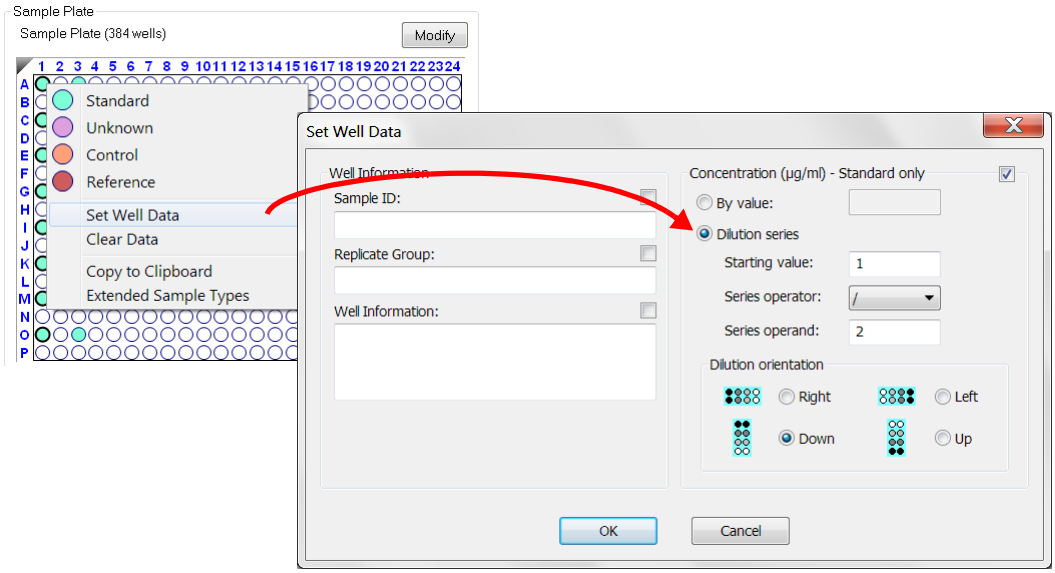


Figure 6-8: *Sample Plate Map—Setting a Dilution Series*

2. Select the **Dilution Series** option and enter the starting concentration value.
3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 6-10).

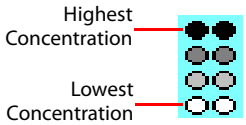


Figure 6-9: *Concentration Representation in Dilution Series*

4. Click **OK**.

   The **Sample Plate Table** will display the standard concentrations entered.

Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

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

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

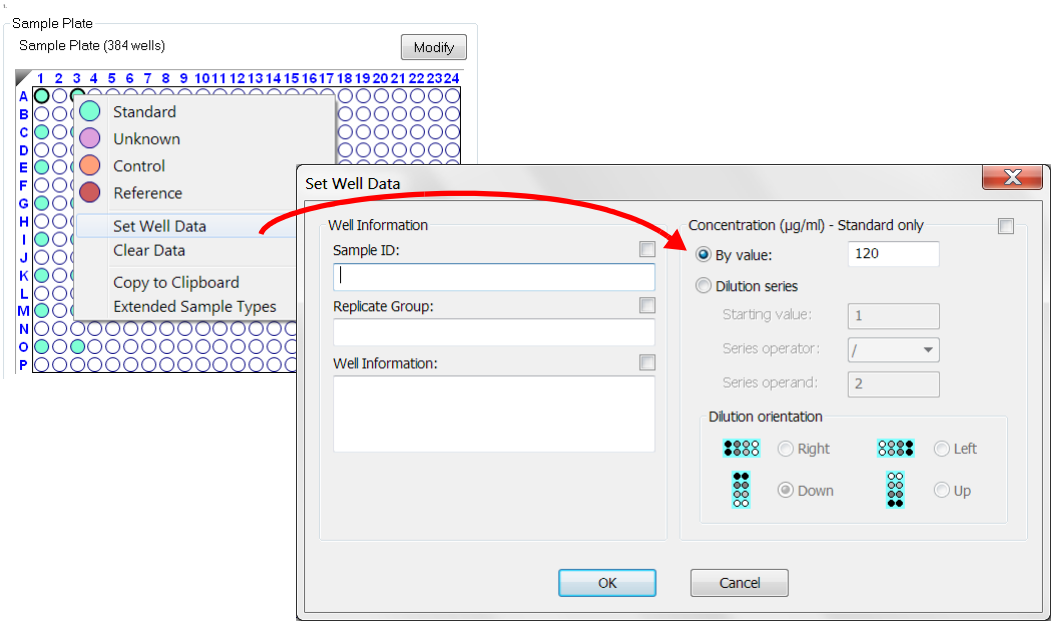


Figure 6-10: *Sample Plate Map—Assigning a Standard Concentration*

2. Select the **By value** option and enter the starting concentration value.
3. Click **OK**. The **Sample Plate Table** will display the standard concentrations entered.

Editing an Individual Standard Concentration

To enter or edit an individual standard concentration, in the **Conc** column of the **Sample Plate Table**, double-click the value and enter a new value (see Figure 6-11).

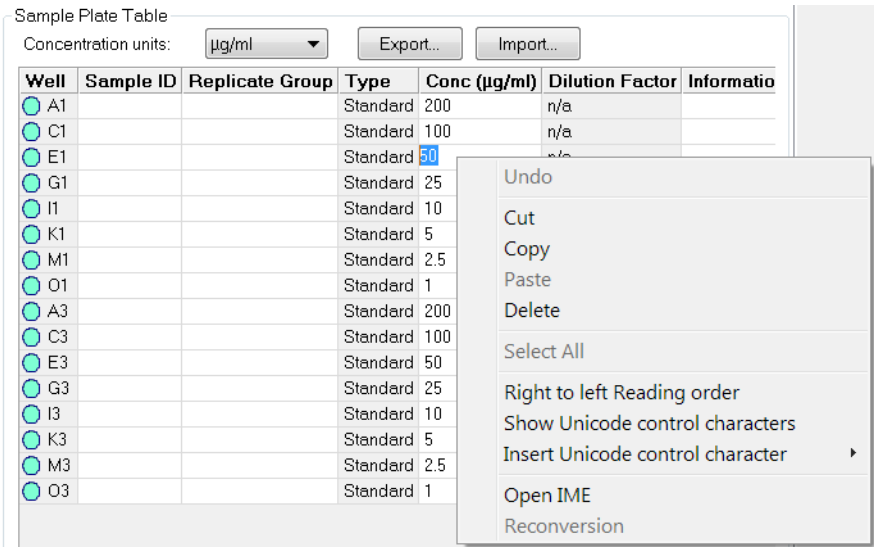


Figure 6-11: *Sample Plate Table—Shortcut Menu of Edit Commands*

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

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

Designating Unknowns

To designate unknowns in the **Sample Plate Map**, select the wells to define as unknown, right-click and select **Unknown**. The unknown wells are marked in the plate map and the **Sample Plate Table** is updated (see Figure 6-12).

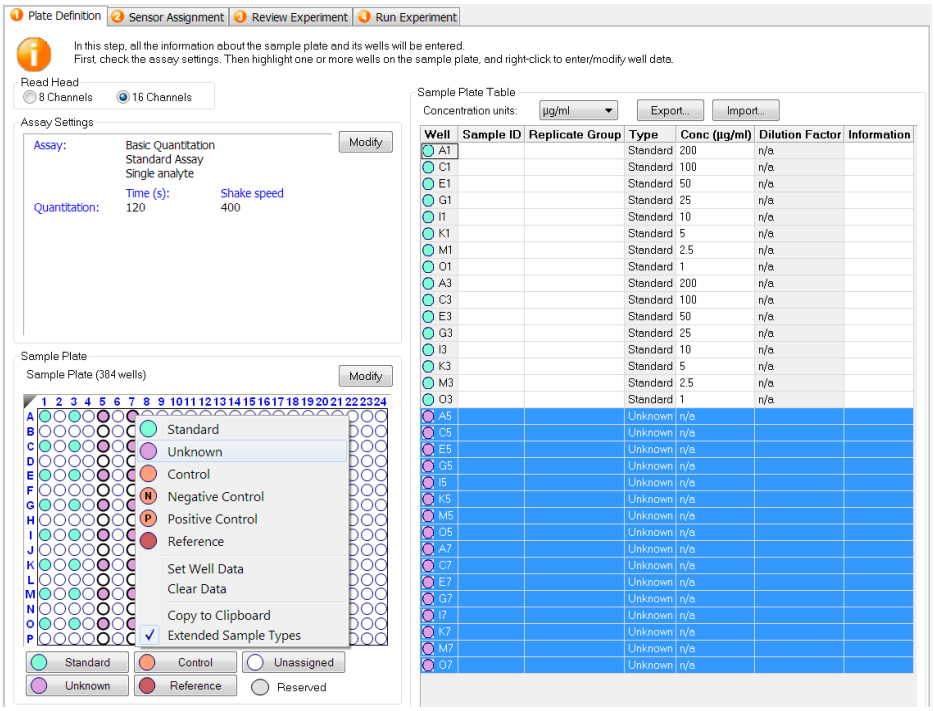


Figure 6-12: *Plate Definition Window—Designate Unknown Wells*

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

Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

1. In the **Sample Plate Map**, select the unknown wells (see Figure 6-12).
2. Right-click and select **Set Well Data**.

   The **Set Well Data** dialog box displays (see Figure 6-13).

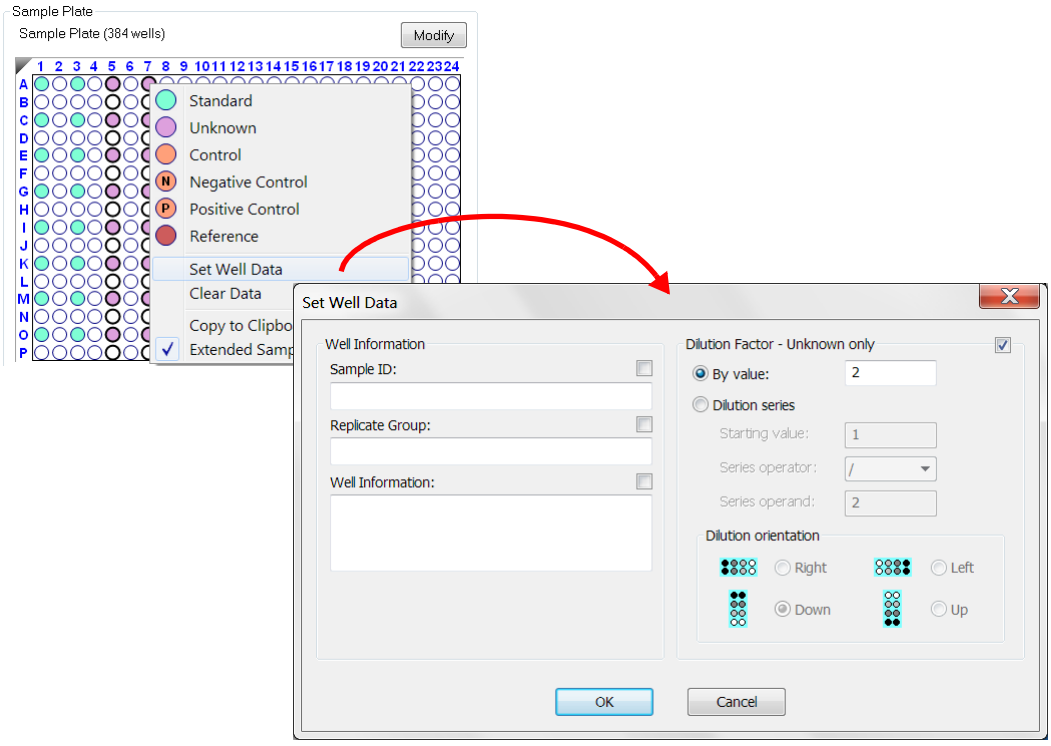


Figure 6-13: *Sample Plate Map—Setting a Dilution Factor or a Serial Dilution*

To assign a dilution factor to selected wells:

1. In the **Set Well Data** dialog box (see Figure 6-13), select the **By Value** option.
2. Enter the dilution factor value and click **OK**.

To assign a serial dilution to selected wells:

1. In the **Set Well Data** dialog box (see Figure 6-13), select the **Dilution series** option.
2. Enter the starting dilution, select a series operator, and enter a series operand.
3. Select the appropriate dilution orientation (see Figure 6-14).

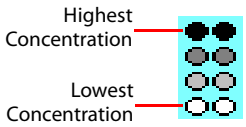


Figure 6-14: *Concentration Representation in Dilution Series*

4. Click **OK**.

   The **Sample Plate Table** will display the dilution factors entered.

Editing a Dilution Factor in the Sample Plate Table

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

1. In the **Sample Plate Table** (see Figure 6-15), double-click a cell in the **Dilution Factor** column for the desired unknown.
2. Enter the new value (the default dilution factor is 1).

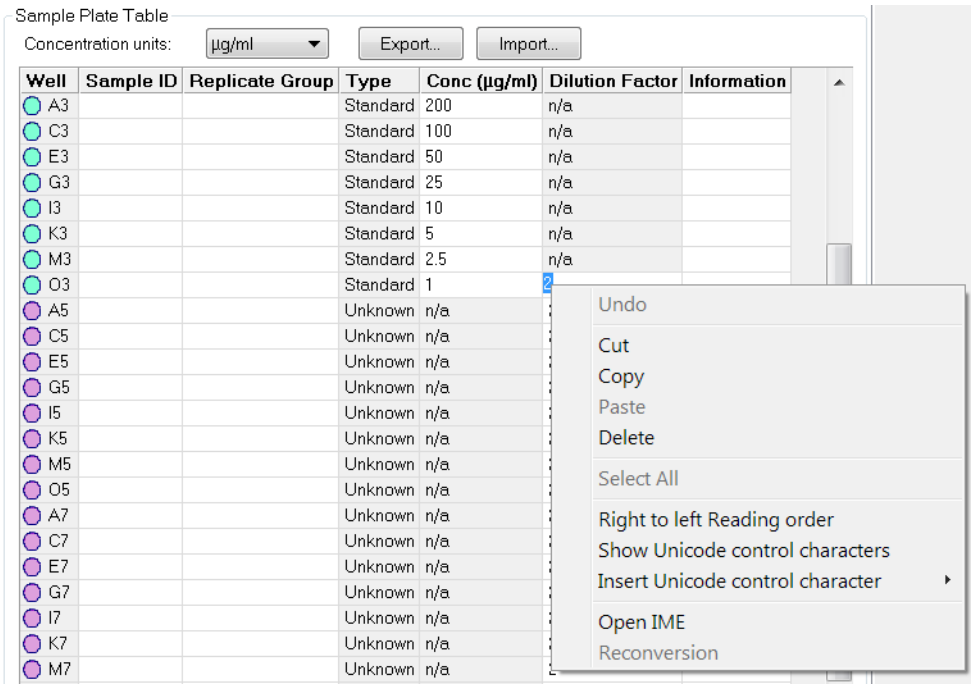


Figure 6-15: *Sample Plate Table—Shortcut Menu of Edit Commands*

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

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

Designating Controls or Reference Wells

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

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

The wells are marked in the **Sample Plate Map** and the **Sample Plate Table** is updated (see Figure 6-15).

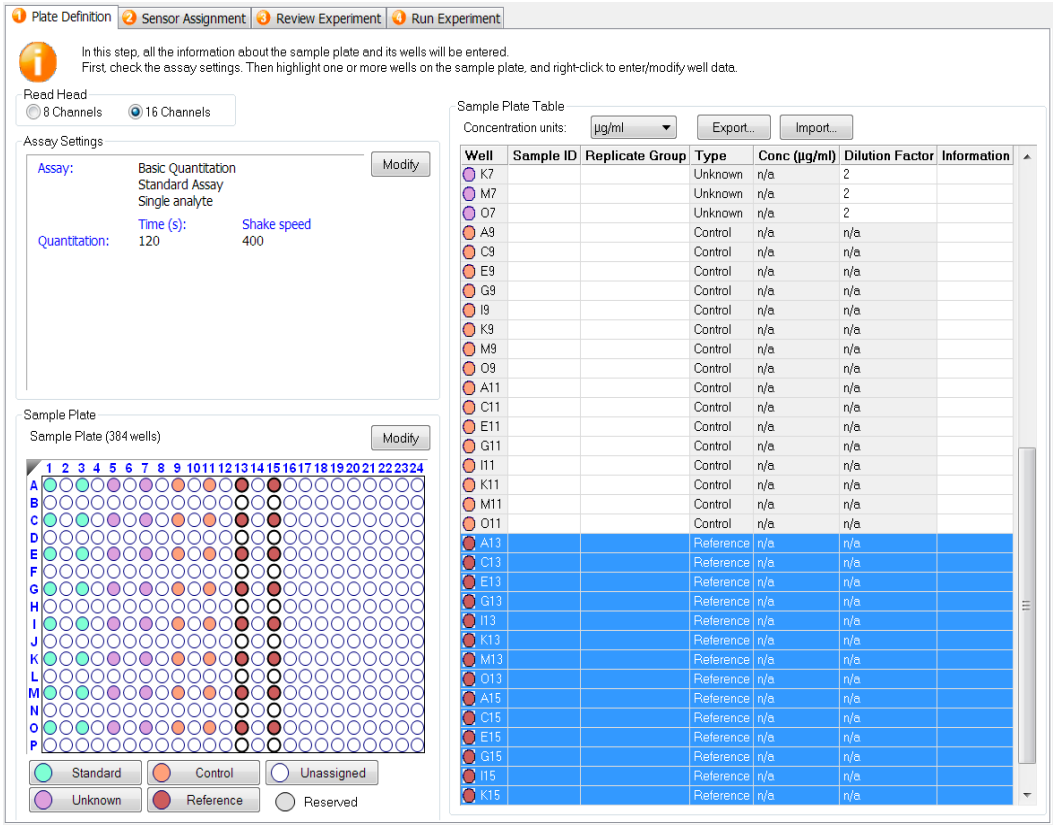


Figure 6-16: *Designate Controls or Reference Wells*

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

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

Annotating Samples

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

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 6-17), enter **Sample ID** and/or **Well Information** and click **OK**.

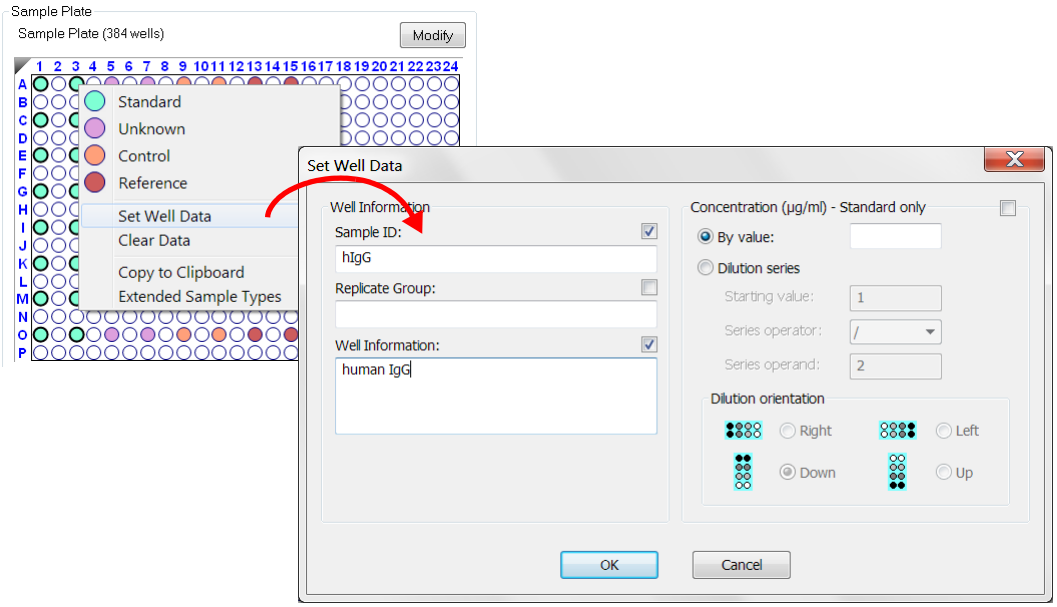


Figure 6-17: *Adding Sample Annotations from the Sample Plate Map*

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 6-18).

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

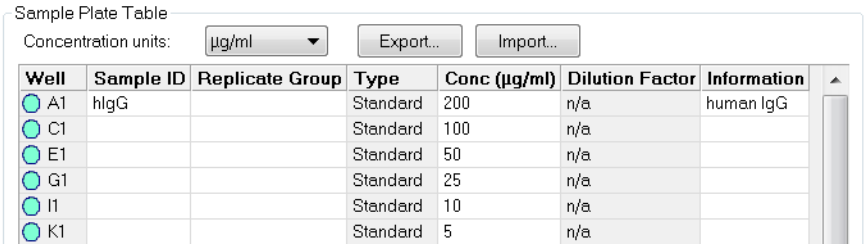


Figure 6-18: *Adding Sample Annotations in the Sample Plate Table*

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

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

Replicate Groups

When samples are assigned to a **Replicate Group**, the Octet System Data Analysis software will automatically calculate statistics for all samples in that group. The average binding rate, average concentration and corresponding standard deviation as well CV% are presented in the **Results** table for each group (see Figure 6-19).

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

Figure 6-19: *Replicate Group Result Table Statistics*

NOTE: *Replicate Group information can also be entered in the Results table in the Octet System Data Analysis software.*

Assigning Replicate Groups in the Sample Plate Map

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

1. Select the samples to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 6-20), enter a name in the **Replicate Group** box and click **OK**.

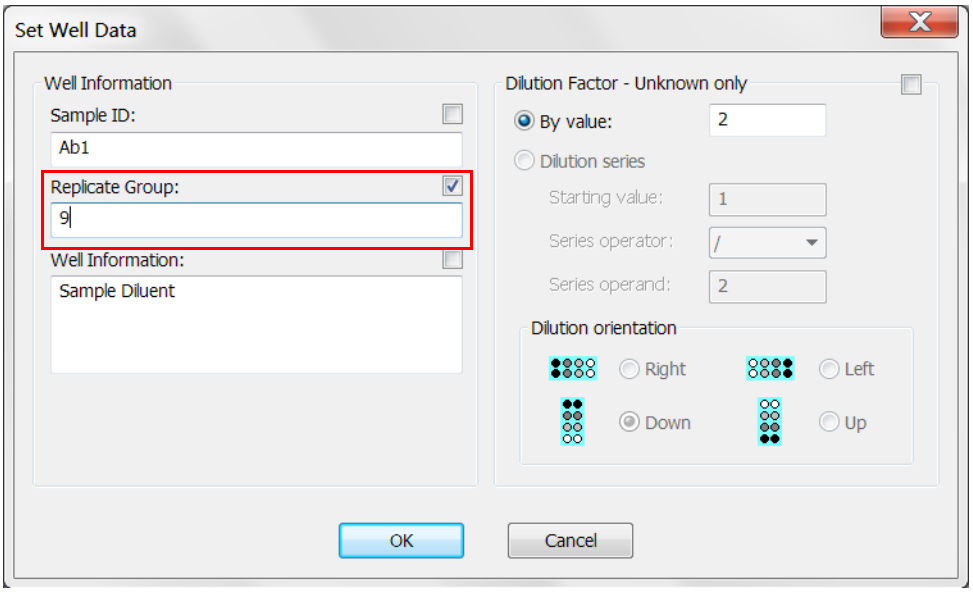


Figure 6-20: *Add Replicate Group from the Sample Plate Map*

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

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

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 6-21).

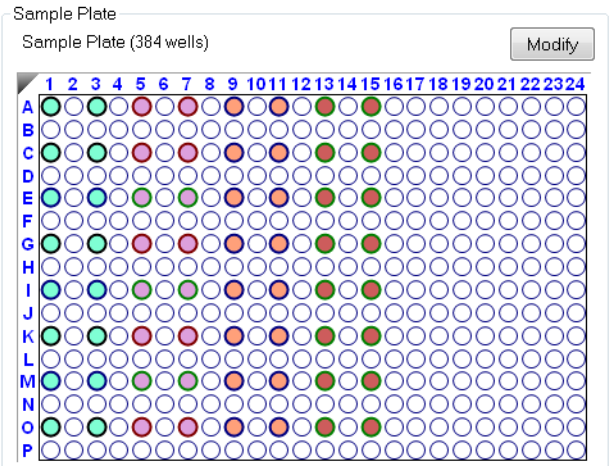


Figure 6-21: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 6-22).

Sample Plate Table

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

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hIgG	1	Standard	200	n/a	human IgG
C1	hIgG	2	Standard	100	n/a	human IgG
E1	hIgG	3	Standard	50	n/a	human IgG
G1	hIgG	4	Standard	25	n/a	human IgG
I1	hIgG	5	Standard	10	n/a	human IgG
K1	hIgG	6	Standard	5	n/a	human IgG
M1	hIgG	7	Standard	2.5	n/a	human IgG
O1	hIgG	8	Standard	1	n/a	human IgG
A3	hIgG	1	Standard	200	n/a	human IgG
C3	hIgG	2	Standard	100	n/a	human IgG
E3	hIgG	3	Standard	50	n/a	human IgG
G3	hIgG	4	Standard	25	n/a	human IgG
I3	hIgG	5	Standard	10	n/a	human IgG
K3	hIgG	6	Standard	5	n/a	human IgG
M3	hIgG	7	Standard	2.5	n/a	human IgG
O3	hIgG	8	Standard	1	n/a	human IgG
A5	Ab1	9	Unknown	n/a	2	Sample Diluent
C5	Ab2	10	Unknown	n/a	2	Sample Diluent
E5	Ab3	11	Unknown	n/a	2	Sample Diluent
G5	Ab4	12	Unknown	n/a	2	Sample Diluent
I5	Ab5	13	Unknown	n/a	2	Sample Diluent
K5	Ab6	14	Unknown	n/a	2	Sample Diluent
M5	Ab7	15	Unknown	n/a	2	Sample Diluent
O5	Ab8	16	Unknown	n/a	2	Sample Diluent
A7	Ab9	9	Unknown	n/a	2	Sample Diluent
C7	Ab10	10	Unknown	n/a	2	Sample Diluent
E7	Ab11	11	Unknown	n/a	2	Sample Diluent
G7	Ab12	12	Unknown	n/a	2	Sample Diluent

Figure 6-22: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 6-23).

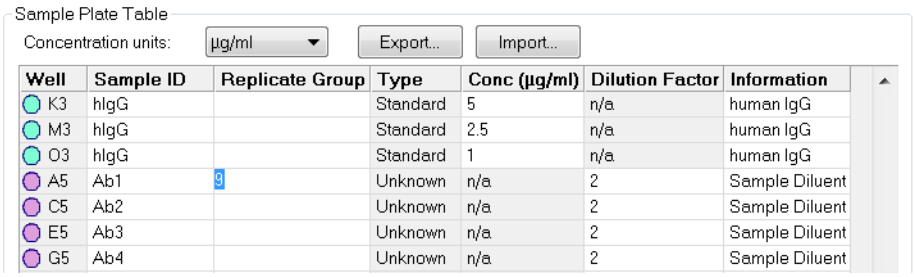


Figure 6-23: *Add Replicate Group from the Sample Plate Table*

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

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

---

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

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

MANAGING SAMPLE PLATE DEFINITIONS

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

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Table** (see Figure 6-24), click **Export**.

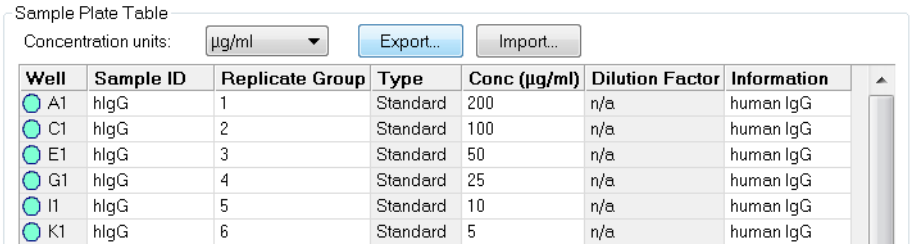


Figure 6-24: *Export Button in Sample Plate Table*

2. In the **Export Plate Definition** window (see Figure 6-24), select a folder, enter a name for the plate (.csv), and click **Save**.

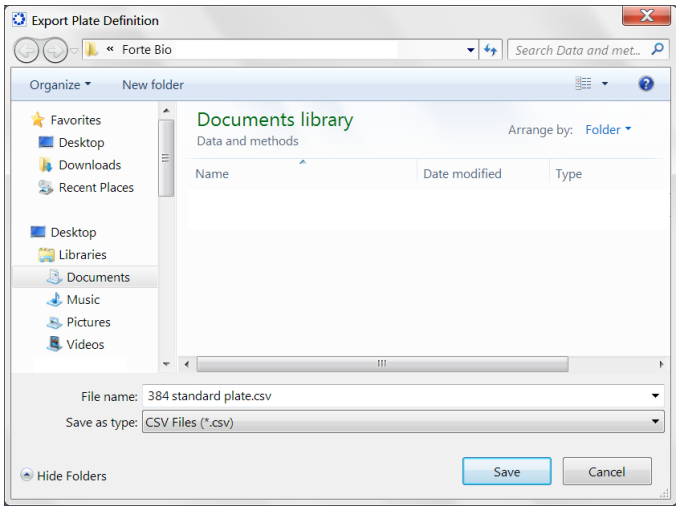


Figure 6-25: *Export Plate Definition Window*

Importing a Plate Definition

To import a plate definition:

1. In the **Sample Plate Table** (see Figure 6-26), click **Import**.

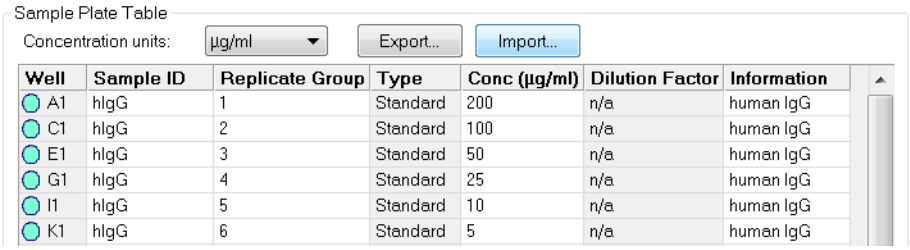


Figure 6-26: *Import Button in Sample Plate Table*

2. In the **Import Plate Definition** window (see Figure 6-28), select the plate definition (.csv), and click **Open**.

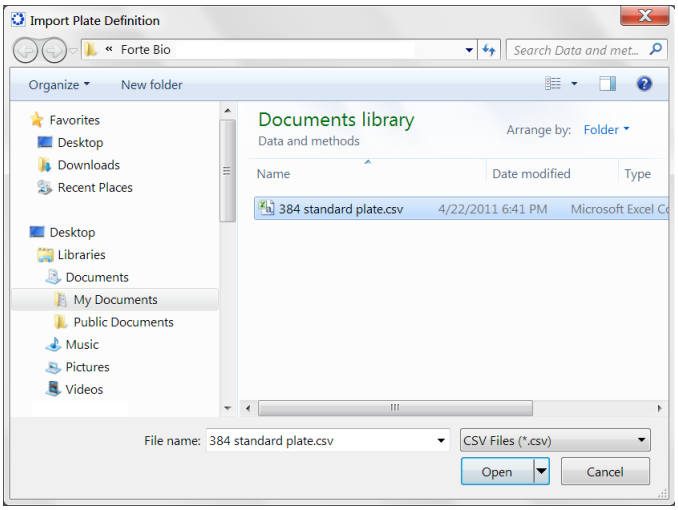


Figure 6-27: *Import Plate Definition Window*

NOTE: *You can also create a .csv file for import. Figure 6-28 shows the appropriate column information layout.*

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

384 Q method export

Figure 6-28: *Example Sample Plate Definition File (.csv)*

WORKING WITH A REAGENT PLATE

You can include an optional reagent plate in a Basic Quantitation with Regeneration or Advanced Quantitation experiment. Using a reagent plate enables higher sample throughput since no reagents are included in the sample plate. A reagent plate can contain:

- Regeneration and neutralization reagents for Basic Quantitation with Regeneration experiments
- Buffers, enzyme solutions, and detection reagents for Advanced Quantitation experiments

An experiment can include any combination of sample and reagent plate formats (96- or 384-well). However, a reagent plate can include only reagent wells (regeneration, neutralization, detection). Wells for standards, unknowns, controls and references can not be assigned to the reagent plate.

NOTE: *The reagent plate format (96- or 384-well) and the read head configuration (8 or 16 channels) determine the reagent plate layout. For more details, see "Read Head Configuration and Plate Layout" on page 143.*

To define a reagent plate:

1. Select the **Reagent Plate** radio button above the plate map to display the **Reagent Plate Map** (Figure 6-29).
2. Click **Modify** to display the **Modify Plates** dialog box.

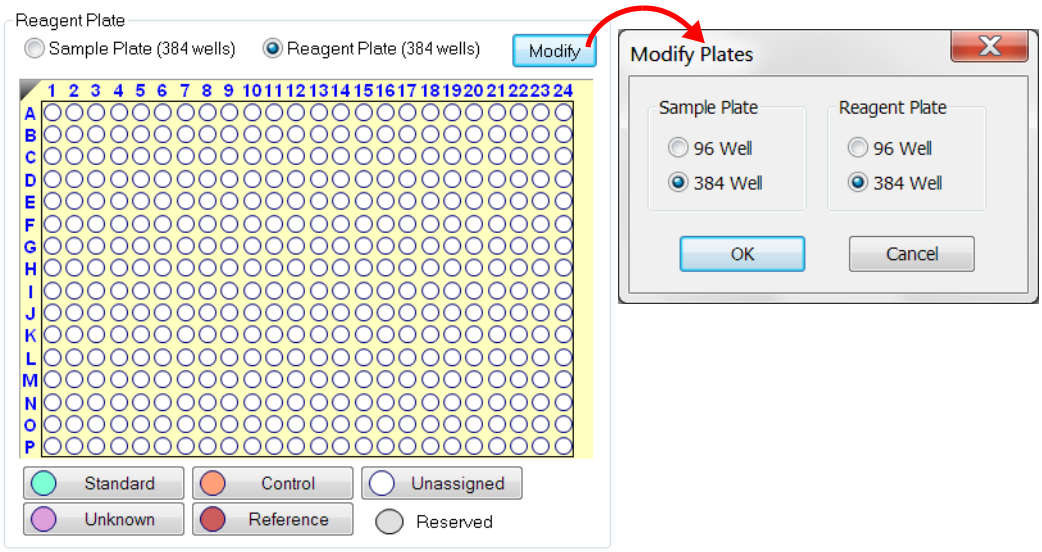
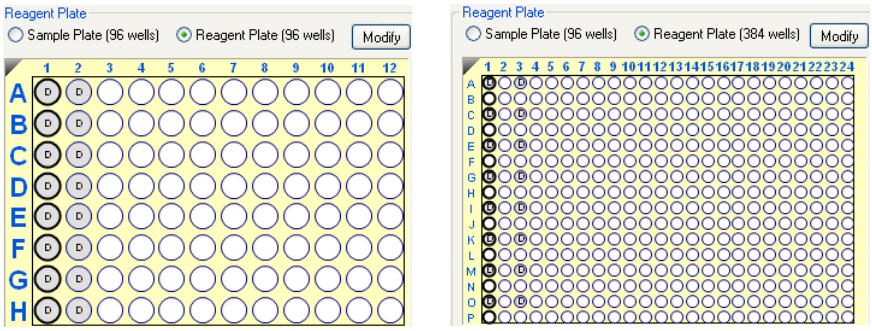


Figure 6-29: *Modifying the Reagent Plate*

3. Select a reagent plate format (**96 Well** or **384 Well**) and click **OK**.
4. In the **Reagent Plate Map**, right-click a column to use and make a selection on the shortcut menu that appears:
   - **Advanced Quantitation**—Select **Detection**.
   - **Basic Quantitation with Regeneration**—Select **Regeneration** or **Neutralization**. Repeat this step to set both the regeneration and neutralization reagent columns.

   The **Reagent Plate Map** then shows where to dispense the reagents in the plate (Figure 6-30).



96-well Format Reagent Plate

384-well Format Reagent Plate

Figure 6-30: *Example Reagent Plate Layouts for an Advanced Quantitation Experiment—16 Channel Read Head*

To remove well designations, select the column(s) and click **Unassigned**, or right-click and choose **Clear Data**.

Saving a Reagent Plate Definition

Exporting and saving a reagent plate definition is done in the same manner as you would for sample plates. For details "Managing Sample Plate Definitions" on page 163.

MANAGING ASSAY PARAMETER SETTINGS

Modifying Assay Parameter Settings

You can modify the assay parameter settings during sample plate definition. However, the changes are only applied to the current experiment. To save modified parameter settings, you must define a new assay. For details on creating a new assay, see "Custom Quantitation Assays" on page 206.

Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box will display (Figure 6-31). The settings available are experiment-dependent.

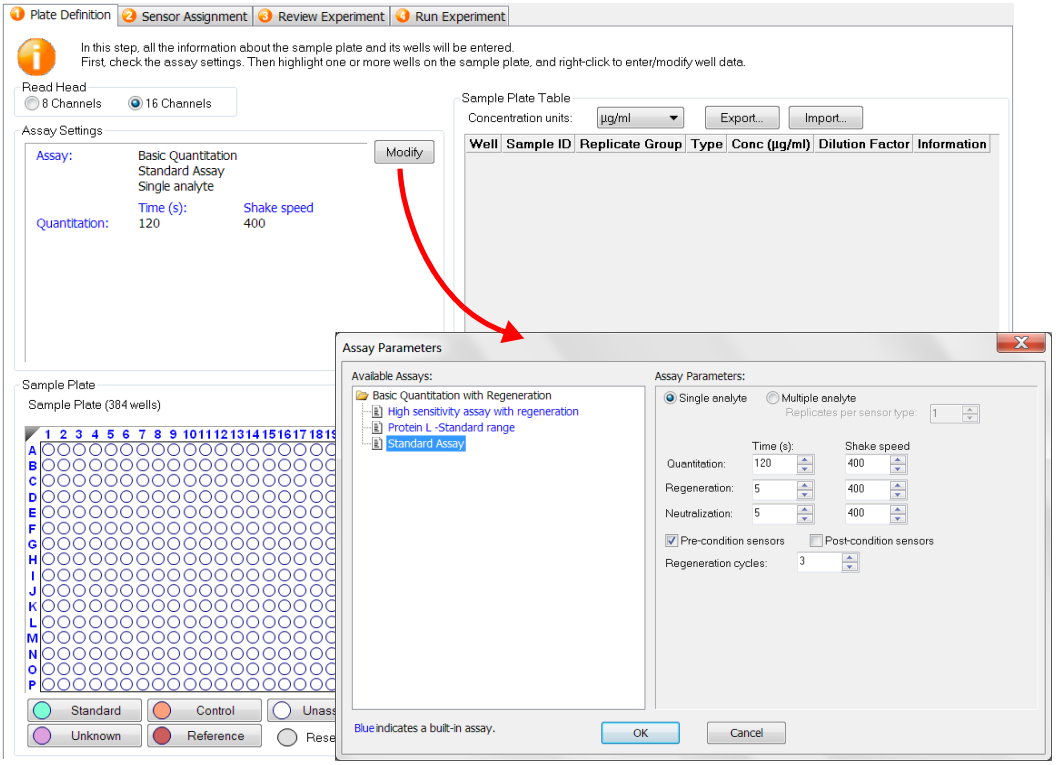


Figure 6-31: *Modifying Assay Parameters*

Basic Quantitation Assay Parameters

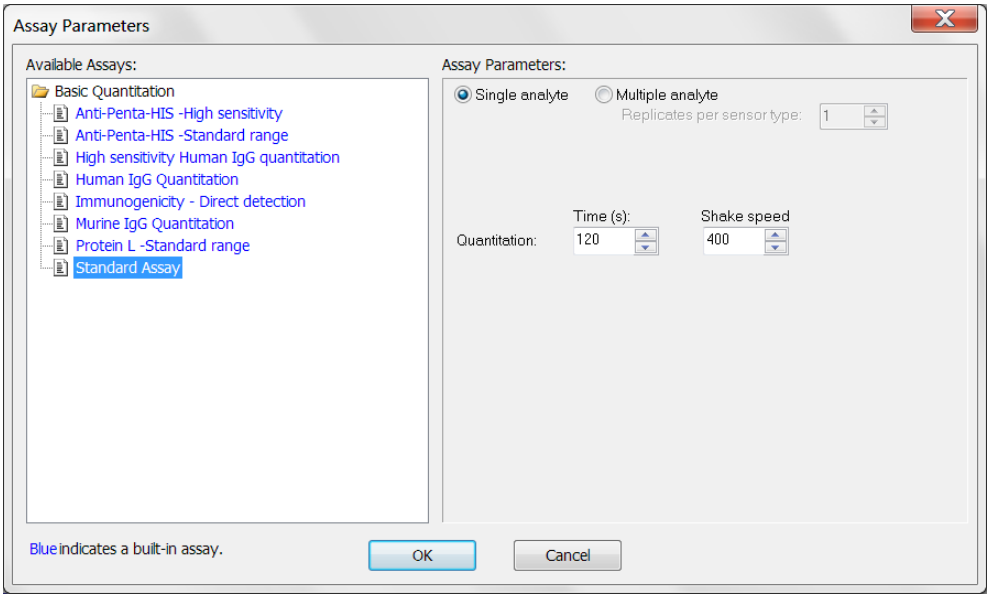


Figure 6-32: *Assay Parameters—Basic Quantitation Assay*

Table 6-6: *Basic Quantitation Assay Parameters*

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

Basic Quantitation with Regeneration Assay Parameters

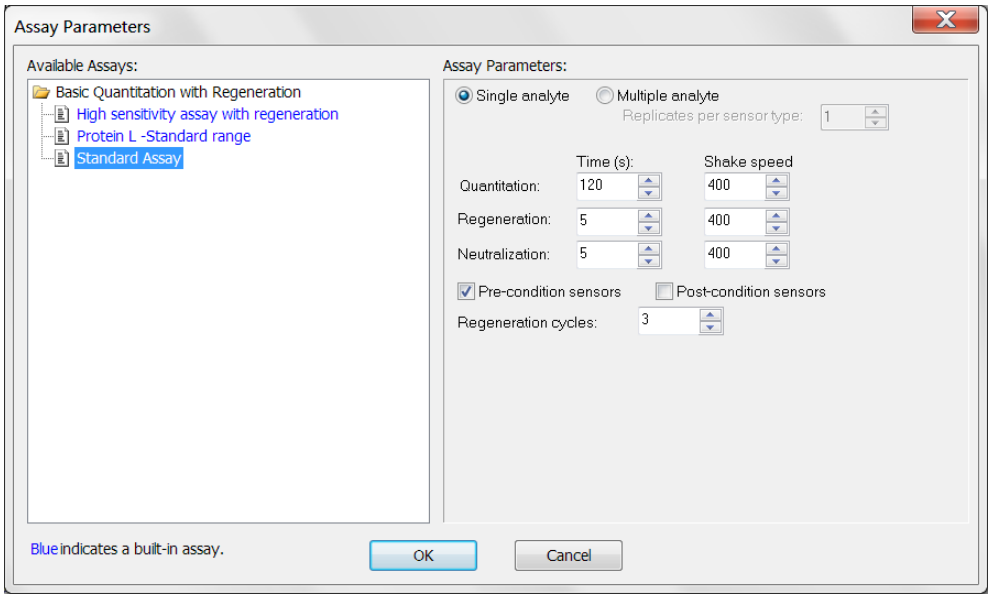


Figure 6-33: *Assay Parameters—Basic Quantitation with Regeneration*

Table 6-7: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	*Description*
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.

Table 6-7: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

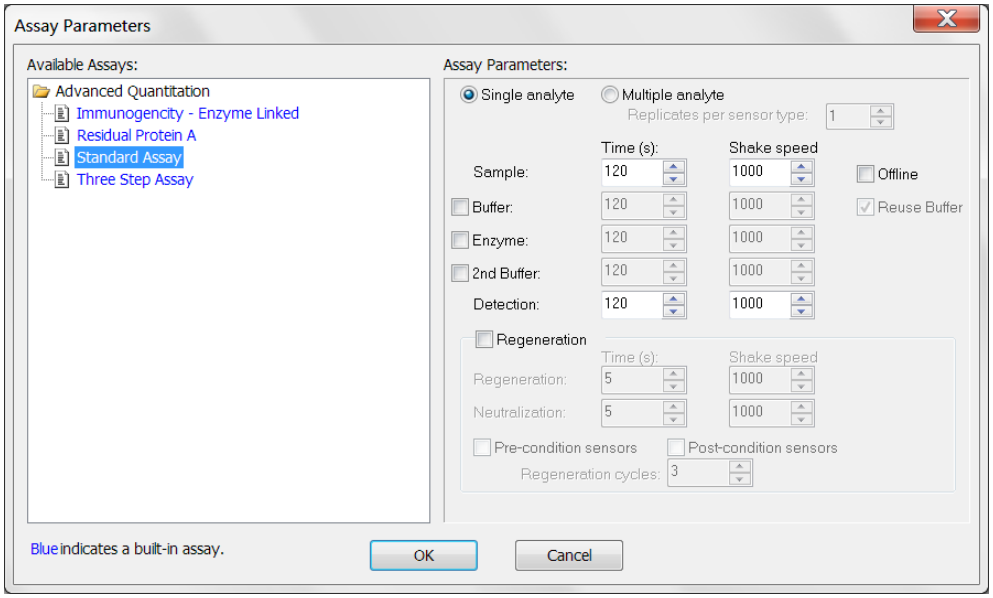


Figure 6-34: Assay Parameters—Advanced Quantitation

Table 6-8: Advanced Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.

Table 6-8: *Advanced Quantitation Assay Parameters*

Parameter	*Description*
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample platform orbital shaking speed (rotations per minute).
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample platform orbital shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample platform orbital shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay. ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.

Table 6-8: *Advanced Quantitation Assay Parameters*

Parameter	*Description*
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse. ***NOTE:*** *In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.*

ASSIGNING BIOSENSORS TO SAMPLES

After the sample plate is defined, biosensors must be assigned to the samples.

NOTE: *When using a 96-well plate with the 8 channel read head, do not put biosensors in columns 2, 4, 6, 8, 10, and 12 if the biosensors will be returned to the biosensor tray and not discarded. If the biosensors will be ejected, biosensors can be placed in all columns.*

Biosensor Assignment in Single-Analyte Experiments

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

NOTE: *For single analyte experiments, the* ***Single Analyte*** *option must be selected in the* ***Assay Parameters*** *dialog box. For more information, please see "Managing Assay Parameter Settings" on page 167.*

Click the **Sensor Assignment** tab, or click the  arrow to access the Sensor Assignment window (see Figure 6-35).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default.

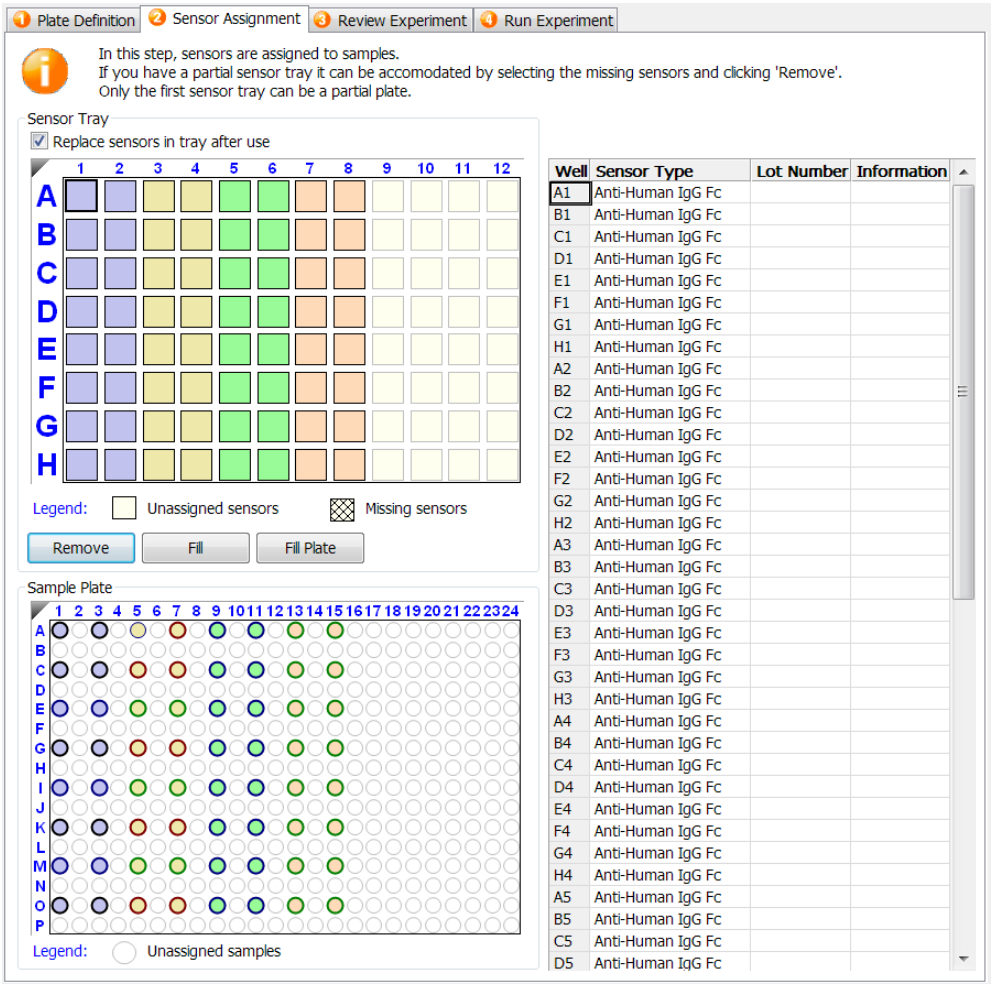


Figure 6-35: *Sensor Assignment Window for Basic Quantitation without Regeneration*

1. Assign biosensors in one of two ways:
   - Select column(s) in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (see Figure 6-35 left).
   - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 6-35 right).

   All wells in the **Sensor Type** column will automatically populate with the biosensor type selected.

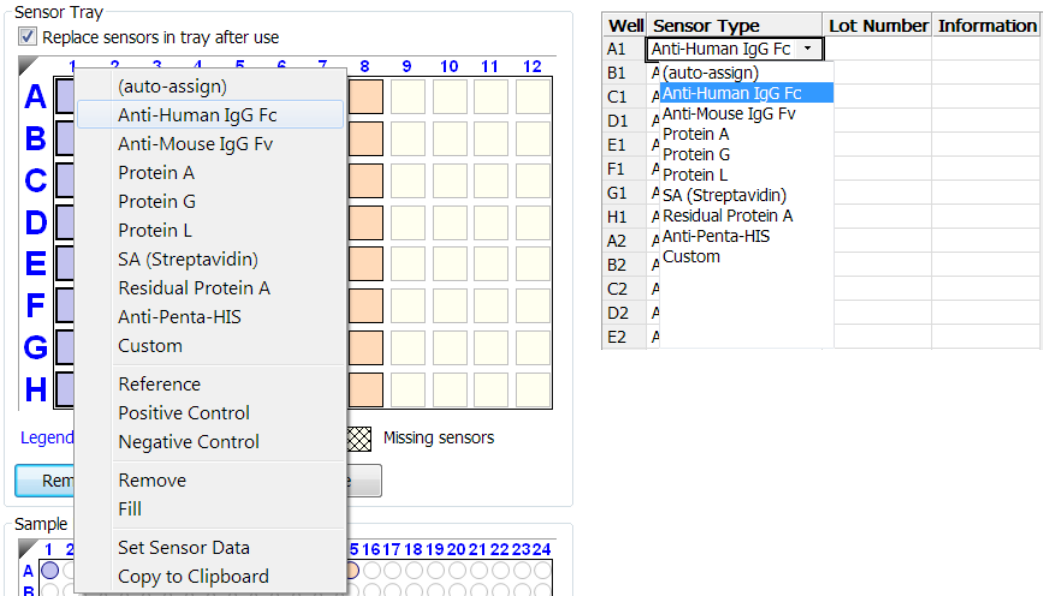


Figure 6-36: *Changing Biosensor Types in the Sensor Tray Map (left) and Sensor Type Column (right)*

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

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

---

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

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

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

5. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 6-37).

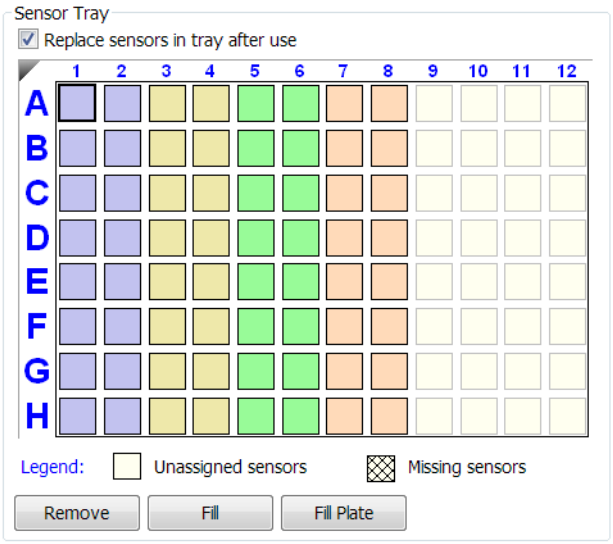


Figure 6-37: *Replace Sensors in Tray After Use Check Box*

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

Biosensor Assignment in Multiple Analyte Experiments

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

NOTE: *For multiple analyte experiments, the* ***Multiple Analyte*** *option must be selected in the* ***Assay Parameters*** *dialog box. For more information, please see "Managing Assay Parameter Settings" on page 167.*

Click the **Sensor Assignment** tab, or click the arrow to access the Sensor Assignment window (see Figure 6-35).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 6-35, **one** replicate had been previously selected with the **Multiple Analyte** assay parameter option.

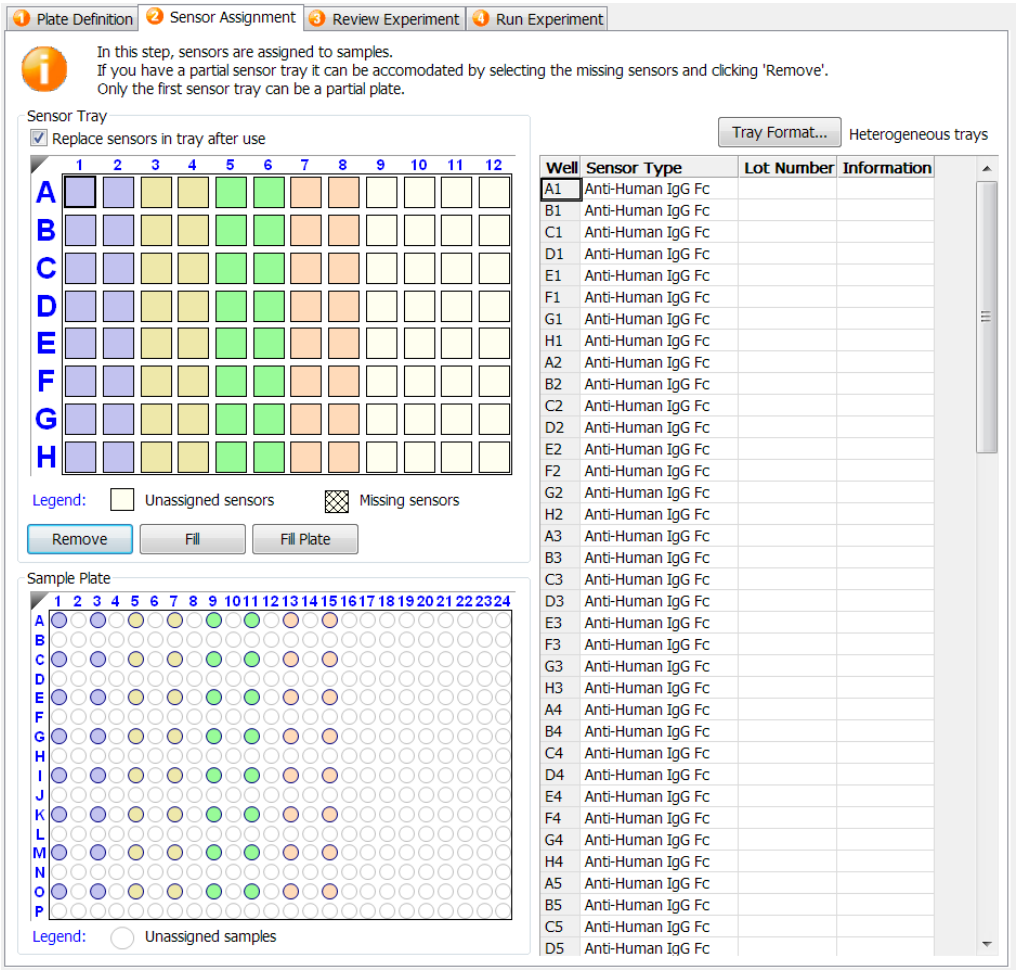


Figure 6-38: *Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option*

There are two ways to assign biosensors:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (see Figure 6-39 left).
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 6-39 right).

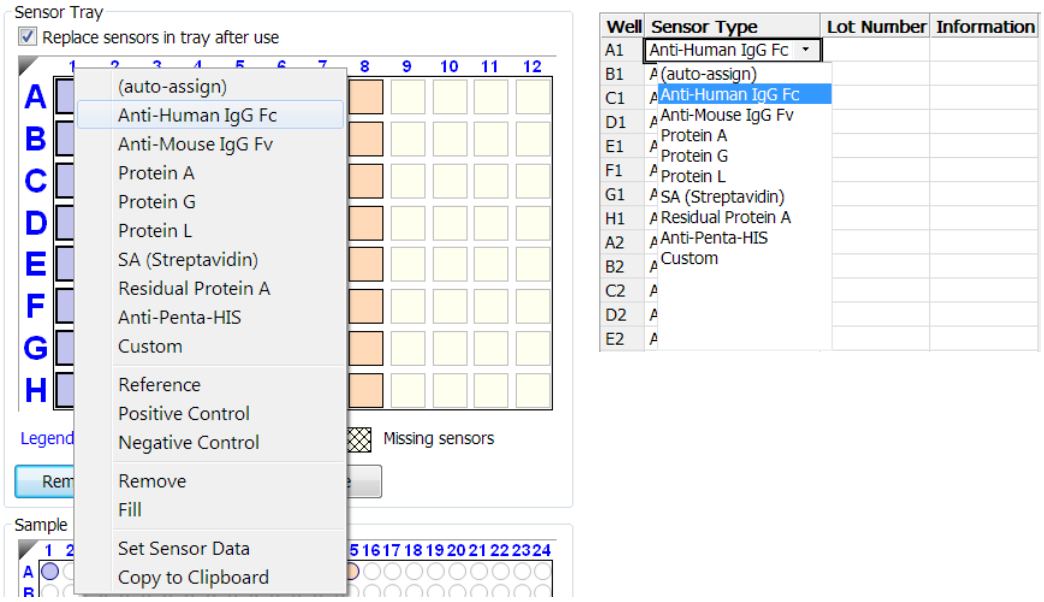


Figure 6-39: *Changing Biosensor Types in the Sensor Tray Map (left) and Sensor Type Column (right)*

Biosensor Assignment Using Heterogeneous Biosensor Trays

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

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

---

1. If Heterogeneous Trays is not displayed next to the **Tray Format** button, click the button.

   The **Tray Format** dialog box displays (see Figure 6-40).

2. Select **Heterogeneous** and click **OK.**

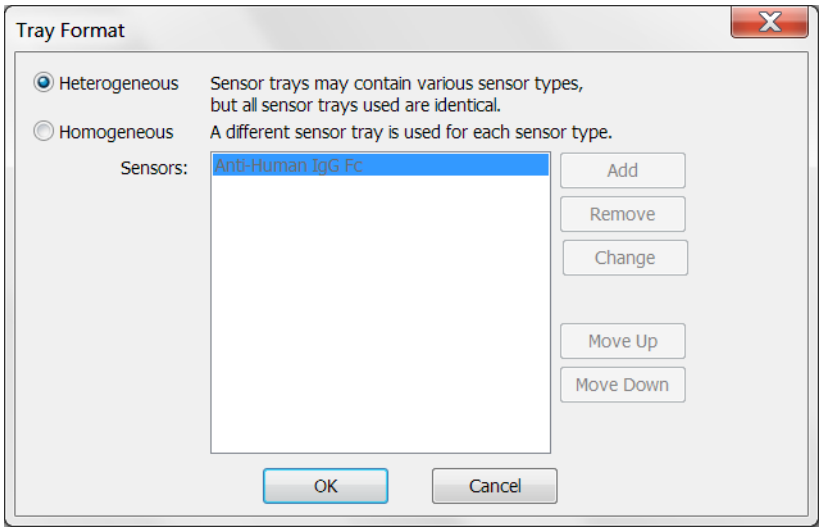


Figure 6-40: Tray Format Dialog Box

The Tray 1 **Sensor Tray Map** will be displayed by default.

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to be used (see Figure 6-41).

   The **Sensor Type** column will update accordingly.

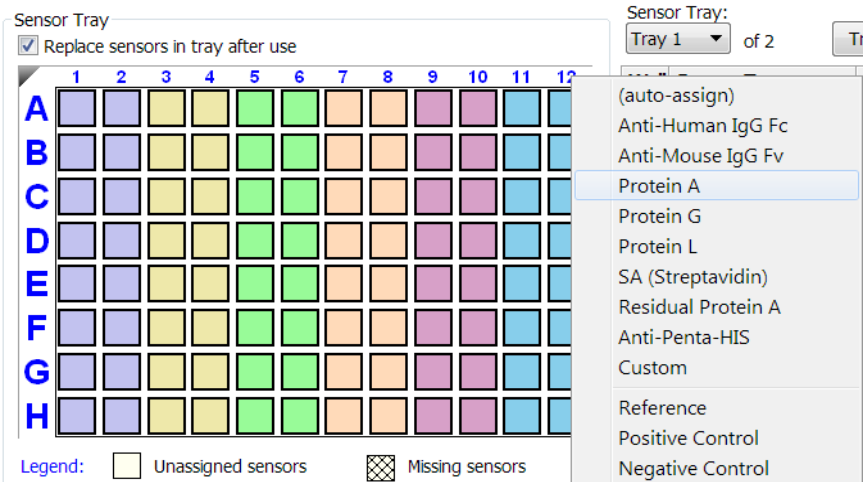


Figure 6-41: Populating the Sensor Tray Map with First Biosensor Type

4. Select the columns in the **Sensor Tray Map** that should contain the second biosensor type, right-click and select the second biosensor type (see Figure 6-43).

   The **Sensor Type** column will update accordingly.

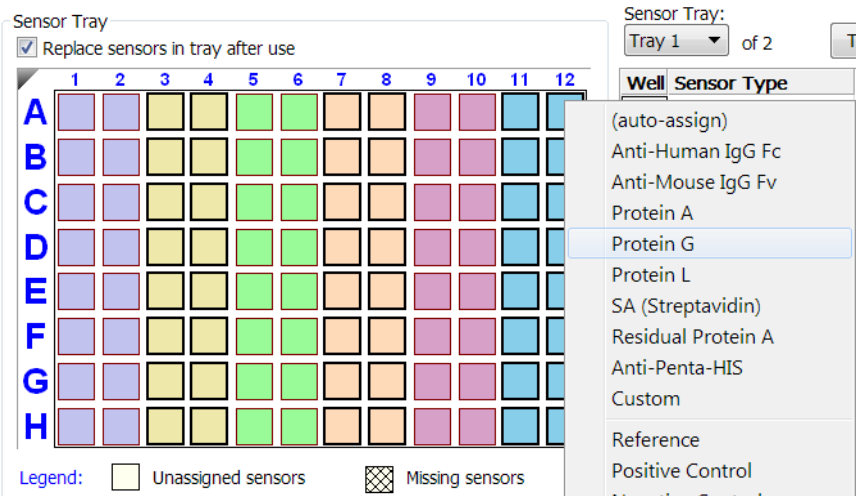


Figure 6-42: *Populating the Sensor Tray Map with Second Biosensor Type*

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

   In the example shown in Figure 6-43, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays will be needed for the experiment.

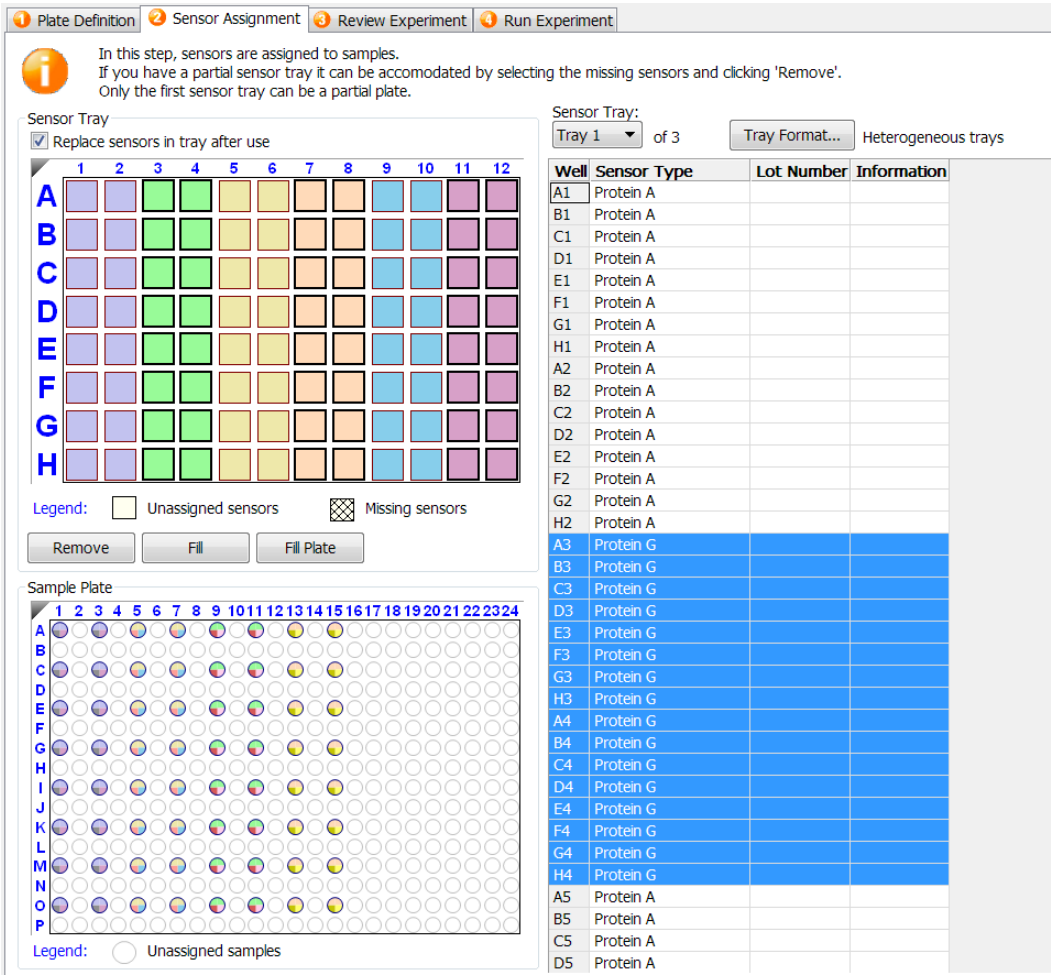


Figure 6-43: *Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types*

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

   The **Sensor Tray Map** and table for the tray selected will be shown and biosensor assignments can be changed as needed (see Figure 6-44).

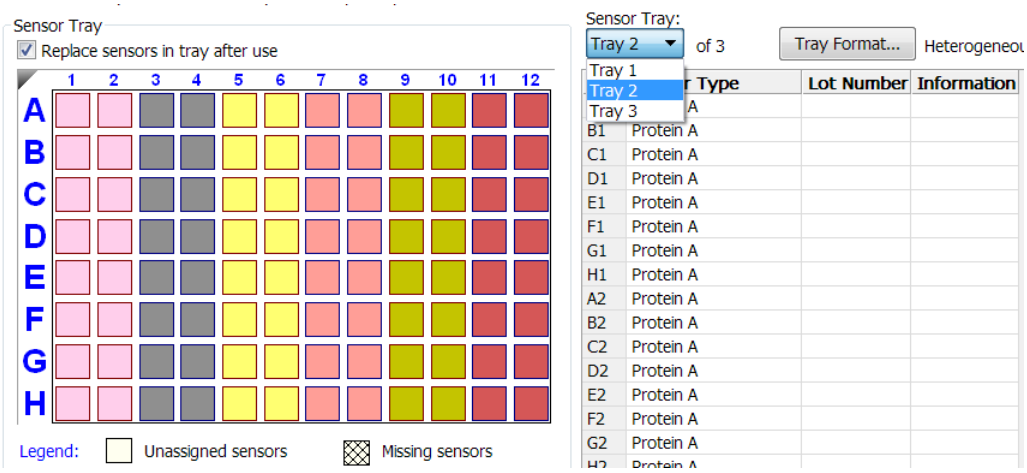


Figure 6-44: *Tray Selection*

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

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

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

---

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

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

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

---

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 6-37).

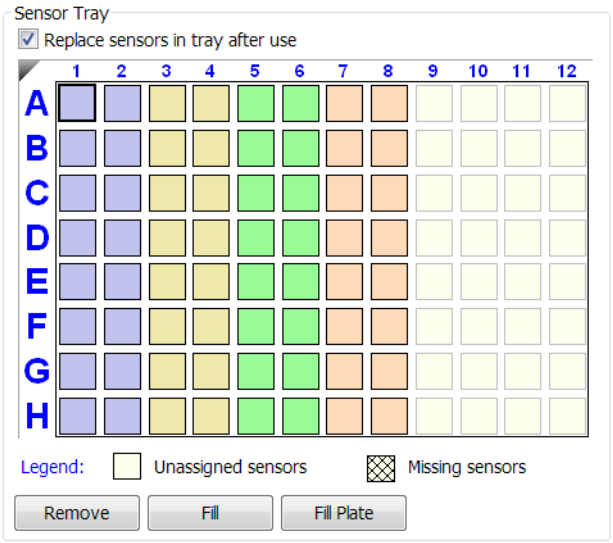


Figure 6-45: *Replace Sensors in Tray After Use Check Box*

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

Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.

NOTE: *Using the **Homogeneous** option will necessitate switching trays during the experiment.*

1. Click **Tray Format**.

   The **Tray Format** dialog box displays (see Figure 6-46) and the **Sensors** box will be populated with the default biosensor type.

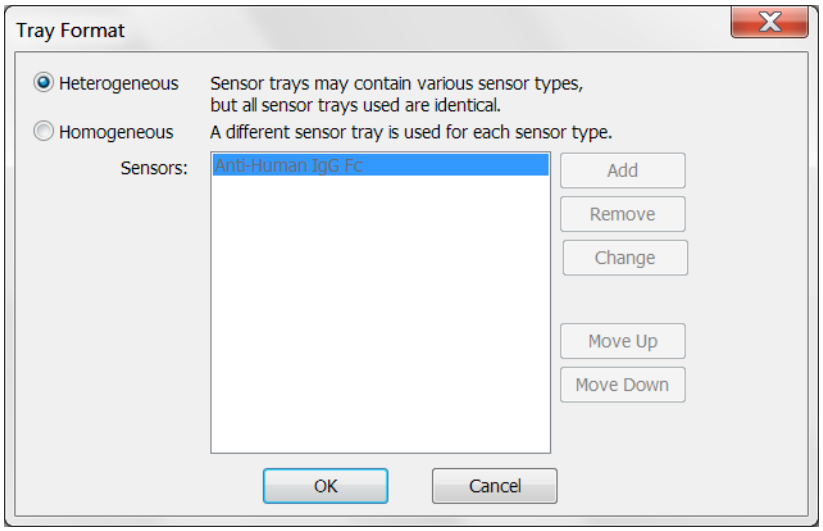


Figure 6-46: *Tray Format Dialog Box*

2. Select **Homogeneous**. Click **Add** to select the first biosensor type (see Figure 6-47).

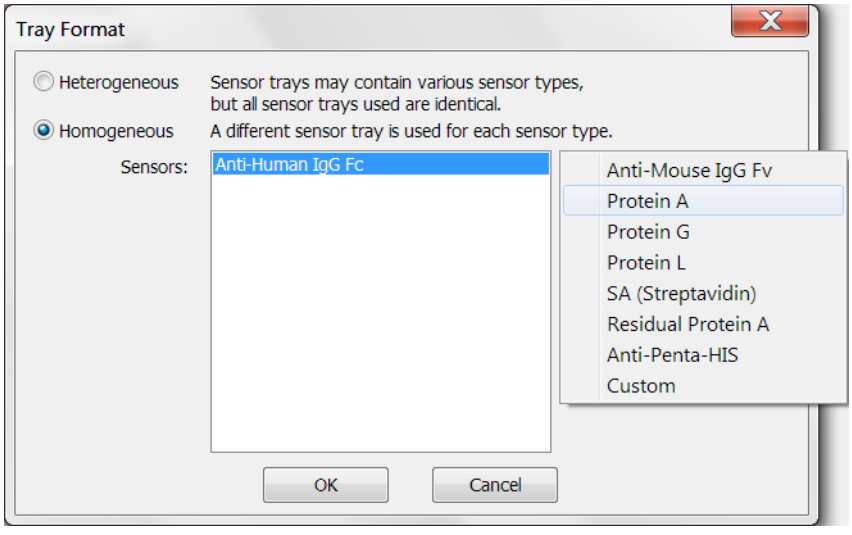


Figure 6-47: *Selecting a Biosensor Type in the Tray Format Dialog Box*

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

The order of biosensor types listed in the **Sensor** box will be used as the default tray assignment (see Figure 6-48).

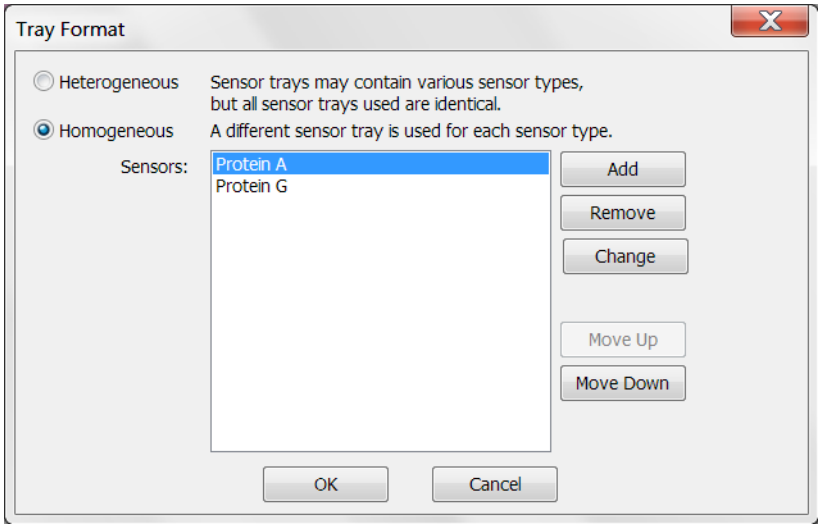


Figure 6-48: *Biosensor Types List Order in Sensor Box*

5. Click **OK**.

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

   In the example shown in Figure 6-49, Protein A and Protein G biosensor types will be used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) will be needed for the experiment. The Tray 1 **Sensor Tray Map** will be displayed by default.

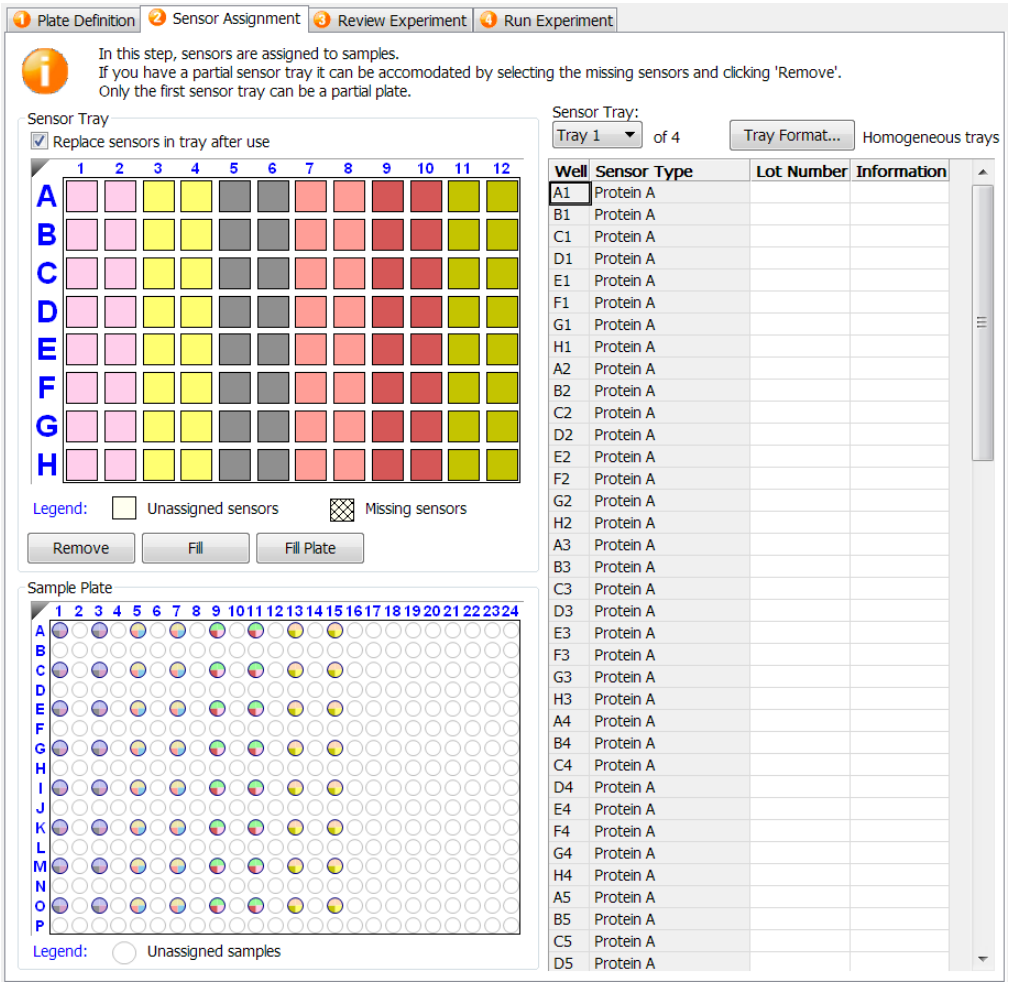


Figure 6-49: *Biosensor Assignment using Homogeneous Trays and Two Biosensor Types*

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

   The **Sensor Tray Map** and table for the tray selected will be shown (see Figure 6-44).

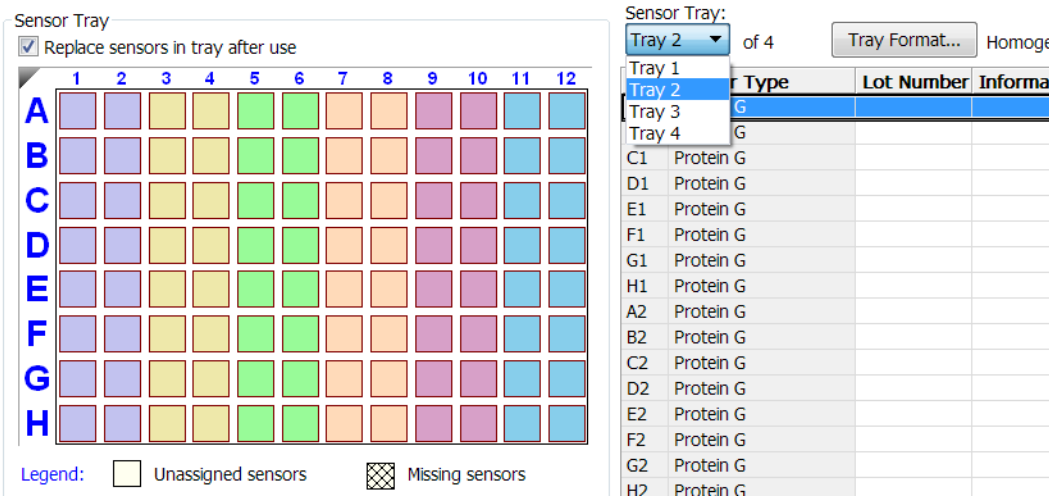


Figure 6-50: *Tray Selection*

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

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

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

---

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

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

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

---

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 6-37).

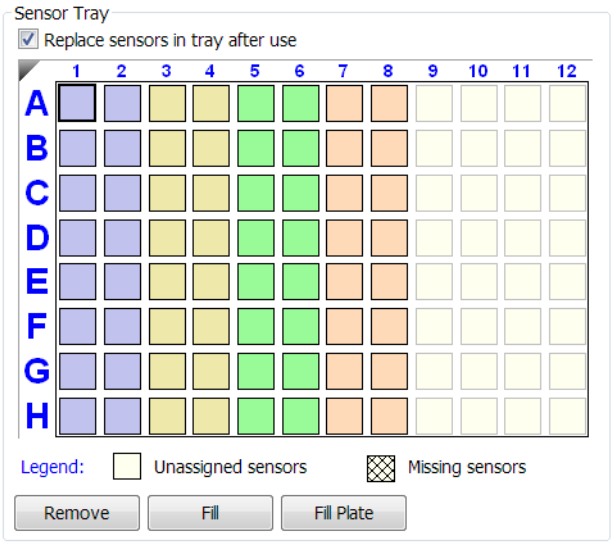


Figure 6-51: *Replace Sensors in Tray After Use Check Box*

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

Biosensor Regeneration

For Basic Quantitation with Regeneration experiments only, the **Sensor Assignment** tab includes the **Regenerations** parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The specified number of regeneration cycles determines the minimum number of cycles required for each column of sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by each column of sensors is represented by a pie chart (Figure 6-52).

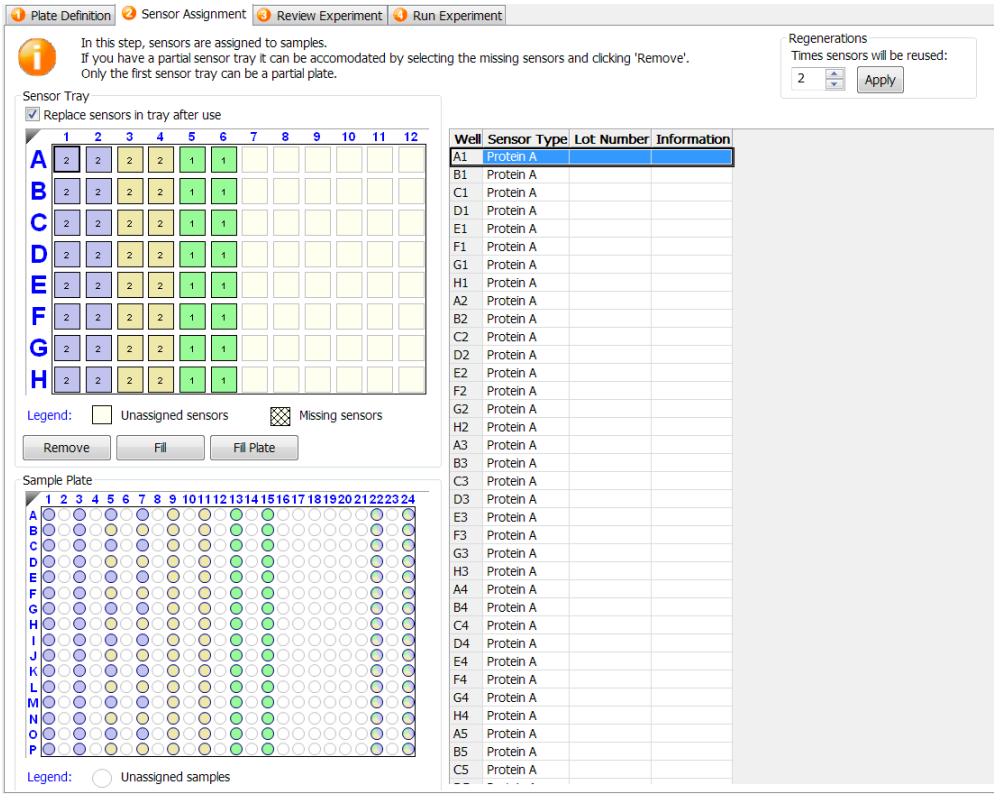


Figure 6-52: Fractional Use of Regeneration and Neutralization Wells

Using Partial Biosensor Trays

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

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

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

2. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 6-53). In the example shown, Tray 1 is a partial tray that does not contain enough biosensors for the assay. To designate a second tray, select Tray 2 from the **Sensor Tray** drop-down list (Figure 6-53 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 6-53 bottom).

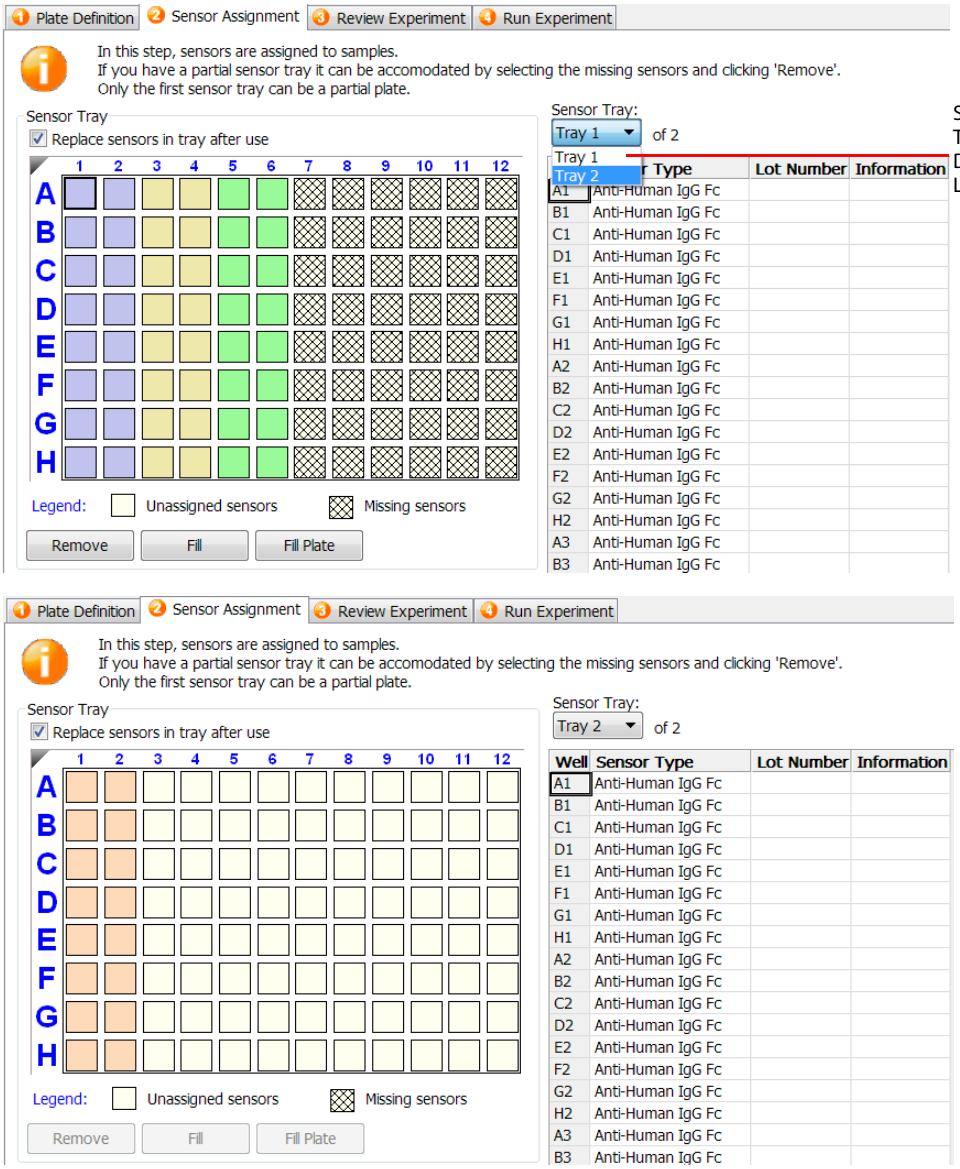


Figure 6-53: *Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray*

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

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

REVIEWING EXPERIMENTS

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

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

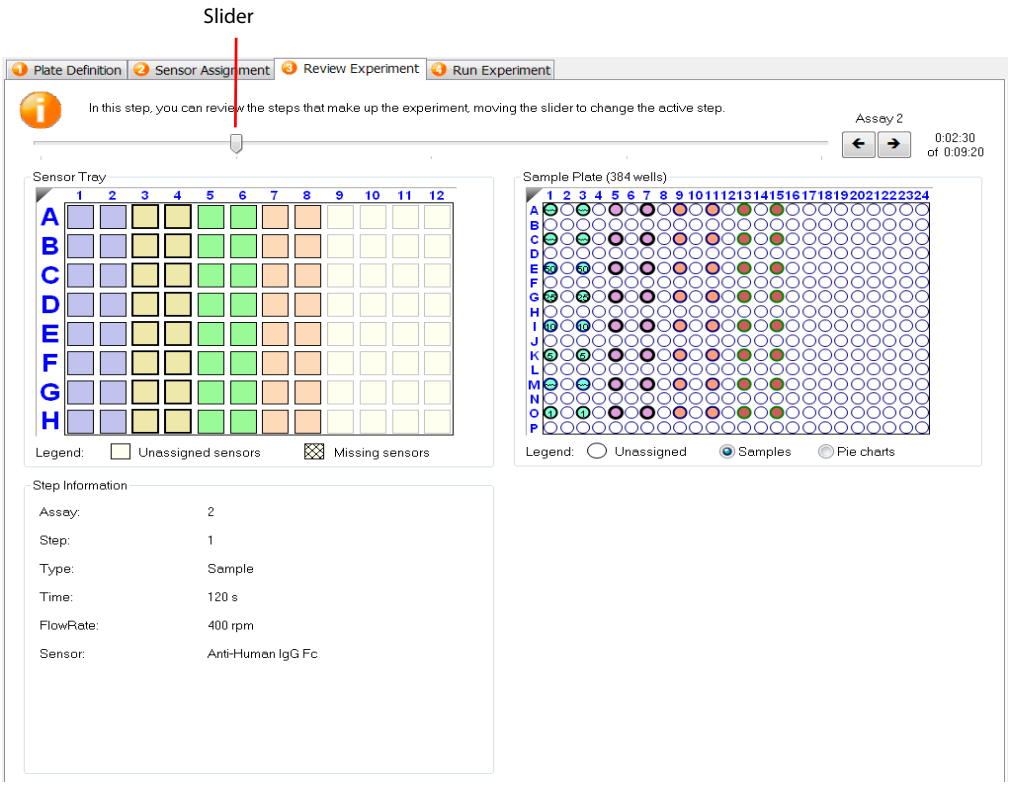


Figure 6-54: *Review Experiment Window*

SAVING EXPERIMENTS

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

To manually save an experiment method:

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

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

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, click **Experiment** > **Templates** > **Quantitation** > **Experiment Name** (see Figure 6-55).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.

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

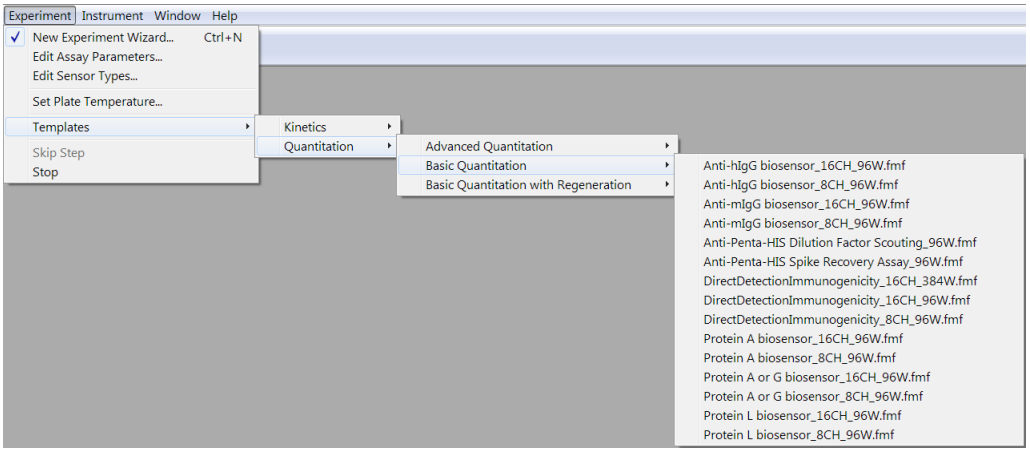


Figure 6-55: *Experiments in the Template Folder*

RUNNING A QUANTITATION EXPERIMENT

IMPORTANT: *Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare the biosensors, see the appropriate biosensor product insert.*

Loading the Biosensor Tray, Sample and Reagent Plates

To load the biosensor tray, sample plate, and reagent plate:

1. Open the Octet instrument door (lift the handle up) and present the instrument stage (click the **Present Stage** button).
2. Place the biosensor tray, sample plate, and reagent plate on the appropriate stage so that well A1 is located at the upper right corner (see Figure 6-56):
   a. Place the rehydration plate and biosensor tray on the biosensor stage (left platform).
   b. Place the sample plate on the sample stage (middle platform).
   c. Optional: Place the reagent plate on the reagent stage (right platform) if you are using a reagent plate.

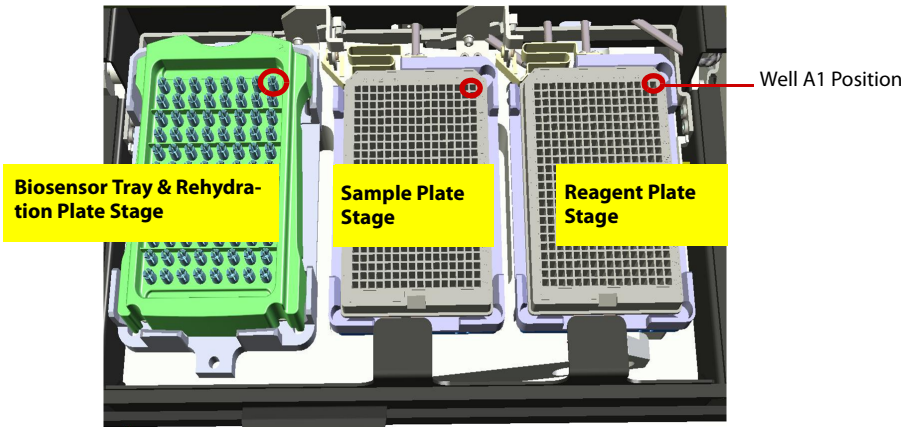


Figure 6-56: *Octet Instrument t Stage Platform*

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

3. Click to close the Octet instrument door.
4. Allow the plate to equilibrate.

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

Starting an Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow to access the Run Experiment window (see Figure 6-57).

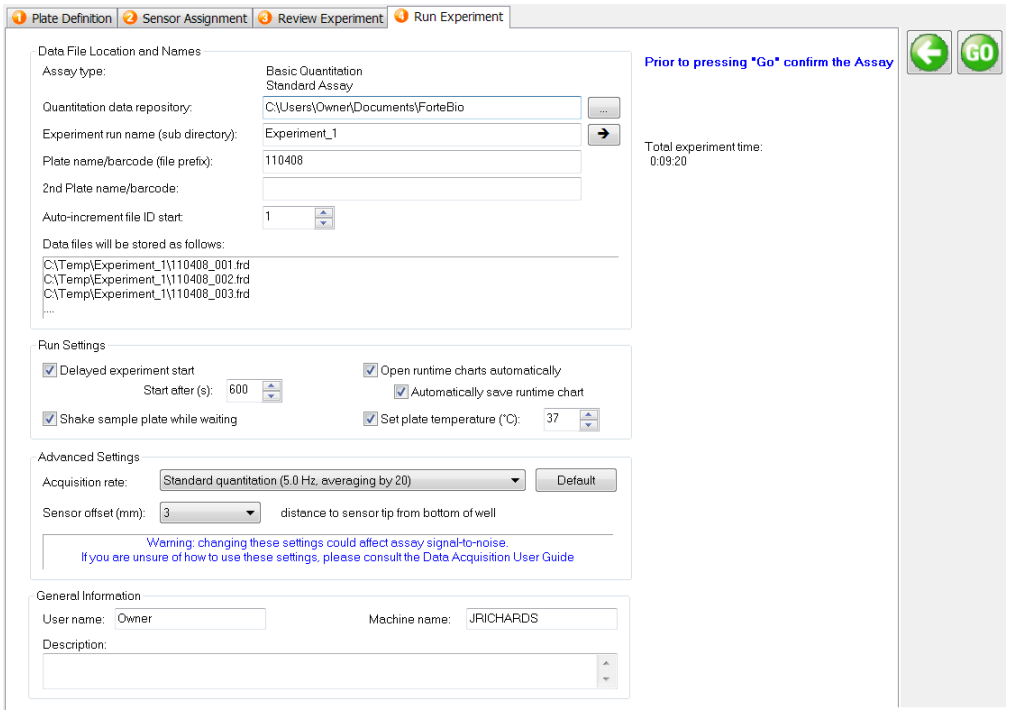


Figure 6-57: *Run Experiment Window—Octet RED384*

2. Confirm the defaults or enter new settings. See "Run Experiment Window Settings" on page 196 for more information on experimental settings.

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

---

3. To start the experiment, click GO.

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

If you selected the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time and the experiment progress (see Figure 6-58).

NOTE: *For more details about the **Runtime Binding Chart**, see "Managing Runtime Binding Charts" on page 199.*

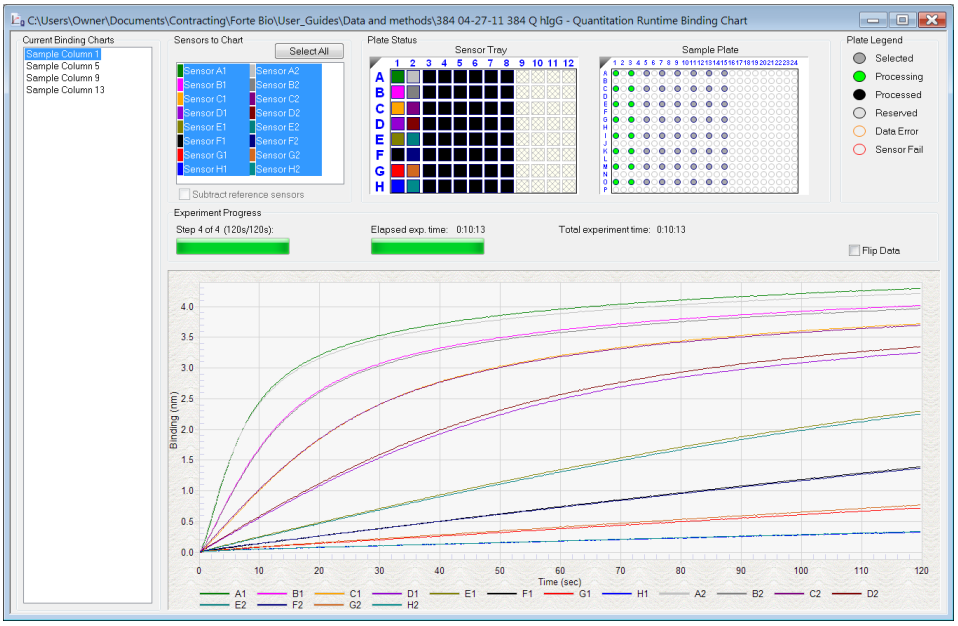


Figure 6-58: *Runtime Binding Chart*

4. Optional: Click **View** > **Instrument Status** to view the log file (see Figure 6-59).

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

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

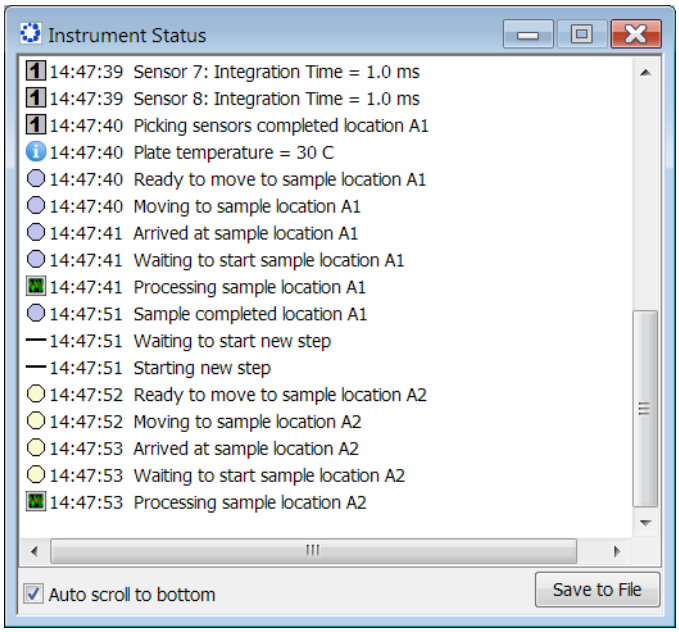


Figure 6-59: *Instrument Status Log*

Run Experiment Window Settings

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

Table 6-9: *Data File Location and Name*

Item	*Description*
Assay type	The name of the selected assay.
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click **Browse** to select another data location. ***NOTE:*** *It is recommended that you save the data to the local machine first, then transfer to a network drive.*
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software generates one data file for each biosensor.
Plate name/ barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.

Table 6-9: *Data File Location and Name (Continued)*

Item	*Description*
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

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

Table 6-10: *Run Settings*

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

Advanced settings are available for Octet RED384 and Octet QK384 systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per second and is reported in Hertz (per second). A higher acquisition rate generates more

data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

The following **Advanced Settings** are available for the Octet384 system:

Table 6-11: *Advanced Settings Octet RED384*

Item	*Description*
Acquisition rate	• High sensitivity quantitation (2.0 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second. • Standard quantitation (5.0 Hz, averaging by 20)—The average of 50 data frames is reported as one data point. Five data points are reported per second. • High concentration quantitation (10.0 Hz, averaging by 5)—The average of 5 data frames is reported as one data point. Ten data points are reported per second.
Sensor off set (mm)	Recommended sensor offset: Quantitation—3 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

The following **Advanced Settings** are available for the OctetQK384 system:

Table 6-12: *Advanced Settings Octet QK384*

Item	*Description*
Acquisition rate	• High sensitivity quantitation (0.3 Hz, averaging by 40)—The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. • Standard quantitation (0.6 Hz, averaging by 5)—The average of 5 data frames is reported as one data point. One data point is reported every 1.6 seconds.
Sensor off set (mm)	Recommended sensor offset: Quantitation—3 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

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

Table 6-13: *General Settings*

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

Stopping an Experiment

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

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

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

MANAGING RUNTIME BINDING CHARTS

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 6-60). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

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

To selectively display acquisition data for a particular acquisition step:

1. Click the corresponding **Sample Column** number.
2. Select a sub-set of sensors for a displayed column in the **Sensors to Chart** box (see Figure 6-60).

WARNING: *Do not close the* ***Runtime Binding Chart*** *window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the* ***Runtime Binding Chart*** *as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.*

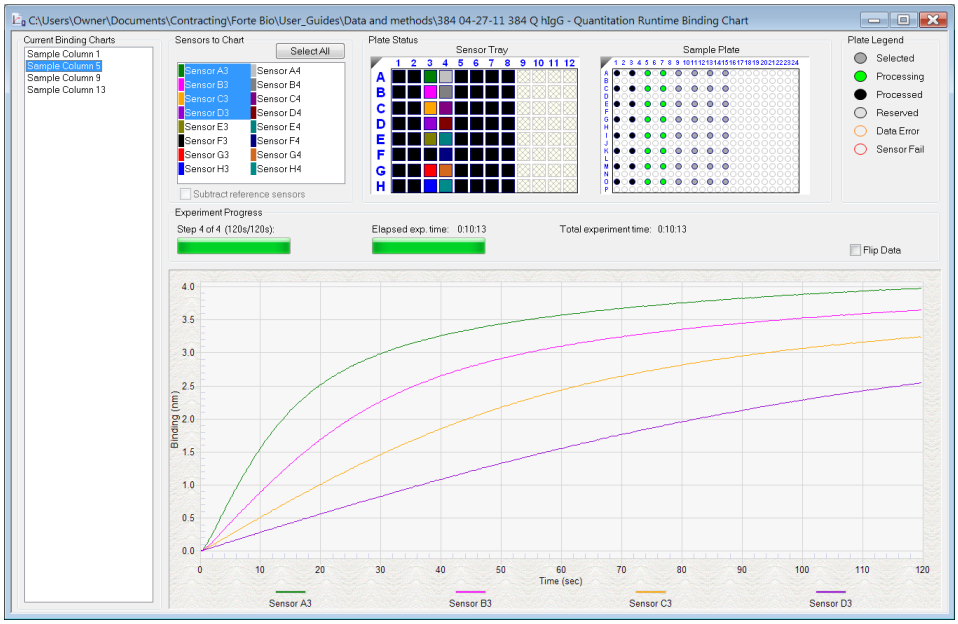


Figure 6-60: *Runtime Binding Chart Window*

Opening a Runtime Binding Chart

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

1. Click **File** > **Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data during acquisition in the chart by clicking the **Subtract reference sensors** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

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

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 6-61).

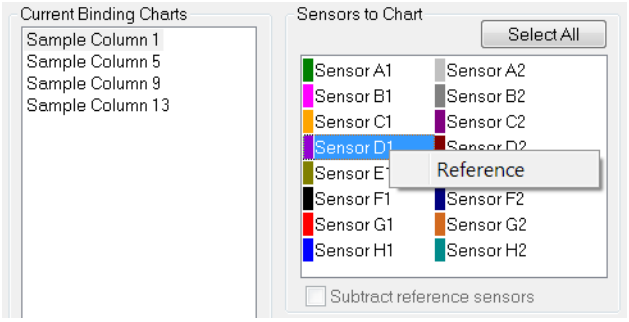


Figure 6-61: *Designating a Reference Biosensor in the Runtime Binding Chart*

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 6-64).

2. Click the **Subtract reference sensors** check box (see Figure 6-64).

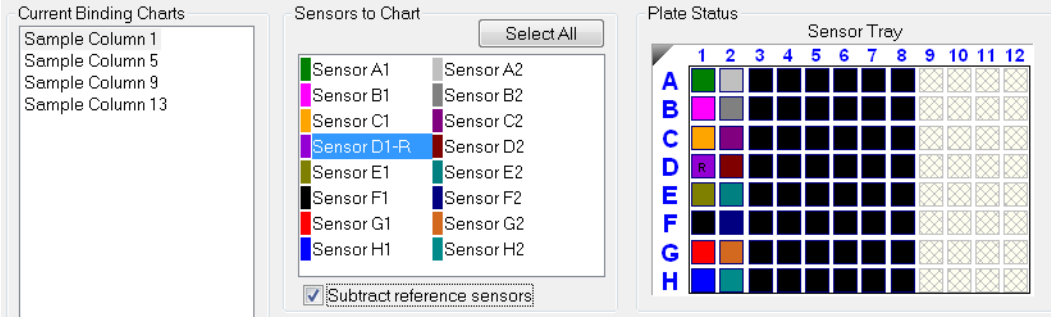


Figure 6-62: *Subtract Reference Sensors check box in the Runtime Binding Chart*

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

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 6-63). Uncheck the box to return to the default data display.

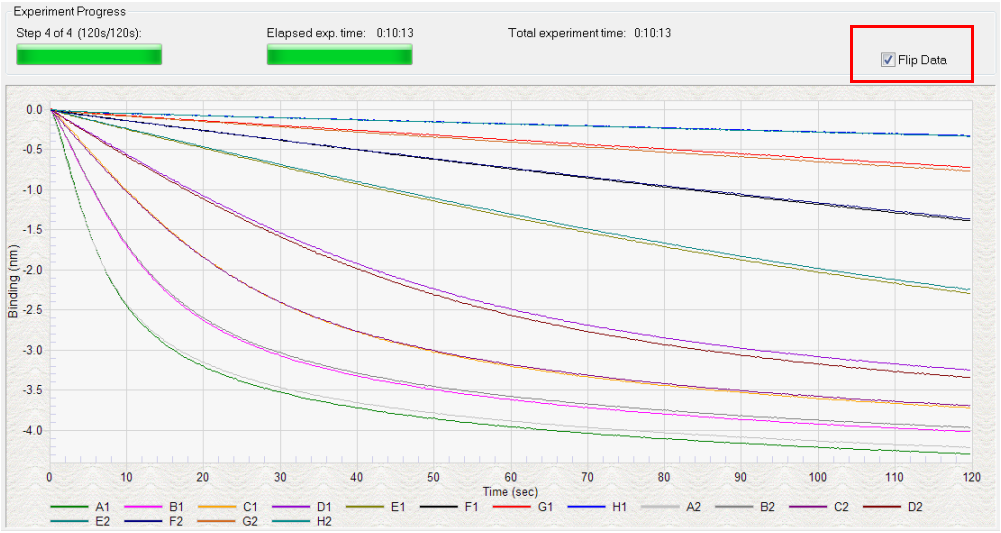


Figure 6-63: *Data Inverted Using Flip Data Function*

Magnifying the Runtime Binding Chart

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

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

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

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

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

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

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 6-64), select one of the following legends:
   - Sensor Location
   - Sample ID
   - Sensor Information
   - Concentration/Dilution

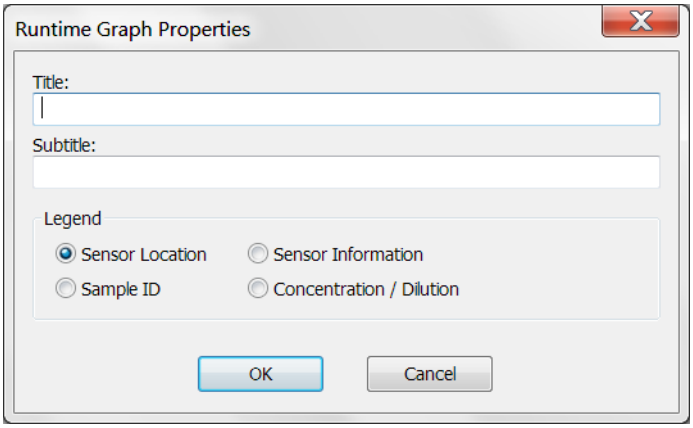


Figure 6-64: *Selecting a Runtime Binding Chart Legend*

---

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

---

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

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

Exporting or Printing the Runtime Binding Chart

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

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 6-65), select the export options and click **Export**.

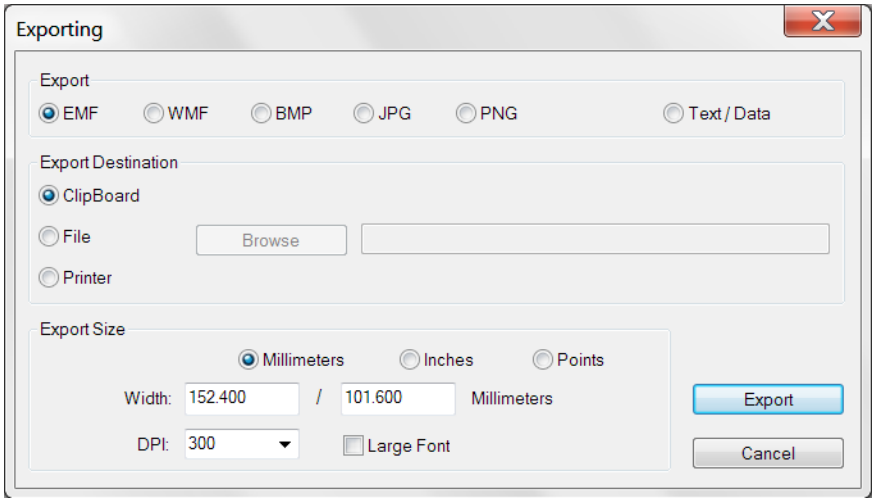


Figure 6-65: *Exporting Dialog Box*

Table 6-14: *Runtime Binding Chart Export Options*

Task	*Export*	*Option*	*Export Destination*	*Result*
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click **File** > **Browse** to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click **File** > **Browse** to select a folder and enter a file name.	Creates a graphic image.

Table 6-14: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it if necessary.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Table 6-15: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > **Open Method File** 	Enables you to select and open a method file (.fmf)
File > **Save Method File**  or 	Saves one method file or all method files. Saves a method file before the experiment is run.
File > **Save Method File As**	Saves a method file to a new name so that the original file is not overwritten.

CUSTOM QUANTITATION ASSAYS

Defining a Custom Assay

To define a custom assay:

1. Click **Experiment** > **Edit Assay Parameters**.

   The **Edit Assay Parameters** dialog box appears (see Figure 6-66).

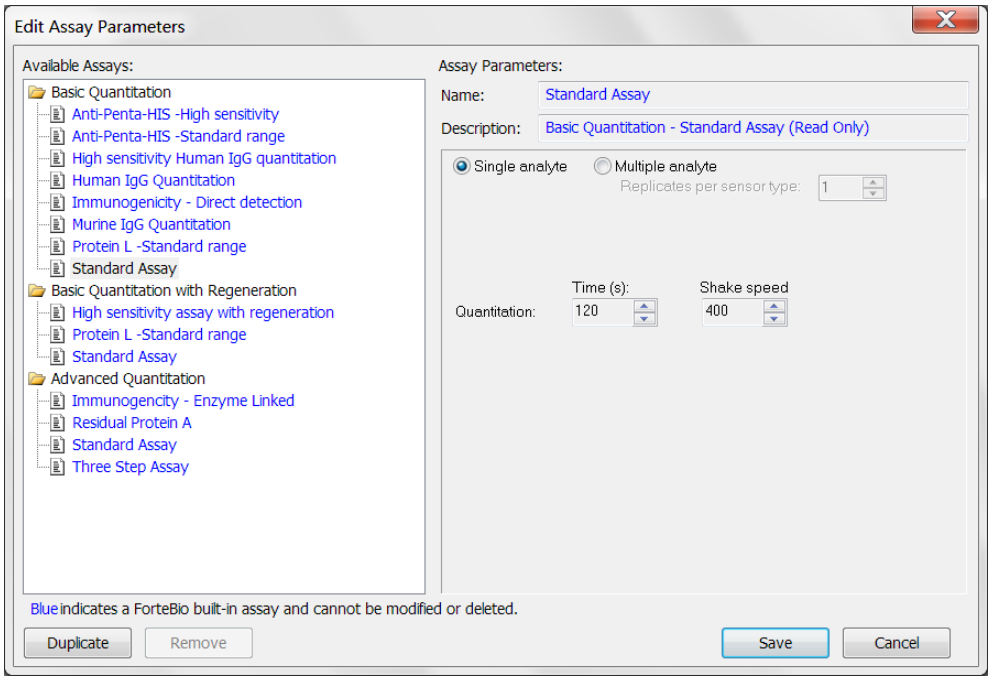


Figure 6-66: *Edit Assay Parameters Dialog Box*

2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
3. Click **Duplicate**.
4. In the **New Assay** dialog box (see Figure 6-67 top), enter an **Assay name**.
5. Optional: In the **Assay Description**, enter information about the assay.
6. Click **Save**.

   The new assay appears in the directory tree of available assays (see Figure 6-67 bottom).

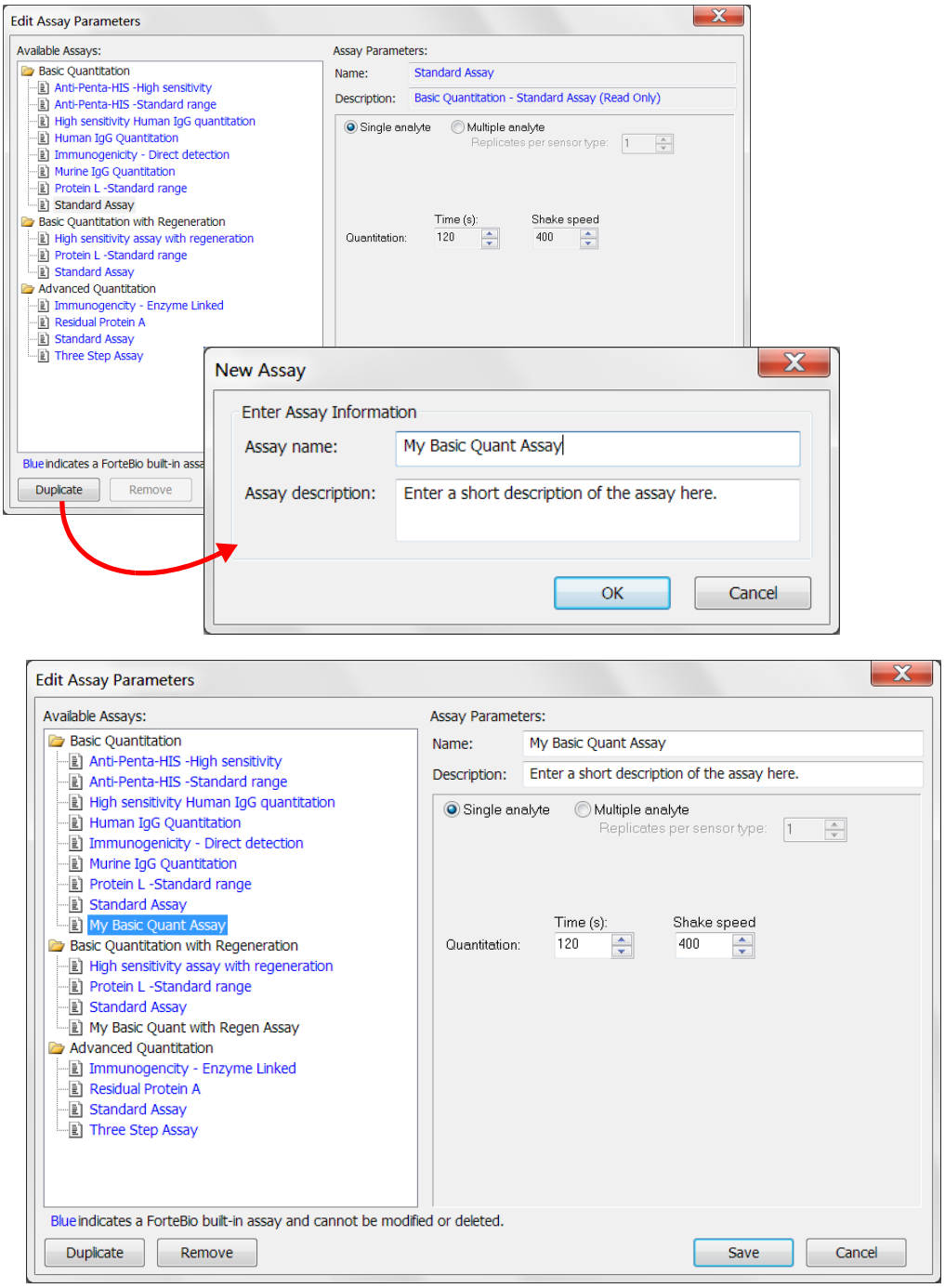


Figure 6-67: *Defining a New Assay*

Editing Assay Parameters

To edit assay parameters:

1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 6-67 bottom).
2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
3. Click **Save** to accept the new parameter values. The new assay is added to the system.

NOTE: *Not all parameters are available for all of the assays.*

Basic Quantitation Assay Parameters

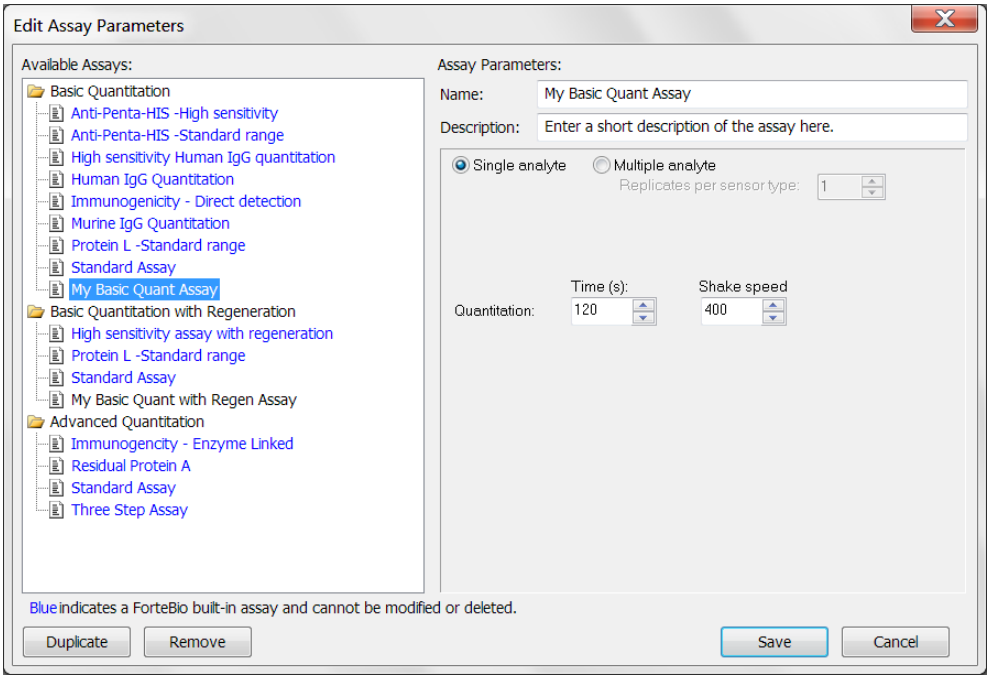


Figure 6-68: *Assay Parameters—Basic Quantitation Assay*

Table 6-16: *Basic Quantitation Assay Parameters*

Parameter	*Description*
Single analyte	For single-analyte experiments using only one biosensor type per sample well.

Table 6-16: *Basic Quantitation Assay Parameters (Continued)*

Parameter	*Description*
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample. ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Quantitation Shake speed (rpm)	The sample platform orbital shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

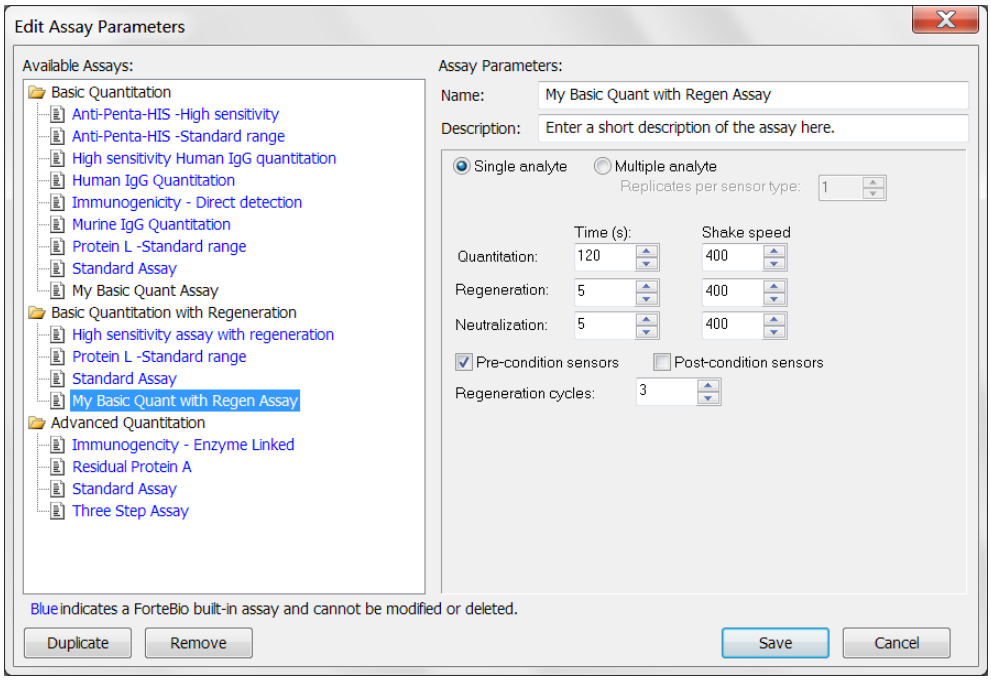


Figure 6-69: *Assay Parameters—Basic Quantitation with Regeneration*

Table 6-17: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	*Description*
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 6-17: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	*Description*
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

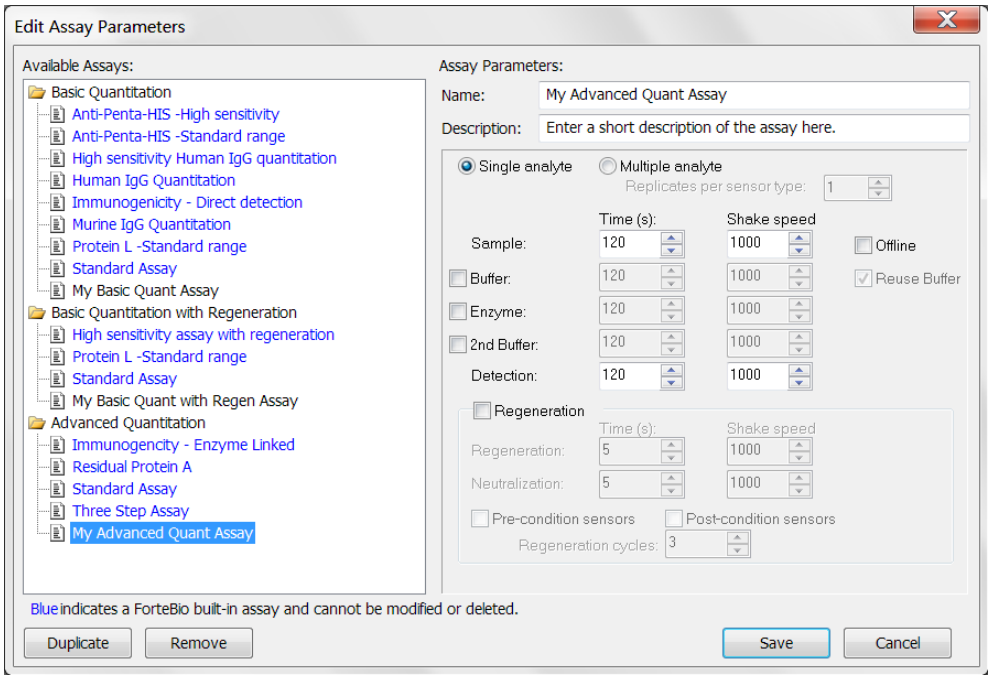


Figure 6-70: *Assay Parameters—Advanced Quantitation*

Table 6-18: *Advanced Quantitation Assay Parameters*

Parameter	*Description*
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample platform orbital shaking speed (rotations per minute). ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample platform orbital shaking speed (rotations per minute).

Table 6-18: *Advanced Quantitation Assay Parameters*

Parameter	*Description*
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample platform orbital shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample platform orbital shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay. ***NOTE:*** *A subset of data points may be selected for processing during data analysis.*
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.
Post-condition sensors	Post-conditions biosensors after Basic Quantitation with Regeneration, allowing re-racked biosensors to be stored in a regenerated state.

Table 6-18: *Advanced Quantitation Assay Parameters*

Parameter	Description
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse. ***NOTE:*** *In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.*

Selecting a Custom Assay

You can select a custom assay when you define a sample plate.

To select a custom assay:

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

   The **Edit Assay Parameters** dialog box displays (see Figure 6-71).

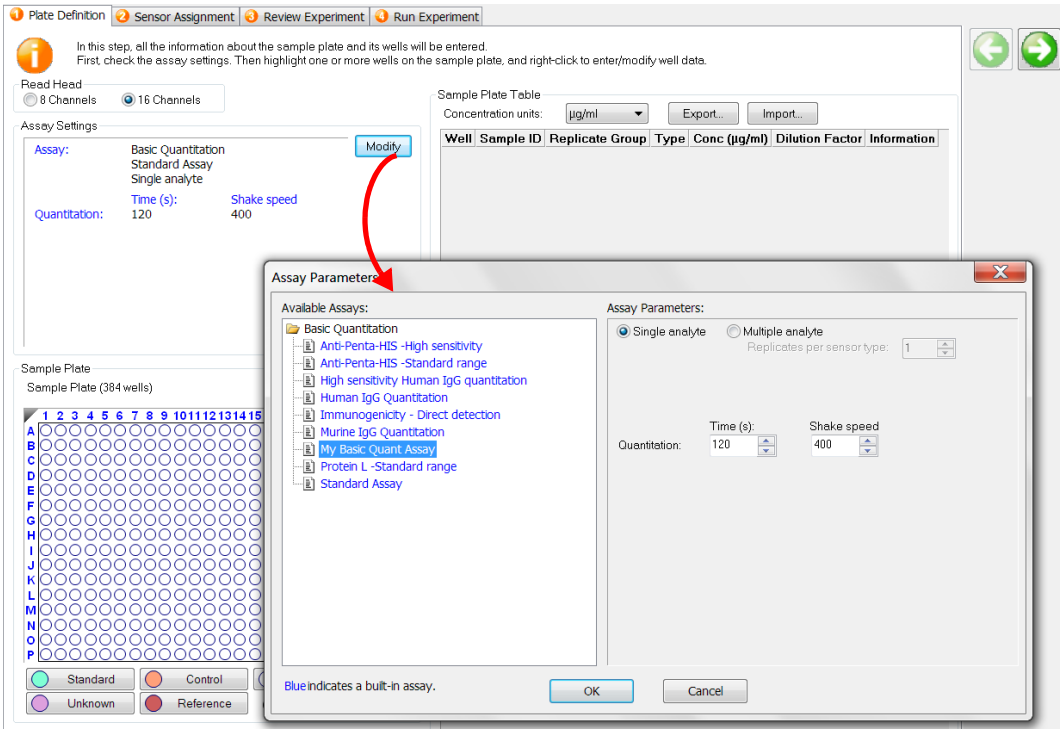


Figure 6-71: *Selecting a Custom Assay*

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

CHAPTER 7:

Kinetics Experiments: Octet RED96, QK^e and QK

INTRODUCTION

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 7-1) to set up and analyze a quantitation experiment.

Table 7-1: *Setting Up and Analyzing a Kinetic Experiment*

Software	*Step*	*See*
Data Acquisition 	1. Select a kinetics experiment in the **Experiment Wizard** or open a method file (.fmf).	"Starting a Basic Kinetics Experiment" on page 217
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 218
	3. Specify assay steps.	"Defining a Kinetic Assay" on page 236
	4. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 249
	5. Run the experiment.	"Running a Kinetics Experiment" on page 260
Data Analysis 	6. View and process the raw data. 7. Analyze the data.	*Octet System Data Analysis Software User Guide*

NOTE: *Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the* ***Options*** *window when the software is relaunched.*

STARTING A BASIC KINETICS EXPERIMENT

You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see "Managing Experiment Method Files" on page 274.
- On the menu bar, click **Experiment** > **Templates** > **Kinetics**.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Starting an Experiment Using the Experiment Wizard

1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button , or click **Experiment** > **New Experiment Wizard** (**Ctrl**+**N**) from the **Main Menu**.
2. In the **Experiment Wizard**, click **New Kinetics Experiment** (see Figure 7-1, left).
3. Click the arrow button( ). The **Basic Kinetics Experiment** window displays (Figure 7-1, right).

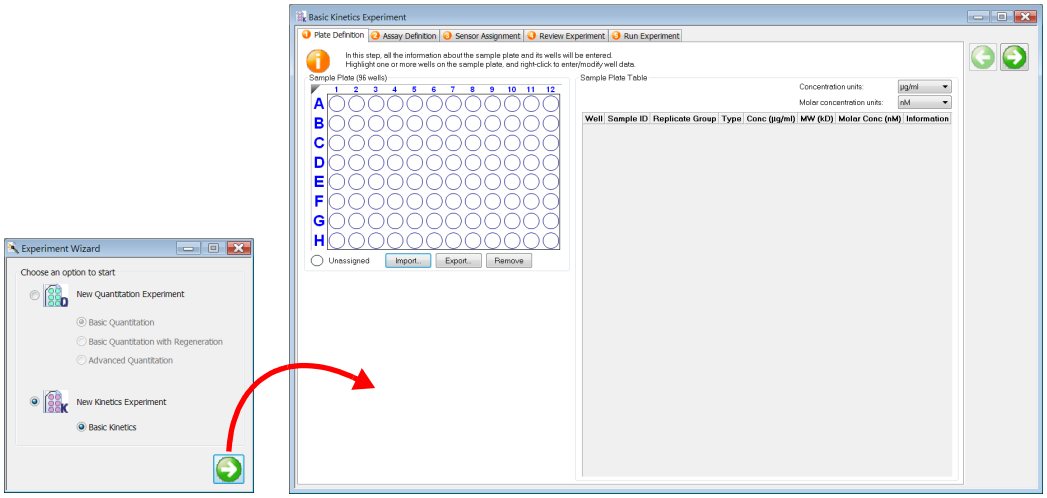


Figure 7-1: *Starting a Kinetics Experiment with the Experiment Wizard*

DEFINING THE SAMPLE PLATE

The steps to define a sample plate include:

Step	See Page
4. Designate the samples.	218
5. Save the sample plate definition (optional).	232

Designating Samples

NOTE: *It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 7-2 will be included in the assay.*

Table 7-2 displays the well types that can be assigned to a plate map.

Table 7-2: *Types of Sample Wells*

Icon	Description
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.
Controls	A control sample, either positive or negative, of known analyte composition. • Positive Control: A control sample that contains analyte of known concentration • Negative Control: A control sample known not to contain analyte
Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
Activation	Activation reagent. Makes the biosensor competent for binding.
Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
Load	Ligand to be immobilized (loaded) on the biosensor surface.
Wash	Wash buffer.
Regeneration	Regeneration reagents dissociate the analyte from the ligand.

Selecting Wells in the Sample Plate Map

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

- Click a column header or select adjacent column headers by click-hold-drag. To select non-adjacent columns, hold the **Ctrl** key and click the column header (Figure 7-2 left).
- Click a row header or select adjacent row headers by click-hold-drag (Figure 7-2, center).
- Click a well or draw a box around a group of wells (Figure 7-2, right).

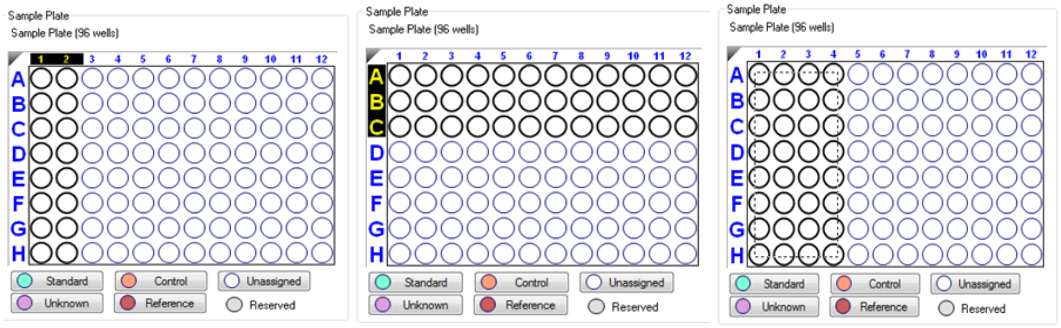


Figure 7-2: *Selecting Wells in the Sample Plate Map*

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

Designating Well Types

In the **Sample Plate Map**, select the wells, right-click and select a sample type (see Figure 7-24).

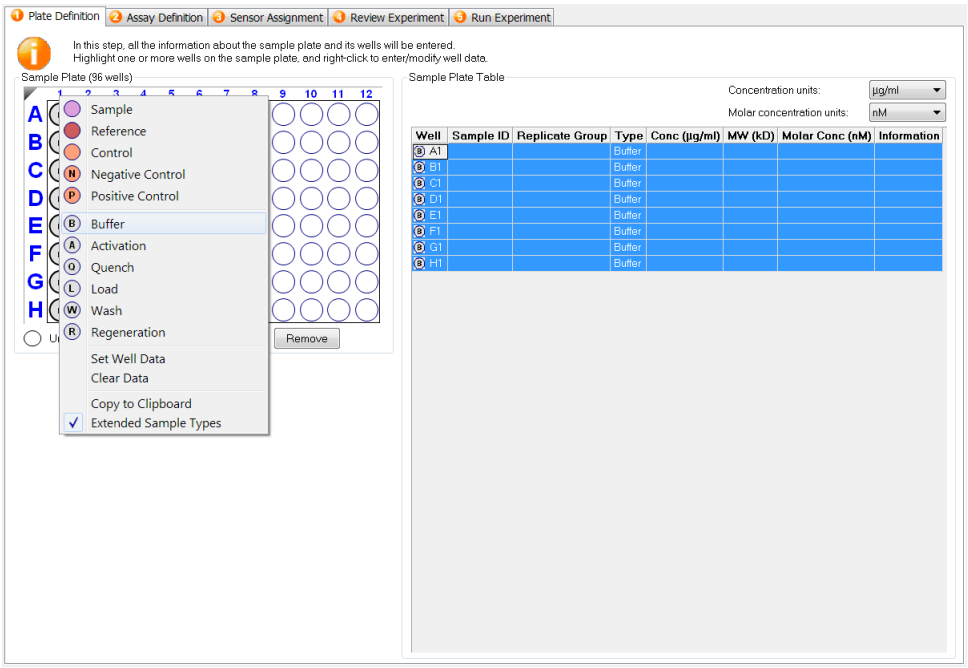


Figure 7-3: *Designating a Well Type in the Plate Definition Window*

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 7-4).

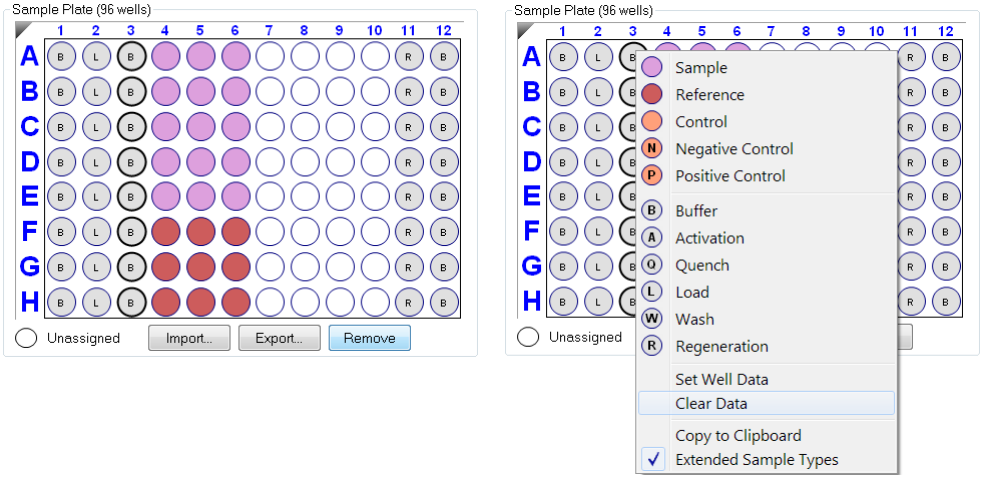


Figure 7-4: *Clearing Sample Data from a Sample Plate*

Entering Sample Information

NOTE: *You must specify sample (analyte) concentration and molecular weight, otherwise the Octet System Data Acquisition software cannot compute a K_D value. If the sample concentration is not specified, only k_d and k_{obs} are calculated. You can also annotate any well with* ***Sample ID*** *or* ***Well Information,*** *and assign* ***Replicate Groups****.*

Assigning Molecular Weight and Molar Concentration

1. In the **Sample Plate Map**, select the sample wells, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box, enter the analyte molecular and molar concentration (Figure 7-5).

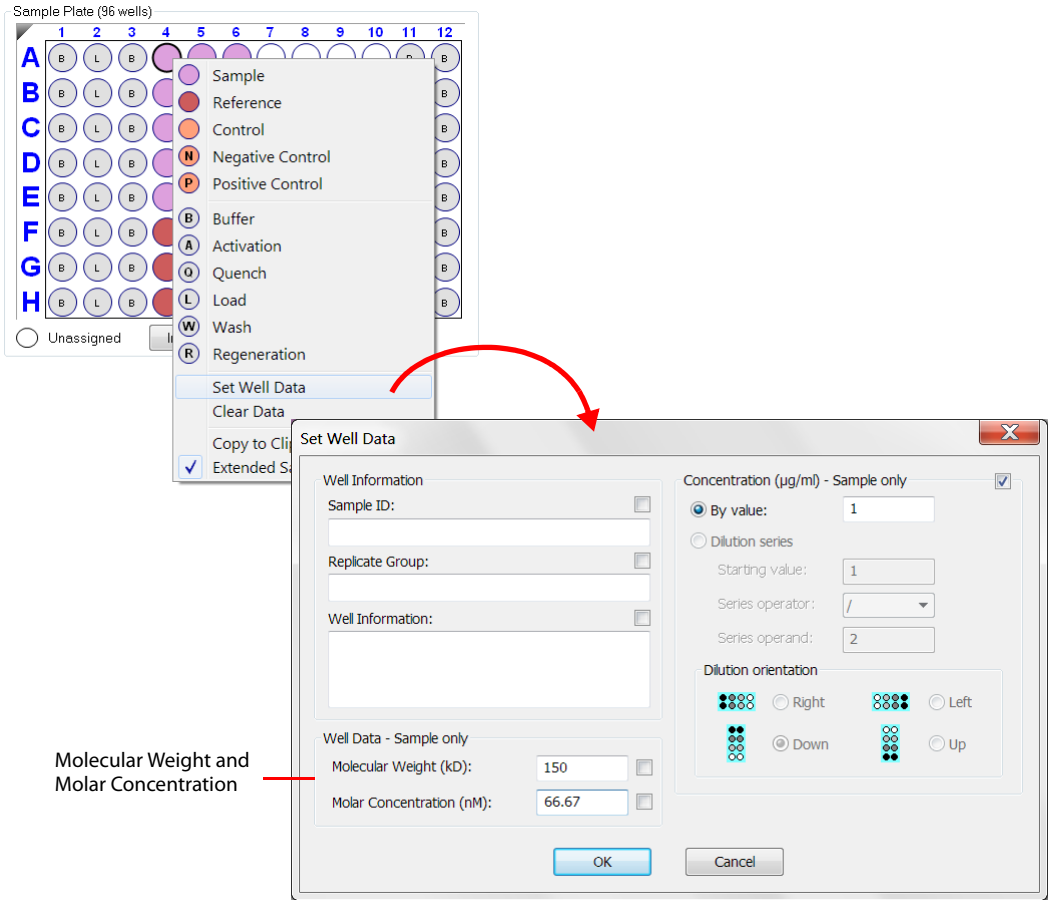
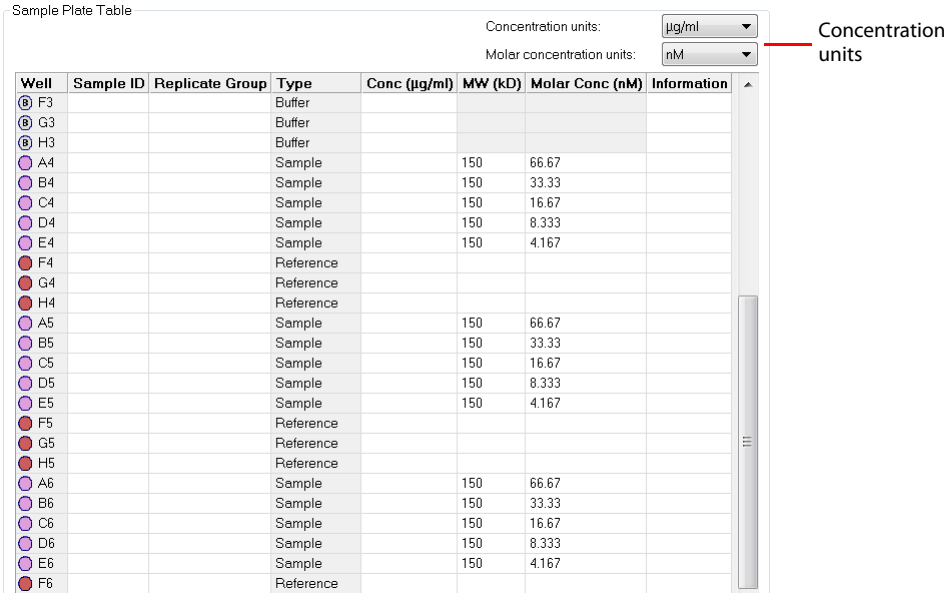


Figure 7-5: *Entering Molecular Weight and Molar Concentration from the Sample Plate Map*

The information displays in the **Sample Plate Table** (see Figure 7-6).

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



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
F3			Buffer				
G3			Buffer				
H3			Buffer				
A4			Sample		150	66.67	
B4			Sample		150	33.33	
C4			Sample		150	16.67	
D4			Sample		150	8.333	
E4			Sample		150	4.167	
F4			Reference				
G4			Reference				
H4			Reference				
A5			Sample		150	66.67	
B5			Sample		150	33.33	
C5			Sample		150	16.67	
D5			Sample		150	8.333	
E5			Sample		150	4.167	
F5			Reference				
G5			Reference				
H5			Reference				
A6			Sample		150	66.67	
B6			Sample		150	33.33	
C6			Sample		150	16.67	
D6			Sample		150	8.333	
E6			Sample		150	4.167	
F6			Reference				

Figure 7-6: *Entering Molecular Weight and Molar Concentration from the Plate Table*

Assigning User Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the desired wells, right-click and select **Set Well Data.**The **Set Well Data** dialog box displays (see Figure 7-7).
2. Select the **By value** option and enter the starting concentration value.

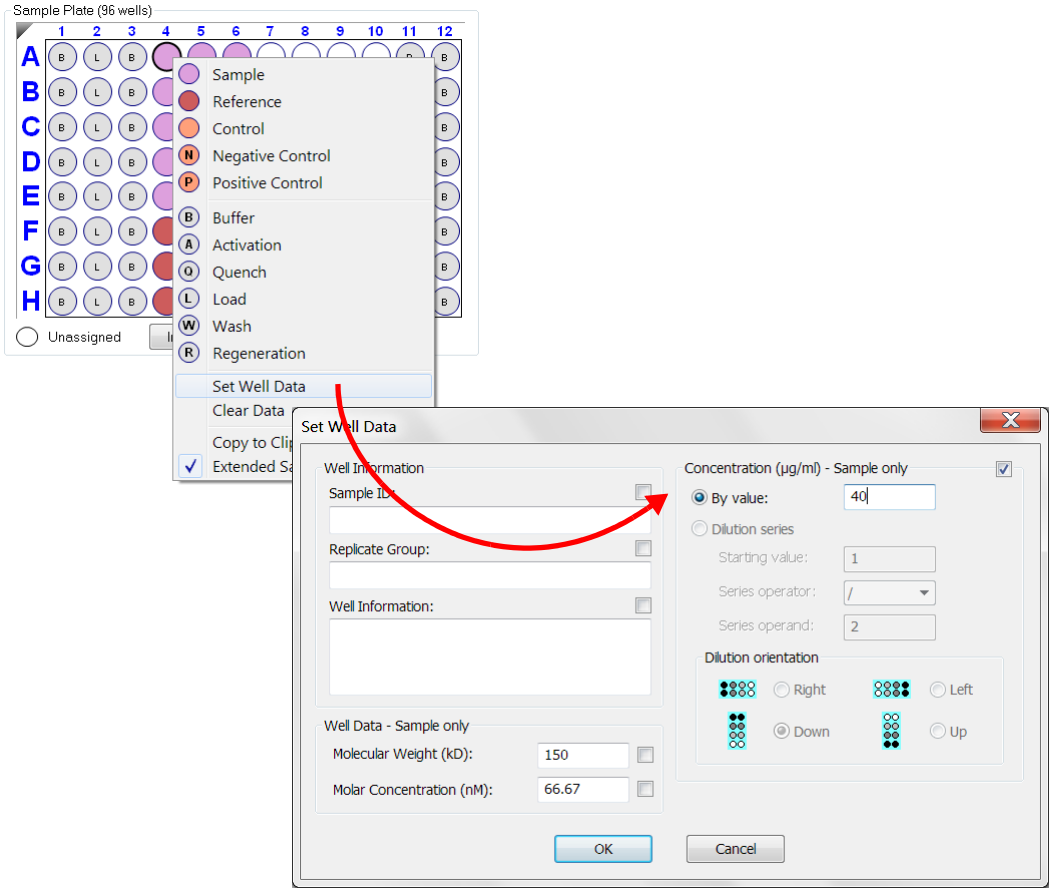


Figure 7-7: *Sample Plate Map—Assigning Sample Concentrations by Value*

3. Click **OK**. The **Sample Plate Table** will display the entered concentration.

Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

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

   The **Set Well Data** dialog box displays (see Figure 7-8)

2. Select the **Dilution Series** option and enter the starting concentration value.

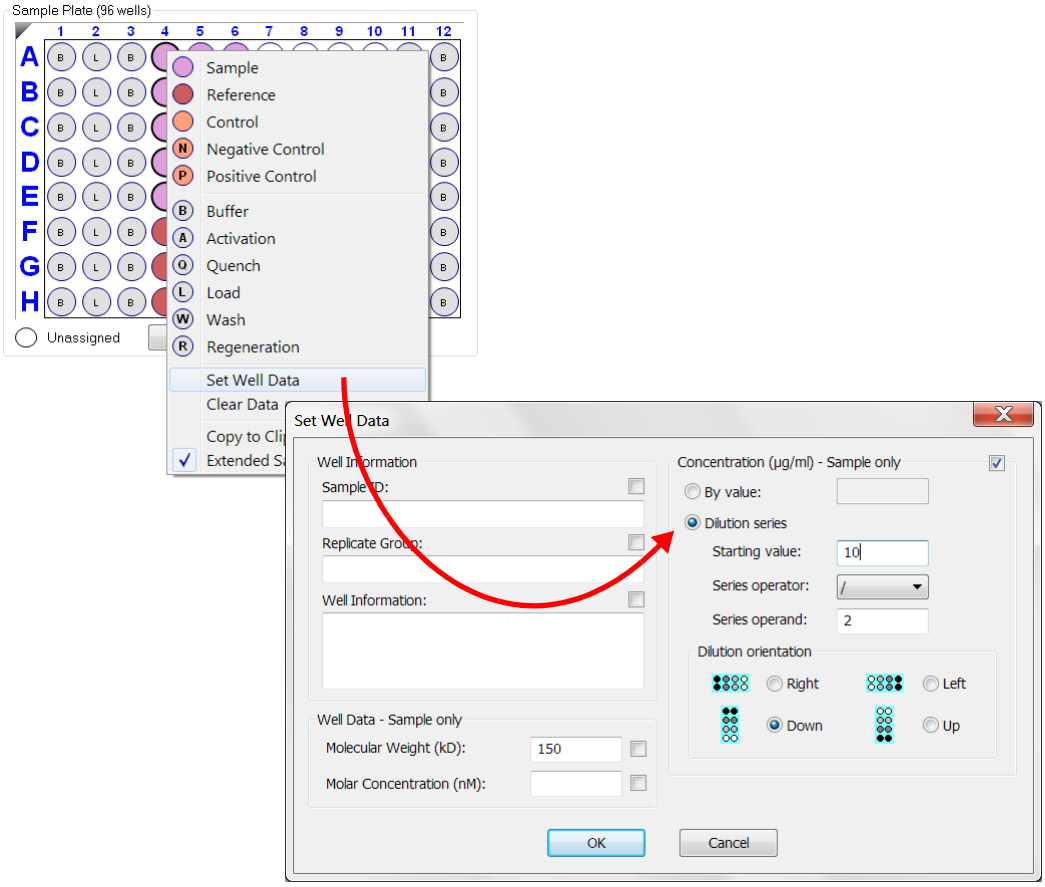


Figure 7-8: *Sample Plate Map—Assigning Sample Concentrations Using Dilution Series*

3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 7-9).

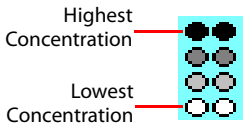


Figure 7-9: *Concentration Representation in Dilution Series*

4. Click **OK**.

   The **Sample Plate Table** displays the standard concentrations.

Annotating Samples

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

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 7-10), enter the **Sample ID** and/or **Well Information** and click **OK**.

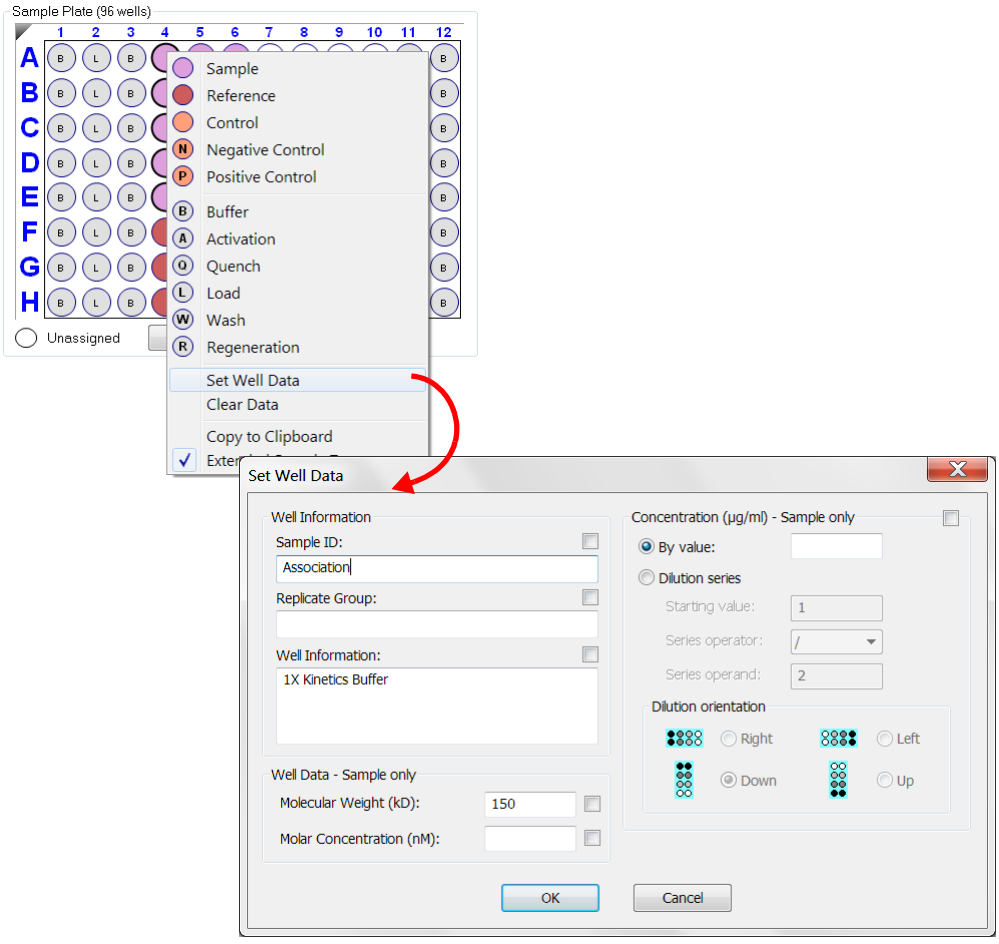


Figure 7-10: *Add Sample Annotations from the Sample Plate Map*

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 7-11).

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

Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
G3	Dissociation		Buffer				1X Kinetics Buffer
H3	Dissociation		Buffer				1X Kinetics Buffer
A4	Association		Sample	10	150	66.67	1X Kinetics Buffer
B4	Association		Sample	5	150	33.33	1X Kinetics Buffer
C4	Association		Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association		Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association		Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association		Reference				1X Kinetics Buffer
G4	Association		Reference				1X Kinetics Buffer
H4	Association		Reference				1X Kinetics Buffer

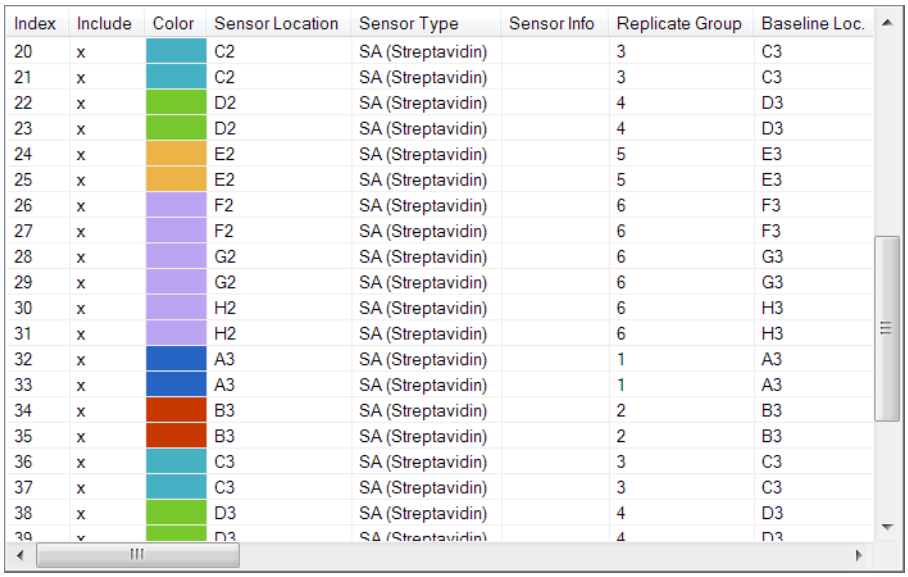
***Figure 7-11:** Add Sample Annotations in the Sample Plate Table*

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

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

Replicate Groups

Replicate Groups enable data to be organized into custom groups during data analysis (see Figure 7-12).



Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.
20	x		C2	SA (Streptavidin)		3	C3
21	x		C2	SA (Streptavidin)		3	C3
22	x		D2	SA (Streptavidin)		4	D3
23	x		D2	SA (Streptavidin)		4	D3
24	x		E2	SA (Streptavidin)		5	E3
25	x		E2	SA (Streptavidin)		5	E3
26	x		F2	SA (Streptavidin)		6	F3
27	x		F2	SA (Streptavidin)		6	F3
28	x		G2	SA (Streptavidin)		6	G3
29	x		G2	SA (Streptavidin)		6	G3
30	x		H2	SA (Streptavidin)		6	H3
31	x		H2	SA (Streptavidin)		6	H3
32	x		A3	SA (Streptavidin)		1	A3
33	x		A3	SA (Streptavidin)		1	A3
34	x		B3	SA (Streptavidin)		2	B3
35	x		B3	SA (Streptavidin)		2	B3
36	x		C3	SA (Streptavidin)		3	C3
37	x		C3	SA (Streptavidin)		3	C3
38	x		D3	SA (Streptavidin)		4	D3
39	x		D3	SA (Streptavidin)		4	D3

Figure 7-12: *Replicate Group Color-Coding*

NOTE: *Replicate Group information can also be entered in the Octet System Data Analysis software.*

Assigning Replicate Groups in the Sample Plate Map

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

1. Select the samples you wish to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 7-13), enter a name in the **Replicate Group** box and click **OK**.

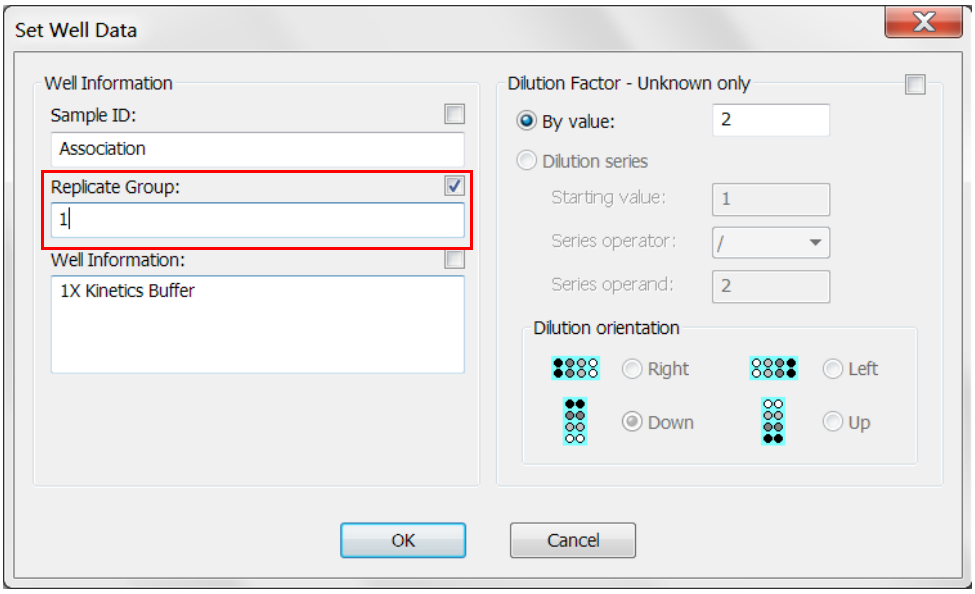


Figure 7-13: *Add Replicate Group from the Sample Plate Map*

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 7-14).

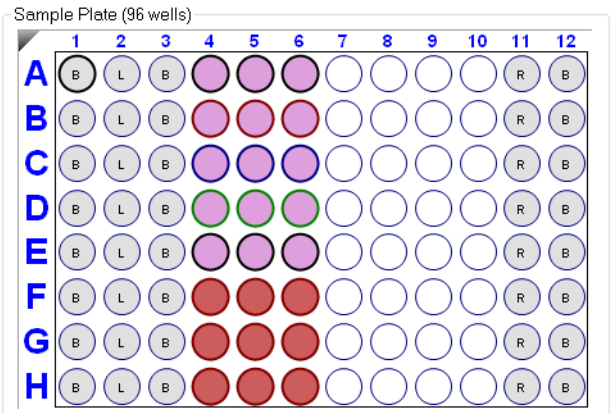


Figure 7-14: *Replicate Groups Displayed in Sample Plate Map*

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 7-15)

Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

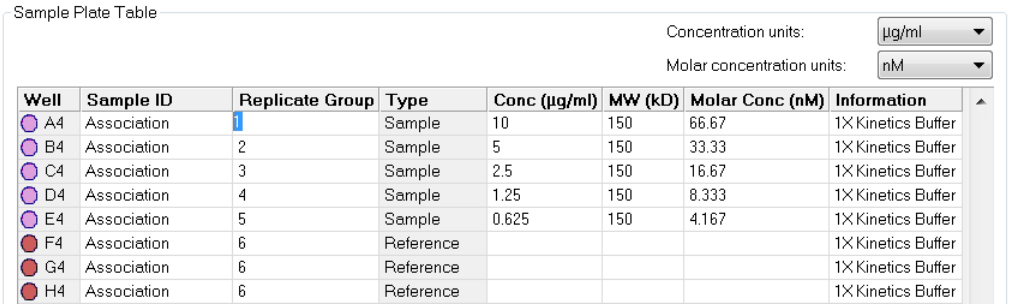
Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association	6	Reference				1X Kinetics Buffer
G4	Association	6	Reference				1X Kinetics Buffer
H4	Association	6	Reference				1X Kinetics Buffer
A5	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B5	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C5	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D5	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E5	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F5	Association	6	Reference				1X Kinetics Buffer
G5	Association	6	Reference				1X Kinetics Buffer
H5	Association	6	Reference				1X Kinetics Buffer

Figure 7-15: *Replicate Groups in Sample Plate Table*

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 7-16).


Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association	6	Reference				1X Kinetics Buffer
G4	Association	6	Reference				1X Kinetics Buffer
H4	Association	6	Reference				1X Kinetics Buffer

Figure 7-16: *Add Replicate Group from the Sample Plate Table*

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

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

---

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

Editing the Sample Table

Changing Sample Well Designations

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection (see Figure 7-17).

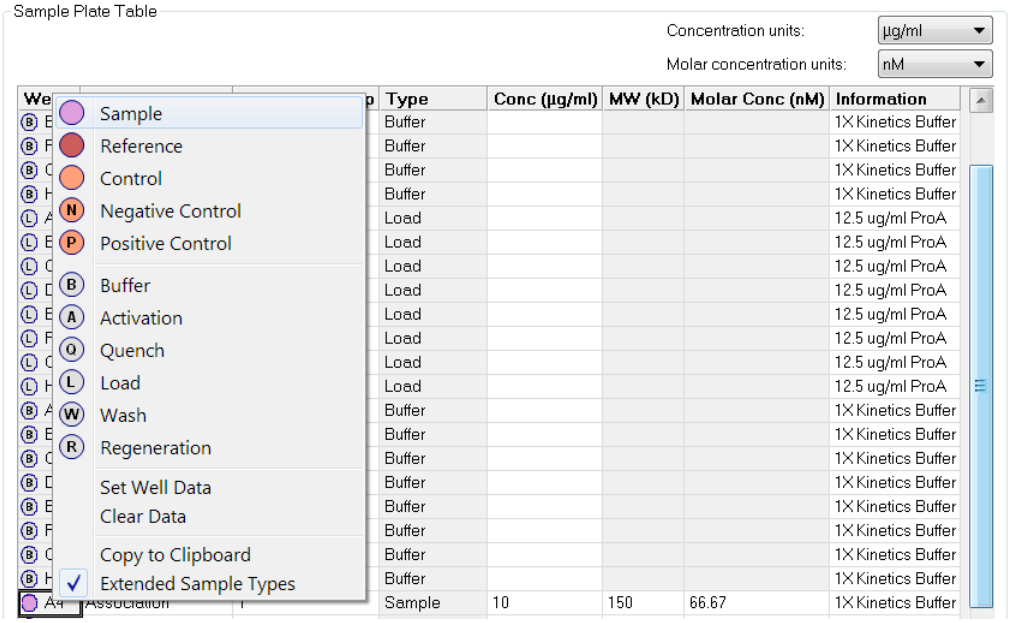


Figure 7-17: *Sample Plate Table—Well Designation*

Editing Sample Information

To edit sample data in the **Sample Plate Table**, double-click a value and enter a new value (see Figure 7-18).

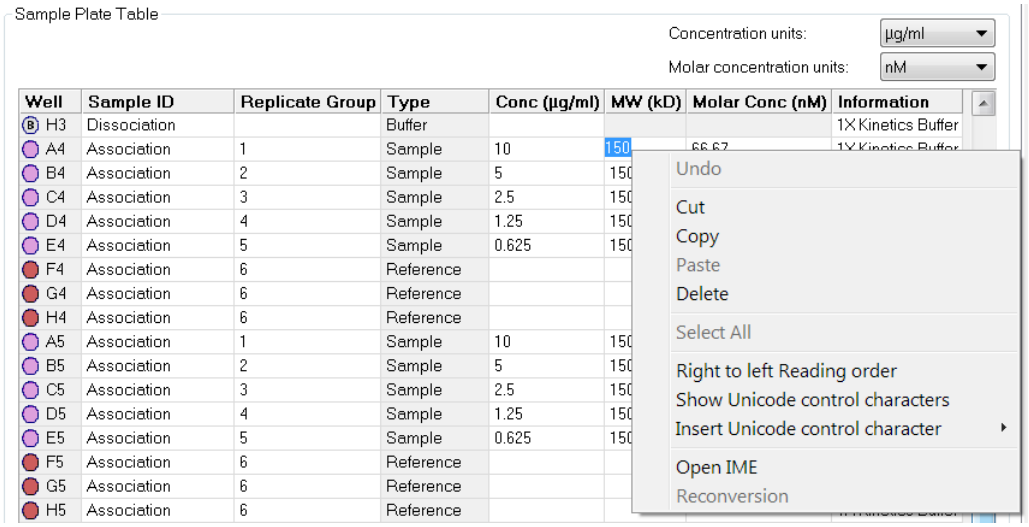


Figure 7-18: *Sample Plate Table—Editing Sample Data*

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

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

MANAGING SAMPLE PLATE DEFINITIONS

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

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Map**, click **Export** (see Figure 7-19).

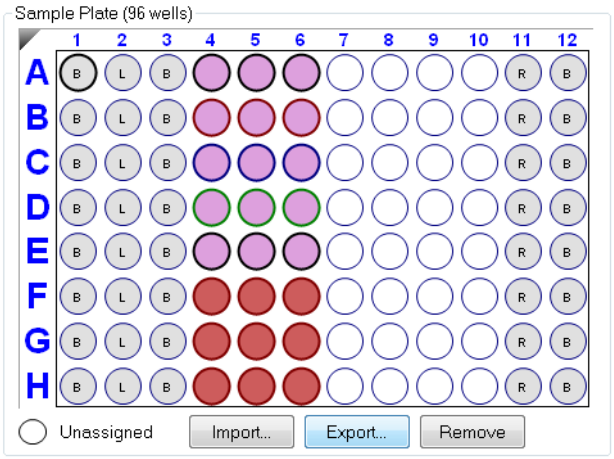


Figure 7-19: *Sample Plate Map— Export Button*

2. In the **Export Plate Definition** window (see Figure 7-20), select a folder, enter a name for the plate (.csv), and click **Save**.

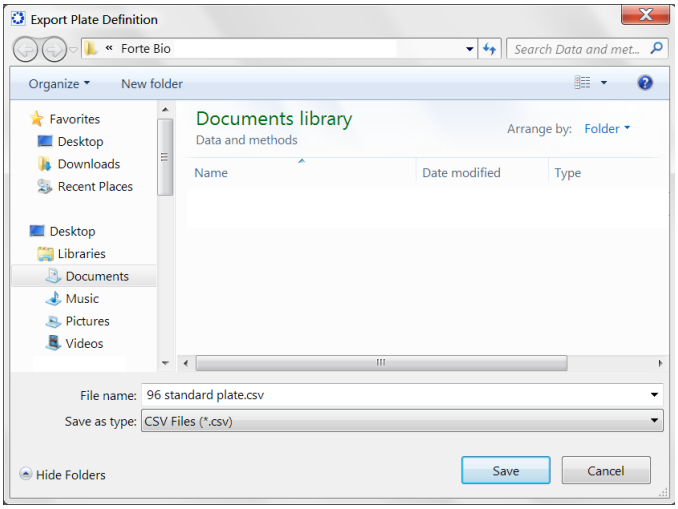


Figure 7-20: *Export Plate Definition Window*

Importing a Plate Definition

To import a plate definition:

1. In the Sample Plate Definition window (see Figure 7-19: on page 233), click **Import**.

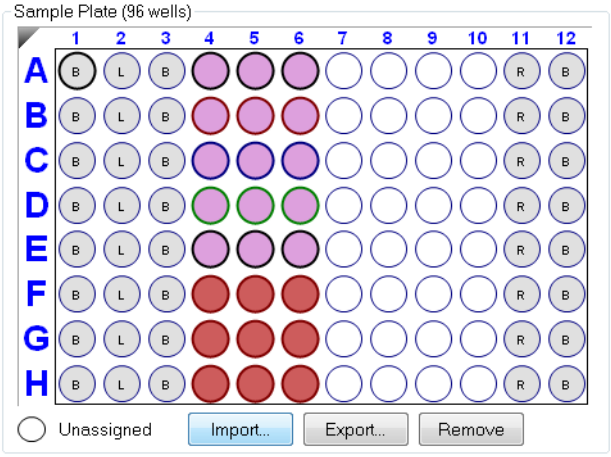


Figure 7-21: *Sample Plate Map— **Import** Button*

2. In the **Import Plate Definition** window (see Figure 7-22), select the plate definition (.csv), and click **Open**.

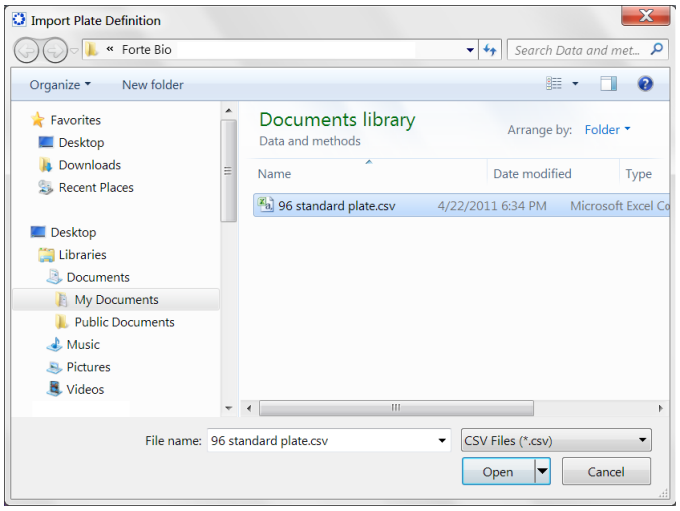
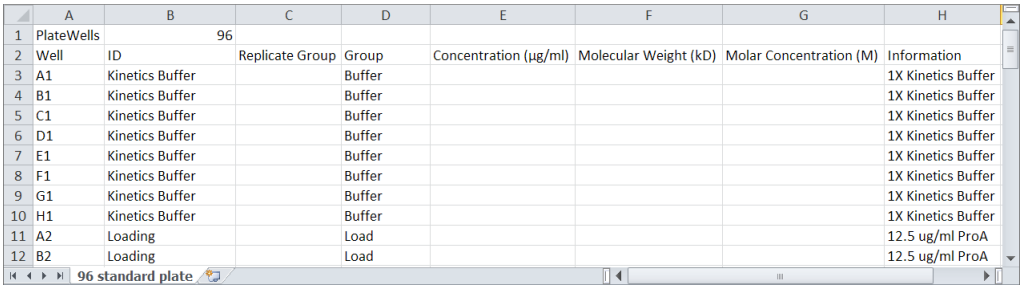


Figure 7-22: *Import Plate Definition Window*

NOTE: *You can also create a .csv file for import. Figure 7-23 shows the appropriate column information layout.*



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

96 standard plate

Figure 7-23: *Example Plate Definition File (.csv)*

DEFINING A KINETIC ASSAY

After the sample plate is defined, the assay must be defined. The steps to define a kinetic assay include:

Step	*See Page*
1. Define the step types.	236
2. Build the assay by assigning a step type to a column(s) in the sample plate.	241
3. Save the sample plate definition (optional).	232

Defining Step Types

Table 7-3 lists the example step types to define a kinetic assay. Use these examples as a starting point to create your own step types.

Table 7-3: *Sample Step Types for Kinetic Assays*

Step Type	*Step Description*
Association	Calculates the k_{obs}. Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the k_d. Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm. ***IMPORTANT:*** *An assay must include a baseline step followed by a set of association/dissociation steps to be analyzed. The Octet System Data Analysis software recognizes the baseline/association/dissociation step series during processing. Data cannot be processed if this sequence is not included in the assay setup.*
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor. ***NOTE:*** *This step may be performed offline (outside the Octet instrument).*

Table 7-3: Sample Step Types for Kinetic Assays (Continued)

Step Type	Step Description
Custom	Can be used for an activity not included in any of the above step types.
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.

Creating Step Types

Click the **Assay Definition** tab, or click the  arrow to access the Assay Definition window (see Figure 7-24). The **Step Data List** shows the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay steps:

1. Click **Add**.
2. In **Assay Step Definition** dialog box (Figure 7-24), specify the step information:
   a. Choose a step type.
   b. Optional: Edit the step name.
   c. Set the step time and shake speed (**Time** range: 2 to 48,000 seconds, **Shake speed** range: 100 to 1,500 rpm or 0).

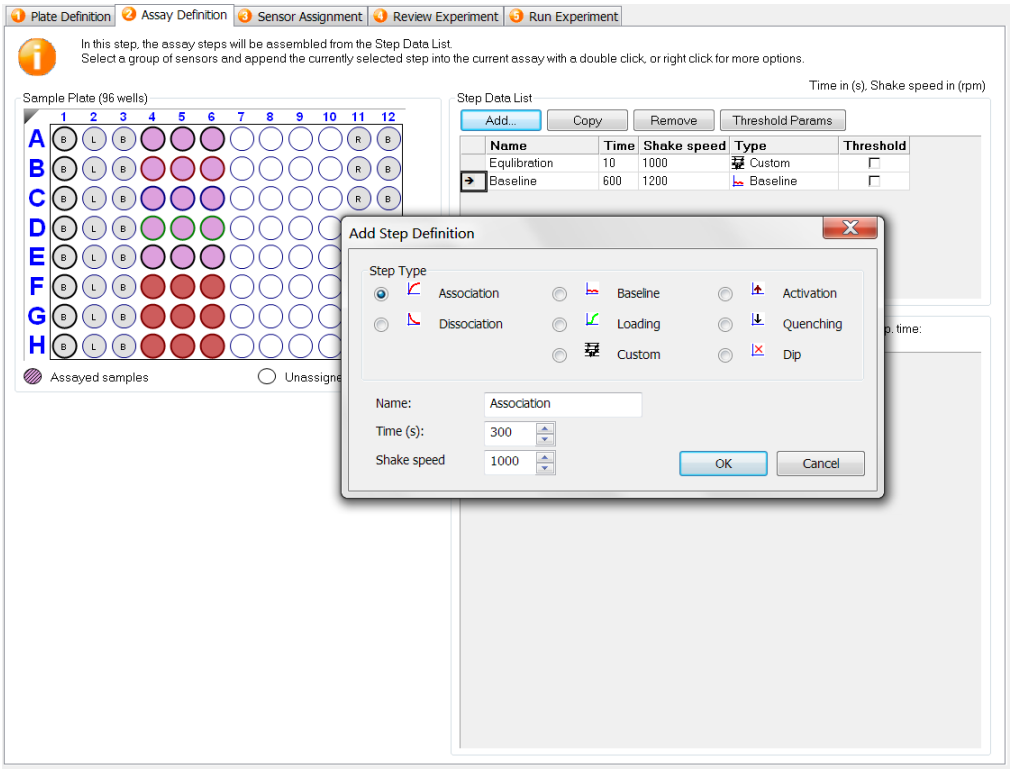


Figure 7-24: *Creating an Assay Step Type*

3. Apply a threshold to the step:
   a. In the **Step Data List**, click the **Threshold** check box.

      The **Threshold Parameters** dialog box displays (see Figure 7-25).
   b. Set the threshold parameters (refer to Table 7-4 for the parameter definitions).

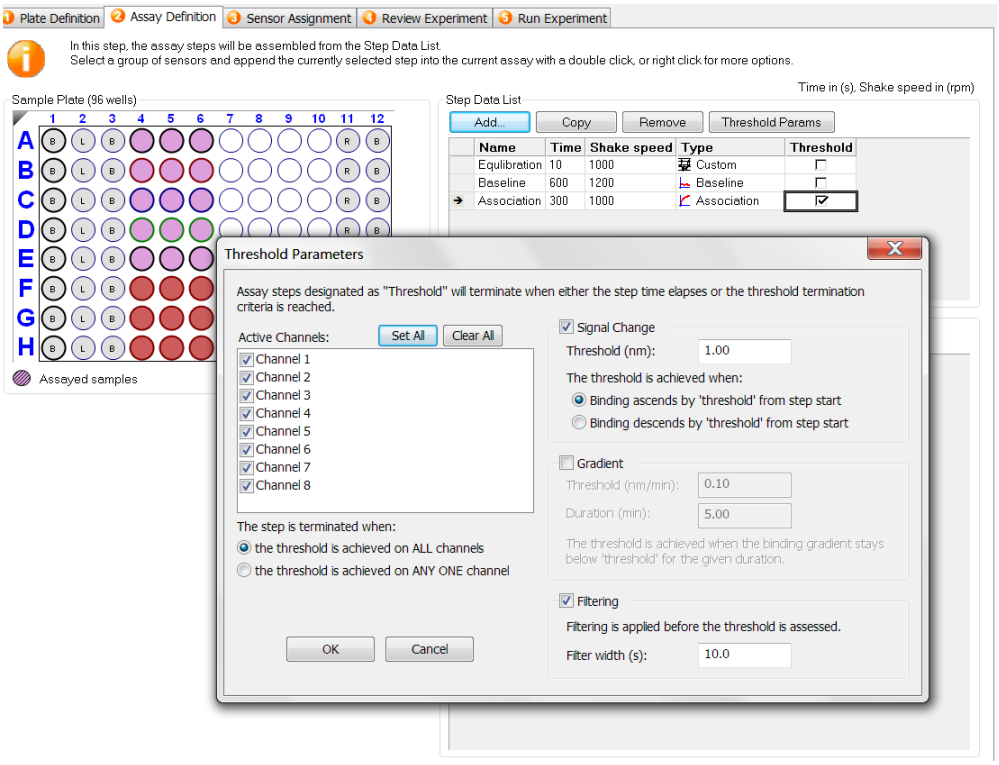


Figure 7-25: *Setting Assay Step Threshold Parameters*

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

Table 7-4: *Threshold Parameters*

Item	*Description*
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step: • **The threshold is achieved on ALL channels** • **The threshold is achieved on ANY ONE channel**
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

4. Click **OK** to save the newly-defined step. The new step type appears in the **Step Data List**.
5. Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 7-26).

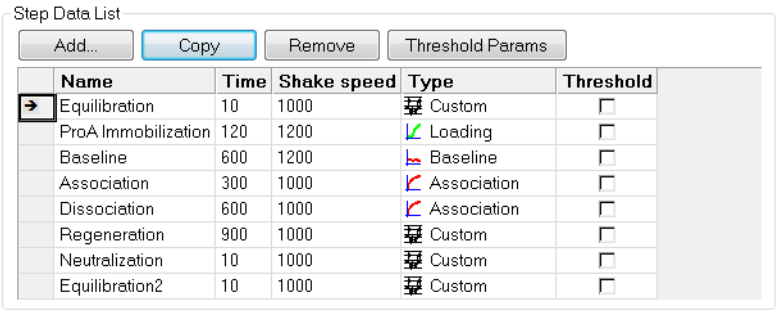


Figure 7-26: *Step Data List—Displaying Step Types*

6. To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the **Step Data List.**

To define a step type by editing an existing one:

1. Double-click the cell in the step's **Name, Time** or **Shake speed** column and then enter a new value. Or, right-click the cell to display a shortcut menu of editing commands (see Figure 7-27, left).

NOTE: *Keyboard commands can also be used (**Ctrl**+**x**=cut, **Ctrl**+**c**=copy, **Ctrl**+**v**=paste, **Ctrl**+**z**=undo).*

---

2. Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 7-27, right).

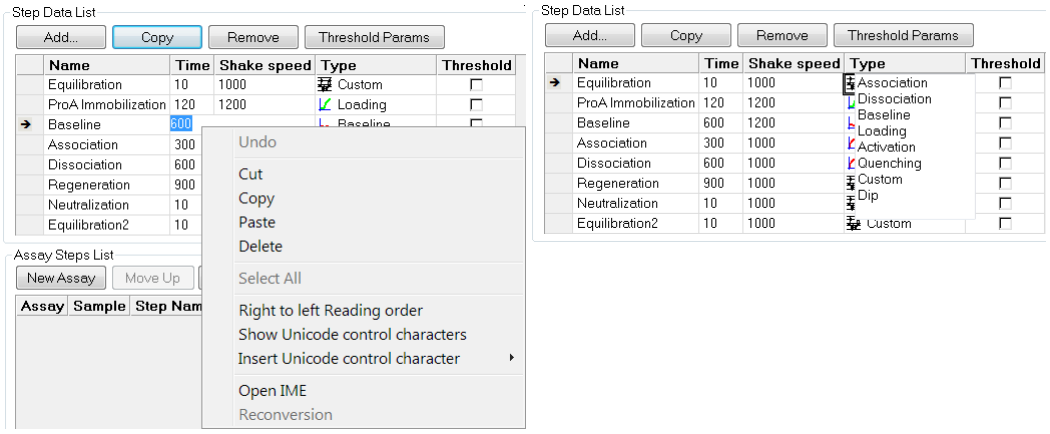


Figure 7-27: *Editing a Step Value (left) or Step Type (right)*

Building an Assay

After creating the different step types that the assay will use, step types are assigned to columns in the Sample Plate or Reagent Plate maps.

To build an assay:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, double-click the column that is associated with the selected step type. For information about sample plate wells, mouse over a well to view a tool tip (see Figure 7-28).

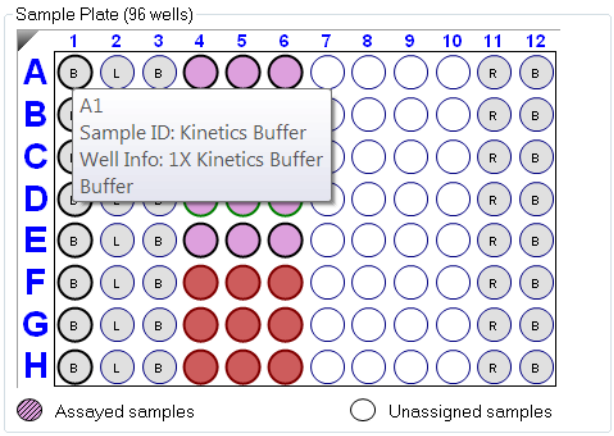


Figure 7-28: *Tool Tip of Well Information*

The selected wells are marked with hatching (for example,) and the step appears in the **Assay Steps List** (see Figure 7-29) with an associated **Assay Time**.

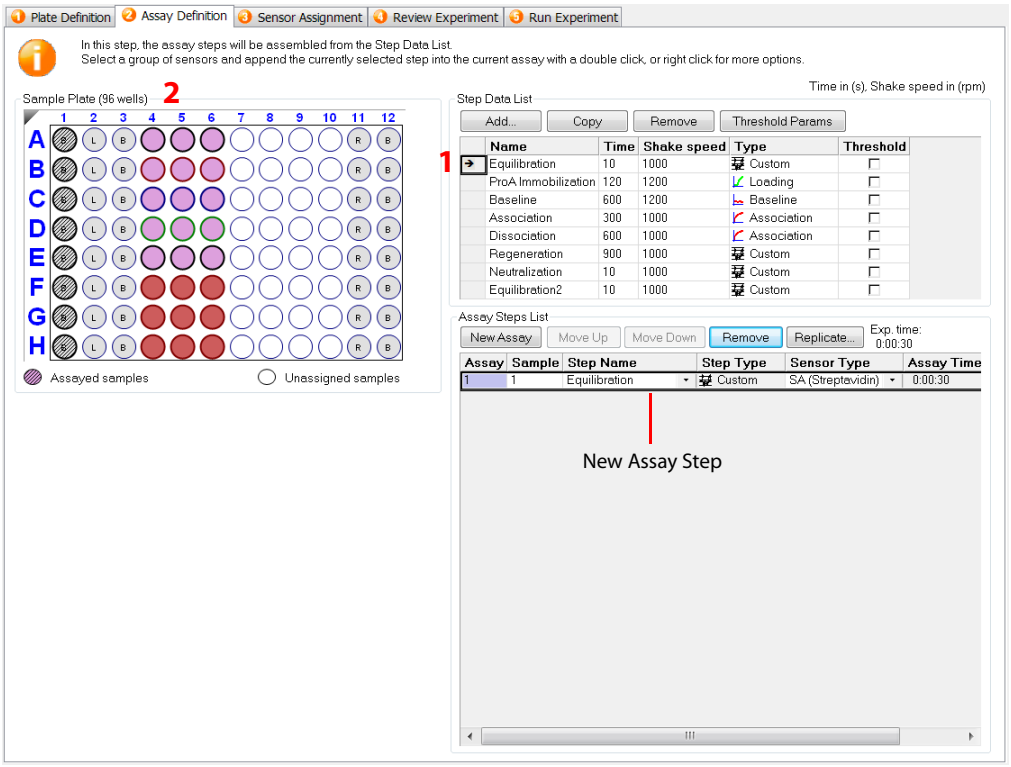


Figure 7-29: *Assigning a Step Type to a Column in the Sample Plate*

3. Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 7-30).

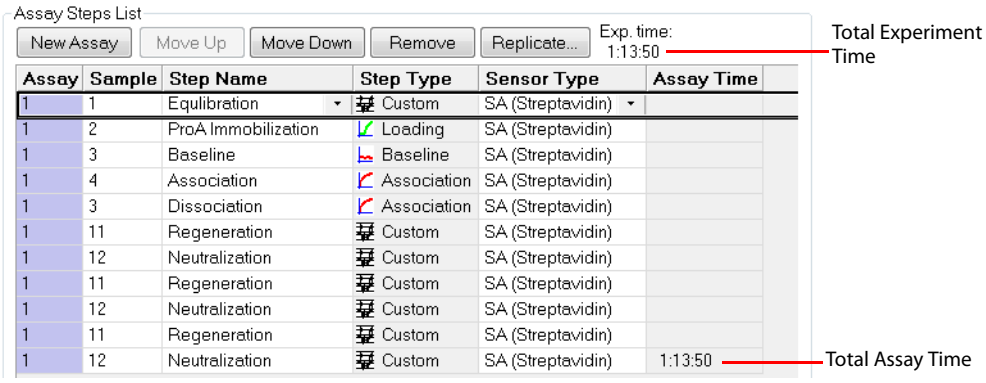


Figure 7-30: *Experiment and Assay Time Updates as Steps Are Added to the Assay*

IMPORTANT: *If you intend to analyze the data from a sample using the* ***Inter-step correction*** *feature in the Octet System Data Acquisition software, the assay must use the same well to perform baseline and dissociation for the sample.*

Replicating Steps within an Assay

To copy steps and add them to an assay:

1. In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 7-31, step rows 1–4 are selected).
   - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
   - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
2. In the **Replicate Steps** dialog box (see Figure 7-31), click the **Append to current assay** option.
3. Click the **Offset steps** check box and set the options, as appropriate. (For more details on offset options, see Table 7-5.)

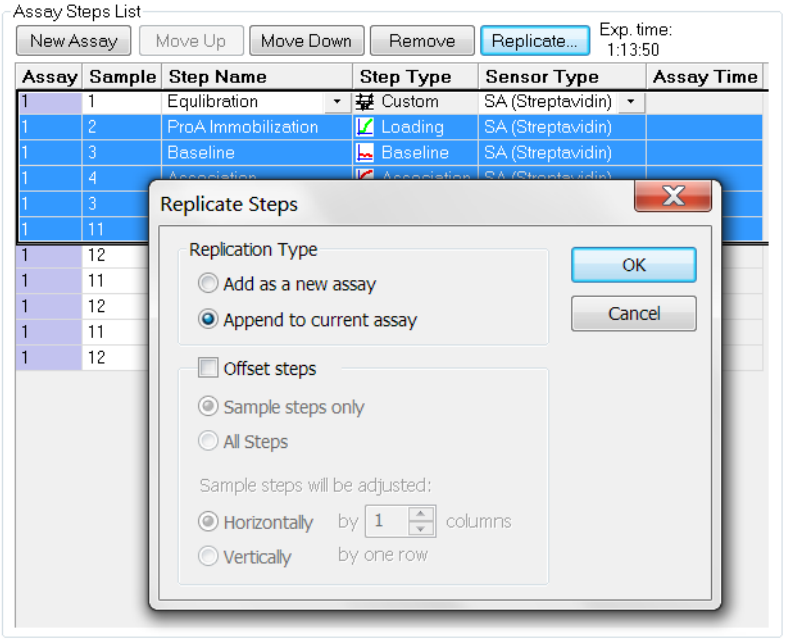


Figure 7-31: *Replicating Assay Steps by Appending*

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

Table 7-5: *Replicate Steps Options .*

Item	*Description*
Add as a new assay	Adds the replicate step(s) as a new assay to the **Assay Steps List**.
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
Sample steps only	Applies the offset to the sample plate only.
All steps	Applies the offset to the sample plate and reagent plate. ***NOTE:*** *Reagent plates are only available when using an Octet384 or Octet QK384 instrument.*
Sample steps will be adjusted horizontally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter **1**. Enter **0** to apply the step(s) to the same columns.
Sample steps will be adjusted vertically by one row	Applies only to the Octet384 or Octet QK384 instruments.

Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensor column:

1. Select a column in the **Sample Plate Map**.
2. Right-click to view the shortcut menu and select **Start New Assay** (see Figure 7-32).
3. Add steps to the assay as described earlier.

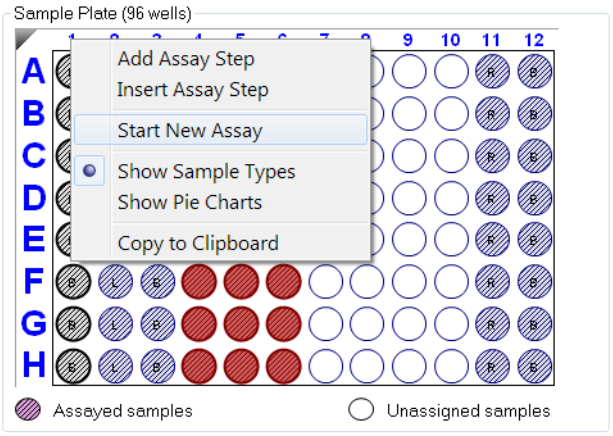


Figure 7-32: *Start New Assay*

Inserting or Adding an Assay Step

To insert an assay step:

1. Select a step in the **Step Data List**.
2. In the **Assay Steps List**, select the row above where you want to insert the step.
3. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Insert Assay Step**.

   The step is inserted into the **Assay Steps List**.

To add an assay step:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, right-click the column to which the step will be applied, and select **Add Assay Step**.

   The step is added to the end of the **Assay Steps List**.

Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow ( ) for any step in the assay and select a sensor type from the drop-down list (Figure 7-33). The biosensor type will automatically update for every assay step.

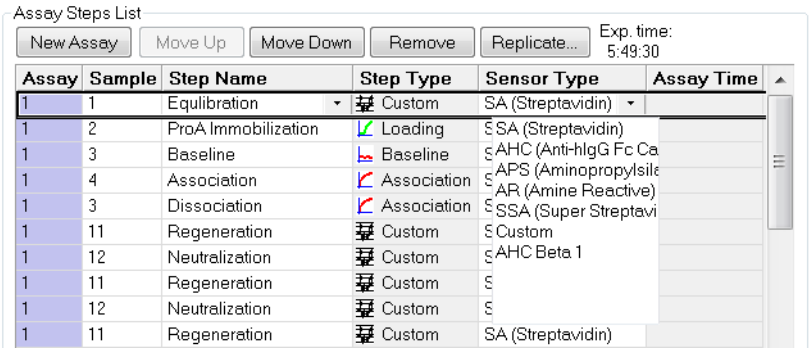


Figure 7-33: *Selecting an Assay Sensor Type*

NOTE: *The **Sensor Type** for the assay must be selected or changed from the **Assay Steps List**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.*

Editing an Assay

To edit the step type or the biosensor type:

1. In the **Assay Steps List**:
   - To change the step type, click the **Step Name** arrow ( ) and select a step name from the drop-down list (Figure 7-34, top).
   - To change the biosensor type, click the **Sensor Type** arrow ( ) for any step in the assay and select a sensor type from the drop-down list (Figure 7-34, bottom). The biosensor type will automatically update for every assay step.

NOTE: *The **Step Name** drop-down list includes only the step types defined in the **Step Data List**.*

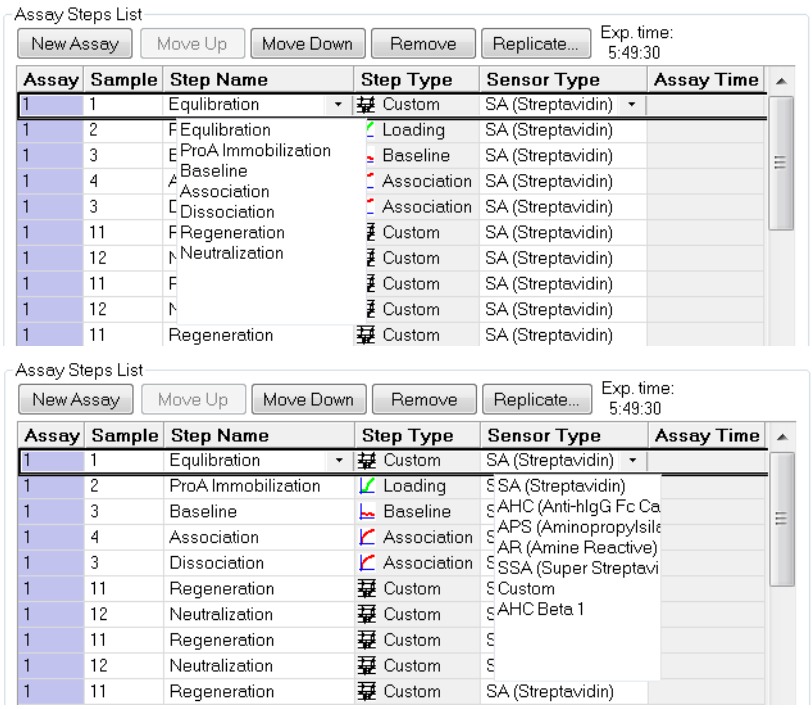


Figure 7-34: *Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List*

To reorder or remove an assay step:

1. Select a step (row) in the **Assay Steps List**.
2. Click the **Move Up**, **Move Down**, or **Remove** button located above the list.

IMPORTANT: *An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. The Octet System Data Acquisition software recognizes the baseline/association/dissociation set of steps.*

Adding an Assay Through Replication

A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

1. In the **Assay Steps List**, select the steps to copy and click **Replicate**.
   - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
   - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 7-35).

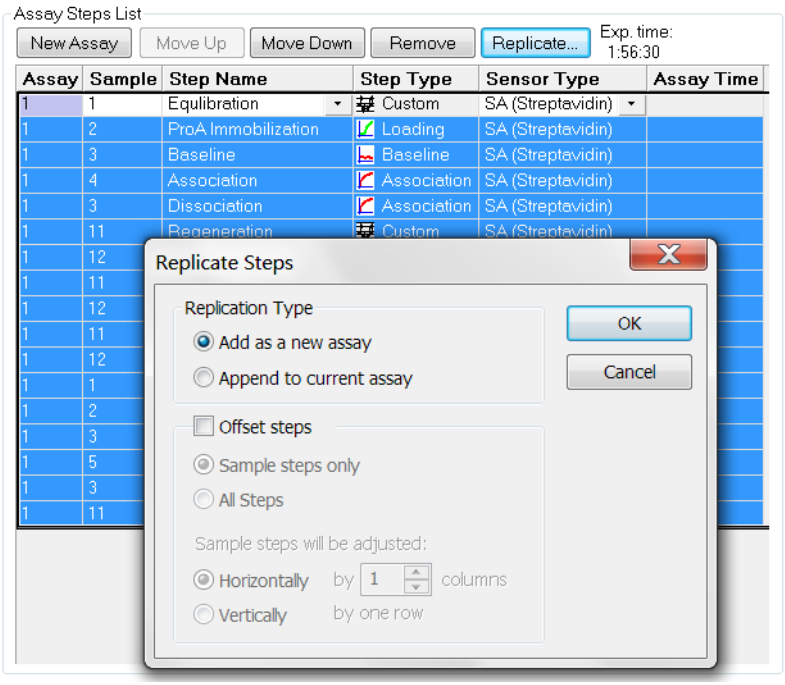


Figure 7-35: *Adding a Replicate Assay to a Plate*

3. Click the **Offset steps** check box and set the options as appropriate (see Table 7-5 on page 244 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the **Offset steps** option. If the replicate assay uses a different sample column, select **Offset steps** and the appropriate options.
   - **Sample steps only** offsets the sample wells by the value specified under **Sample steps will be adjusted**. The offset will not be applied to reagent wells such as buffer, loading, regeneration, neutralization and detection.
   - **All Steps** offsets all wells in the assay, including sample and reagent wells, by the value specified under **Sample steps will be adjusted**.
4. Click **OK**. The new assay appears in the **Assay Steps List**.
5. Continue to add assay steps as needed.

ASSIGNING BIOSENSORS TO SAMPLES

After you define the sample plate and assay(s), click the **Sensor Assignment** tab, or click the arrow to access the Sensor Assignment window. The color-coded **Sensor Tray** and **Sample Plate Map** show the locations of the biosensors associated with the samples Figure 7-36).

NOTE: *If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.*

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 7-36, the experiment includes three assays in the same wells. The use of those wells by three different biosensors is indicated by the pie chart colors.

NOTE: *The **Sensor Type** for the assay must be first be defined in the **Assay Steps List** on the **Assay Definition Tab**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.*

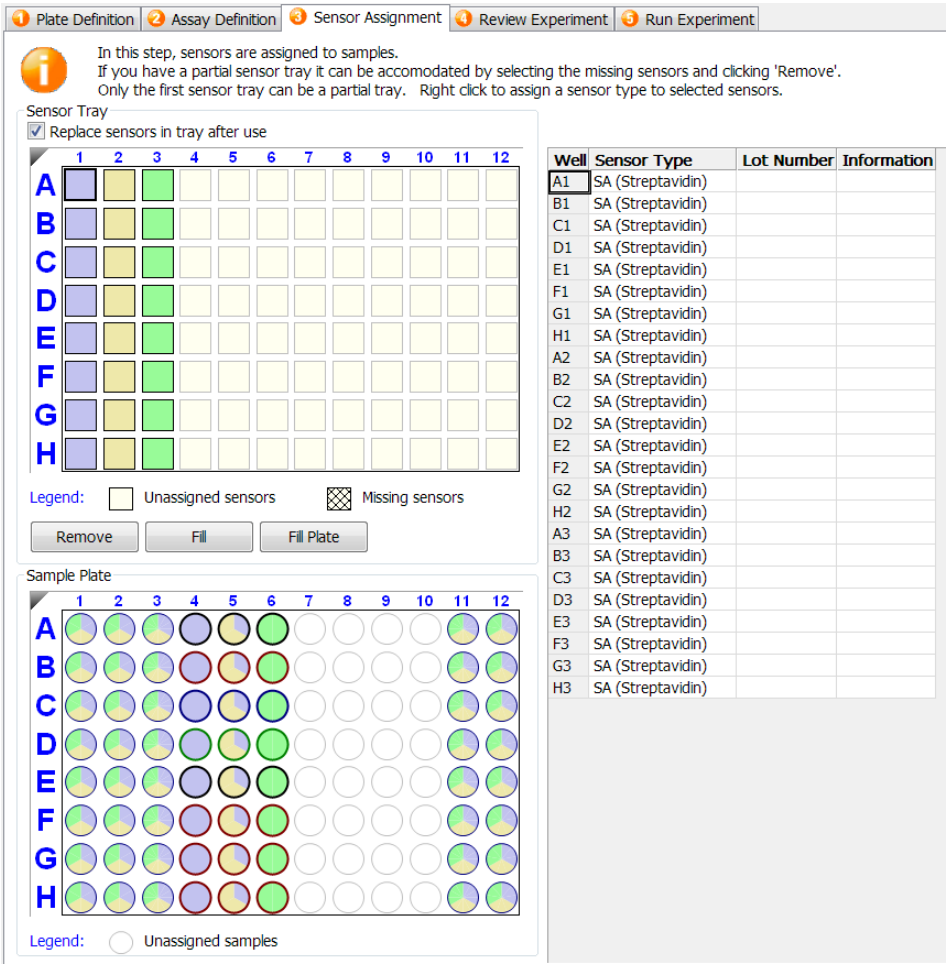


Figure 7-36: *Sensor Assignment Window*

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 7-37).

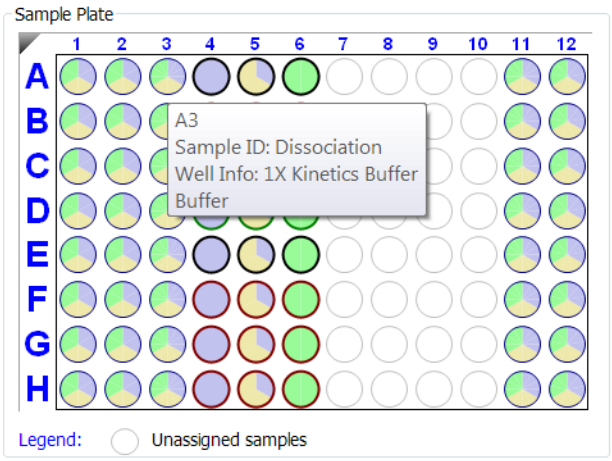


Figure 7-37: *Tool Tip of Well Information*

Replacing the Biosensors in the Biosensor Tray

After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 7-38).

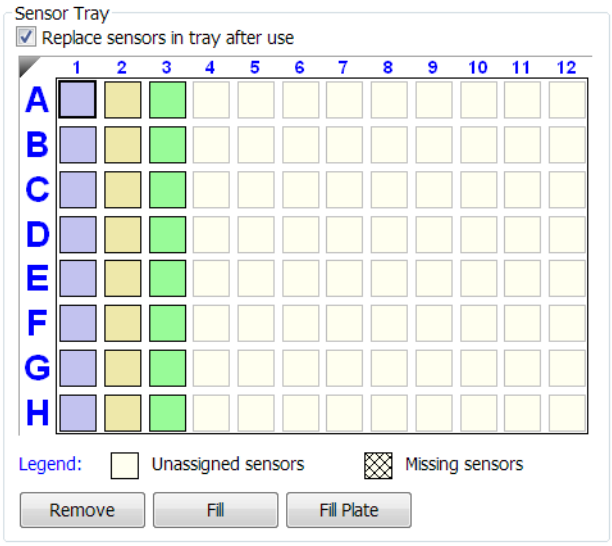


Figure 7-38: *Replace Sensors in Tray After Use Check Box*

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

Entering Biosensor Information

To enter information about a biosensor:

1. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered (see Figure 7-39).
2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 7-39).

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

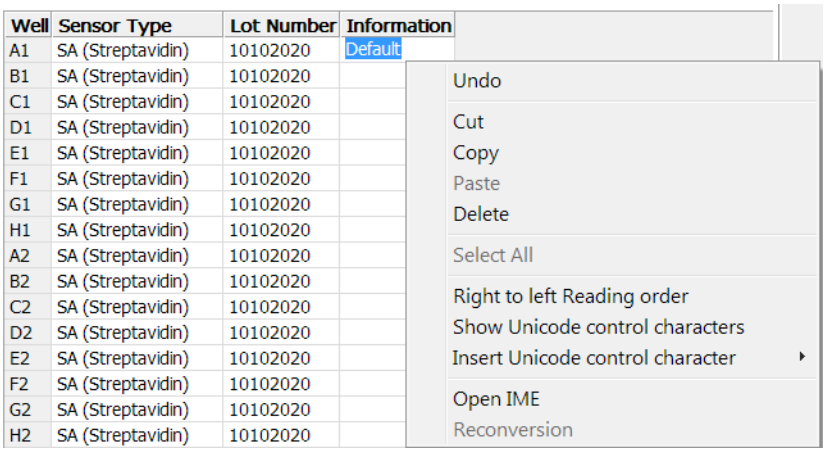


Figure 7-39: *Entering or Editing Biosensor Information*

Changing the Biosensor Location

If you prefer to not use the default biosensor columns, you can select other column(s) to use. There are two ways to do this:

- **Method 1—**In the **Sensor Tray Map**, **Remove** the columns you do not want to use. The software automatically selects the next available column(s).
- **Method 2—**Remove all columns from the **Sensor Tray Map**, then select the columns you want to use.

Method 1

1. In the **Sensor Tray Map** (see Figure 7-40), select the columns to not use and click **Remove**. Or, right-click the selection and select **Remove** (Figure 7-40 left). The software automatically selects the next available biosensor columns in the tray (Figure 7-40 right).

2. Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

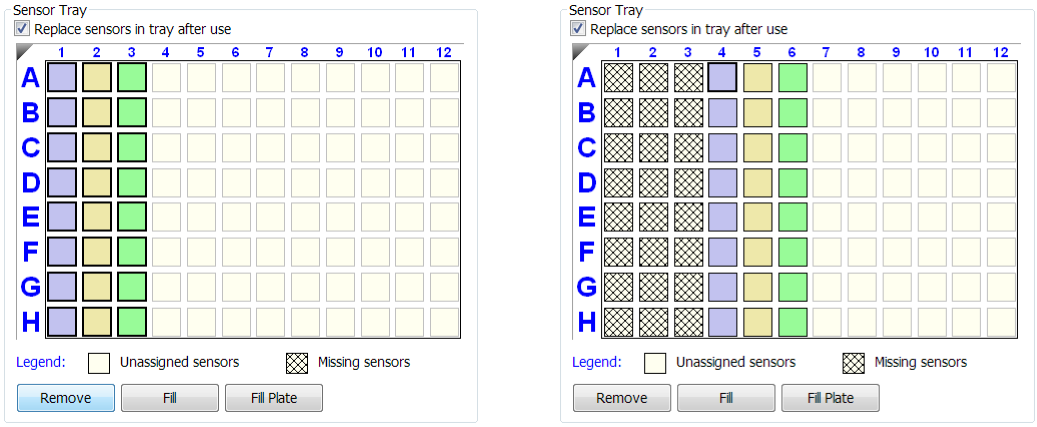


Figure 7-40: *Changing Biosensor Location (Method 1)*

Method 2

1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 7-41 top left). Or, right-click the selection and select **Remove**. All columns will be shown as **Missing** (Figure 7-41 top right).
2. Select the column(s) to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 7-41 bottom left). The software fills the selected columns in the tray (Figure 7-41 bottom right).

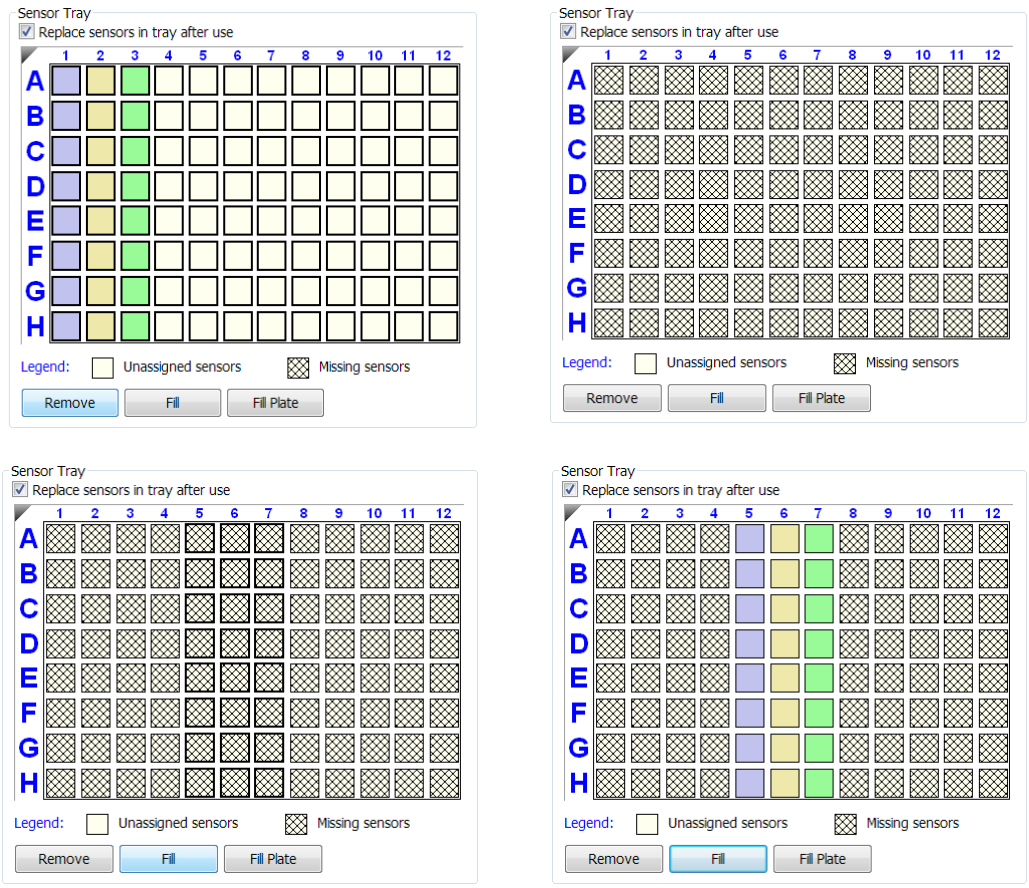


Figure 7-41: *Changing Biosensor Location (Method 2)*

Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

Using Heterogenous Trays

If heterogenous biosensor trays will be used, the column location of each biosensor type in the tray can be identified in the **Sensor Assignment Tab**. Assignment of biosensors that will not be used in the assay enables the software to auto-assign the biosensors that will be used in the assay by biosensor type.

There are two ways to change the biosensor type:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (Figure 7-42 left). The associated wells in the **Sensor Type** column will automatically populate with the biosensor type selected.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (Figure 7-42 right). All other wells in the same column of the **Sensor Tray Map** as the selected cell will automatically populate with the biosensor type selected.

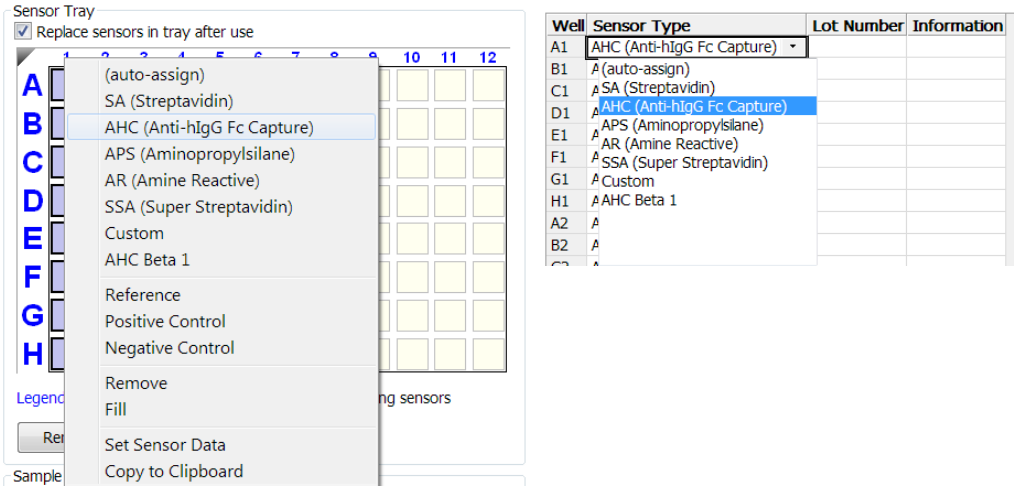


Figure 7-42: Sensor Assignment Window—Changing the Biosensor Type

The biosensor types shown in the **Sensor Assignment** window were specified previously in the **Assay Definition** window, and default locations are assigned automatically. To assign biosensor types for heterogenous trays:

1. Select the column location of the biosensor type (see Figure 7-43).

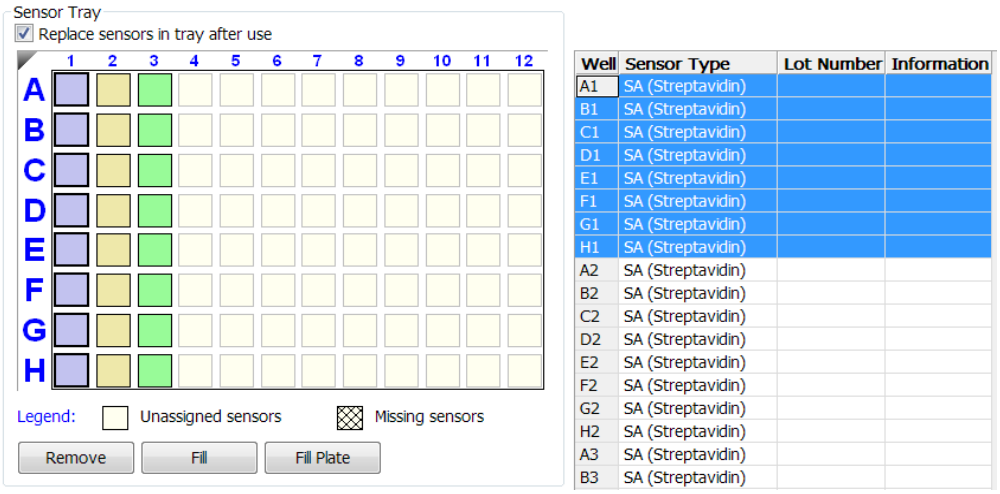


Figure 7-43: Selecting a Sensor Tray Column

2. Right-click in the **Sensor Tray Map** or click in a cell in the **Sensor Type** table column and select a biosensor type from the drop-down list. The biosensor type associated with the assay will shift location accordingly (see Figure 7-44). In the example shown, Streptavidin is the **Sensor Type** used for the current assay. Column 1 was reassigned as AHC according to the heterogeneous tray being used.

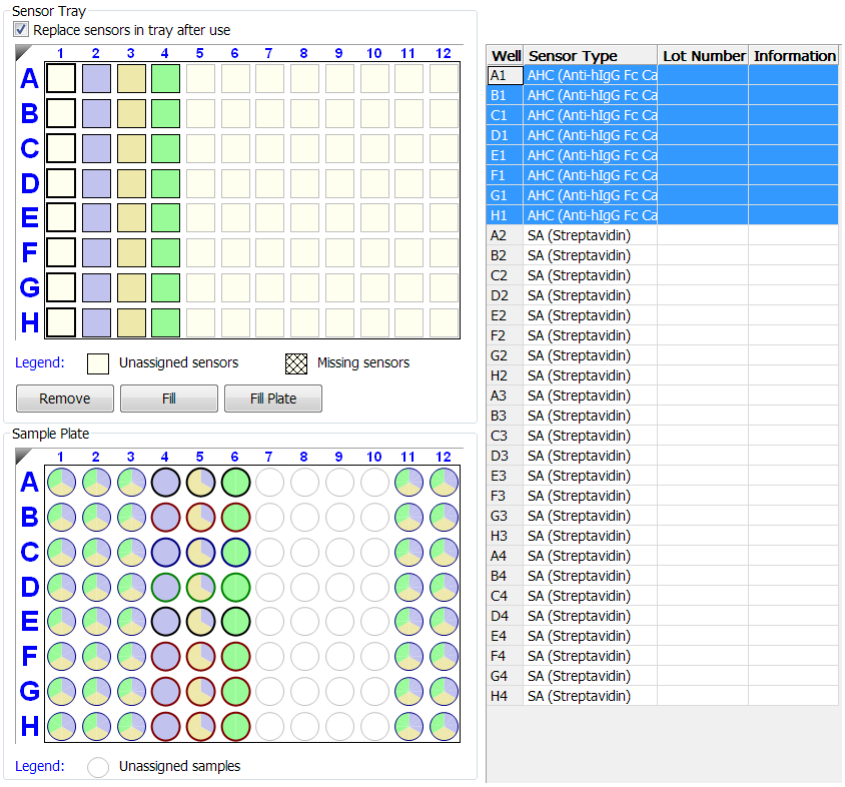


Figure 7-44: *Assay Sensor Type Reassignment*

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

IMPORTANT: *Ensure that the biosensor types selected in the* ***Assay Definition*** *window have assigned column(s) in the* ***Sensor Assignment*** *window or the experiment cannot be run.*

Using Partial Biosensor Trays

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 7-45) includes three assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 7-45 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 7-45 bottom). If necessary, change the location of these biosensors.

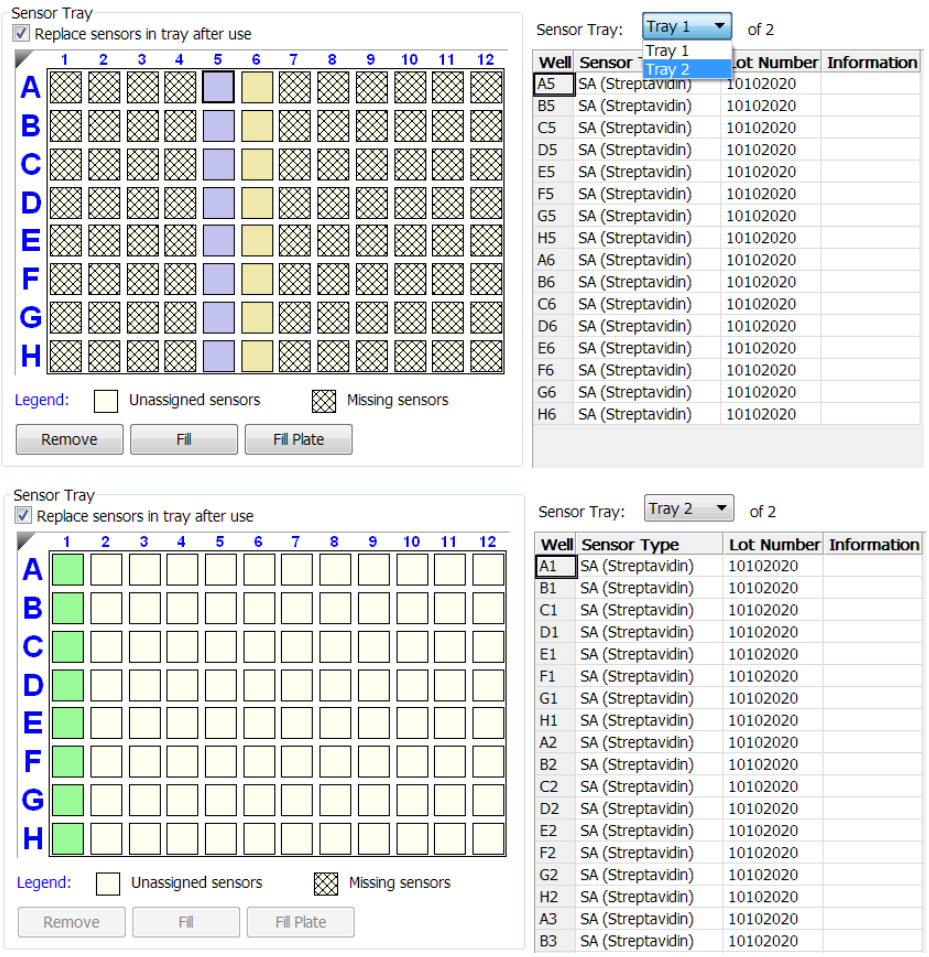


Figure 7-45: *Example Experiment Using Two Biosensor Trays*

NOTE: *Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet instrument.*

Reference Biosensors

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

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

Changing the Biosensor Type

The biosensor type used in the assay must be selected in the **Assay Definition** window. To change the biosensor type:

1. Click the **Assay Definition Tab.**
2. In the **Assay Steps List**, click the cell in the **Sensor Type** column to change.
3. Select from the drop-down list (see Figure 7-46).

IMPORTANT: *Ensure that the same biosensor types are selected in both the Assay Definition and the Sensor Assignment windows or the experiment cannot be run.*

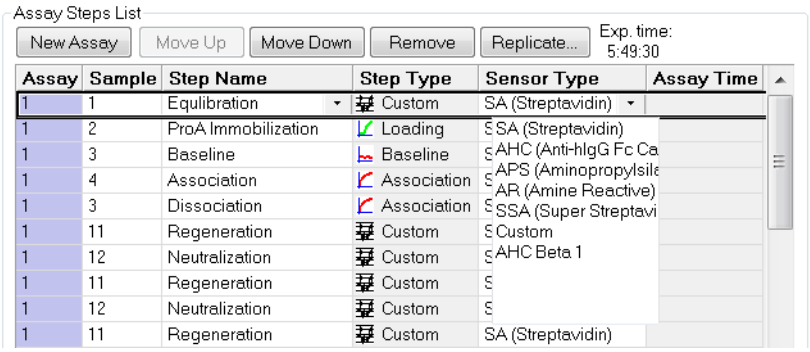


Figure 7-46: *Assay Definition Window—Changing the Biosensor Type*

REVIEWING EXPERIMENTS

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

In the **Review Experiment** window (Figure 7-47), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the ← → arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

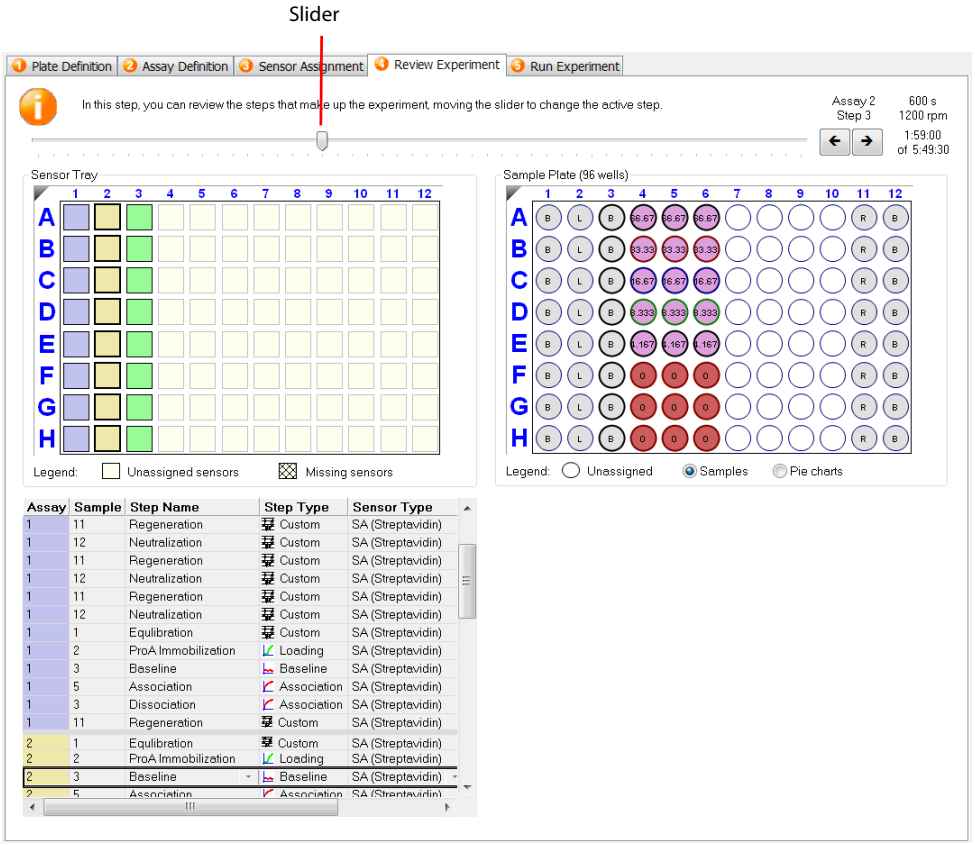


Figure 7-47: *Review Experiment Window*

SAVING EXPERIMENTS

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

To manually save an experiment:

1. Click **Save Method File** (), or on the main menu, click **File** > **Save Method File**.

   If there is more than one open experiment and you want to save all of them, click **Save All Methods Files** .

2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.

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

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment** > **Templates** > **Kinetics** > **Experiment Name** (Figure 7-48).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.

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

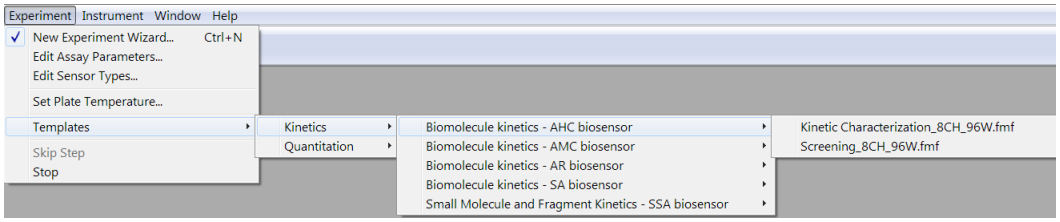


Figure 7-48: *Saved Experiments in the Template Folder*

RUNNING A KINETICS EXPERIMENT

IMPORTANT: *Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.*

Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

1. Open the Octet instrument door (lift the handle up).
2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 7-49).
3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 7-49).

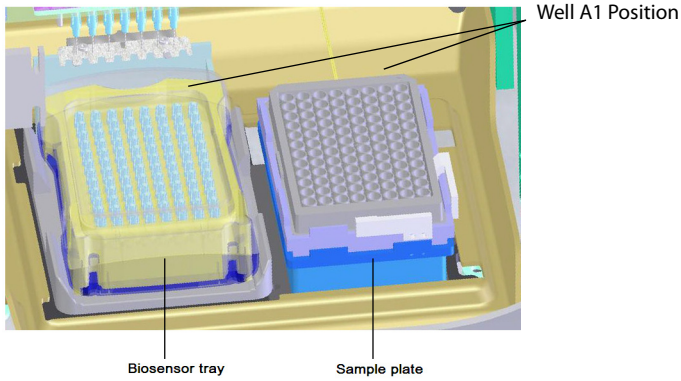


Figure 7-49: *Biosensor Stage (left) and Sample Stage (right)*

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

---

4. Close the Octet instrument door.
5. Allow the plate to equilibrate.

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

Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow ( ) to access the Run Experiment window (see Figure 7-50).

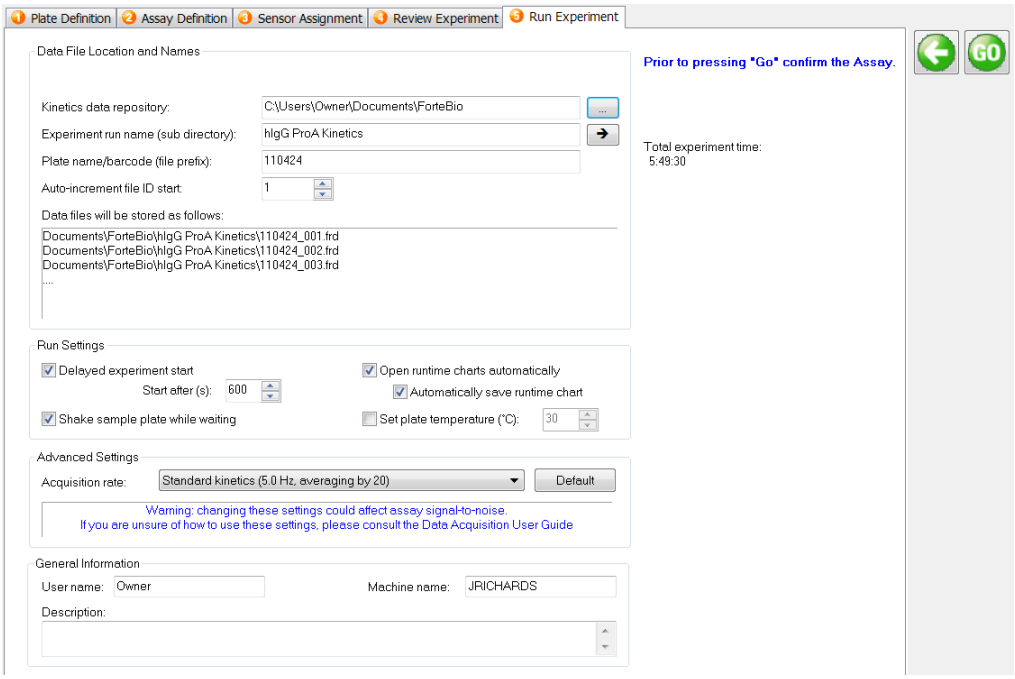


Figure 7-50: *Run Experiment Window—Octet RED96*

2. Confirm the default settings or enter new settings. See "Run Experiment Window Settings" on page 264 for more information on experimental settings.

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

---

3. To start the experiment, click GO.

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

   If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 7-51).

NOTE: *For more details about the **Runtime Binding Chart**, see "Managing the Runtime Binding Chart" on page 267.*

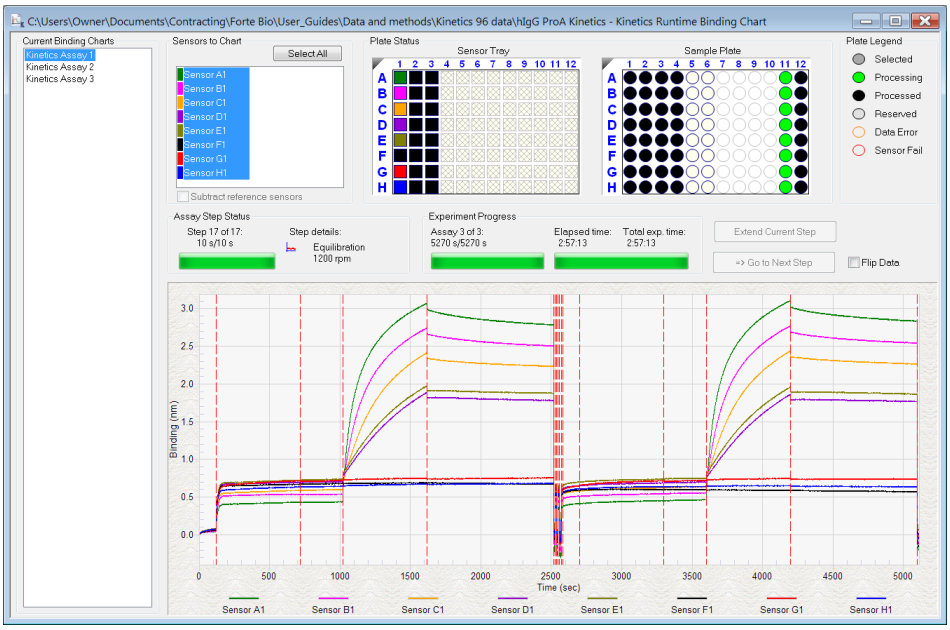


Figure 7-51: Runtime Binding Chart

4. Optional: Click **View** > **Instrument Status** to view the log file (see Figure 7-52).

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

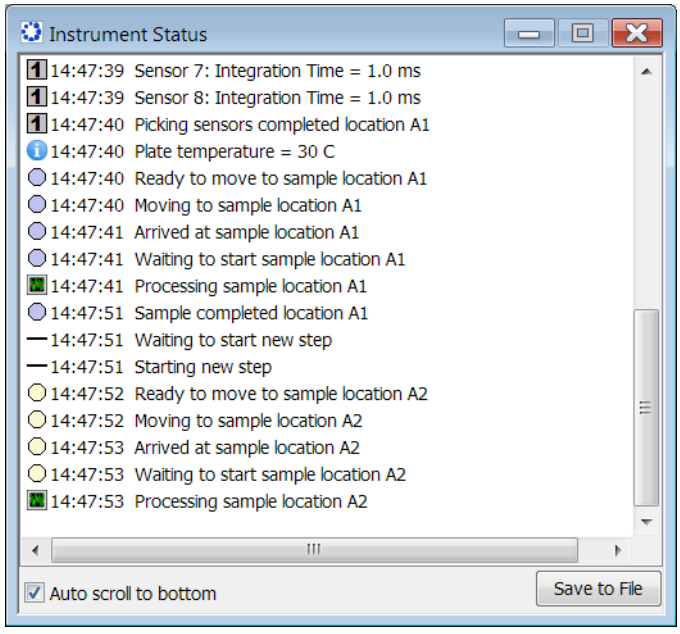


Figure 7-52: Instrument Status Log

WARNING: *Do not open the Octet instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.*

Run Experiment Window Settings

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

Table 7-6: *Data File Location and Name*

Item	*Description*
Assay type	The name of the selected assay.
Kinetics data repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click **Browse** to select another data location. ***NOTE:*** *It is recommended that you save the data to the local machine first, then transfer to a network drive.*
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/ barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

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

Table 7-7: *Run Settings*

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

Advanced settings are available for the Octet QK^{e}, Octet RED and Octet RED96 systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per minute and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise

and thus, better signal-to-noise ratios. The choice of a setting should be determined based upon consideration of the binding rate and the amount of signal generated in your assay, and some experimentation with the settings.

Table 7-8: *Advanced Settings Octet QK^e, Octet RED and Octet RED96*

Item	*Description*
Acquisition rate Octet QK^e	• High sensitivity kinetics (0.3 Hz, averaging by 40) - The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. • Standard kinetics (0.6 Hz, averaging by 5) - The average of five data frames is reported as one data point. One data point is reported every 1.6 seconds.
Acquisition rate Octet RED96	• High sensitivity kinetics (2 Hz, averaging by 50): - The average of 50 data frames is reported as one data point. Two data points are reported per second. • Standard kinetics (5 Hz, averaging by 20 - The average of 20 data frames is reported as one data point.
Sensor offset (mm) - Octet QK^e only	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets acquisition rate and sensor offset to the defaults.

Stopping an Experiment

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

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

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

MANAGING THE RUNTIME BINDING CHART

If the **Open runtime charts automatically** check box is selected in the Run Experiment window (Figure 7-53), the Runtime Binding Charts are automatically displayed when data acquisition starts. The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

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

To selectively display data for particular assay:

1. Click the corresponding **Assay** number.
2. Select a subset of sensors for a displayed column under **Sensors to Chart** box (see Figure 7-53).

WARNING: *Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.*

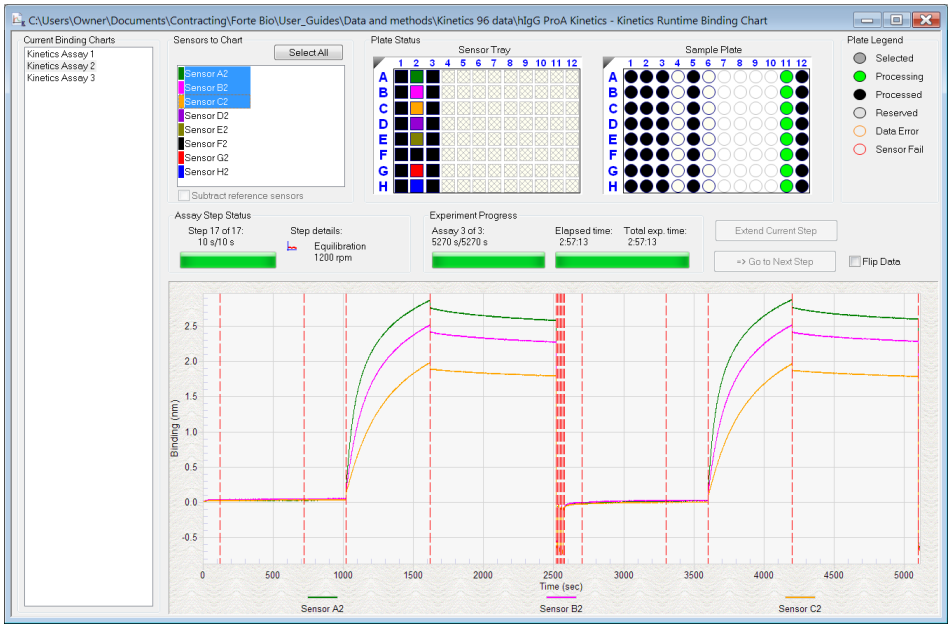


Figure 7-53: *Runtime Binding Chart Window*

Opening the Runtime Binding Chart

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

1. Click **File** > **Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

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

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 7-54).

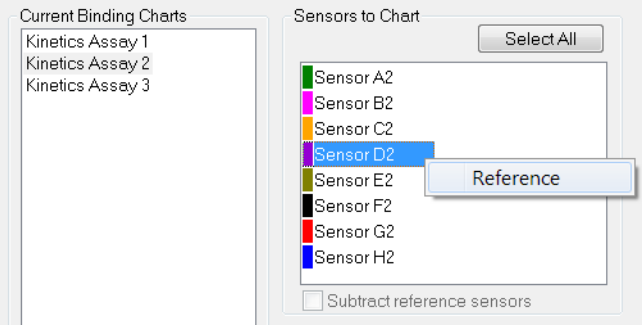


Figure 7-54: *Designating a Reference Biosensor in the Runtime Binding Chart*

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 7-55).

2. Click the **Subtract reference sensors** check box (see Figure 7-55).

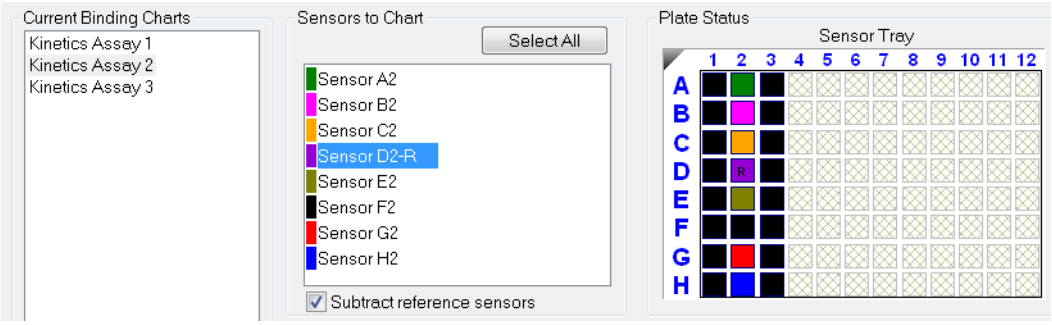


Figure 7-55: *Subtract Reference Sensors check box in the Runtime Binding Chart*

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

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 7-56). Uncheck the box to return to the default data display.

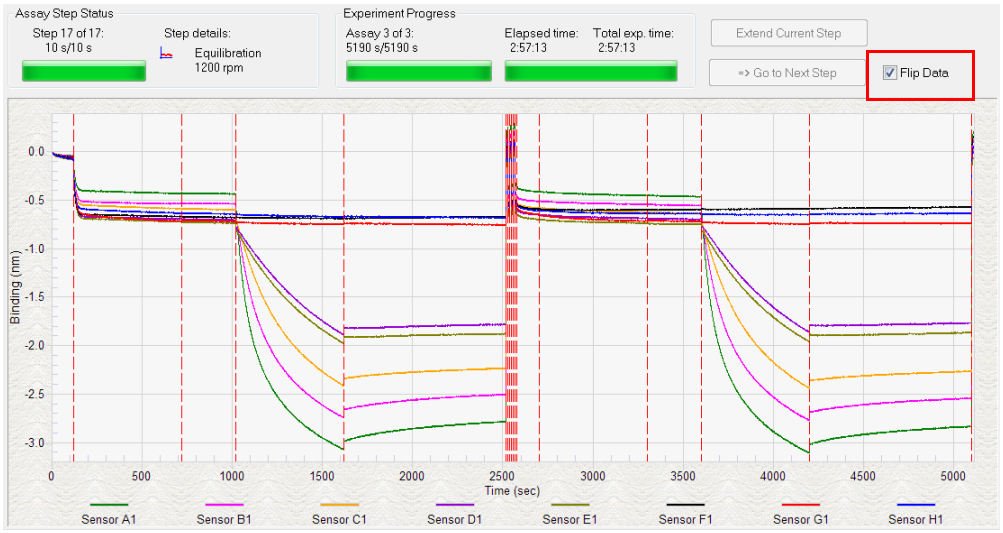


Figure 7-56: *Data Inverted Using Flip Data Function*

Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 7-57), right-click a step and select **Align to Step <*number*>.**

To remove the step alignment, right-click the step and select **Unaligned**.

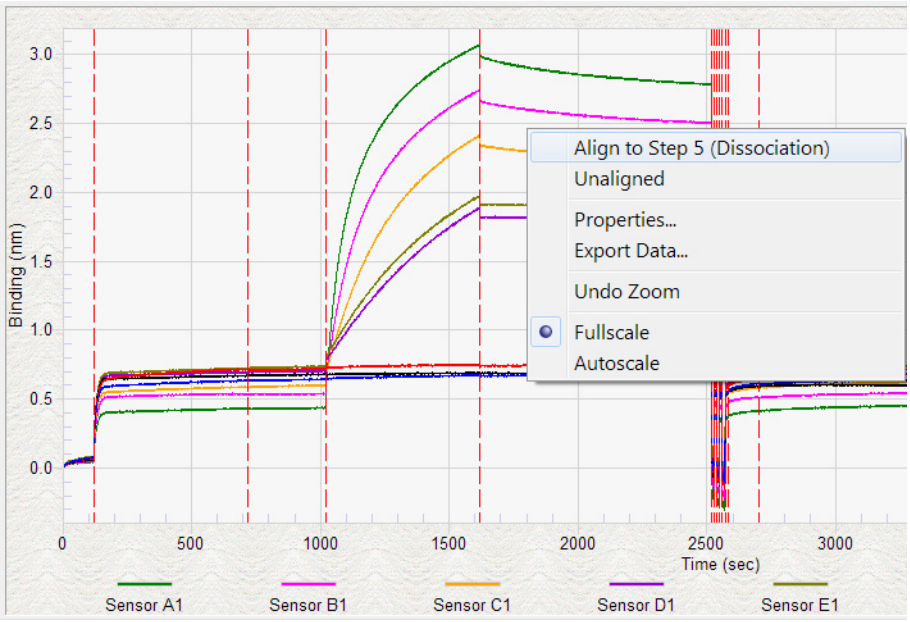


Figure 7-57: *Runtime Binding Chart—Aligning the Data to a User-Selected Step*

Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.

NOTE: *If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.*

To extend the duration of the active step:

1. In the chart window, click the **Extend Current Step** button.
2. In the **Extend Current Step** dialog box (see Figure 7-58), enter the number of seconds to extend the step and click **OK**.

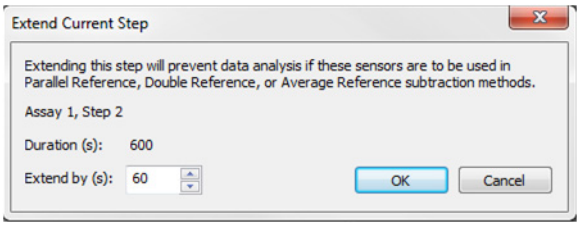


Figure 7-58: *Extend Current Step Dialog Box*

Terminating a Step to Begin the Next Step

To terminate a step and begin the next step in the assay:

1. In the chart window, click the **Go to Next Step** button.
2. In the **Data Acquisition** dialog box, click **OK**.

Magnifying the Runtime Binding Chart

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

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

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

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

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

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

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 7-59), select one of the following legends:
   - Sensor Location
   - Sample ID
   - Sensor Information
   - Concentration/Dilution

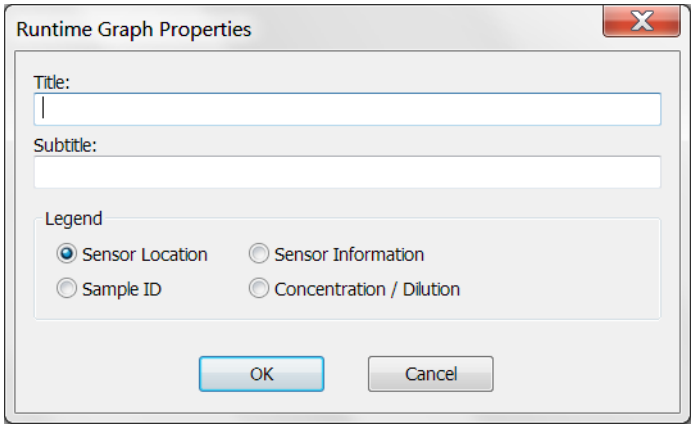


Figure 7-59: *Selecting a Runtime Binding Chart Legend*

---

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

---

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

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

Exporting or Printing the Runtime Binding Chart

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

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 7-60), select the export options and click **Export**.

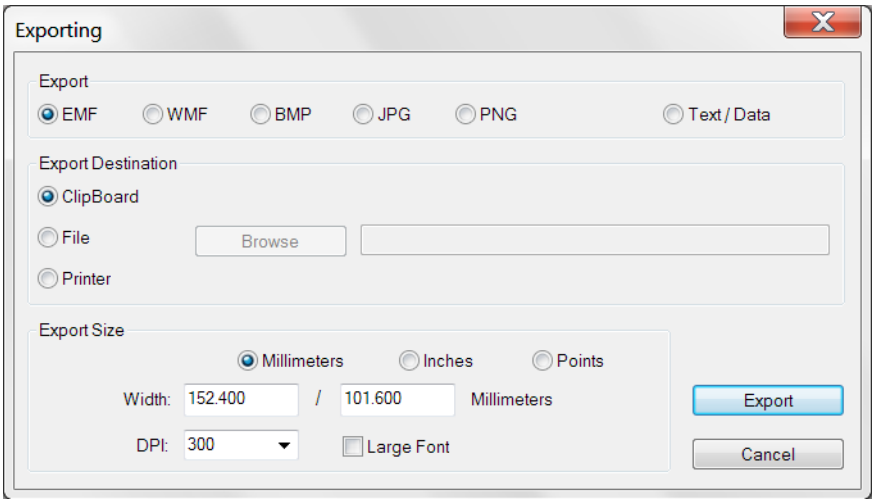


Figure 7-60: *Exporting Dialog Box*

Table 7-9: *Runtime Binding Chart Export Options*

Task	*Export*	*Option*	*Export Destination*	*Result*
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click **File** > **Browse** to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click **File** > **Browse** to select a folder and enter a file name.	Creates a graphic image.
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it if necessary.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Table 7-10: *Managing Experiment Method Files*

Menu Bar Command/ Toolbar Button	*Description*
File > **Open Method File**	Enables you to select and open a method file (.fmf)
File > **Save Method File** or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > **Save Method File As**	Saves a method file to a new name so that the original file is not overwritten.

CHAPTER 8:

Kinetics Experiments: Octet RED384 and QK384

INTRODUCTION

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 8-1) to set up and analyze a quantitation experiment.

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

Software	*Step*	*See*
Data Acquisition 	1. Select a kinetics experiment in the **Experiment Wizard** or open a method file (.fmf).	"Starting a Basic Kinetics Experiment" on page 277
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 278
	3. Define a or import a reagent plate (optional).	"Working with a Reagent Plate" on page 299
	4. Specify assay steps.	"Defining a Kinetic Assay" on page 302
	5. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 313
	6. Run the experiment.	"Running a Kinetics Experiment" on page 324
Data Analysis 	7. View and process the raw data. 8. Analyze the data.	*Octet System Data Analysis Software User Guide*

NOTE: *Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the* ***Options*** *window when the software is relaunched.*

STARTING A BASIC KINETICS EXPERIMENT

You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File** > **Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see "Managing Experiment Method Files" on page 338.
- On the menu bar, click **Experiment** > **Templates** > **Kinetics**.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Starting an Experiment Using the Experiment Wizard

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

1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button  , or click **Experiment** > **New Experiment Wizard** (**Ctrl+N**) from the **Main Menu**.
2. In the **Experiment Wizard**, click **New Kinetics Experiment** (see Figure 8-1, left).
3. Click the arrow button().

   The **Basic Kinetics Experiment** window displays (Figure 8-1, right).

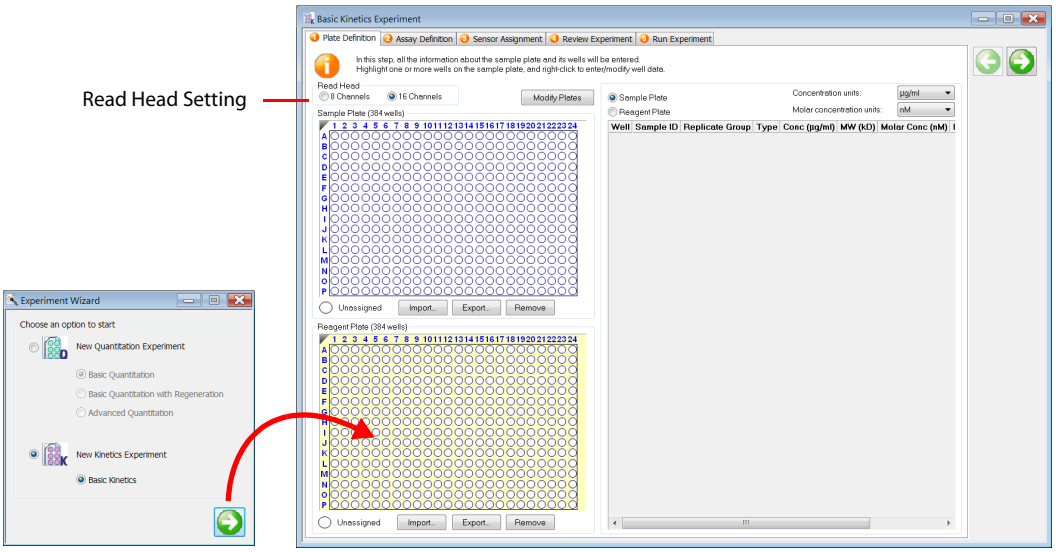


Figure 8-1: *Starting a Kinetics Experiment with the Experiment Wizard*

DEFINING THE SAMPLE PLATE

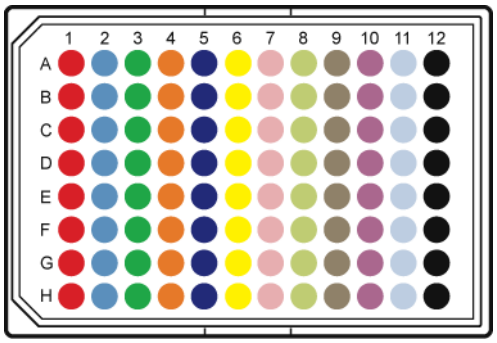
The steps to define a sample plate include:

Step	*See Page*
1. Select the instrument read head configuration (8 or 16 channels).	278
2. Select the sample plate format (96 or 384 wells).	280
3. Designate the samples.	280
4. Save the sample plate definition (optional).	296

Read Head Configuration and Plate Layout

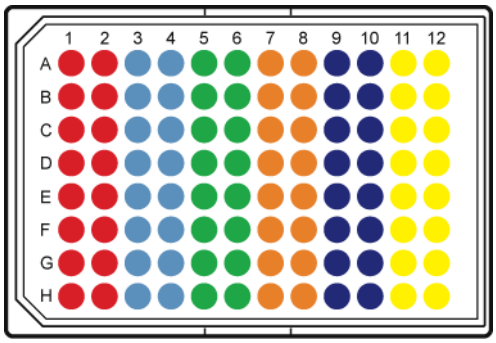
The Octet read head contains the collection optics. If the read head is set to 8 channels, one column of 8 biosensors interrogate 8 plate wells. If the read head is set to 16 channels, two columns of biosensors interrogate 16 wells (see Figure 8-2). The read head configuration and the plate format (96 or 384 wells) determine the plate layout (see example Figure 8-2).

8 Channel Read Head



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

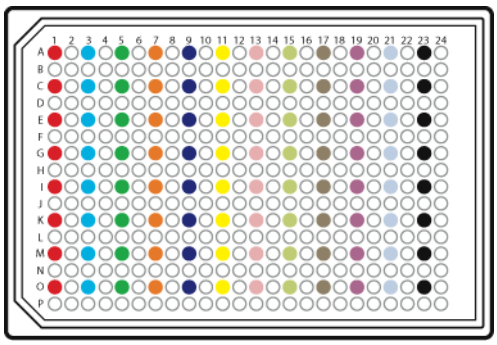
16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.

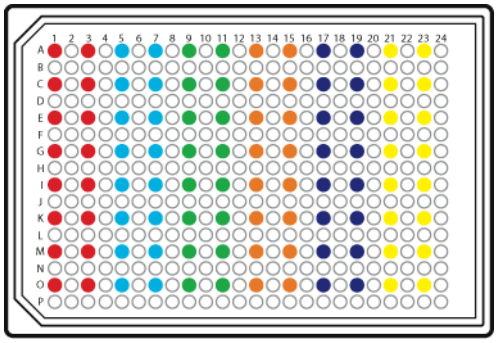
Figure 8-2: *Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head*

8 Channel Read Head



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

16 Channel Read Head



Biosensors interrogate 16 wells in two columns. Columns 1 & 2 are interrogated at the same time. Columns 3 & 4 are interrogated at the same time, and so on.

Figure 8-3: *Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head*

NOTE: *Keep the read head configuration in mind when laying out the sample plate.While reading a 384-well sample plate, both the 8 channel and 16 channel read heads can freely step through the plate by either moving left or right to step across columns or step one row up or down.*

Changing the Sample Plate Format

To change the sample plate format:

1. Click **Modify** (above the plate map).
2. In the **Modify Plates** dialog box, select **96 Well** or **384 Well** format.

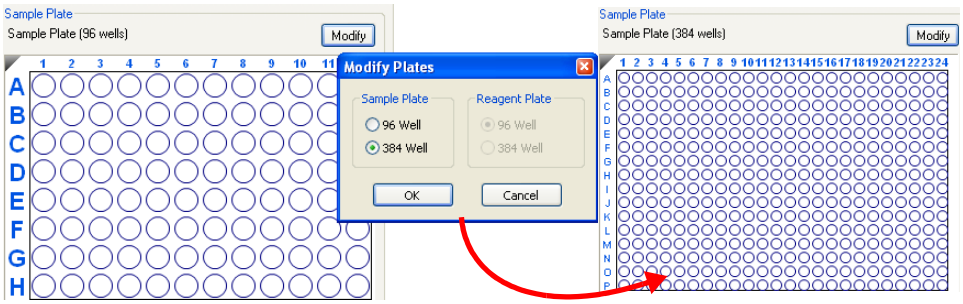


Figure 8-4: *Changing the Sample Plate Format*

Designating Samples

NOTE: *It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 8-2 will be included in the assay.*

Table 8-2 displays the well types that can be assigned to a plate map.

Table 8-2: *Types of Sample Wells*

Icon	*Description*
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.
Controls	A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis. • Positive Control: A control sample that contains analyte of known concentration • Negative Control: A control sample known not to contain analyte
Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.

Table 8-2: Types of Sample Wells

Icon	Description
(A) Activation	Activation reagent. Makes the biosensor competent for binding.
(Q) Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
(L) Load	Ligand to be immobilized (loaded) on the biosensor surface.
(W) Wash	Wash buffer.
(R) Regeneration	Regeneration reagents dissociate the analyte from the ligand.

Selecting Wells in the Sample Plate Map

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

- Click a column header or select adjacent column headers by click-hold-drag (Figure 8-5 left). To select non-adjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 8-5, center).
- Click a well or draw a box around a group of wells(Figure 8-5, right).

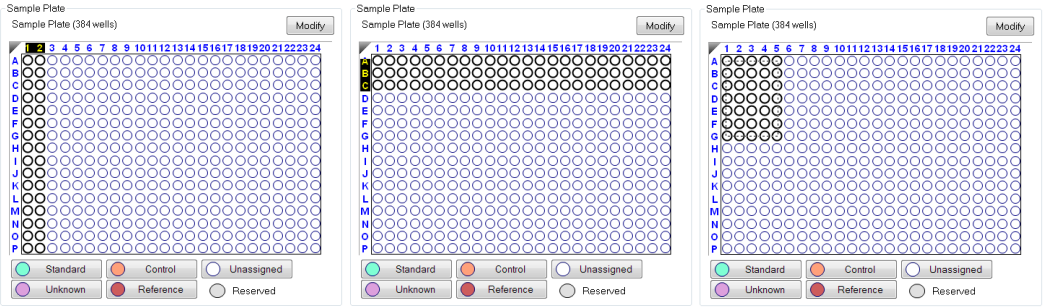


Figure 8-5: Selecting Wells in the Sample Plate Map

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

Designating Well Types

In the **Sample Plate Map**, select the wells, right-click and select a sample type. (Figure 8-6).

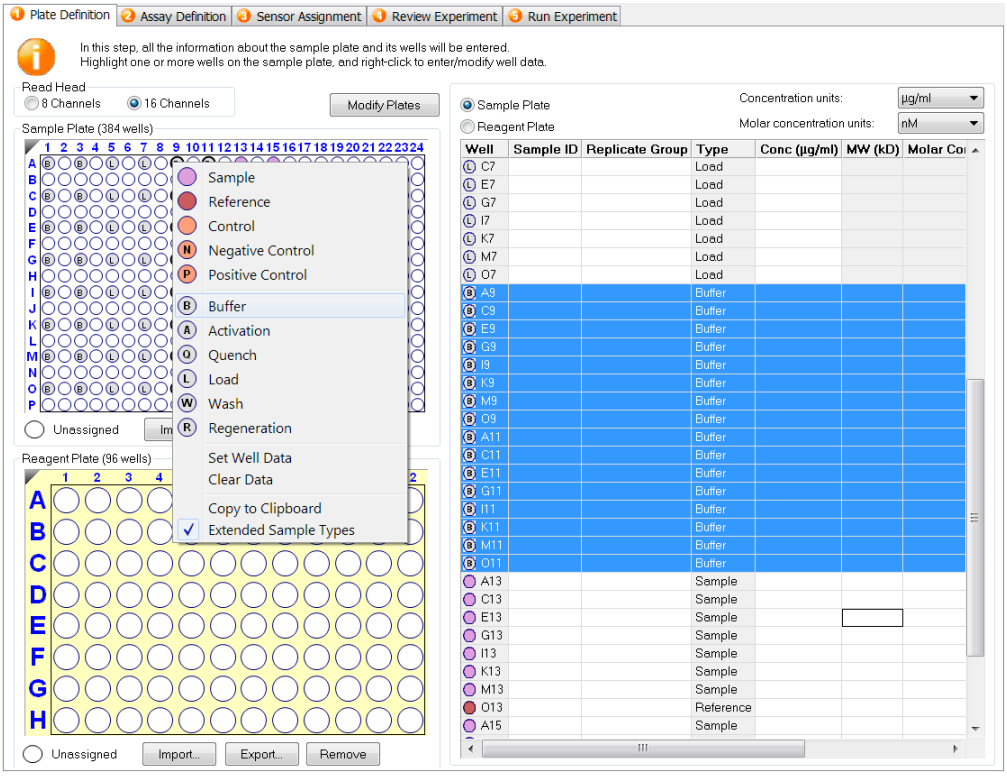


Figure 8-6: *Designating a Well Type in the Plate Definition Window*

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 8-7).

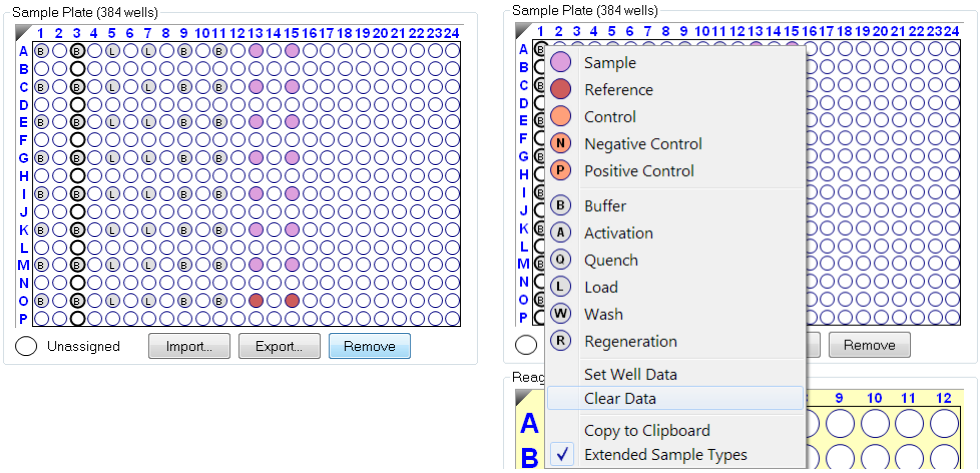


Figure 8-7: *Clearing Sample Data from a Sample Plate*

Entering Sample Information

***NOTE**: You must specify sample (analyte) concentration and molecular weight; otherwise, the Octet System Data Acquisition software cannot compute a K_D value. If the sample concentration is not specified, only k_d and k_{obs} are calculated. You can also annotate any well with **Sample ID** or **Well Information,** and assign **Replicate Groups**.*

Assigning Molecular Weight and Molar Concentration

1. In the **Sample Plate Map**, select the sample wells, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box, enter the analyte molecular and molar concentration (Figure 8-8).

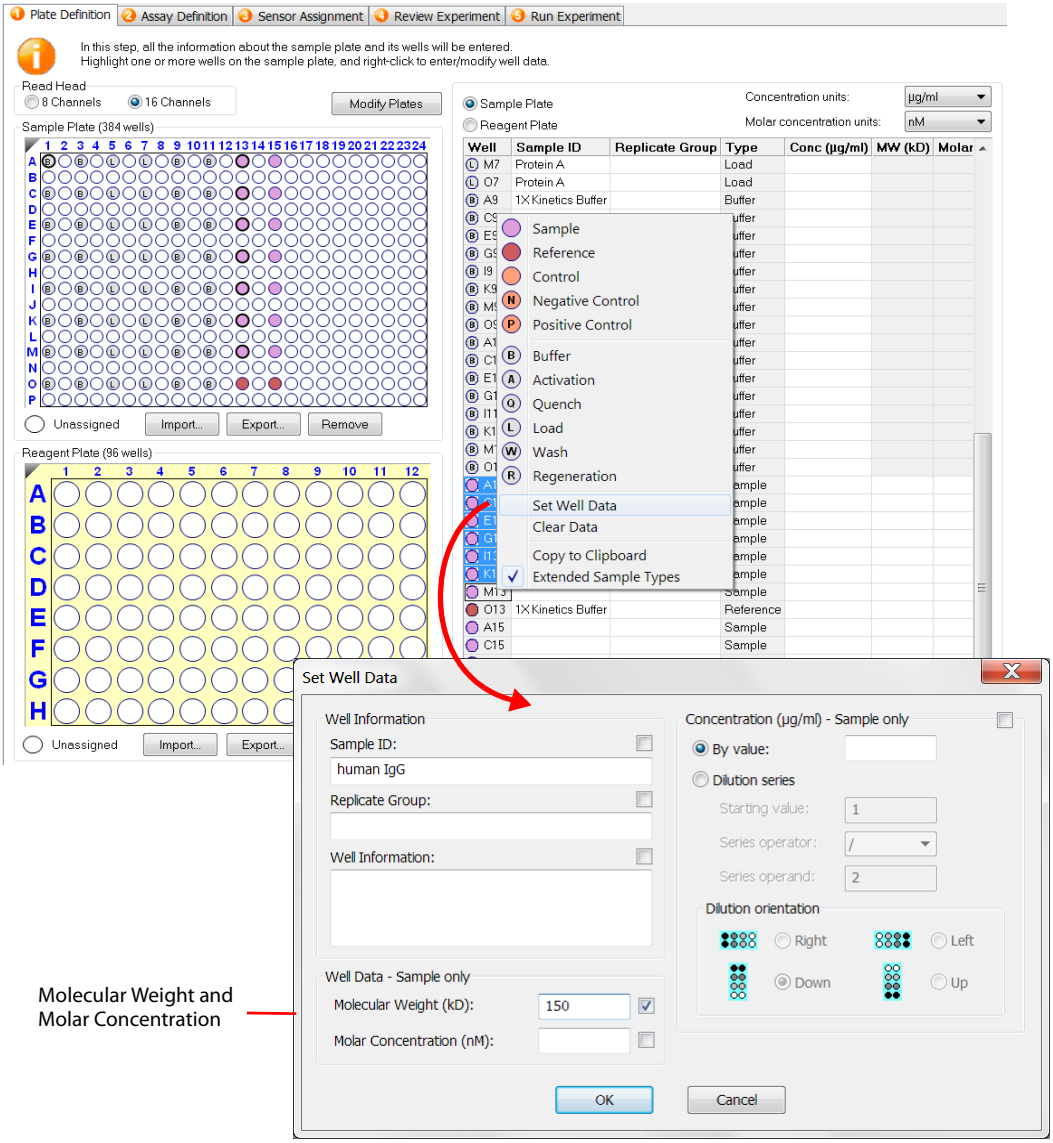
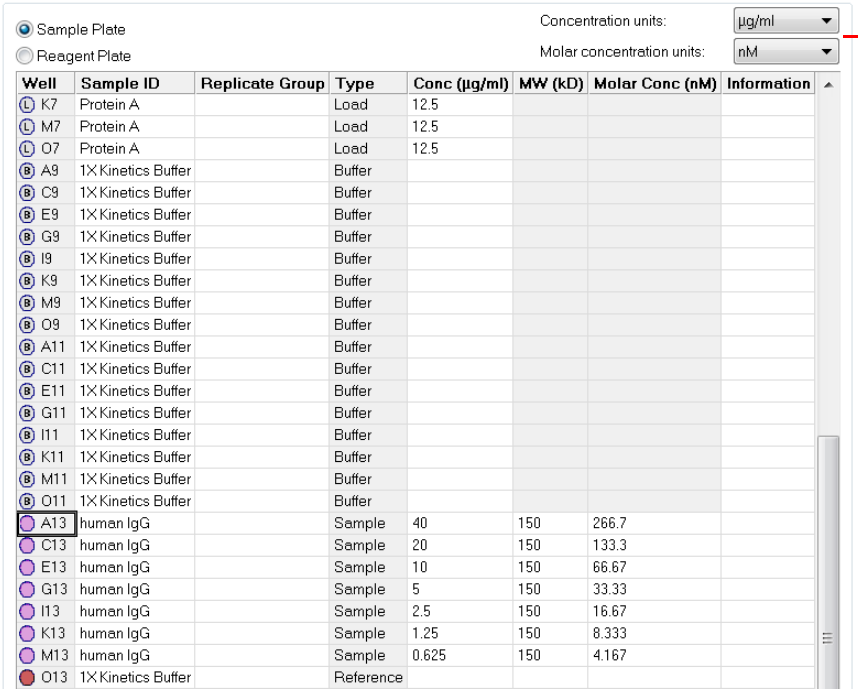


Figure 8-8: *Entering Molecular Weight and Molar Concentration from the Sample Plate Map*

The information displays in the **Sample Plate Table** (see Figure 8-9).

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



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
K7	Protein A		Load	12.5			
M7	Protein A		Load	12.5			
O7	Protein A		Load	12.5			
A9	1X Kinetics Buffer		Buffer				
C9	1X Kinetics Buffer		Buffer				
E9	1X Kinetics Buffer		Buffer				
G9	1X Kinetics Buffer		Buffer				
I9	1X Kinetics Buffer		Buffer				
K9	1X Kinetics Buffer		Buffer				
M9	1X Kinetics Buffer		Buffer				
O9	1X Kinetics Buffer		Buffer				
A11	1X Kinetics Buffer		Buffer				
C11	1X Kinetics Buffer		Buffer				
E11	1X Kinetics Buffer		Buffer				
G11	1X Kinetics Buffer		Buffer				
I11	1X Kinetics Buffer		Buffer				
K11	1X Kinetics Buffer		Buffer				
M11	1X Kinetics Buffer		Buffer				
O11	1X Kinetics Buffer		Buffer				
A13	human IgG		Sample	40	150	266.7	
C13	human IgG		Sample	20	150	133.3	
E13	human IgG		Sample	10	150	66.67	
G13	human IgG		Sample	5	150	33.33	
I13	human IgG		Sample	2.5	150	16.67	
K13	human IgG		Sample	1.25	150	8.333	
M13	human IgG		Sample	0.625	150	4.167	
O13	1X Kinetics Buffer		Reference				

Figure 8-9: *Entering Molecular Weight and Molar Concentration from the Plate Table*

Assigning User Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the desired wells, right-click and select **Set Well Data.**The **Set Well Data** dialog box displays (see Figure 8-10).
2. Select the **By value** option and enter the starting concentration value.

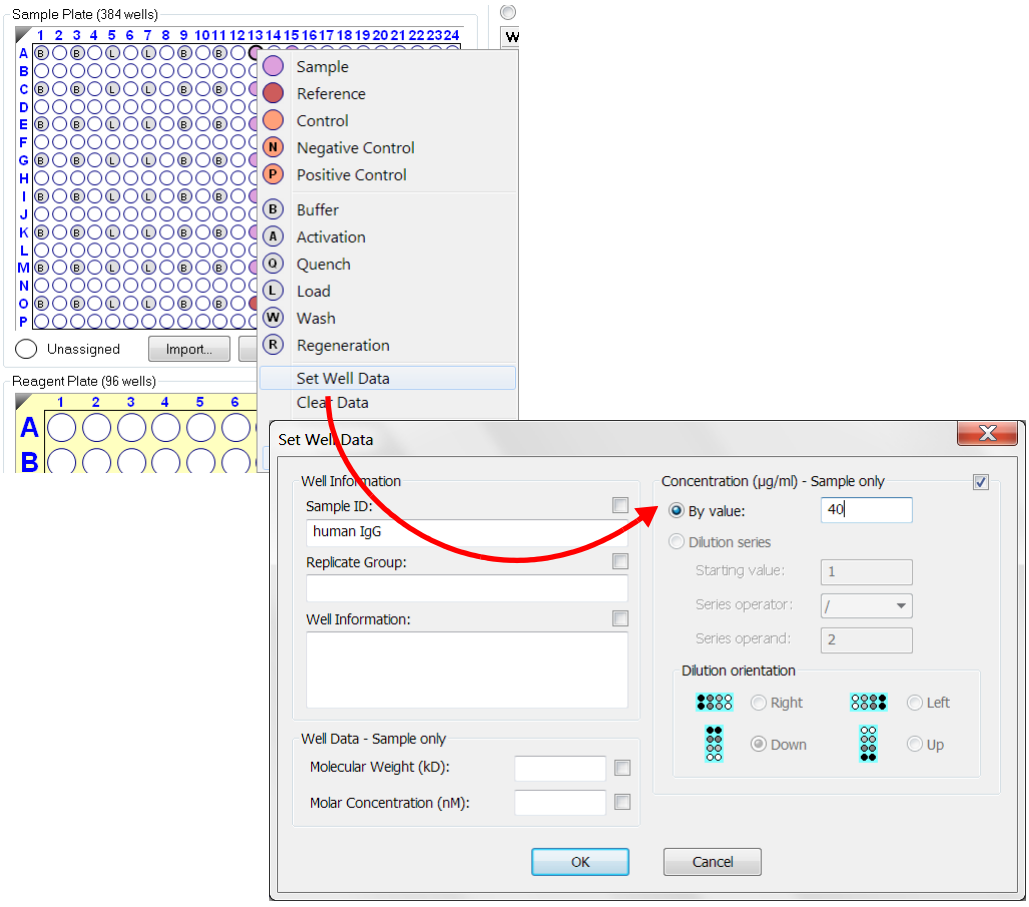


Figure 8-10: *Sample Plate Map—Assigning Sample Concentrations by Value*

3. Click **OK**. The **Sample Plate Table** will display the entered concentration.

Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

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

   The **Set Well Data** dialog box displays (see Figure 8-11)

2. Select the **Dilution Series** option and enter the starting concentration value.

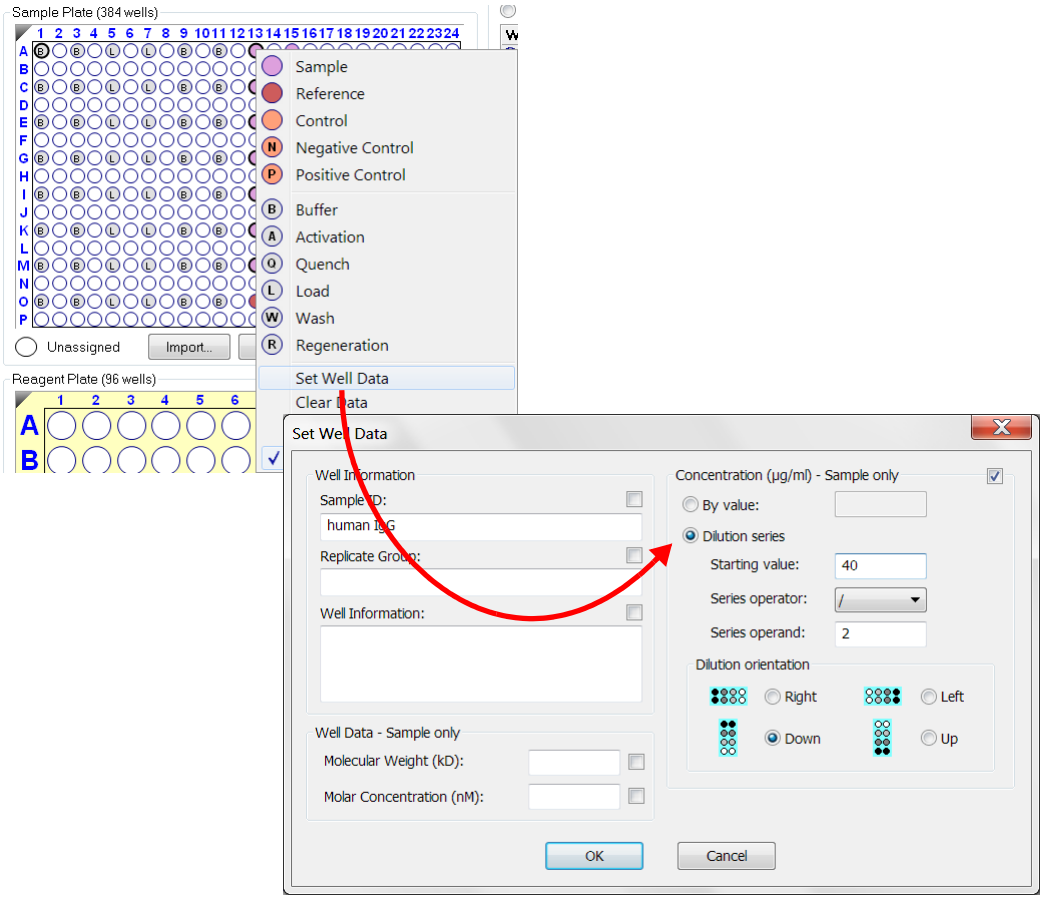


Figure 8-11: *Sample Plate Map—Assigning Sample Concentrations Using Dilution Series*

3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 8-12).

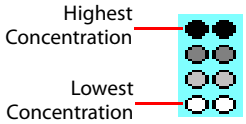


Figure 8-12: *Concentration Representation in Dilution Series:*

4. Click **OK**.

   The **Sample Plate Table** displays the standard concentrations.

Annotating Samples

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

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 8-13), enter the **Sample ID** and/or **Well Information** and click **OK**.

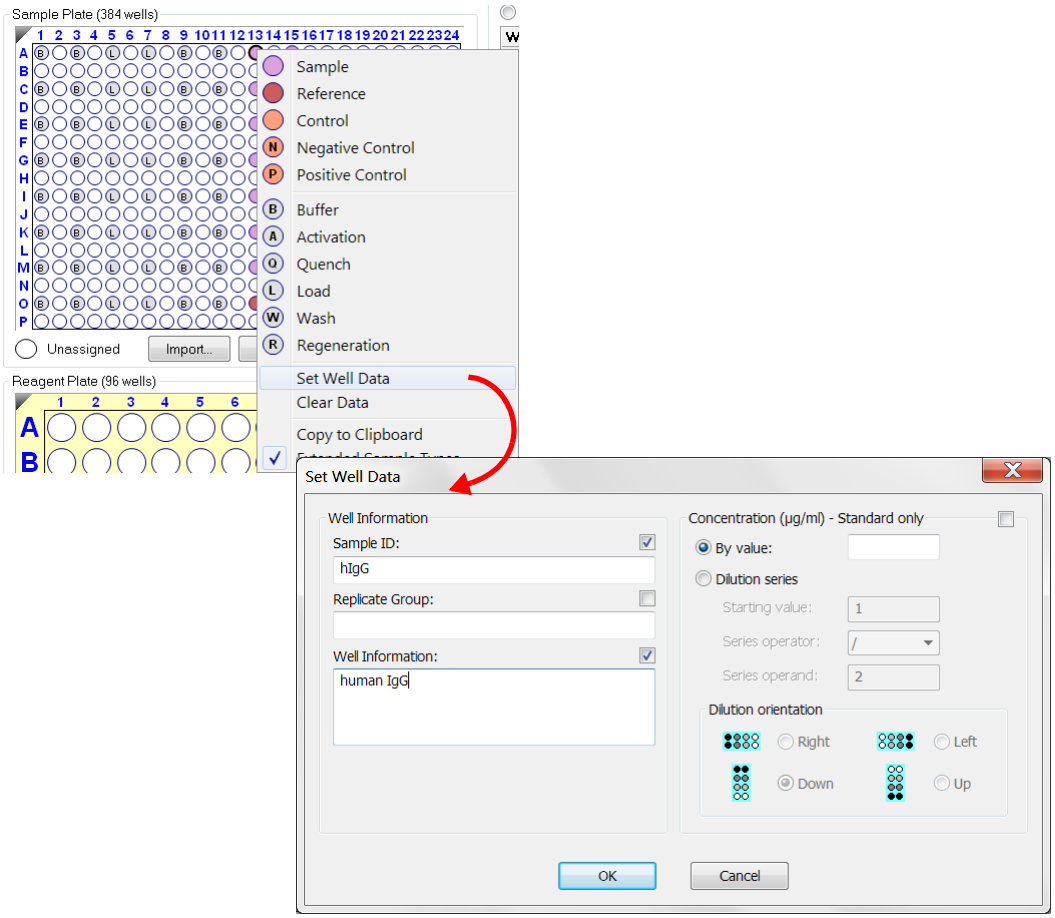


Figure 8-13: *Add Sample Annotations from the Sample Plate Map*

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 8-14).

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

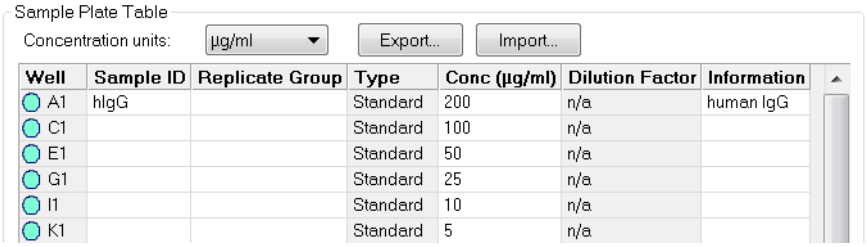


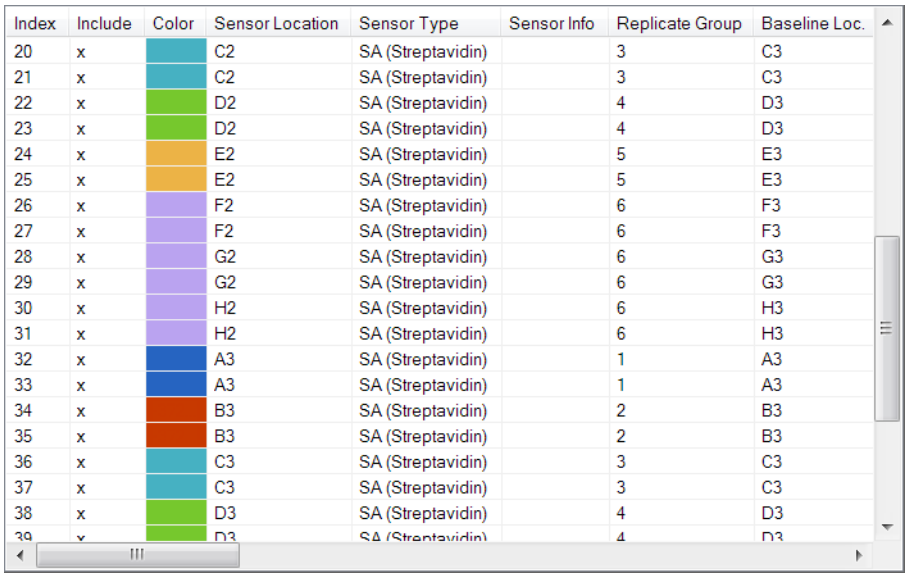
***Figure 8-14:** Add Sample Annotations in the Sample Plate Table*

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

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

Replicate Groups

Replicate Groups enable data to be organized into custom groups during data analysis (see Figure 8-15).



Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.
20	x		C2	SA (Streptavidin)		3	C3
21	x		C2	SA (Streptavidin)		3	C3
22	x		D2	SA (Streptavidin)		4	D3
23	x		D2	SA (Streptavidin)		4	D3
24	x		E2	SA (Streptavidin)		5	E3
25	x		E2	SA (Streptavidin)		5	E3
26	x		F2	SA (Streptavidin)		6	F3
27	x		F2	SA (Streptavidin)		6	F3
28	x		G2	SA (Streptavidin)		6	G3
29	x		G2	SA (Streptavidin)		6	G3
30	x		H2	SA (Streptavidin)		6	H3
31	x		H2	SA (Streptavidin)		6	H3
32	x		A3	SA (Streptavidin)		1	A3
33	x		A3	SA (Streptavidin)		1	A3
34	x		B3	SA (Streptavidin)		2	B3
35	x		B3	SA (Streptavidin)		2	B3
36	x		C3	SA (Streptavidin)		3	C3
37	x		C3	SA (Streptavidin)		3	C3
38	x		D3	SA (Streptavidin)		4	D3
39	x		D3	SA (Streptavidin)		4	D3

Figure 8-15: *Replicate Group Color-Coding*

NOTE: *Replicate Group information can also be entered in the Octet System Data Analysis software.*

Assigning Replicate Groups in the Sample Plate Map

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

1. Select the samples you wish to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 8-16), enter a name in the **Replicate Group** box and click **OK**.

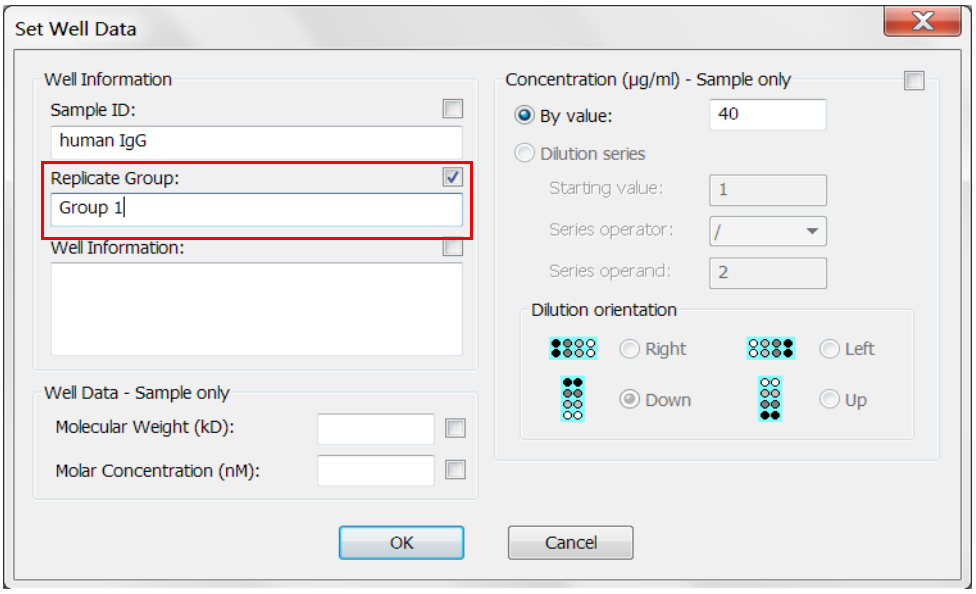


Figure 8-16: *Add Replicate Group from the Sample Plate Map*

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 8-17).

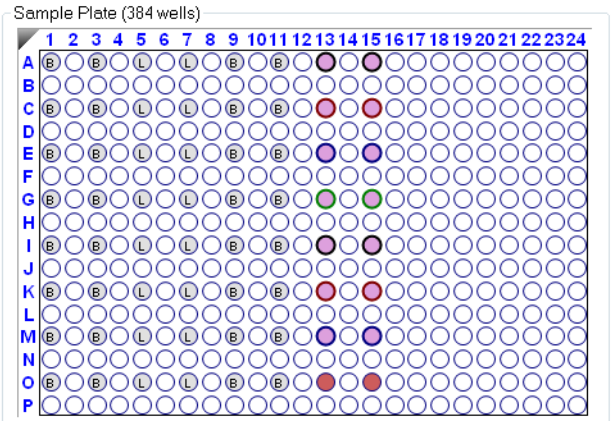
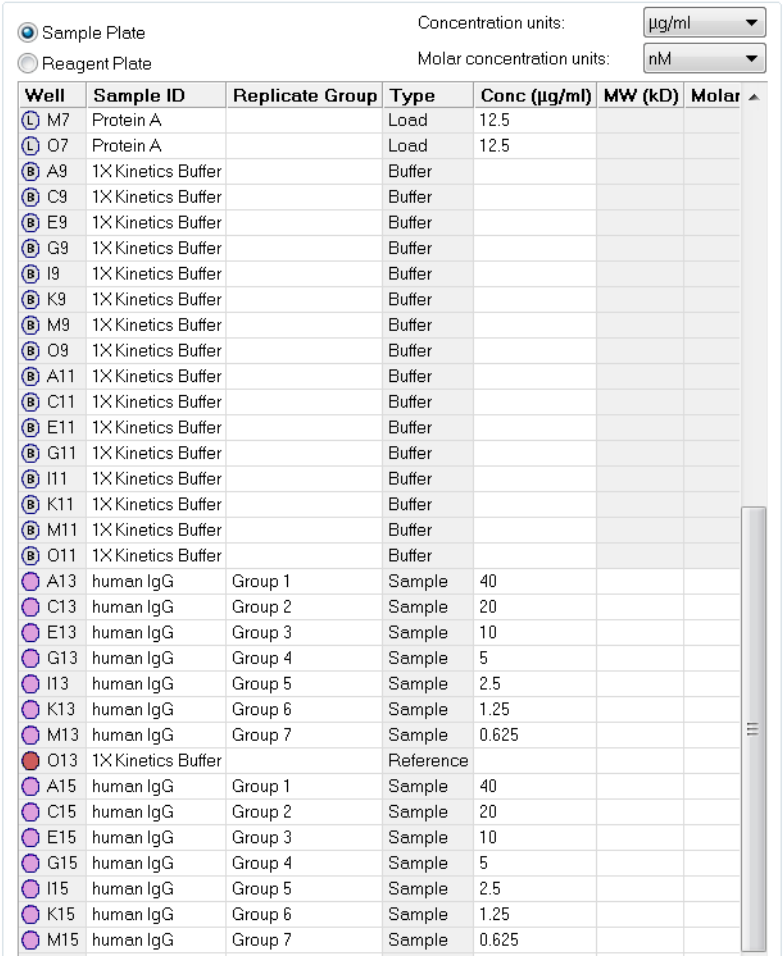


Figure 8-17: *Replicate Groups Displayed in Sample Plate Map*

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 8-18)



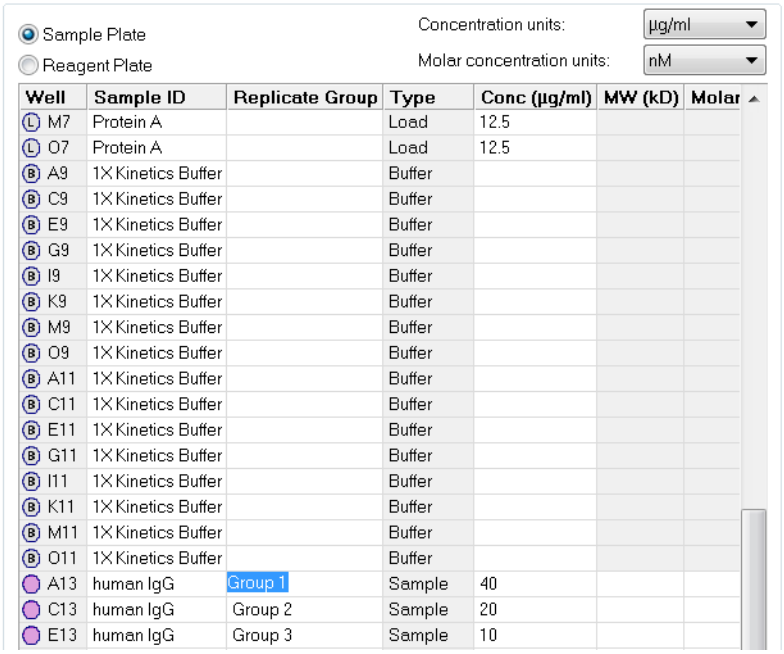
Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar
M7	Protein A		Load	12.5		
O7	Protein A		Load	12.5		
A9	1X Kinetics Buffer		Buffer			
C9	1X Kinetics Buffer		Buffer			
E9	1X Kinetics Buffer		Buffer			
G9	1X Kinetics Buffer		Buffer			
I9	1X Kinetics Buffer		Buffer			
K9	1X Kinetics Buffer		Buffer			
M9	1X Kinetics Buffer		Buffer			
O9	1X Kinetics Buffer		Buffer			
A11	1X Kinetics Buffer		Buffer			
C11	1X Kinetics Buffer		Buffer			
E11	1X Kinetics Buffer		Buffer			
G11	1X Kinetics Buffer		Buffer			
I11	1X Kinetics Buffer		Buffer			
K11	1X Kinetics Buffer		Buffer			
M11	1X Kinetics Buffer		Buffer			
O11	1X Kinetics Buffer		Buffer			
A13	human IgG	Group 1	Sample	40		
C13	human IgG	Group 2	Sample	20		
E13	human IgG	Group 3	Sample	10		
G13	human IgG	Group 4	Sample	5		
I13	human IgG	Group 5	Sample	2.5		
K13	human IgG	Group 6	Sample	1.25		
M13	human IgG	Group 7	Sample	0.625		
O13	1X Kinetics Buffer		Reference			
A15	human IgG	Group 1	Sample	40		
C15	human IgG	Group 2	Sample	20		
E15	human IgG	Group 3	Sample	10		
G15	human IgG	Group 4	Sample	5		
I15	human IgG	Group 5	Sample	2.5		
K15	human IgG	Group 6	Sample	1.25		
M15	human IgG	Group 7	Sample	0.625		

Figure 8-18: *Replicate Groups in Sample Plate Table*

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 8-19).



Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar
M7	Protein A		Load	12.5		
O7	Protein A		Load	12.5		
A9	1X Kinetics Buffer		Buffer			
C9	1X Kinetics Buffer		Buffer			
E9	1X Kinetics Buffer		Buffer			
G9	1X Kinetics Buffer		Buffer			
I9	1X Kinetics Buffer		Buffer			
K9	1X Kinetics Buffer		Buffer			
M9	1X Kinetics Buffer		Buffer			
O9	1X Kinetics Buffer		Buffer			
A11	1X Kinetics Buffer		Buffer			
C11	1X Kinetics Buffer		Buffer			
E11	1X Kinetics Buffer		Buffer			
G11	1X Kinetics Buffer		Buffer			
I11	1X Kinetics Buffer		Buffer			
K11	1X Kinetics Buffer		Buffer			
M11	1X Kinetics Buffer		Buffer			
O11	1X Kinetics Buffer		Buffer			
A13	human IgG	Group 1	Sample	40		
C13	human IgG	Group 2	Sample	20		
E13	human IgG	Group 3	Sample	10		

Figure 8-19: *Add Replicate Group from the Sample Plate Table*

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

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

---

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

IMPORTANT: *The Octet System Data Analysis software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.*

Editing the Sample Table

Changing Sample Well Designations

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection (see Figure 8-20).

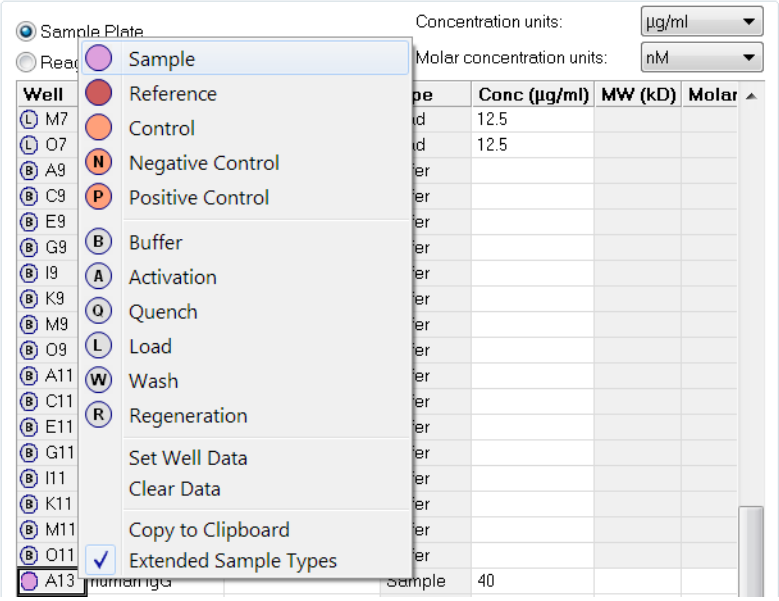


***Figure 8-20:** Sample Plate Table—Well Designation*

Editing Sample Information

To edit sample data in the **Sample Plate Table**, double-click a value and enter a new value (see Figure 8-21).

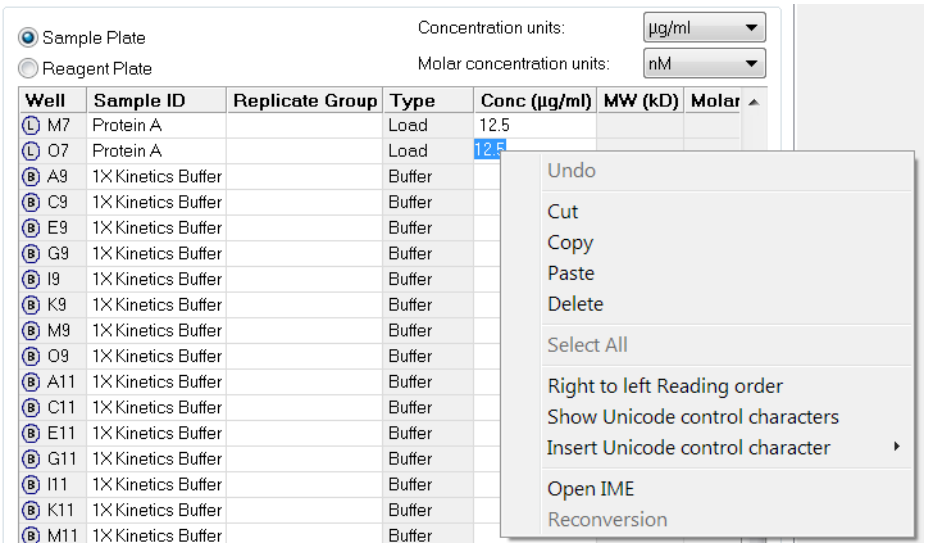


Figure 8-21: *Sample Plate Table—Editing Sample Data*

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

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

MANAGING SAMPLE PLATE DEFINITIONS

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

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Map**, click **Export** (see Figure 8-22).

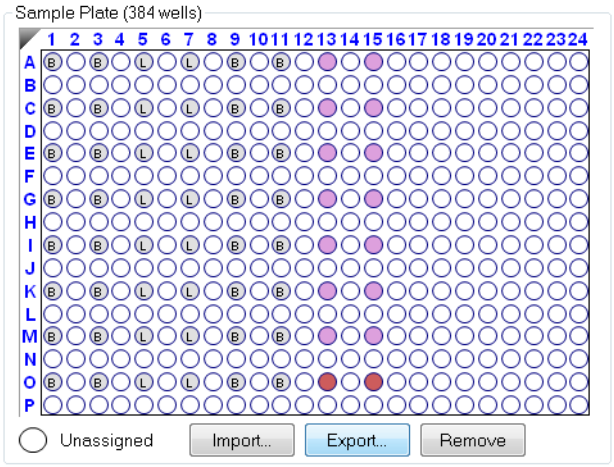


***Figure 8-22:** Sample Plate Map — Export Button*

2. In the **Export Plate Definition** window (see Figure 8-23), select a folder, enter a name for the plate (.csv), and click **Save**.

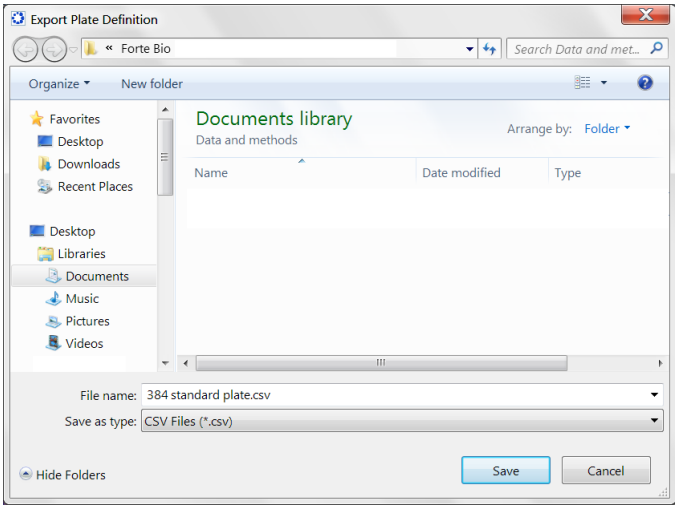


***Figure 8-23:** Export Plate Definition Window*

Importing a Plate Definition

To import a plate definition:

1. In the Plate Definition window (see Figure 8-22: on page 297), click **Import**.

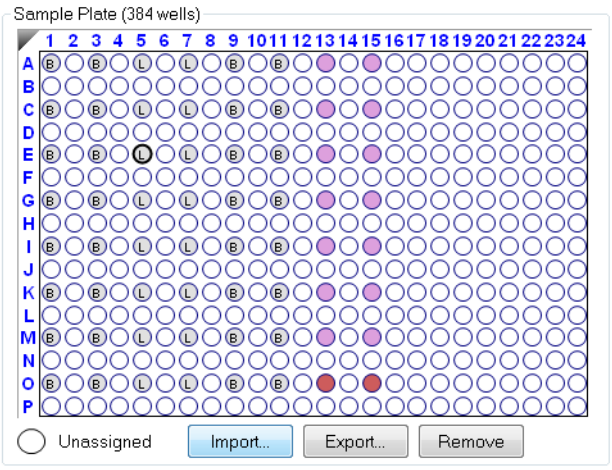


Figure 8-24: *Sample Plate Map— **Import** Button*

2. In the **Import Plate Definition** window (see Figure 8-25), select the plate definition (.csv), and click **Open**.

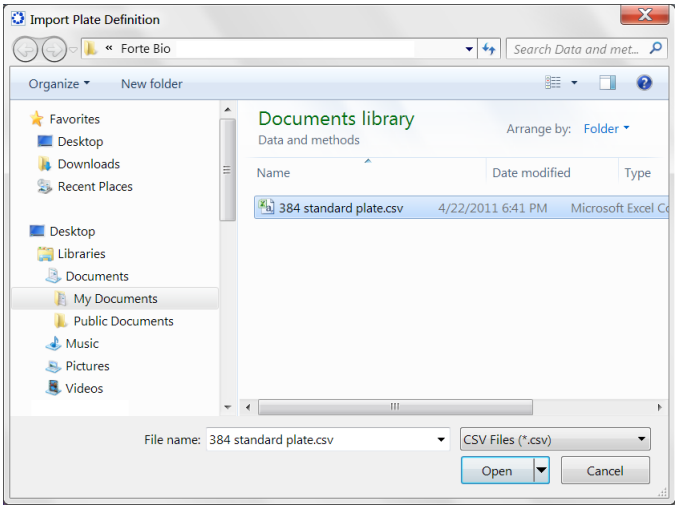


Figure 8-25: *Import Plate Definition Window*

NOTE: *You can also create a .csv file for import. Figure 8-26 shows the appropriate column information layout.*

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

384 K method 4 export

Figure 8-26: *Example Plate Definition File (.csv)*

WORKING WITH A REAGENT PLATE

You can include an optional reagent plate in a Basic Kinetics experiment. Using a reagent plate enables higher sample throughput since no reagents are included in the sample plate. An experiment can include any combination of sample and reagent plate formats (96- or 384-well). The reagent plate can be used for reagents but not samples, references or controls.

NOTE: *The reagent plate format (96- or 384-well) and the read head configuration (8 or 16 channels) determine the reagent plate layout. For more details, see "Read Head Configuration and Plate Layout" on page 278.*

To modify a reagent plate:

3. Click **Modify Plates** above the **Sample Plate Map**. The **Modify Plates** dialog box displays (see Figure 8-27).

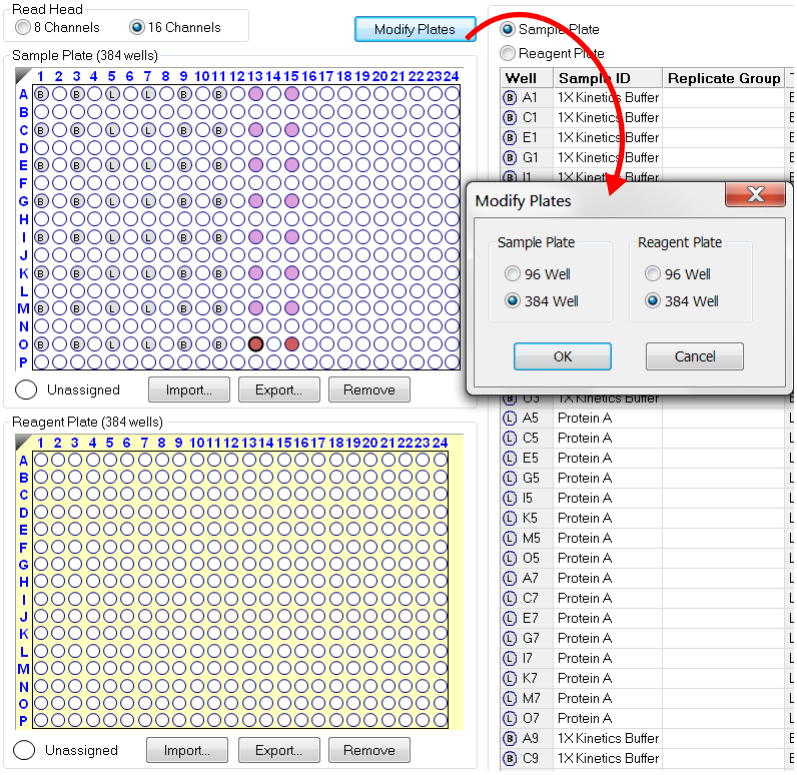


Figure 8-27: *Modifying the Reagent Plate*

4. Select a reagent plate format (**96 Well** or **384 Well**) and click **OK**.
5. Select the **Reagent Plate** radio button above the plate table. This will display the **Reagent Plate Table**.
6. In the **Reagent Plate Map**, right-click a column to use and select **Buffer**, **Activation**, **Quench**, **Load**, **Wash**, or **Regeneration** from the shortcut menu (see Figure 8-28). The well designations appear in the **Reagent Plate Table**. Repeat this step to define other wells in the reagent plate.

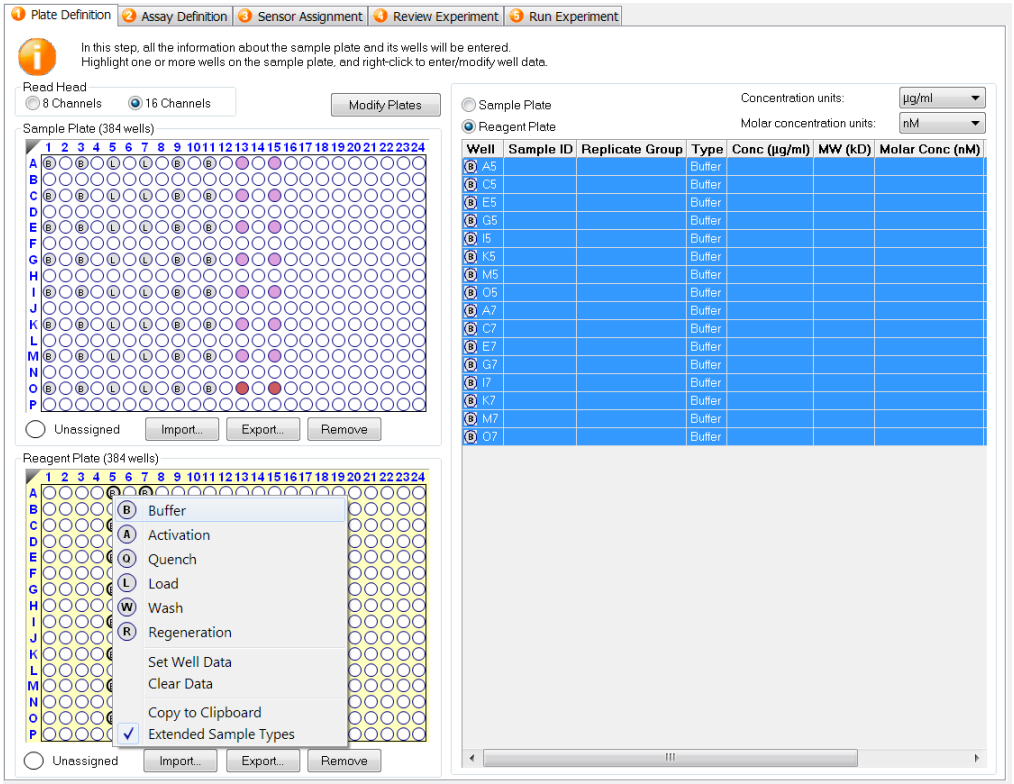


Figure 8-28: *Defining Wells in the Reagent Plate*

7. Optional: Enter well data or reagent information in the **Reagent Plate Table**.

To remove well designations, select the column(s) and click **Remove**, or right-click and choose **Clear Data**.

Saving a Reagent Plate Definition

Exporting and saving reagent plate definition is done in the same manner as you would for sample plates. For details “Managing Sample Plate Definitions” on page 296.

DEFINING A KINETIC ASSAY

After the sample plate is defined, the assay must be defined. The steps to define a kinetic assay include:

Step	*See Page*
1. Define the step types.	302
2. Build the assay by assigning a step type to a column(s) in the sample plate.	306
3. Save the sample plate definition (optional).	296

Defining Step Types

Table 8-3 lists the example step types to define a kinetic assay. Use these examples as a starting point to create your own step types.

Table 8-3: *Sample Step Types for Kinetic Assays .*

Step Type	*Step Description*
Association	Calculates the k_{obs}. Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the k_d. Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm. ***IMPORTANT:*** *An assay must include a baseline step followed by a set of association/dissociation steps to be analyzed. The Octet System Data Analysis software recognizes the baseline/association/dissociation step series during processing. Data cannot be processed if this sequence is not included in the assay setup.*
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor. ***NOTE:*** *This step may be performed offline (outside the Octet instrument).*

Table 8-3: Sample Step Types for Kinetic Assays (Continued).

Step Type	Step Description
Custom	Can be used for an activity not included in any of the above step types.
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.

Creating Step Types

Click the **Assay Definition** tab, or click the arrow to access the Assay Definition window (see Figure 8-29). The **Step Data List** shows the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay steps:

1. Click **Add**.
2. In **Assay Step Definition** dialog box (Figure 8-29), specify the step information:
   a. Choose a step type.
   b. Optional: Edit the step name.
   c. Set the step time and shake speed (**Time** range: 2 to 48,000 seconds, **Shake speed** range: 100 to 1,500 rpm or 0).

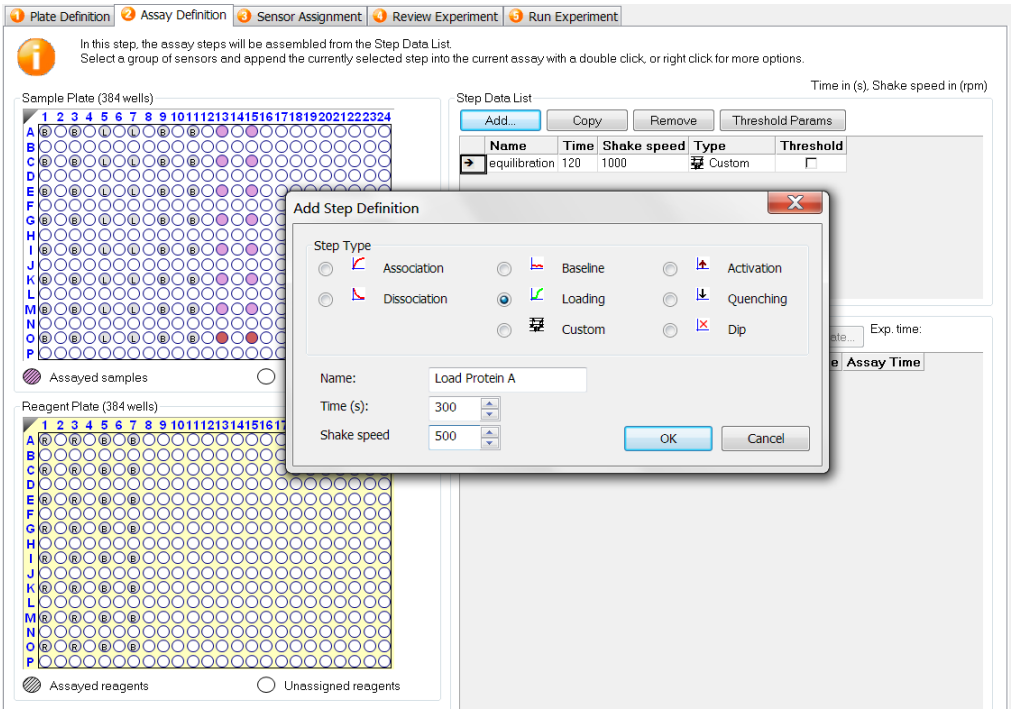


Figure 8-29: Creating an Assay Step Type

3. Apply a threshold to the step:
   a. In the **Step Data List**, click the **Threshold** check box.

      The **Threshold Parameters** dialog box displays (see Figure 8-30).

   b. Set the threshold parameters (refer to Table 8-4 for the parameter definitions).

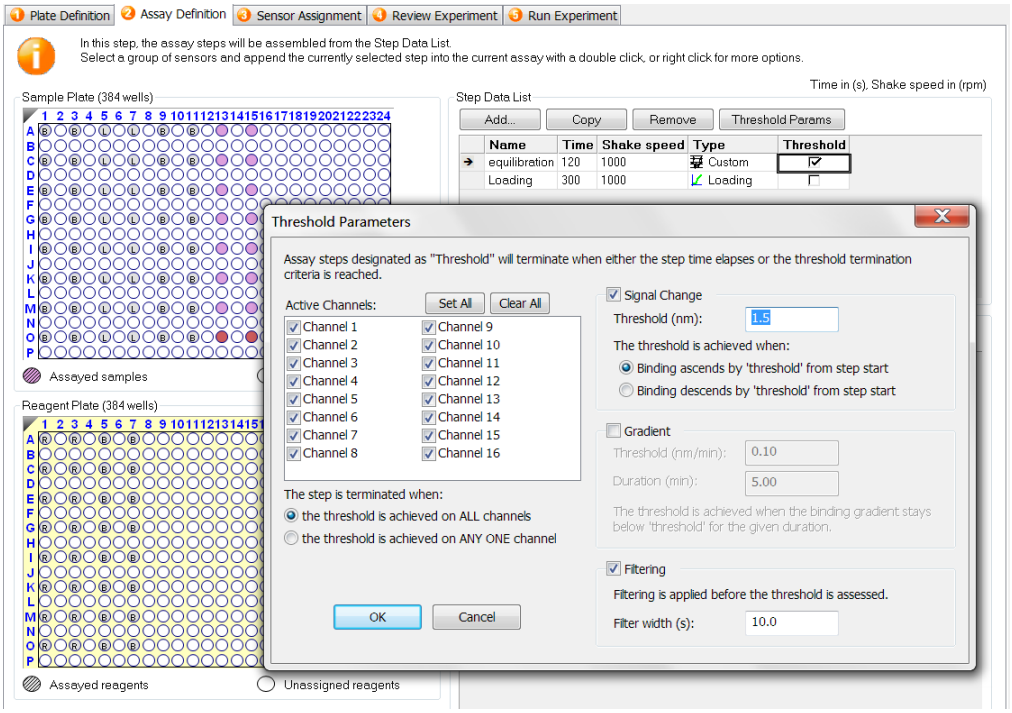


Figure 8-30: *Setting Assay Step Threshold Parameters*

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

Table 8-4: *Threshold Parameters*

Item	*Description*
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step: • **The threshold is achieved on ALL channels** • **The threshold is achieved on ANY ONE channel**
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).

Table 8-4: Threshold Parameters (Continued)

Item	Description
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

4. Click **OK** to save the newly-defined step. The new step type appears in the **Step Data List**.
5. Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 8-31).

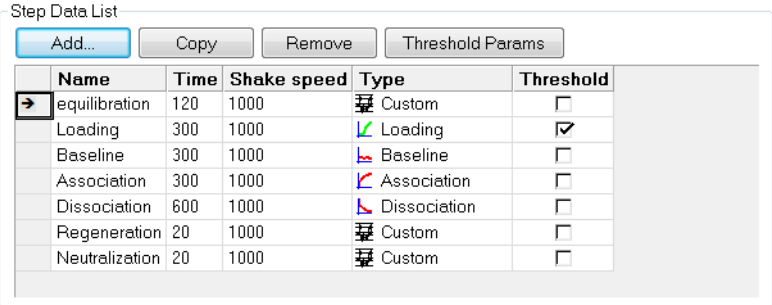


***Figure 8-31:** Step Data List—Displaying Step Types*

6. To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the **Step Data List.**

To define a step type by editing an existing one:

1. Double-click the cell in the step's **Name**, **Time**, or **Shake speed** column and then enter a new value. Or, right-click the cell to display a shortcut menu of editing commands (see Figure 8-32, left).

***NOTE:** Keyboard commands can also be used (**Ctrl**+**x**=cut, **Ctrl**+**c**=copy, **Ctrl**+**v**=paste, **Ctrl**+**z**=undo).*

2. Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 8-32, right).

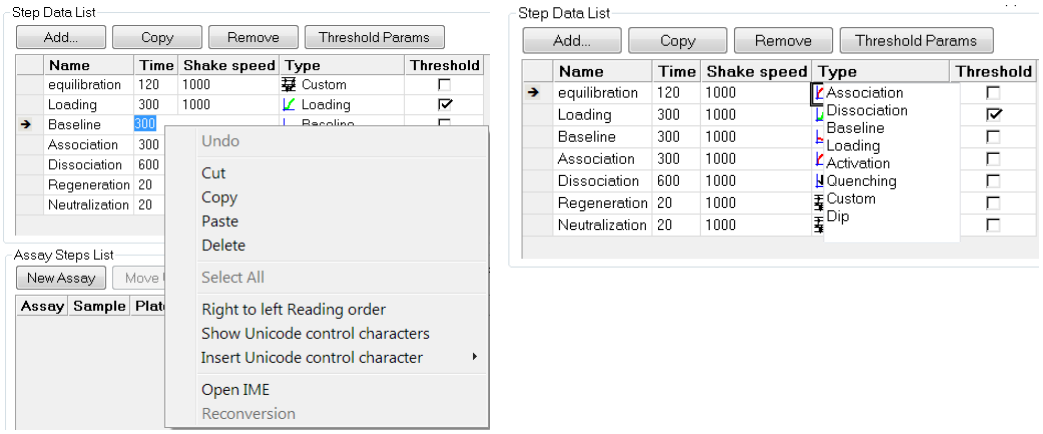


Figure 8-32: *Editing a Step Value (left) or Step Type (right)*

Building an Assay

After creating the different step types that the assay will use, step types are assigned to columns in the Sample Plate or Reagent Plate maps.

To build an assay:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate** or **Reagent Plate Map**, double-click the column that is associated with the selected step type. For information about sample or reagent plate wells, mouse over a well to view a tool tip (see Figure 8-33).

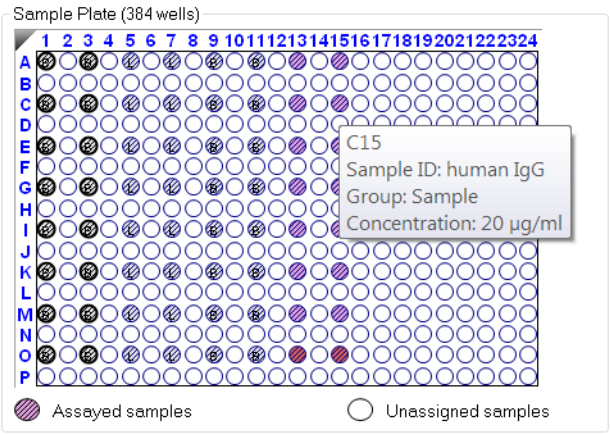


Figure 8-33: *Tool Tip of Well Information*

The selected wells are marked with hatching (for example,) and the step appears in the **Assay Steps List** (see Figure 8-34) with an associated **Assay Time**.

***NOTE:** In the **Assay Steps List**, Plate 1 is the Sample Plate and Plate 2 is the Reagent Plate.*

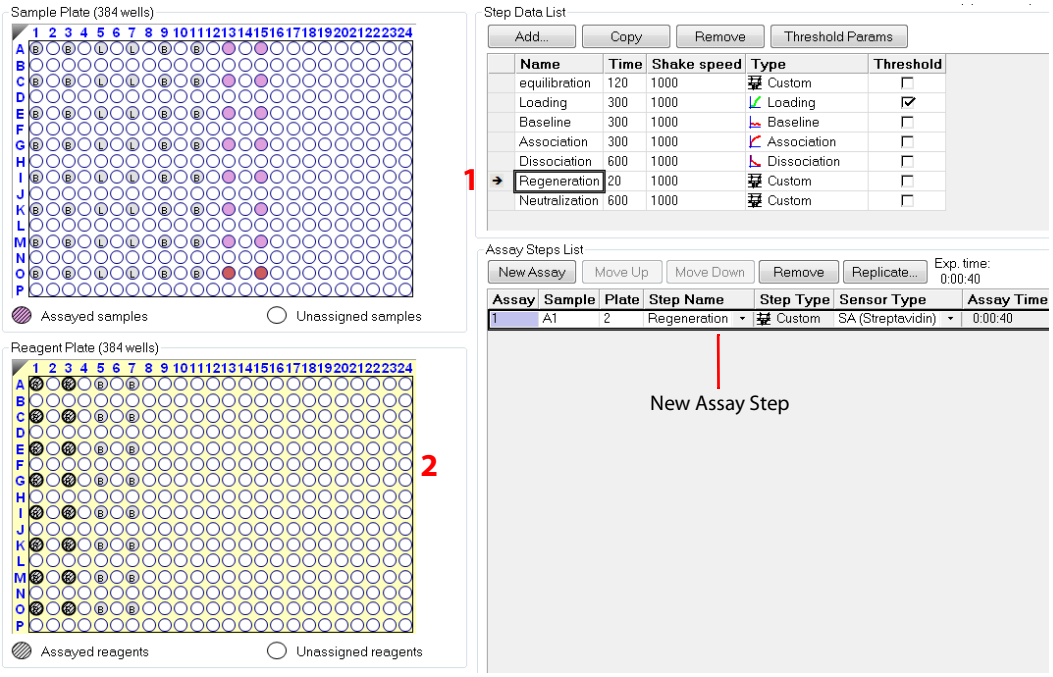


***Figure 8-34:** Assigning a Step Type to a Column in the Sample Plate*

3. Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 8-35).

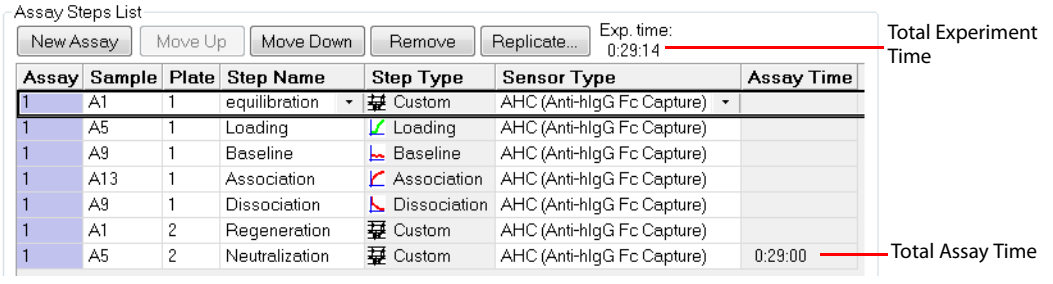


***Figure 8-35:** Experiment and Assay Time Updates as Steps Are Added to the Assay*

IMPORTANT: *If you intend to analyze the data from a sample using the* ***Inter-step correction*** *feature in the Octet System Data Acquisition software, the assay must use the same well to perform baseline and dissociation for the sample.*

Replicating Steps Within an Assay

To copy steps and add them to an assay:

1. In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 8-36, step rows 1–4 are selected).
   - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
   - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
2. In the **Replicate Steps** dialog box (see Figure 8-36), click the **Append to current assay** option.
3. Click the **Offset steps** check box and set the options, as appropriate. (For more details on offset options, see Table 8-5.)

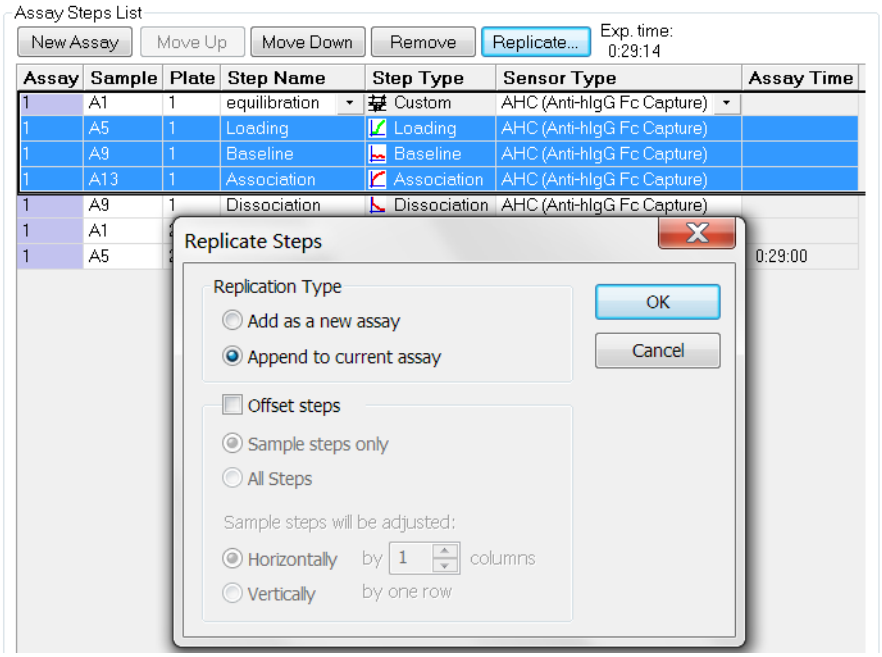


Figure 8-36: *Replicating Assay Steps by Appending*

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

Table 8-5: *Replicate Steps Options .*

Item	*Description*
Add as a new assay	Adds the replicate step(s) as a new assay to the **Assay Steps List.**
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
Sample steps only	Applies the offset to the sample plate only.
All steps	Applies the offset to the sample plate and reagent plate.
Sample steps will be adjusted horizontally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter **1**. Enter **0** to apply the step(s) to the same columns.
Sample steps will be adjusted vertically by one row	Choose this option to put the replicate step in the same column, but the next row.

Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensor column:

1. Select a column in the **Sample Plate Map**.
2. Right-click to view the shortcut menu and select **Start New Assay** (see Figure 8-37).
3. Add steps to the assay as described earlier.

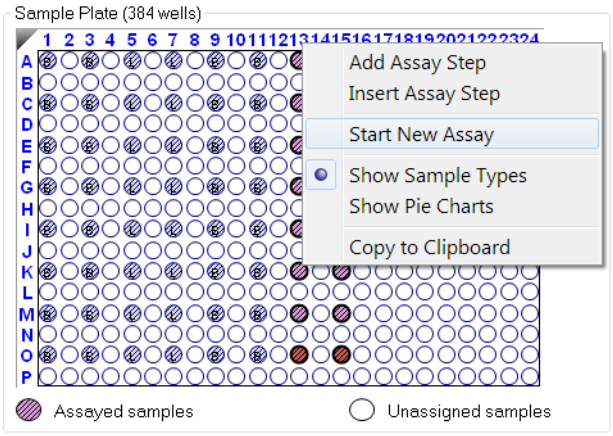


Figure 8-37: *Start New Assay*

Inserting or Adding an Assay Step

To insert an assay step:

1. Select a step in the **Step Data List**.
2. In the **Assay Steps List**, select the row above where you want to insert the step.
3. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Insert Assay Step**.

   The step is inserted into the **Assay Steps List**.

To add an assay step:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Add Assay Step**.

   The step is added to the end of the **Assay Steps List**.

Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow ( ) for any step in the assay and select a sensor type from the drop-down list (Figure 8-38). The biosensor type will automatically update for every assay step.

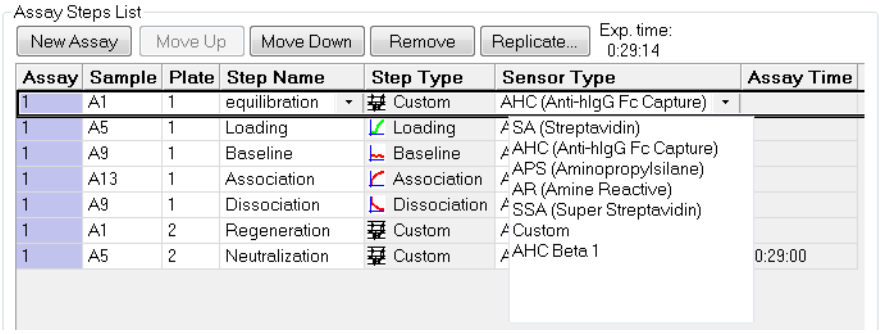


Figure 8-38: *Selecting an Assay Sensor Type*

NOTE: *The **Sensor Type** for the assay must be selected or changed from the **Assay Steps List**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.*

Editing an Assay

To edit the step type or the biosensor type:

In the **Assay Steps List**:

- To change the step type, click the **Step Name** arrow ( ) and select a step name from the drop-down list (Figure 8-39, top).
- To change the biosensor type, click the **Sensor Type** arrow ( ) for any step in the assay and select a sensor type from the drop-down list (Figure 8-39, bottom). The biosensor type will automatically update for every assay step.

***NOTE:** The **Step Name** drop-down list includes only the step types defined in the **Step Data List**.*

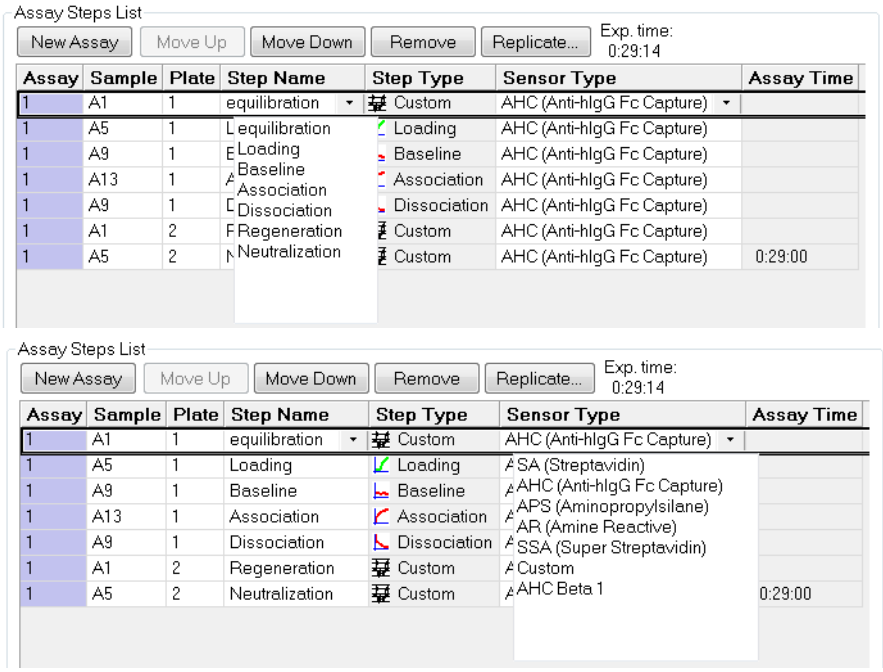


***Figure 8-39:** Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List*

To reorder or remove an assay step:

1. Select a step (row) in the **Assay Steps List**.
2. Click the **Move Up**, **Move Down**, or **Remove** button located above the list.

***IMPORTANT:** An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. The Octet System Data Acquisition software recognizes the baseline/association/dissociation set of steps.*

Adding an Assay Through Replication

A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

1. In the **Assay Steps List**, select the steps to copy and click **Replicate**.
   - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
   - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 8-40).

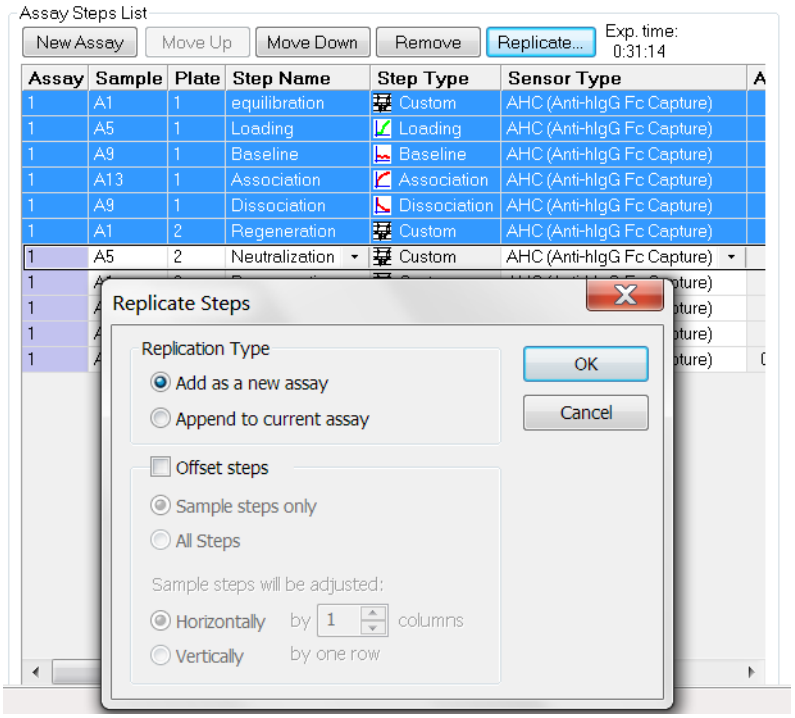


Figure 8-40: *Adding a Replicate Assay to a Plate*

3. Click the **Offset steps** check box and set the options as appropriate (see Table 8-5 on page 309 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the **Offset steps** option. If the replicate assay uses a different sample column, select **Offset steps** and the appropriate options.
   - **Sample steps only** offsets the sample wells by the value specified under **Sample steps will be adjusted**. The offset will not be applied to reagent wells such as buffer, loading, regeneration, neutralization and detection.

- **All Steps** offsets all wells in the assay, including sample and reagent wells, by the value specified under **Sample steps will be adjusted**.

4. Click **OK**. The new assay appears in the **Assay Steps List**.
5. Continue to add assay steps as needed.

ASSIGNING BIOSENSORS TO SAMPLES

After you define the sample plate and assay(s), click the **Sensor Assignment** tab or click the arrow  to access the Sensor Assignment window. The color-coded **Sensor Tray** and **Sample Plate Map** show the locations of the biosensors associated with the samples Figure 8-41).

NOTE: *When using a 96-well plate with the 8 channel read head, do not put biosensors in columns 2, 4, 6, 8, 10, and 12 if the biosensors will be returned to the biosensor tray and not discarded. If the biosensors will be ejected, biosensors can be placed in all columns.*

NOTE: *If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.*

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 8-41, the experiment includes two assays in the same wells. The use of those wells by two different biosensors is indicated by the pie chart colors.

NOTE: *The **Sensor Type** for the assay must be selected or changed from the **Assay Steps List** in the **Assay Definition Tab**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.*

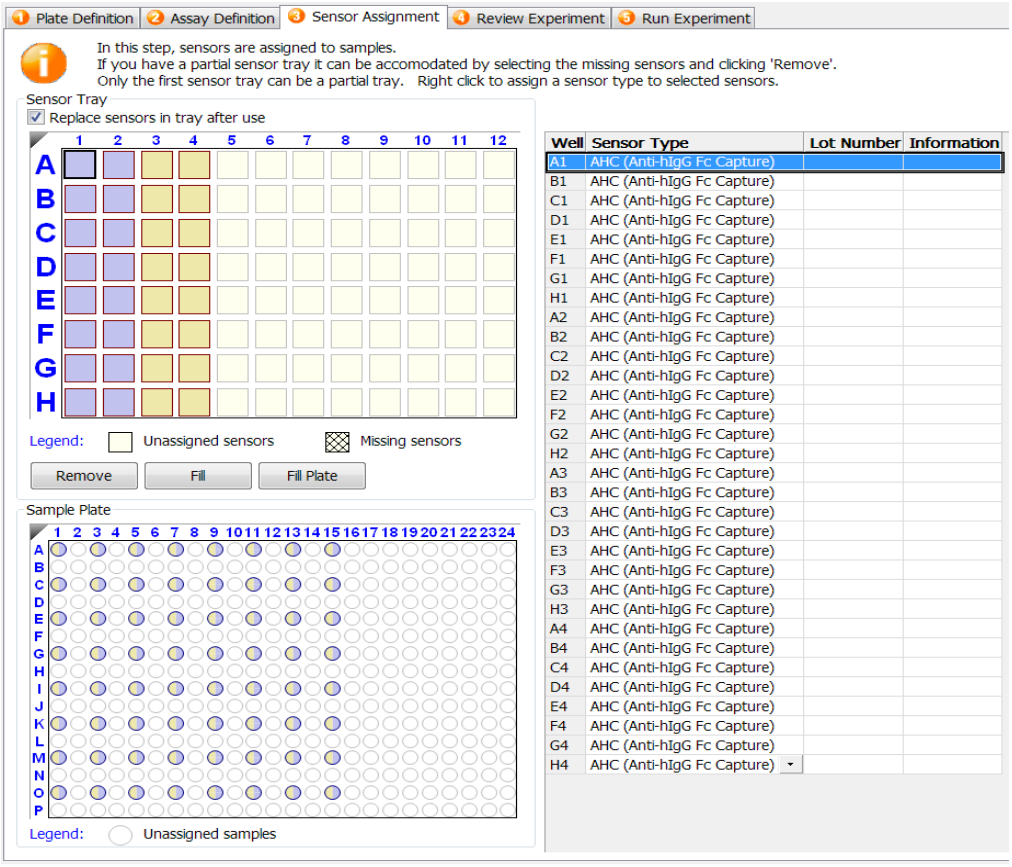


Figure 8-41: *Sensor Assignment Window*

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 8-42).

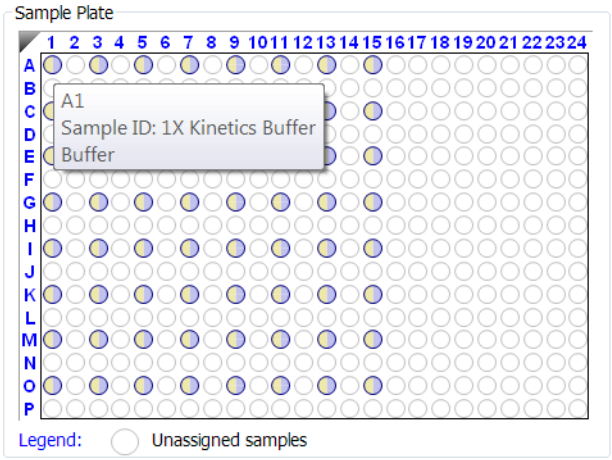


Figure 8-42: *Tool Tip of Well Information*

Replacing the Biosensors in the Biosensor Tray

After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 8-43).

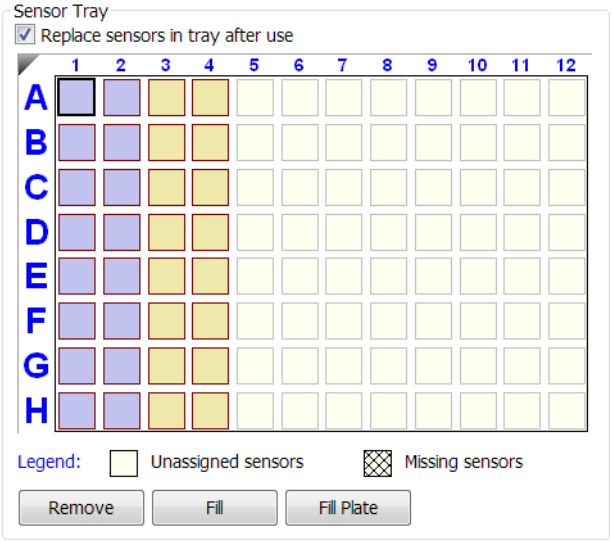


Figure 8-43: *Replace Sensors in Tray After Use Check Box*

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

Entering Biosensor Information

To enter information about a biosensor:

1. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered (see Figure 8-44).
2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 8-44).

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

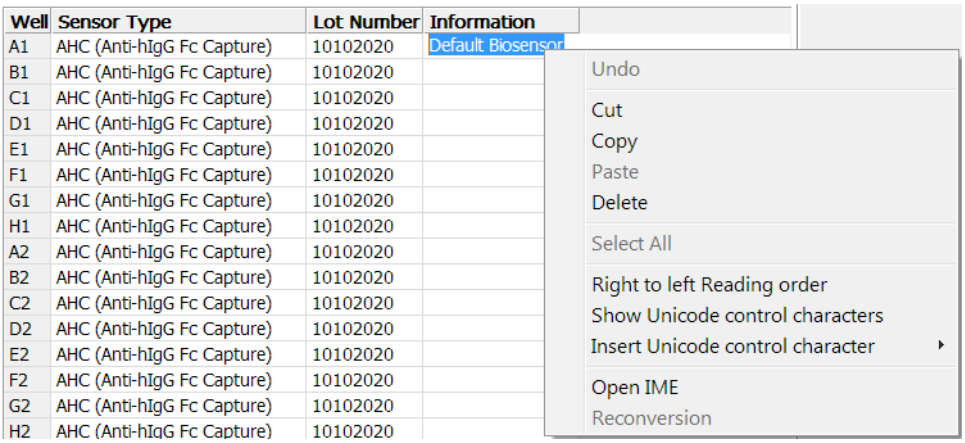


Figure 8-44: *Entering or Editing Biosensor Information*

Changing the Biosensor Location

If you prefer to not use the default biosensor columns, you can select other column(s) to use. There are two ways to do this:

- **Method 1—**In the **Sensor Tray Map**, **Remove** the columns you do not want to use. The software automatically selects the next available column(s).
- **Method 2—Remove** all columns from the **Sensor Tray Map**, then select the columns you want to use.

Method 1

In the **Sensor Tray Map**, select the columns to not use and click **Remove**. Or, right-click the selection and select **Remove** (Figure 8-45 left).The software automatically selects the next available biosensor columns in the tray (Figure 8-45 right).

Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

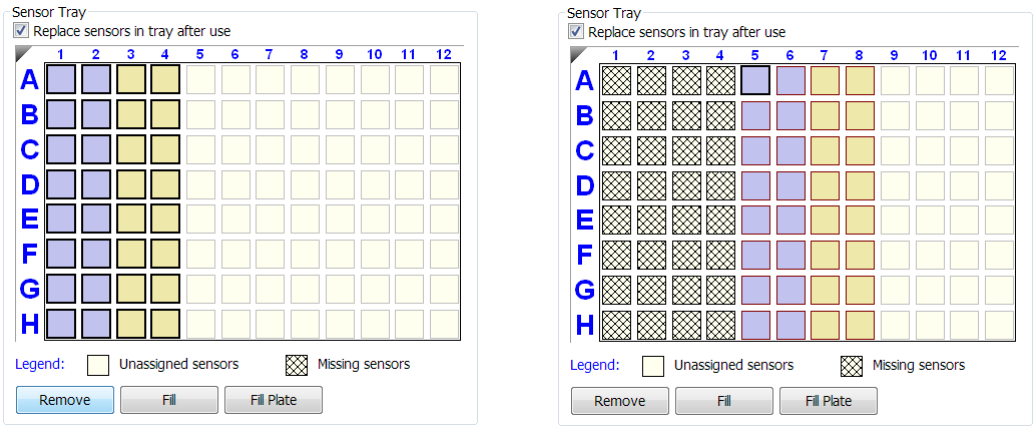


Figure 8-45: *Changing Biosensor Location (Method 1)*

Method 2

1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 8-46 top left). Or, right-click the selection and select **Remove**. All columns will be shown as **Missing** (Figure 8-46 top right).
2. Select the column(s) to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 8-46 bottom left). The software fills the selected columns in the tray (Figure 8-46 bottom right).

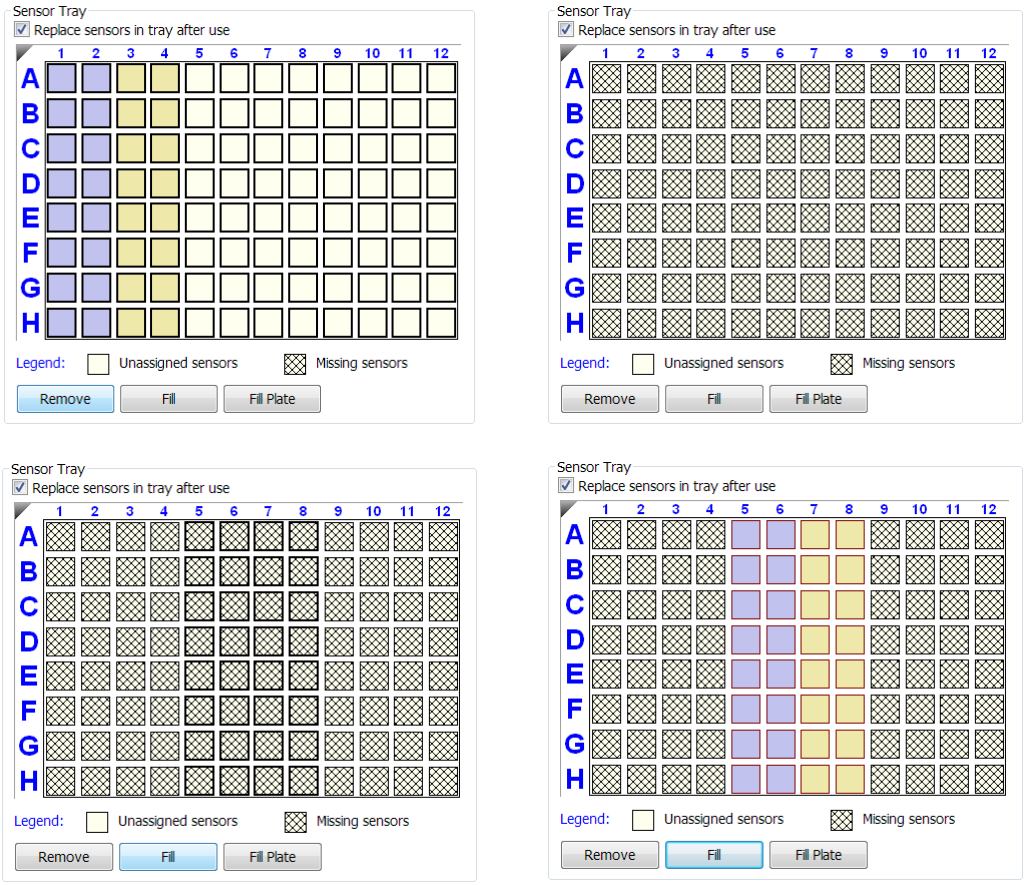


Figure 8-46: *Changing Biosensor Location (Method 2)*

Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

Using Heterogenous Trays

If heterogenous biosensor trays will be used, the column location of each biosensor type in the tray can be identified in the **Sensor Assignment Tab**. Assignment of biosensors that will not be used in the assay enables the software to auto-assign the biosensors that will be used in the assay by biosensor type.

There are two ways to change the biosensor type:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (Figure 8-47 left). The associated wells in the **Sensor Type** column will automatically populate with the biosensor type selected.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (Figure 8-47 right). All other wells in the same column of the **Sensor Tray Map** as the selected cell will automatically populate with the biosensor type selected.

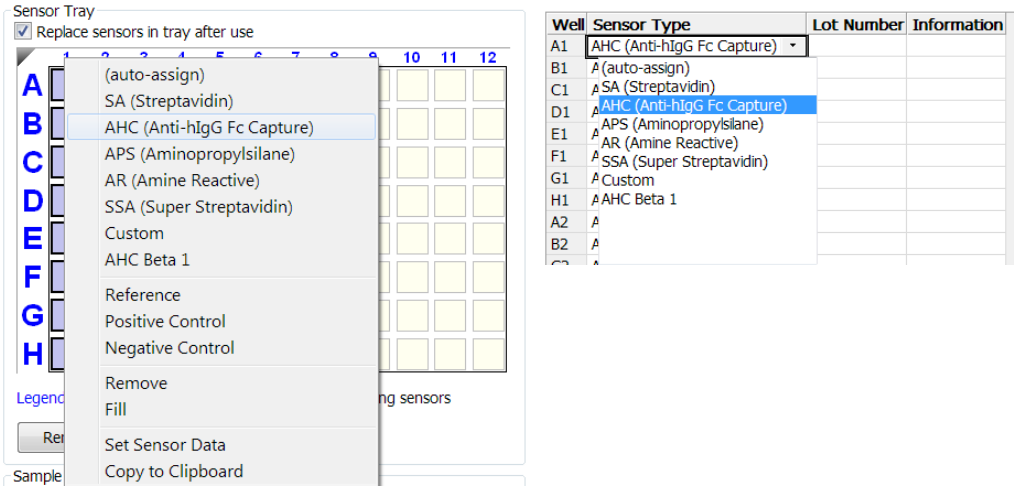


Figure 8-47: *Sensor Assignment Window—Changing the Biosensor Type*

The biosensor types shown in the **Sensor Assignment** window were specified previously in the **Assay Definition** window, and default locations are assigned automatically. To assign biosensor types for heterogenous trays:

1. Select the column location of the biosensor type (see Figure 8-48).

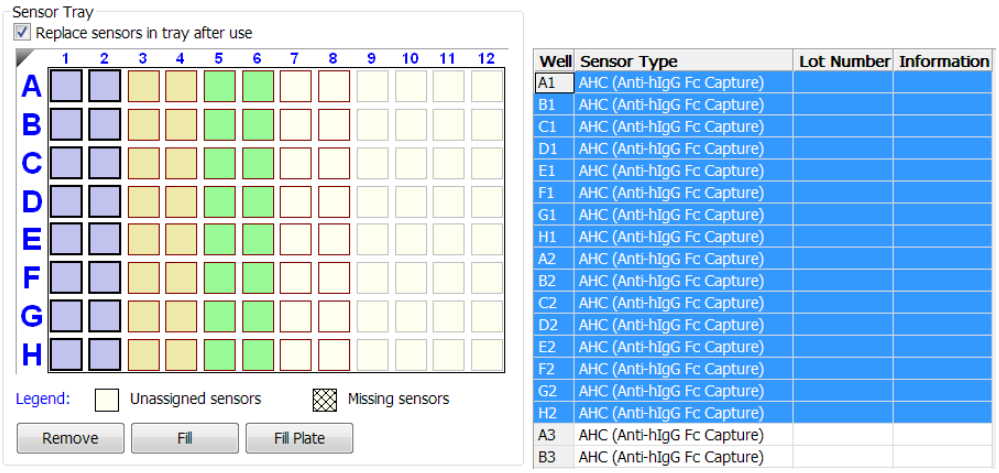


Figure 8-48: *Selecting a Sensor Tray Column*

2. Right-click in the **Sensor Tray Map** or click in a cell in the **Sensor Type** table column and select a biosensor type from the drop-down list. The biosensor type associated with the assay will shift location accordingly (see Figure 8-49). In the example shown, AHC is the **Sensor Type** used for the current assay. Columns 1 and 2 were reassigned as Streptavidin according to the heterogeneous tray being used.

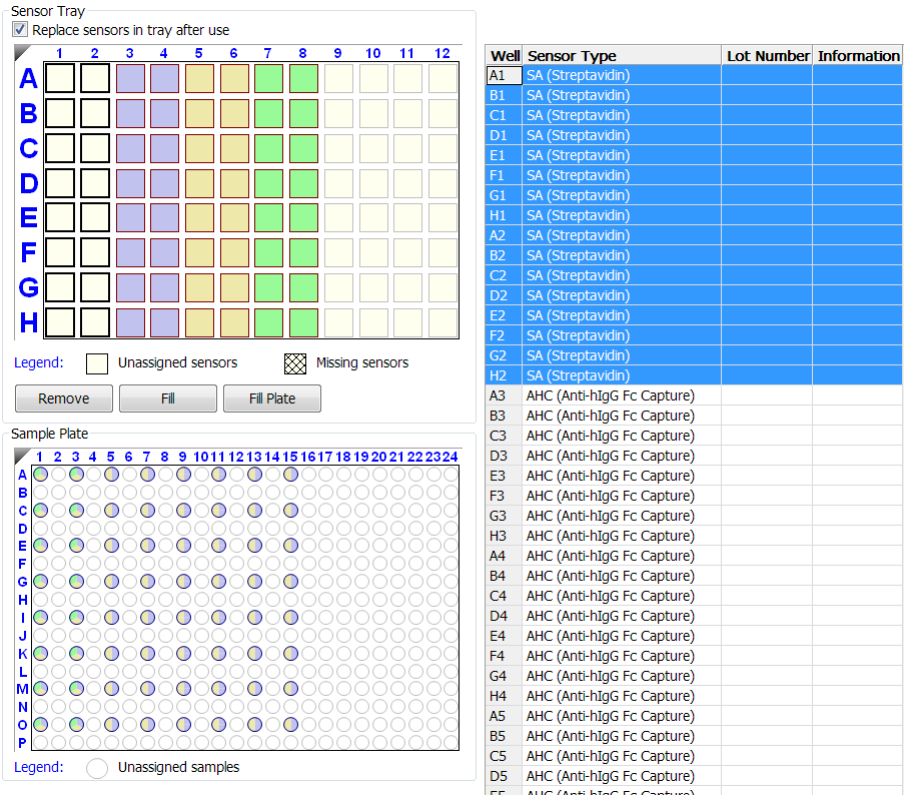


Figure 8-49: *Assay Sensor Type Reassignment*

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

IMPORTANT: *Ensure that the biosensor types selected in the* ***Assay Definition*** *window have assigned column(s) in the* ***Sensor Assignment*** *window or the experiment cannot be run.*

Using Partial Biosensor Trays

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 8-50) includes two assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 8-50 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 8-50 bottom). If necessary, change the location of these biosensors.

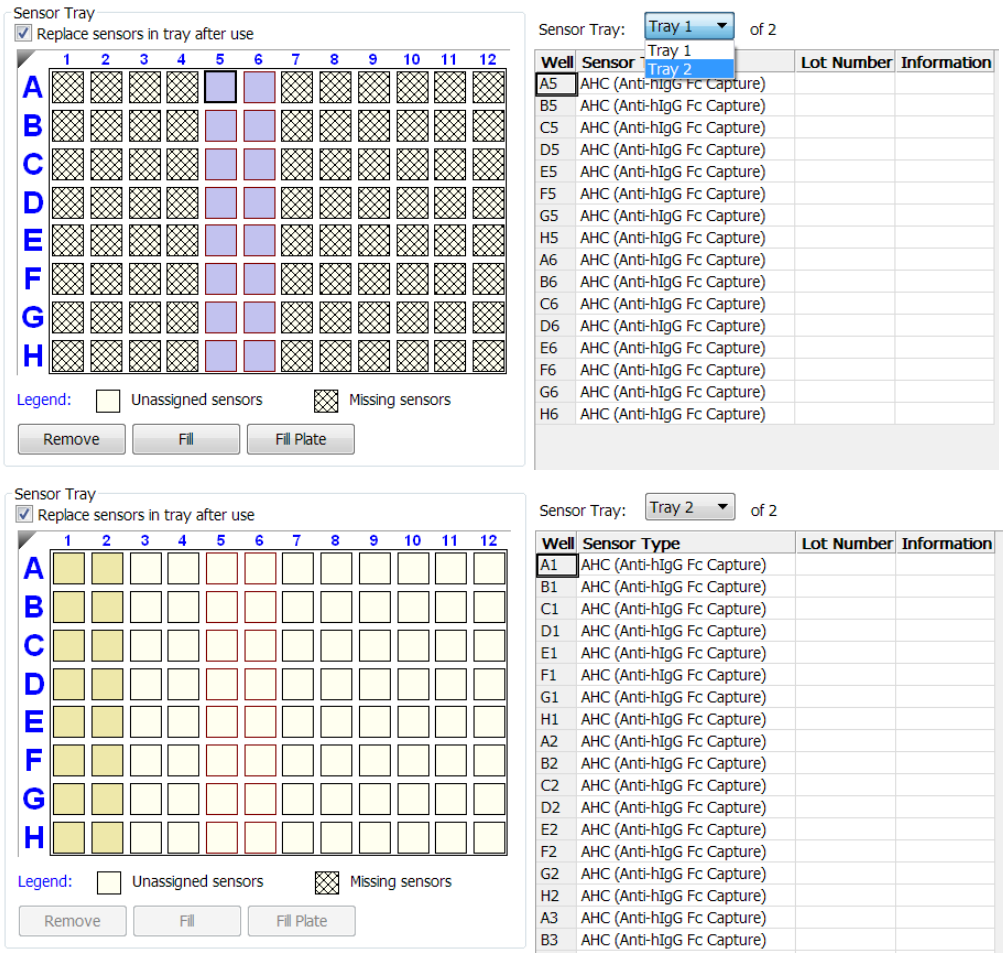


Figure 8-50: *Example Experiment Using Two Biosensor Trays*

NOTE: *Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet instrument.*

Reference Biosensors

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

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

Changing the Biosensor Type

The biosensor type used in the assay must be selected in the **Assay Definition** window. To change the biosensor type:

1. Click the **Assay Definition Tab.**
2. In the **Assay Steps List**, click the cell in the **Sensor Type** column to change.
3. Select from the drop-down list (see Figure 8-51).

IMPORTANT: *Ensure that the same biosensor types are selected in both the Assay Definition and the Sensor Assignment windows or the experiment cannot be run.*

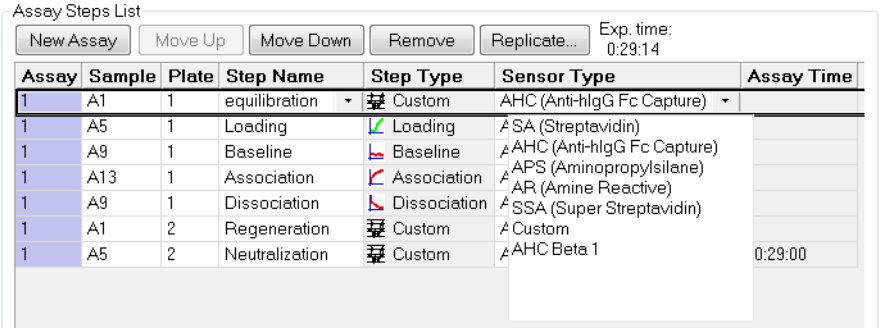


Figure 8-51: *Assay Definition Window—Changing the Biosensor Type*

REVIEWING EXPERIMENTS

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

In the **Review Experiment** window (Figure 8-52), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the ← → arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

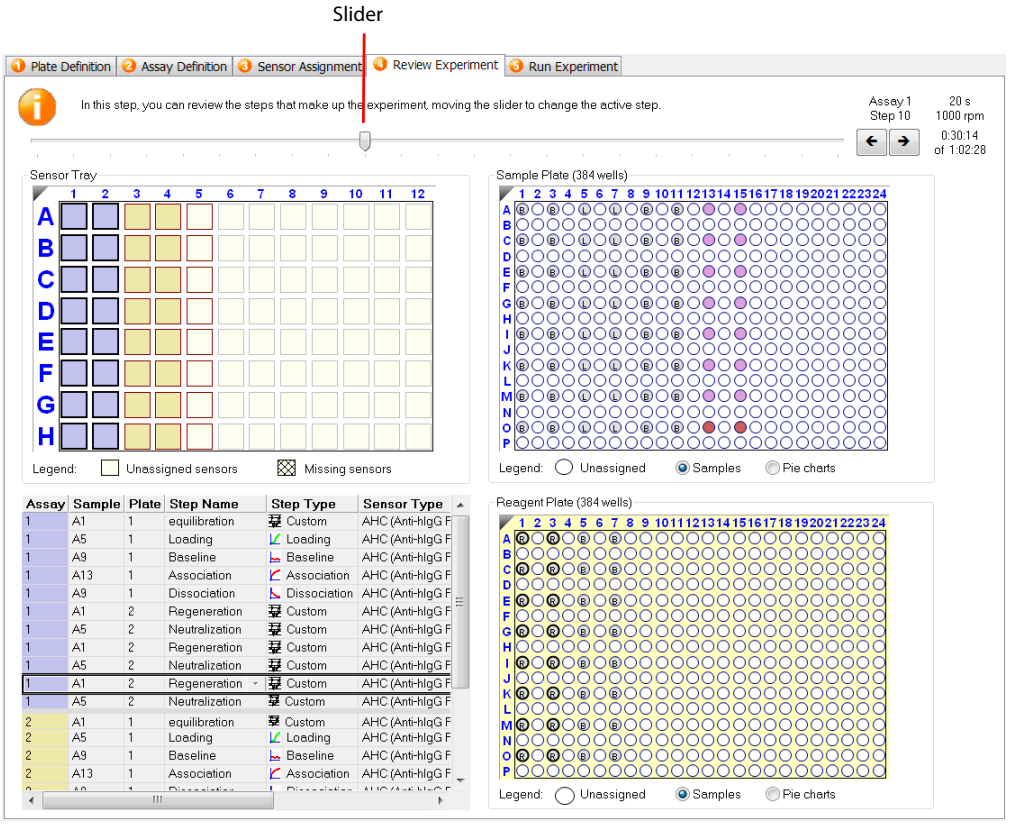


Figure 8-52: *Review Experiment Window*

SAVING EXPERIMENTS

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

To manually save an experiment:

1. Click **Save Method File** (), or on the main menu, click **File** > **Save Method File**.

   If there is more than one open experiment and you want to save all of them, click **Save All Methods Files** .

2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.

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

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment** > **Templates** > **Kinetics** > **Experiment Name** (see Figure 8-53).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.

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

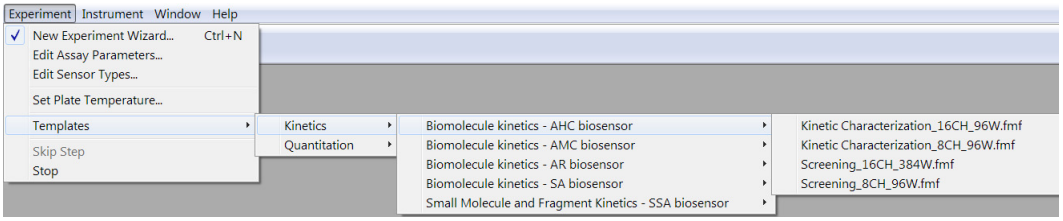


Figure 8-53: *Saved Experiments in the Template Folder*

RUNNING A KINETICS EXPERIMENT

IMPORTANT: *Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.*

Loading the Biosensor Tray, Sample, and Reagent Plates

To load the biosensor tray, sample plate, and reagent plate:

1. Open the Octet instrument door (lift the handle up) and present the instrument stage (click the **Present Stage** button).
2. Place the biosensor tray, sample plate, and reagent plate on the appropriate stage so that well A1 is located at the upper right corner (see Figure 8-54):
   a. Place the rehydration plate and biosensor tray on the biosensor stage (left plat-

form).

b. Place the sample plate on the sample stage (middle platform).

c. Place the reagent plate on the reagent stage (right platform).

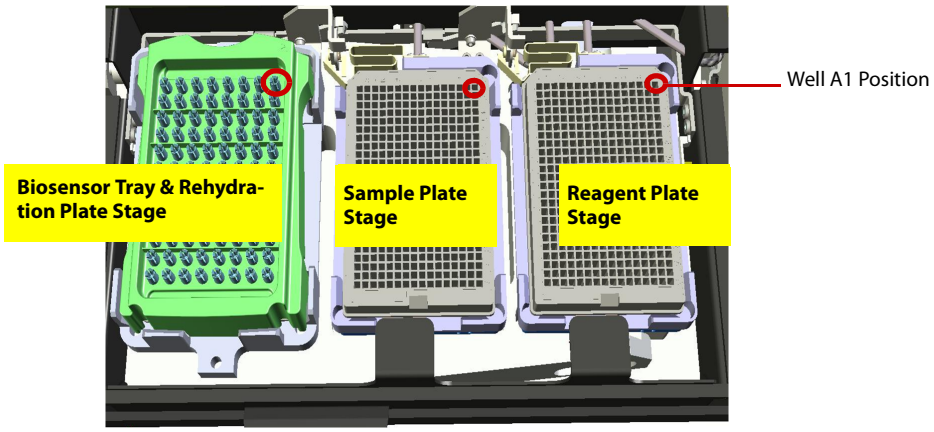


Figure 8-54: *Octet Instrument Stage Platform*

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

3. Click to close the Octet instrument door.
4. Allow the plate to equilibrate.

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

Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow ( ) to access the Run Experiment window (see Figure 8-55).

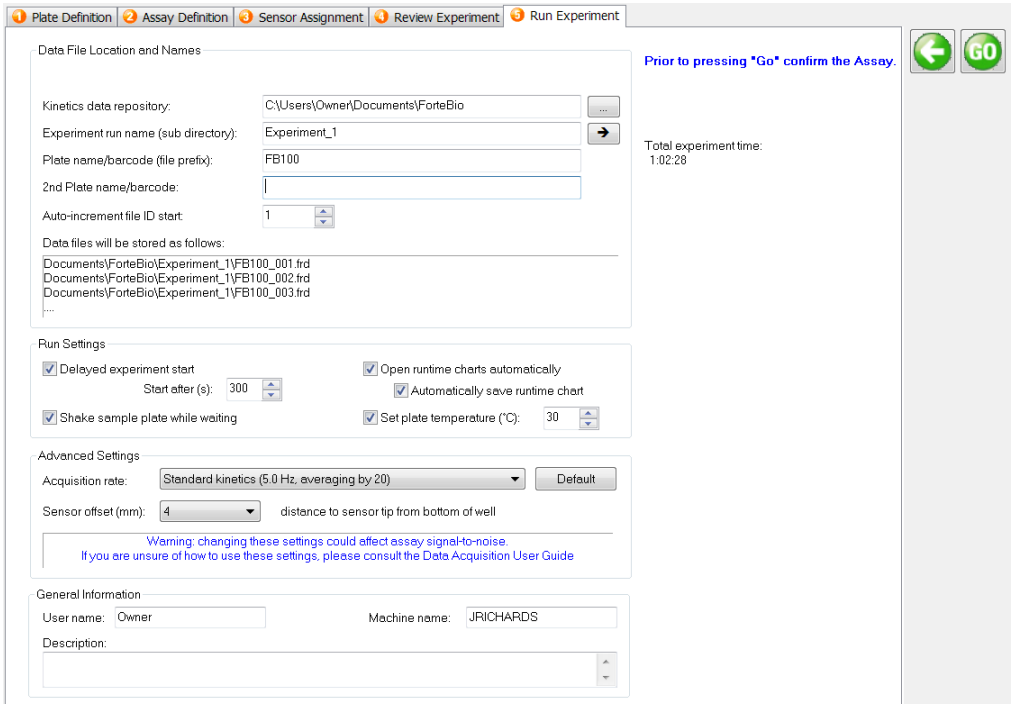


Figure 8-55: *Run Experiment Tab—Octet RED384*

2. Confirm the default settings or enter new settings. See "Run Experiment Window Settings" on page 328 for more information on experimental settings.

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

---

3. To start the experiment, click GO.

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

   If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 8-56).

NOTE: *For more details about the **Runtime Binding Chart**, see "Managing the Runtime Binding Chart" on page 331.*

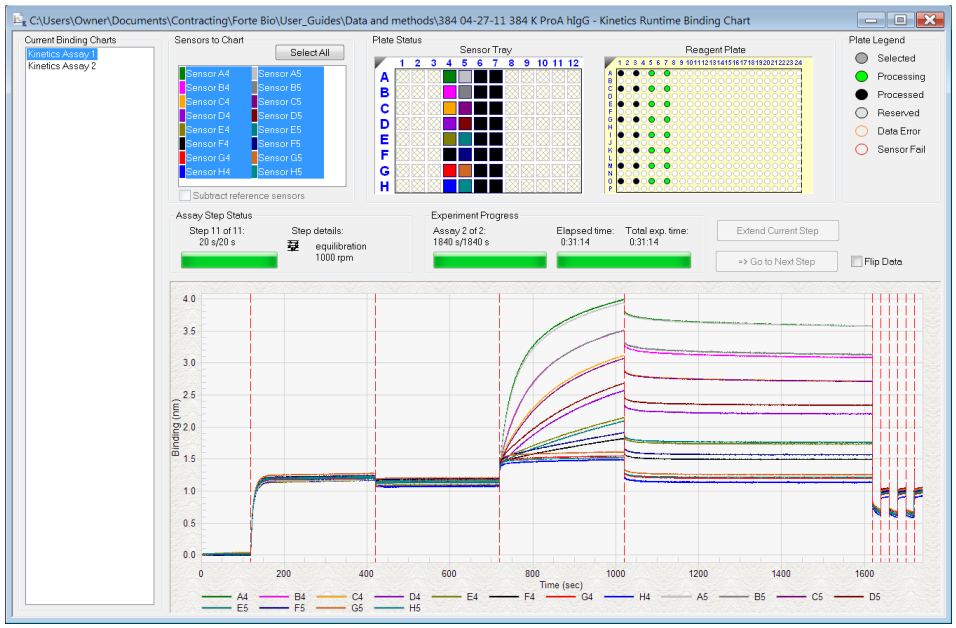


Figure 8-56: *Runtime Binding Chart*

4. Optional: Click **View** > **Instrument Status** to view the log file (see Figure 8-57).

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

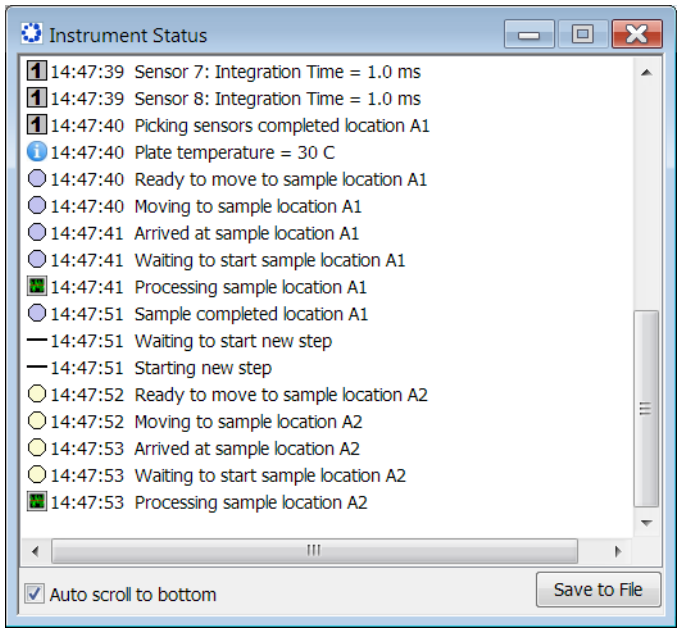


Figure 8-57: *Instrument Status Log*

WARNING: *Do not open the Octet instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.*

Run Experiment Window Settings

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

Table 8-6: *Data File Location and Name*

Item	*Description*
Assay type	The name of the selected assay.
Kinetics data repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click **Browse** to select another data location. ***NOTE:*** *It is recommended that you save the data to the local machine first, then transfer to a network drive.*
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/ barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

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

Table 8-7: *Run Settings*

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

Advanced settings are available for Octet RED384 and Octet QK384 systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per second and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

The following **Advanced Settings** are available for the Octet384 system:

Table 8-8: *Advanced Settings Octet RED384*

Item	*Description*
Acquisition rate	• High sensitivity kinetics (2.0 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second. • Standard kinetics (5.0 Hz, averaging by 20)—The average of 50 data frames is reported as one data point. Five data points are reported per second. • Fast kinetics (10.0 Hz, averaging by 5)- The average of 5 data frames is reported as one data point. Ten data points are reported per second.
Sensor off set (mm)	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

The following **Advanced Settings** are available for the OctetQK384 system:

Table 8-9: *Advanced Settings Octet QK384*

Item	*Description*
Acquisition rate	• High sensitivity kinetics (0.3 Hz, averaging by 40) - The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. • Standard kinetics (0.6 Hz, averaging by 5) - The average of 5 data frames is reported as one data point. One data point is reported every 1.6 seconds.
Sensor off set (mm)	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

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

Table 8-10: *General Settings*

Item	*Description*
Machine name	The computer name that controls the Octet instrument and acquires the data.
User name	The user logon name.

Table 8-10: General Settings (Continued)

Item	Description
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

Stopping an Experiment

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

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

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

MANAGING THE RUNTIME BINDING CHART

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 8-58). The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

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

To selectively display data for particular assay:

1. Click the corresponding **Assay** number.
2. Select a subset of sensors for a displayed column under **Sensors to Chart** box (see Figure 8-58).

WARNING: *Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.*

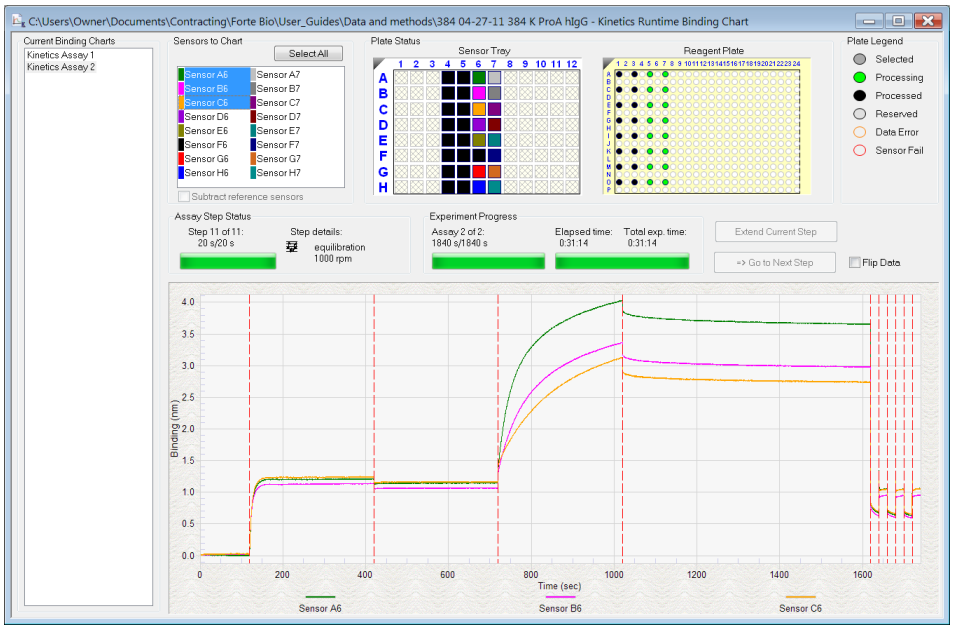


Figure 8-58: *Runtime Binding Chart Window*

Opening the Runtime Binding Chart

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

1. Click **File** > **Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

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

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 8-59).

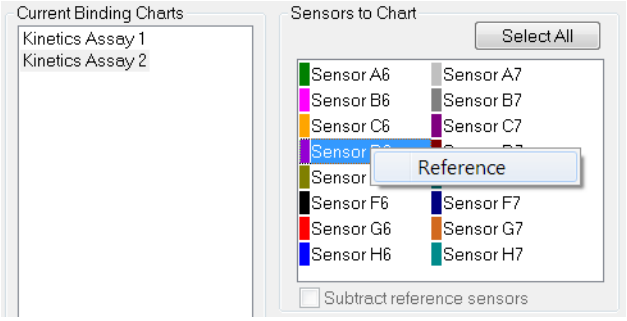


Figure 8-59: *Designating a Reference Biosensor in the Runtime Binding Chart*

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 8-60).

2. Click the **Subtract reference sensors** check box (see Figure 8-60).

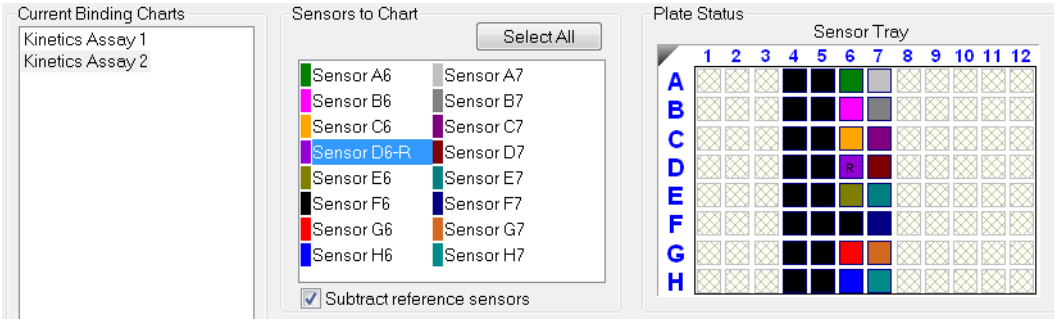


Figure 8-60: *Subtract Reference Sensors check box in the Runtime Binding Chart*

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

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 8-61). Uncheck the box to return to the default data display.

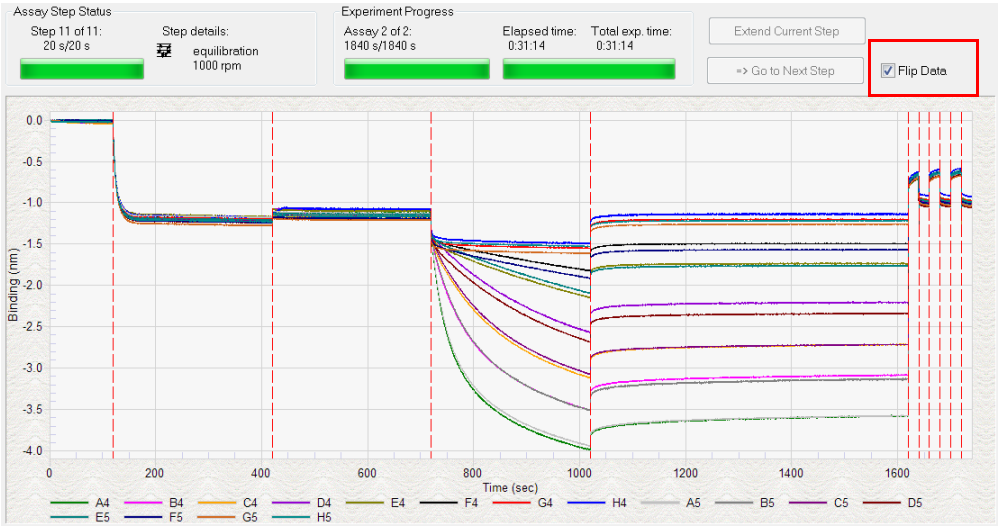


Figure 8-61: *Data Inverted Using Flip Data Function*

Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 8-62), right-click a step and select **Align to Step <*number*>**.

To remove the step alignment, right-click the step and select **Unaligned**.

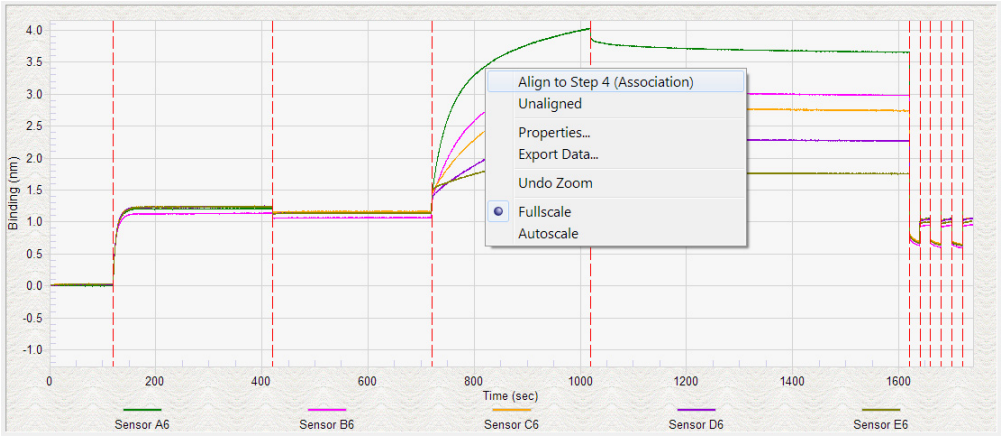


Figure 8-62: *Runtime Binding Chart—Aligning the Data to a User-Selected Step*

Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.

NOTE: *If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.*

To extend the duration of the active step:

1. In the chart window, click the **Extend Current Step** button.
2. In the **Extend Current Step** dialog box (see Figure 8-63), enter the number of seconds to extend the step and click **OK**.

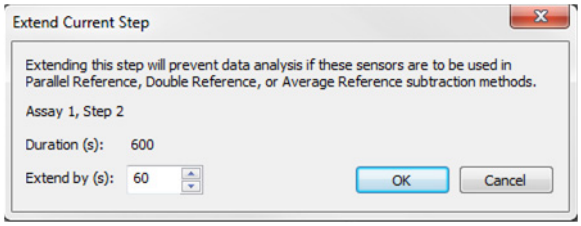


Figure 8-63: *Extend Current Step Dialog Box*

To terminate a step and begin the next step in the assay:

1. In the chart window, click the **Go to Next Step** button.
2. In the **Data Acquisition** dialog box, click **OK**.

Magnifying the Runtime Binding Chart

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

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

Scaling a Runtime Binding Chart

To scale the Runtime Binding Chart:

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

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

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

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

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

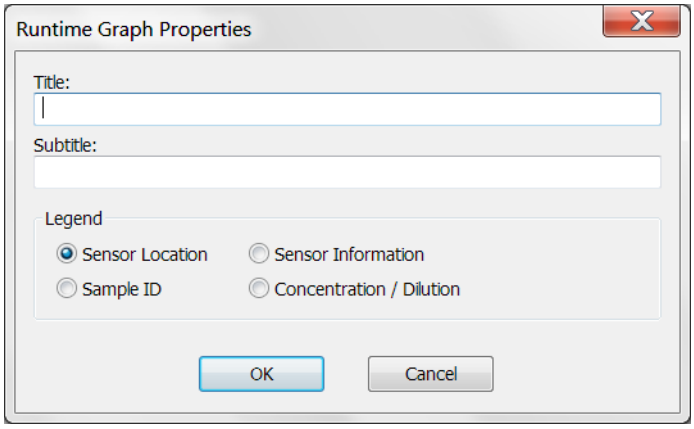


Figure 8-64: *Selecting a Runtime Binding Chart Legend*

---

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

---

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

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

Exporting or Printing the Runtime Binding Chart

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

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 8-65), select the export options and click **Export**.

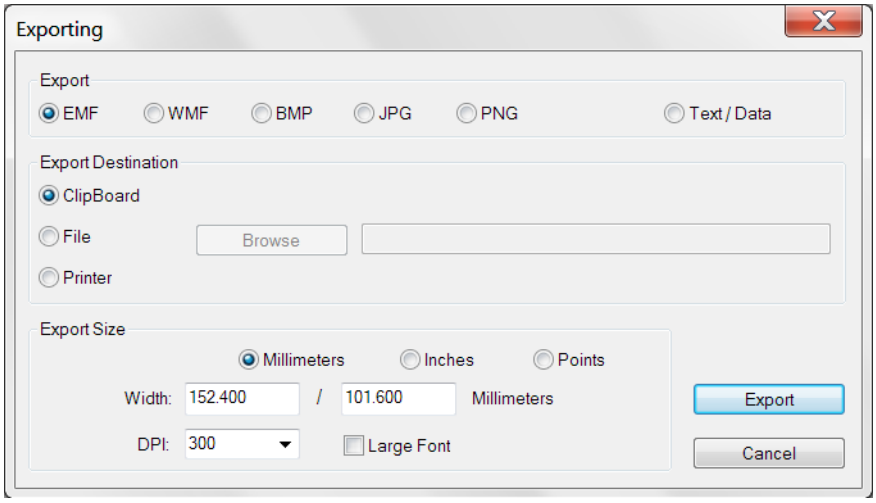


***Figure 8-65:** Exporting Dialog Box*

***Table 8-11:** Runtime Binding Chart Export Options*

Task	*Export*	*Option*	*Export Destination*	*Result*
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click **File** > **Browse** to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click **File** > **Browse** to select a folder and enter a file name.	Creates a graphic image.

Table 8-11: *Runtime Binding Chart Export Options (Continued)*

Task	*Export*	*Option*	*Export Destination*	*Result*
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it if necessary.

NOTE: *When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.*

Table 8-12: *Managing Experiment Method Files*

Menu Bar Command/ Toolbar Button	*Description*
File > **Open Method File**	Enables you to select and open a method file (.fmf)
File > **Save Method File** or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > **Save Method File As**	Saves a method file to a new name so that the original file is not overwritten.

CHAPTER 9:

Maintenance

OCTET RED96 AND OCTET QK SYSTEMS

Cleaning the Octet Instrument

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

To clean the Octet instrument:

1. Turn off the power to the instrument
2. Open the system door.
3. Wipe the biosensor and sample platform (Figure 9-1).
4. Carefully wipe the eight biosensor pickup tips.
5. Allow the surfaces to dry for at least one minute with the door open.

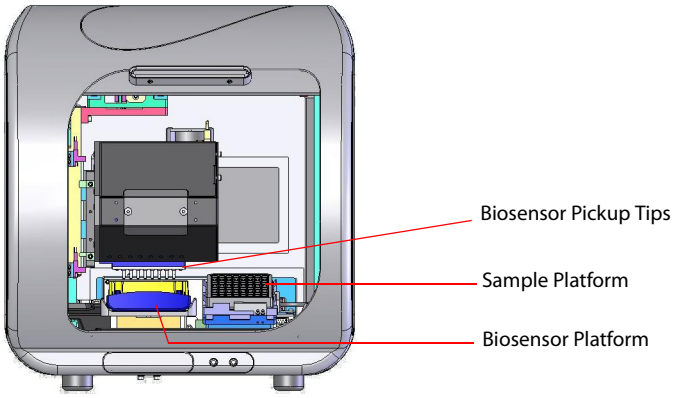


Figure 9-1: *Octet Instrument*

Emptying the Waste Container

To empty the waste container:

1. Press on the container to open it (Figure 9-2).
2. Pull the container out and completely remove it from the instrument.
3. Remove the container insert with the biosensor tips and dispose of both in a biohazard container suitable for sharp objects.

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

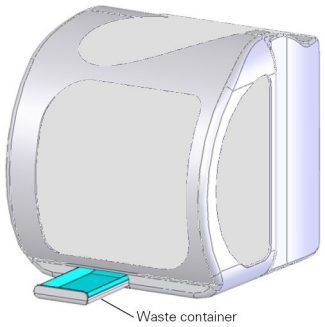


Figure 9-2: *Waste Container for the Octet Instrument*

Replacing Fuses (Octet RED96 and Octet QK^e Systems only)

Two replaceable fuses are located on the left back panel of the Octet instrument power supply (Figure 9-3).

***WARNING**: Turn off and unplug the instrument before replacing the fuses.*

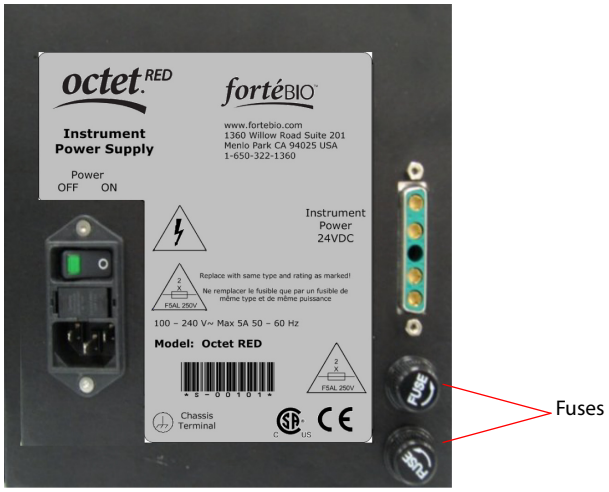


Figure 9-3: *Octet Instrument Power Supply Back Panel*

To replace the fuses:

1. Using a small screwdriver, gently pop the fuse drawer out.
2. Remove the expired fuse and place a new one in the holder.

***WARNING**: Only use 5 amp slow-blow fuses.*

3. Reinstall the fuse drawer.

OCTET RED384 AND OCTET QK384 SYSTEMS

Cleaning the Octet Instrument

***NOTE:** If you use the Octet instrument regularly, clean the interior horizontal surfaces daily with a Kimwipe moistened with a 30–60% isopropyl alcohol solution. Otherwise, clean once a week or as needed.*

To clean the Octet instrument:

1. Present the sample plate stage (Figure 9-4).
2. Turn off the power to the instrument.
3. Open the system door.
4. Wipe the biosensor and sample platform.
5. Allow the surfaces to dry for at least one minute with the door open.

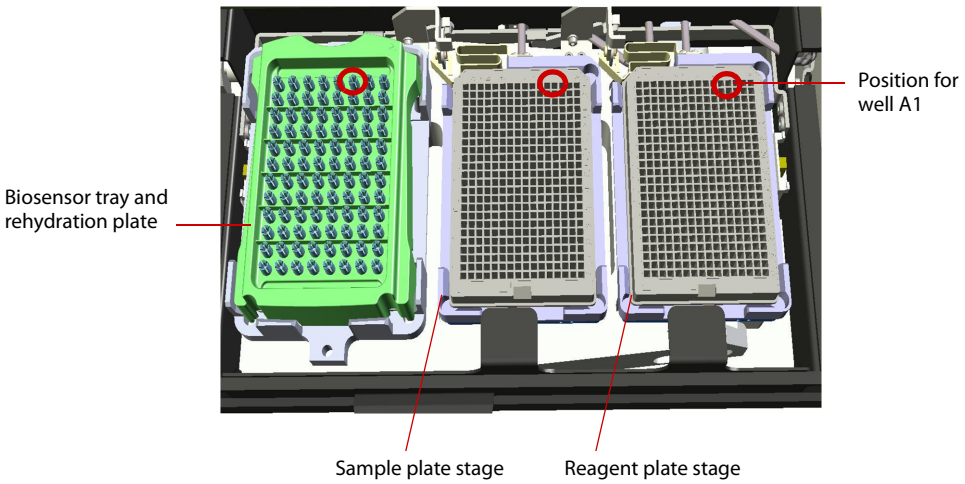


***Figure 9-4:** Octet RED384 and QK384 Stage Platform*

Cleaning the Biosensor Pickup Tips

The biosensor pickup tips hold the biosensors during an assay.

NOTE: *The biosensor pickup tips should be cleaned weekly, or as needed.*

To clean the biosensor pickup tips:

1. Present the sample plate stage.
2. Turn off the power to the instrument.
3. Remove the side panel of the instrument (Figure 9-5).

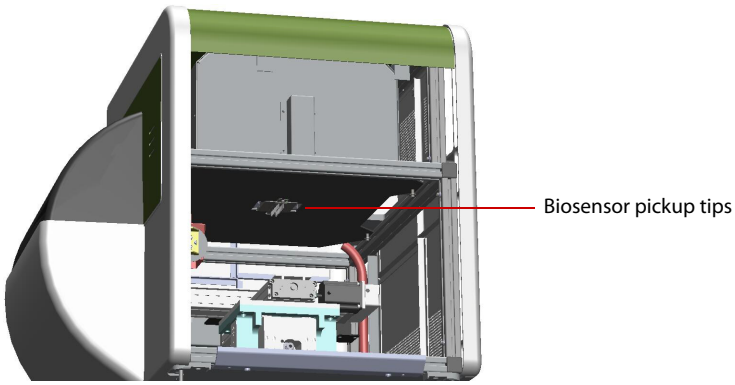


Figure 9-5: *Octet Instrument Side Panel Removed*

4. Gently clean the biosensor pickup tips with a Kimwipe moistened with a 30-60% iso-propyl alcohol solution. Remove any debris left from the biosensor hub.
5. Allow the biosensor pickup tips to dry for at least one minute.
6. Replace the side cover, and then turn on the instrument.

Replacing Fuses

Two replaceable fuses are located on the left back panel of the Octet instrument power supply (Figure 9-6).

WARNING*: Turn off and unplug the instrument before replacing the fuses.*

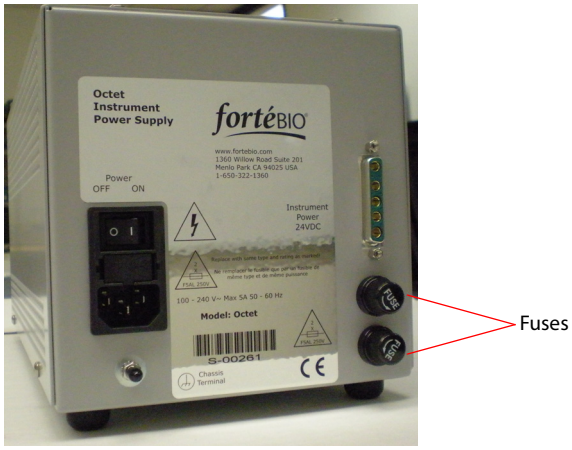


Figure 9-6: *Octet Instrument Power Supply Back Panel*

To replace the fuses:

1. Using a small screwdriver, gently pop the fuse drawer out.
2. Remove the expired fuse and place a new one in the holder.

***WARNING**: Only use 5 amp slow-blow fuses.*

---

3. Reinstall the fuse drawer.

APPENDIX A:

Using Octet384 Systems with an Automation Interface

OVERVIEW

The Octet Data Acquisition software provides support for an automation interface using a COM port (RS-232) or a Transmission Control Protocol/Internet Protocol (TCP/IP) socket/port.

An example application for testing the automation interface, called **AutomationClient.exe**, is included in the applications and Dynamic Link Libraries (DLLs) installed with the Octet Data Acquisition software. The file is located in the C:\Program Files\ForteBio\DataAcquisition directory.

NOTES:
The automation interface can be used with Octet384 systems only.

The examples that follow are illustrated using a TCP/IP connection, but the serial port connection behaves identically.

DESIGN OF THE AUTOMATION INTERFACE

The automation interface is designed to be as universal as possible, making no assumptions about the communication medium or the language of the client application connecting to the Octet System Data Acquisition software.

The following guidelines apply:

- All commands and responses are ASCII strings, one per line.
- All lines are terminated with both carriage-return and line-feed characters ("\r\n").
- Each command starts with the name of the command and may then be followed by required and optional parameters.
- Each parameter starts with a switch definition (a la dos/unix command line) followed by the parameter itself, which allows parameters to be sent in any order.
- The command or response is terminated with a new line (CR/LF) sequence.
- Parameters containing embedded spaces need to be enclosed in double quotes.

Automation Interface Control Setup

Before the Octet System Data Acquisition software can be controlled using an automation interface, the correct automation options must be set. To do this, go to **File** > **Options** (Figure A-1) and select the appropriate port in the **Automation** box.

NOTE: *The Octet System Data Acquisition software can be controlled via automation interface through a serial port (RS-232) or a TCP/IP socket.*

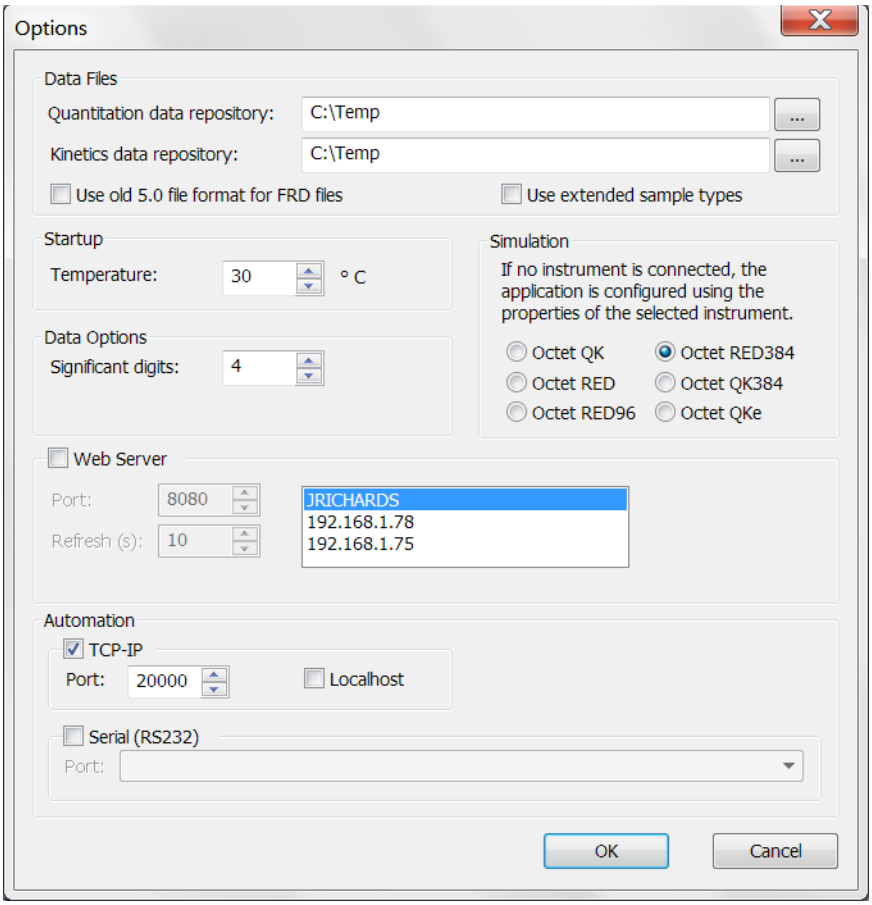


Figure A-1: *Options Dialog Box—Automation Interface Selection*

NOTE: *The* ***Localhost*** *option can be useful in developing the automation client on the same computer that runs the Octet System Data Acquisition software.*

NOTE: *ForteBio recommends that the* ***Data File*** *repositories be set using shared folders addressed by "UNC" folder names so that the internal path used by the Data Acquisition application corresponds to the external path used to access/retrieve the data files recorded during the experiment. Alternatively, the path returned by the* ***GetRunInfo*** *command to access the data files from another computer on the LAN.*

Automation Client Example Application

The **Automation Client** example application can connect to the Octet System Data Acquisition software via serial port (RS-232) port or TCP/IP socket.

To connect the Automation Client example application:

1. In the Octet System Data Acquisition software, go to **File** > **Options** (see Figure A-1).
2. In the **Automation** box, select the communication port to be used (either TCP/IP or RS232, see Figure A-1).
3. Launch **AutomationClient.exe** located in the C:\Program Files\ForteBio\DataAcquisition directory to display the **Automation Client** dialog box (Figure A-2).

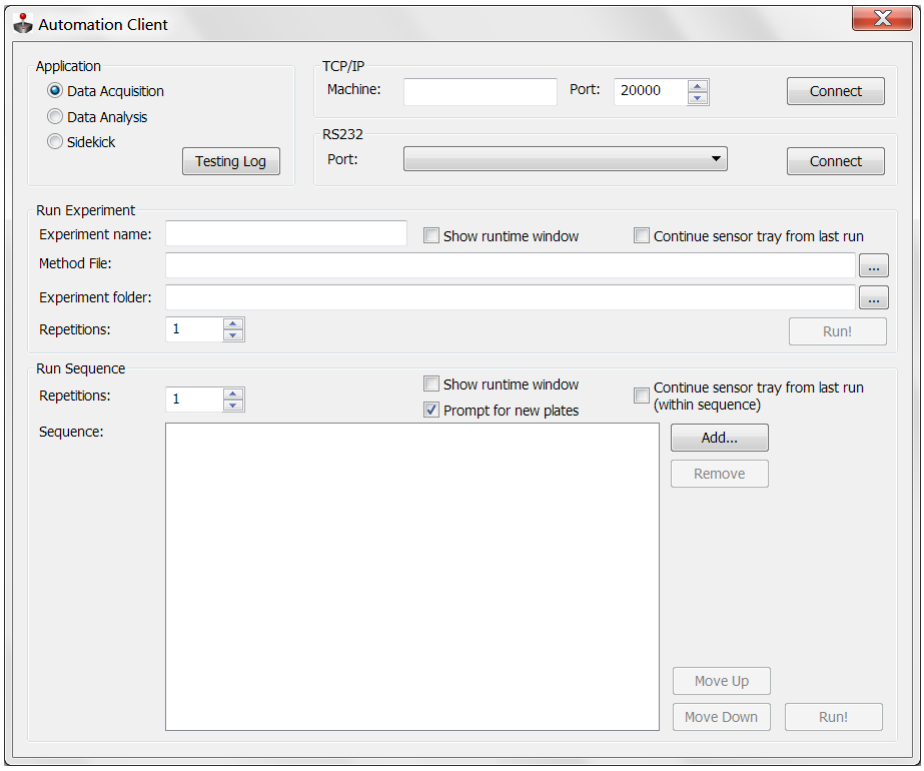


Figure A-2: *Automation Client Window*

4. Select the TCP/IP or RS-232 port selected previously in the Octet Data Acquisition software **Options** dialog box (Figure A-1). To connect locally using **Localhost**, leave the **Machine** field blank.
5. Click **Connect**.

   If the port is successfully opened, the automation client dialog will be minimized and remain minimized, indicating that the connection succeeded and the port is open. Otherwise, the automation client dialog will minimize and come back again, indicating that the connection attempt failed.

6. After a successful connection is established, send the default **Version** command (in the **Send Commands—Command** field) and then click **Send**!.

   A response similar to the following should appear in the **Response** box:

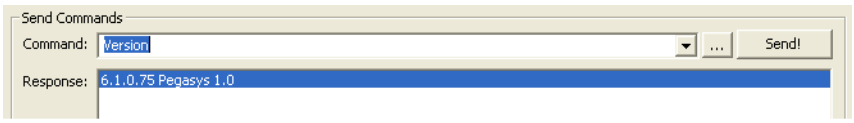


Figure A-3: *Send Commands—Command Field*

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

AUTOMATION COMMANDS

Table A-1 summarizes the commands supported by the Octet System Data Acquisition software automation interface.

NOTE: *The symbolic names are provided for C++ clients who connect using the interface as defined in the AutomationAPI.h header file.*

Table A-1: *Commands Supported by the Automation Interface*

Command	*Symbolic Name*	*Purpose*
Version	AUT_CMD_VERSION	Returns the version of the application being automated, the type of instrument it is controlling, and the automation API version.
Reset	AUT_CMD_RESET	Stops any running experiment and resets the instrument.
GetMethodInfo	AUT_CMD_GETMETHODINFO	Returns information about the resources required by given method file.
Run	AUT_CMD_RUN	Runs an experiment using a given method file.
GetRunInfo	AUT_CMD_GETRUNINFO	Returns information about the experiment currently running.

Table A-1: *Commands Supported by the Automation Interface (Continued)*

Command	*Symbolic Name*	*Purpose*
Stop	AUT_CMD_STOP	Stops a running experiment, ejecting the sensors if necessary.
Status	AUT_CMD_STATUS	Returns status during a running experiment: **OK** = ready **Busy** =running **Waiting** = waiting for a condition to be resolved **Error** = experiment was terminated by an error **Busy** is followed by descriptive information on the progress of the experiment (% complete)
Present	AUT_CMD_PRESENT	(Octet 384 only) Open the door and move the stage to the presentation position.
Resume	AUT_CMD_RESUME	Indicates that the "Waiting" condition has been resolved (new sensor tray installed). Continues the experiment.
Close	AUT_CMD_CLOSE	Closes the door if it is open. Homes the read head on an Octet 384 instrument.
Cleanup	AUT_CMD_CLEANUP	Closes open MDI windows. Only valid when not busy. Useful when using the **Run** command without the **-s** option.

Typical Automation Session

The following example is a typical automation session that illustrates the use of the automation commands to run an experiment.

NOTE: *Commands sent from the client application are designated as* ***SEND:****. Responses received from the Octet System Data Acquisition software are designated as* ***RECV:****.*

Connecting to the Data Acquisition Software

```
SEND:  Version\r\n
RECV:  6.1.0.30 Pegasys 1.0
SEND:  Status\r\n
RECV:  OK
```

Preparing for an Experiment

```
SEND: Cleanup
RECV: OK
SEND:  GetMethodInfo -mC:\MethodFiles\Q001.fmf\r\n
RECV:  OK -p96,0 -t1 -s"Anti-Human IgG Fc"
```

Starting the Experiment

```
SEND:  Version\r\n
RECV:  6.1.0.30 Pegasys 1.0
SEND:  Status\r\n
RECV:  OK
```

Getting Information about the Experiment

```
SEND:  Version\r\n
RECV:  6.1.0.30 Pegasys 1.0
SEND:  Status\r\n
RECV:  OK
```

Monitoring the Experiment

```
bool bBusy = true;
while (bBusy)
{
   Send("Status\r\n");
   response = Recv();

   if (response==OK)
```

```
      bBusy = false;
   else
      Sleep(1000); // sleep for a second
}

SEND:  Status\r\n
RECV:  Running (5%)

SEND:  Status\r\n
RECV:  Running (25%)

SEND:  Status\r\n
RECV:  Running (45%)

SEND:  Status\r\n
RECV:  Running (75%)

SEND:  Status\r\n
RECV:  Running (95%)

SEND:  Status\r\n
RECV:  OK
```

Stopping the Experiment and Presenting the Plate for Unloading

Both the **Stop** and the **Present** commands are asynchronous—they initially return **OK** to indicate that the command was accepted and started OK, but status must be polled until **OK** is returned to indicate completion.

```
SEND:  Stop\r\n
RECV:  OK

SEND:  Status\r\n
RECV:  Busy

SEND:  Status\r\n
RECV:  Busy

SEND:  Status\r\n
RECV:  OK

SEND:  Present\r\n
RECV:  OK
```

```
SEND:  Status\r\n
RECV:  Busy

SEND:  Status\r\n
RECV:  Busy

SEND:  Status\r\n
RECV:  OK
```

Advanced Automation Session

If an experiment is sufficiently complex it may require more than one tray of sensors to complete the experiment. This can be detected at the start of the experiment by checking the **-tN** response from the **GetMethodInfo** command. If *N* is greater than **1**, then the experiment requires more than one tray of sensors to complete.

If this is the case, initially the experiment will start as before, but halfway through the experiment the **Status** command will return **LoadSensors** indicating that the first tray of sensors has been exhausted and another tray of sensors needs to be loaded. At this point, you must issue the **Present** command to allow access to the sensor plate (polled for completion) and then once the new sensor tray is in place, the **Resume** command must be sent to resume the experiment.

Connecting to Data Acquisition

```
SEND:  Version\r\n
RECV:  6.1.0.30 Pegasys 1.0
SEND:  Status\r\n
RECV:  OK
```

Preparing for an Experiment

```
SEND: Cleanup
RECV: OK
SEND:  GetMethodInfo -mC:\MethodFiles\Q002.fmf\r\n
RECV:  OK -p96,0 -t2 -s"Anti-Human IgG Fc"
```

Starting the Experiment

```
SEND:  Run -mC:\MethodFiles\Q002.fmf -bP0001 -s\r\n
RECV:  OK
```

Getting Information about the Experiment

```
SEND:  GetRunInfo\r\n
RECV:  OK -n"Experiment 2" -p"\\fbdata\Quantitation\Experiment 2"\r\n
```

Monitoring the Experiment

```
bool MonitorExperiment(CCmdTransport *pPort)
{
      // Poll the experiment until it is done.
      for (;;)
      {
         Sleep(200);

         if (!SendRecv(pPort, AUT_CMD_STATUS + AUT_EOL, csResp))
            return false;

         int nStart = 0;
         CString csStatus = csResp.Tokenize(" ", nStart);

         if (csStatus == AUT_OK)
            break;                                          // SUCCESS
         else if (csStatus == AUT_STOPPED)
            break;                                          // SUCCESS
         else if (csStatus == AUT_RUNNING)
            ;
         else if (csStatus == AUT_WAITING)
            ;
         else if (csStatus == AUT_LOADSENSORS)
         {
            if (!LoadSensors(pPort))
               return false;
         }
         else if (csStatus == AUT_BUSY)
            ;
         else if (csStatus == AUT_ERROR)
            return false;
      }
   }

bool LoadSensors(CCmdTransport *pPort)
{
   if (!SendRecv(pPort, AUT_CMD_PRESENT + AUT_EOL, csResp))
      return false;

   if (csResp != AUT_OK)
      return false;
```

```
   if (!WaitNotBusy(pPort))
      return false;

   // At this point the robot replaces the sensor tray.
   AfxMessageBox("Robot changes sensor tray...");

   if (!SendRecv(pPort, AUT_CMD_RESUME + AUT_EOL, csResp))
      return false;

   if (csResp != AUT_OK)
      return false;

   return WaitNotBusy(pPort);
}


bool WaitNotBusy(CClientResponder *pPort)
{
   CCountdownTimer Timer(c_uBusyTimeoutMS);
   CString csResp;
   while (!Timer.IsDone())
   {
      Sleep(200);

      if (!SendRecv(pPort, AUT_CMD_STATUS + AUT_EOL, csResp))
         return false;

      int nStart = 0;
      CString csStatus = csResp.Tokenize(" ", nStart);

      if (csStatus == AUT_OK)
         return true;
      else if (csStatus == AUT_STOPPED)
         return false;
      else if (csStatus == AUT_RUNNING)
         return true;
      else if (csStatus == AUT_WAITING)
         return true;
      else if (csStatus == AUT_LOADSENSORS)
         return true;
```

```
      else if (csStatus == AUT_BUSY)
         ;
      else if (csStatus == AUT_ERROR)
         return false;
   }
   TRACE1("Timeout waiting for not busy after %d ms\n",
Timer.GetElapsed());
   return false;
}
```

Automation API.H

```
//
*************************************************************************
***
//
//    Copyright (c) 2011 ForteBio.
//    All rights reserved.
//
//
*************************************************************************
***
// HEADER:  AutomationAPI.h
// PURPOSE: Defines the commands supported by the automation API.
// AUTHOR:  BHI  Nov 2008
//
#ifndef INC_ACQUISITION_AUTOMATIONAPI_H
#define INC_ACQUISITION_AUTOMATIONAPI_H


// NOTES:
// Do not position the Octet instrument such that it is difficult to
disconnect the power.
// The automation interface is string based. Commands and responses are
strings, one per line.
// Each command starts with the name of the command and may then be
followed by required and optional parameters.
// Each parameter starts with a switch definition (a la dos/unix com-
mand line) followed by the parameter itself. This allows parameters to
be sent in any order.
// The command or response is terminated with a new line (CR/LF)
sequence.
// Parameters containing embedded spaces must be enclosed in double
quotes.
// Response items containing embedded spaces will be enclosed in double
quotes.
```

```
// REVISIONS:
// 1.0      First release
// 1.1      Added (-p) plate file parameter to "Run" and "GetMethod-
Info"
// commands
//          Added (-u) use-last-sensor-tray option to the "Run" com-
mand.
//          Added "SetValue" command to set the temperature target.

// Version of the API described in this header file.
const char AUT_API_VERSION[] = "1.1";

// Status return values
const char AUT_OK[]          = "OK";
const char AUT_STOPPED[]     = "Stopped";
const char AUT_RUNNING[]     = "Running";
const char AUT_WAITING[]     = "Waiting";
const char AUT_LOADSENSORS[] = "LoadSensors";
const char AUT_BUSY[]        = "Busy";       // Resetting, Presenting
const char AUT_ERROR[]       = "ERROR";
const char AUT_EOL[]         = "\r\n";

// Parameter switches for the Run command
const char AUT_SWITCH_METHOD      = 'm';     // Method file to load
(required)
const char AUT_SWITCH_FOLDER      = 'f';     // Root folder for exper-
iment data (optional)
const char AUT_SWITCH_EXPERIMENT  = 'e';     // Overide for the exper-
iment name in the FMF file (optional)
const char AUT_SWITCH_PLATEFILE   = 'p';     // Plate file to import
after method file is loaded (optional)
const char AUT_SWITCH_BARCODE     = 'b';     // Bar code of Sample
plate (optional)
const char AUT_SWITCH_BARCODE1    = '1';     // Alias for
AUT_SWITCH_BARCODE (optional)
const char AUT_SWITCH_BARCODE2    = '2';     // Bar code of Reagent
plate (optional)
const char AUT_SWITCH_LOTNUMBER   = 'l';     // Lot number of sensors
(optional)
const char AUT_SWITCH_SILENT      = 's';     // Don't open the runtime
window (optional)
const char AUT_SWITCH_USELAST     = 'u';     // Reuse the sensor tray
as it was left after last run (optional)
const char AUT_SWITCH_VERBOSE     = 'v';     // Send back verbose sta-
tus information
```

```
// Parameter switches for the SetValue command
const char AUT_SWITCH_TEMPERATURE = 't';

// Response parameter switches for the GetMethodInfo command
const char AUT_RESPONSE_PLATEWELLS  = 'p';
const char AUT_RESPONSE_SENSORTRAYS = 't';
const char AUT_RESPONSE_SENSORTYPE  = 's';
const char AUT_RESPONSE_EXPTYPE     = 'e';
const char AUT_RESPONSE_RERACKING   = 'r';

// Response parameter switches for the GetRunInfo command
const char AUT_RESPONSE_EXPNAME     = 'n';
const char AUT_RESPONSE_EXPPATH     = 'p';

const char AUT_CMD_VERSION[] = "Version";
// Returns the version of the app being automated, the hardware plat-
form it controls, and the API version.
// Args: (none)
// Response: App product version (e.g. "6.0.0.120 Pegasys 1.0\r\n")

const char AUT_CMD_RESET[]   = "Reset";
// Stops any running experiment and resets the instrument.
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_GETMETHODINFO[] = "GetMethodInfo";
// Returns info about a method file
// Args:
//   -m <path>      Method file name (required)
// Response:
//   "OK -r<bool> -t<int> -s<name>\r\n"
// e.g. OK -p96,0 -t2 -s"SA (Streptavidin)\r\n"
// Response params:
//   -p<int>,<int>  Sizes of the plates in use                  e.g. -
p384,96
//   -t<int>        Number of sensor trays required (0 .. 5)    e.g. -t2
//   -s<name>       Name of first sensor in the tray            e.g. -
s"SA (Streptavidin)"
//   "Error: load method\r\n"
```

```
//   "Error: bad method\r\n"

const char AUT_CMD_RUN[]     = "Run";
// Runs an experiment
// Args:
//   -m <path>      Method file name (required)
//   -p <path>      Plate file to update sample plate in  method set-
tings (optional)
//   -b <barcode>   Sample plate bar code  (optional)
//   -1 <barcode>   Sample plate bar code  (optional)
//   -2 <barcode>   Reagent plate bar code  (optional)
//   -l <lotnumber> Sensor tray lot number (optional)
//   -s             Silent - does not open the runtime view (optional)
//   -u             Use the state of the sensor tray as it was left
after last run
// Response:
//   "OK\r\n"
//   "Error: not ready\r\n"
//   "Error: bad method\r\n"
//   "Error: bad barcode\r\n"

const char AUT_CMD_GETRUNINFO[] = "GetRunInfo";
// Returns information about an experiment that is currently running
// Args: (none)
// Response:
//   "OK -n"Experiment 1" -p"\\fbdata\Quantitation\Experiment 1"\r\n"
//   "Error: <reason>\r\n"
// Response params:
//   -n<experiment name>  Name of the experiment (folder name in repos-
itory)      e.g. -n"Experiment 1"
//  -p<experiment path> Full path to experiment folder in repository
e.g. -p"\\fbdata\Quantitation\Experiment 1"

const char AUT_CMD_STOP[]    = "Stop";
// Stops a running experiment
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_SETVALUE[] = "SetValue";
// Sets a value
```

```
// Args:
//   -t <temp>      Sets heater target temperature (DegC)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_STATUS[]  = "Status";
// Returns status: OK=ready, Busy=running, Error=Experiment was termi-
nated by an error.
// Busy is followed by descriptive information on the progress of the
experiment (% complete)
// Args: (none)
// Response:
//   "OK\r\n"
//   "Waiting\r\n"
//   "Busy\r\n"
//   "Running (nn%)\r\n"
//   "LoadSensors\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_PRESENT[] = "Present"; // Pegasys only
// Open the door and move the stage to the presentation position.
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"
// N.B.: Poll status waiting for "Waiting" condition to reappear

const char AUT_CMD_RESUME[] = "Resume";
// Indicates that the "Waiting" condition has been resolved (new sensor
tray installed). Continues the experiment.
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"
// Status will indicate busy until door is closed, then will return to
Running state.

const char AUT_CMD_CLOSE[] = "Close";
// Closes the stage if it is open.
// Args: (none)
// Response:
```

```
//   "OK\r\n"
//   "Error: <reason>\r\n"
// Status will indicate busy until door is closed.

const char AUT_CMD_CLEANUP[] = "Cleanup";
// Closes open MDI windows. Only valid when not busy.
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: busy\r\n";

#endif // INC_ACQUISITION_AUTOMATIONAPI_H
```

Analysis Automation API

```
//
*************************************************************************
***
//
//    Copyright (c) 2011 ForteBio.
//    All rights reserved.
//
//
*************************************************************************
***
// HEADER:  AutomationAPI.h
// PURPOSE: Defines the commands supported by the automation API.
// AUTHOR:  BHI  Nov 2008
//
#ifndef INC_ANALYSIS_AUTOMATIONAPI_H
#define INC_ANALYSIS_AUTOMATIONAPI_H

// NOTES:
// * The automation interface is string based. Commands and responses
are
// strings, one per line.
// * Each command starts with the name of the command and may then be
// followed by required and
//   optional parameters.
// * Each parameter starts with a switch definition (a la dos/unix com-
mand
// line) followed by the
//   parameter itself. This allows parameters to be sent in any order.
```

```
// * The command or response is terminated with a new line (CR/LF)
sequence.
// * Parameters containing embedded spaces must be enclosed in double
// quotes.
// * Response items containing embedded spaces will be enclosed in dou-
ble
// quotes.

// Version of thew API described in this header file.
const char AUT_API_VERSION[] = "1.0";

// Status return values
const char AUT_OK[]          = "OK";
const char AUT_RUNNING[]     = "Running";
const char AUT_ERROR[]       = "ERROR";
const char AUT_BUSY[]        = "Busy";
const char AUT_STOPPED[]     = "Stopped"; // Stopped by user.
const char AUT_EOL[]         = "\r\n";

// Parameter switches for the LOAD command
const char AUT_SWITCH_DATASET   = 'd';

// Parameter switches for the ANALYZE command
const char AUT_SWITCH_PARAMS    = 'p';
const char AUT_SWITCH_XMLINFO   = 'x';

// COMMAND API
// ===========

const char AUT_CMD_VERSION[] = "Version";
// Returns the version of the app being automated, and the API version.
// Args: (none)
// Response: App product version (e.g. "6.3.1.12 1.0\r\n")

const char AUT_CMD_LOAD[]    = "Load";
// Loads an experiment
// Args:
//   -d <path>      Path to experiment data files
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"
```

```
const char AUT_CMD_ANALYZE[] = "Analyze";
// Runs an analysis
// Args:
//   -p <path>      Path to parameters (INI file)
//   -x <path>      Path to XML information file (optional, can be mul-
tiple XML info files)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_STATUS[]  = "Status";
// Returns status: OK=ready, Busy=running, Error=Action was terminated
by an error.
// Busy is followed by descriptive information on the progress of the
experiment (% complete)
// Args: (none)
// Response:
//   "OK\r\n"
//   "Busy\r\n"
//   "Running (nn%)\r\n"
//   "Error: <reason>\r\n"

#endif // INC_ANALYSIS_AUTOMATIONAPI_H
```

APPENDIX B:

21 CFR Part 11 Software Administrator Options

INSTALLING THE DATA ACQUISITION 7.0 21 CFR PART 11 SOFTWARE

To install the Data Acquisition 7.0 21 CFR Part 11 software:

1. Insert the software **V7.0 CFR CD (7.00.35/7.0.0.9)** into your CD drive.
   - If the Autoplay dialog box displays, choose to open the CD to view files.
   - If the Autoplay dialog box does not display, navigate to the CD using Windows Explorer.

   Optical drives are typically found under the **D:** or **E:** drive.
2. Double-click **DataAcquisition-CFR-7_0_0_x.exe** to launch the installation wizard (see Figure B-1).

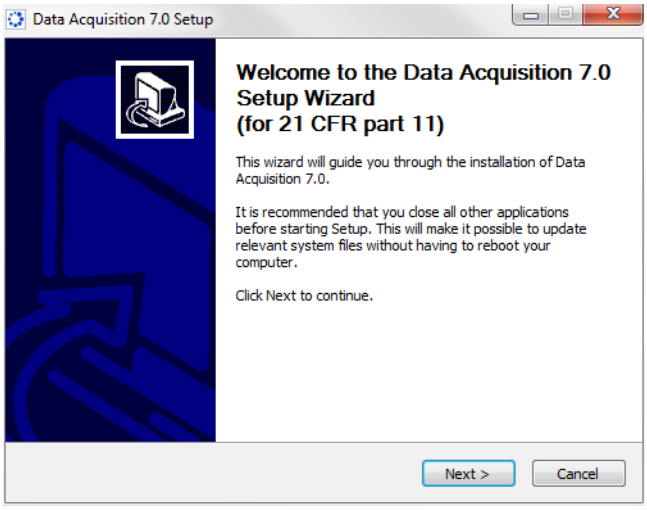


Figure B-1: *Data Acquisition 7.0 (for 21 CFR Part 11) Software Setup Wizard*

3. Click **Next** to display the Choose Install Location dialog box (Figure B-2).

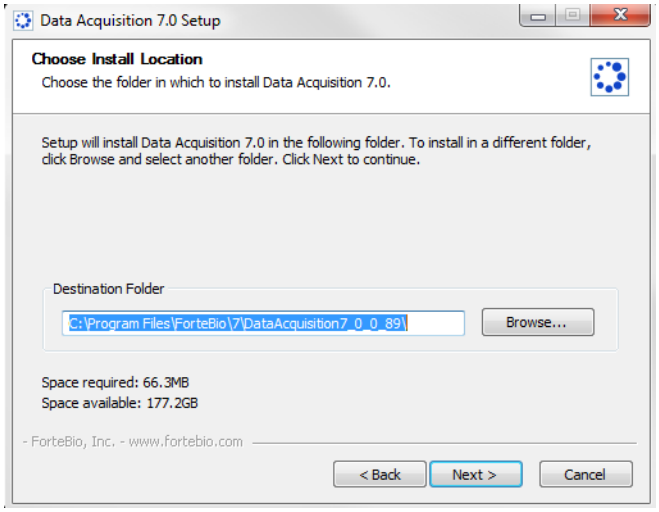


Figure B-2: *Choose Install Location Dialog Box*

The default location for the software on the local machine is **C:\Program Files\ForteBio\DataAcquisition7**.

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

   The Choose Start Menu Folder dialog box displays (Figure B-3).

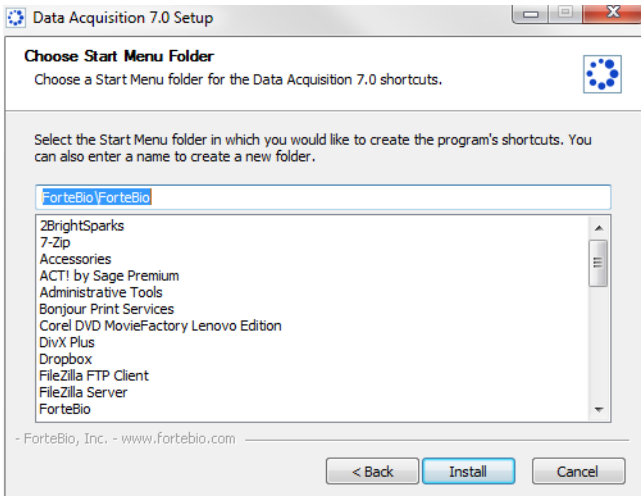


Figure B-3: *Choose Start Menu Folder Dialog Box*

The default Start Menu folder is **ForteBio**.

5. Click **Install**.

   The installation wizard takes a few seconds to install (Figure B-4).

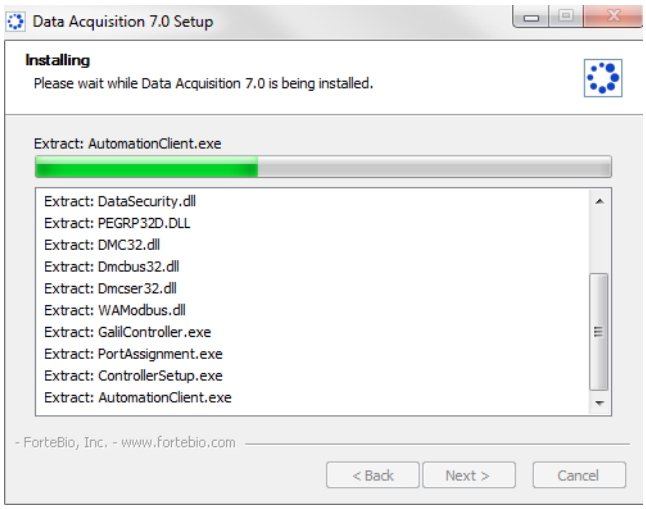


Figure B-4: *Installation Progress*

The installation wizard displays the Completing the Data Acquisition 7.0 Setup Wizard dialog box (Figure B-5).

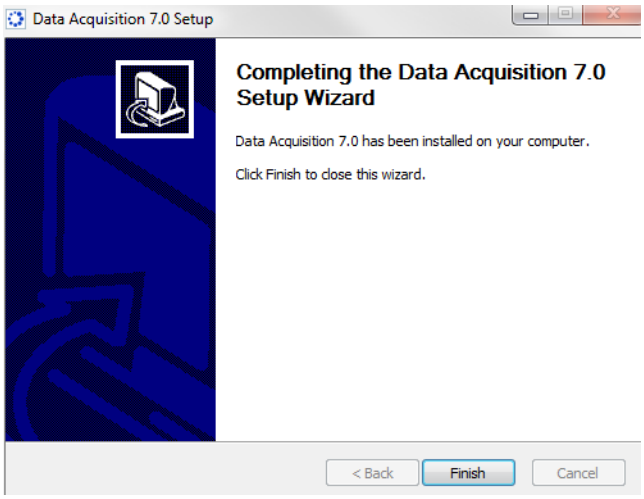


Figure B-5: *Completing the Data Analysis 7.0 Setup*

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

INSTALLING THE DATA ANALYSIS 7.0 21 CFR PART 11 SOFTWARE

To install the Data Analysis 7.0 21 CFR Part 11 software:

1. Insert the software CD into your CD drive.
2. Navigate to the window listing the files located on the installation CD.
3. Double-click **DataAnalysis-CFR-7_0_0_x.exe** to launch the installation wizard (see Figure B-6).

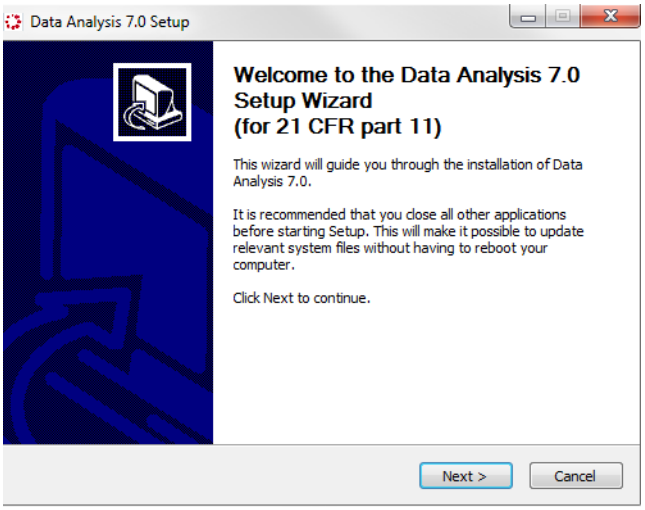


Figure B-6: *Data Analysis 7.0 (for 21 CFR Part 11) Software Setup Wizard*

4. Click **Next** to display the Choose Install Location dialog box (Figure B-7).

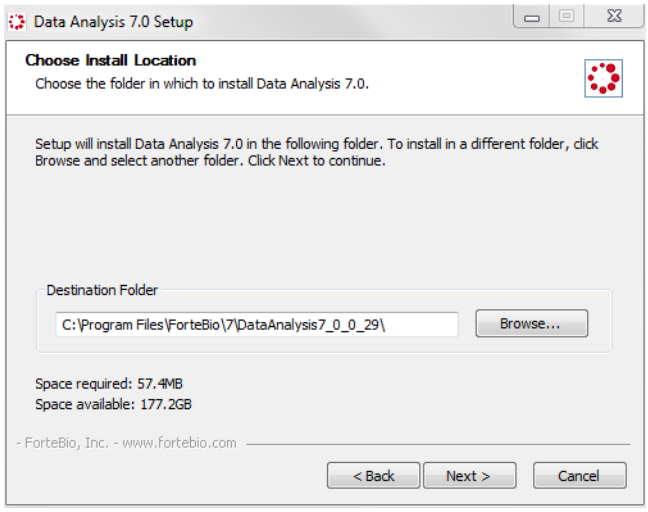


Figure B-7: *Choose Install Location Dialog Box*

The default location for the software on the local machine is **C:\Program Files\Forte-Bio\DataAnalysis7**.

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

   The Choose Start Menu Folder dialog box displays (Figure B-8).

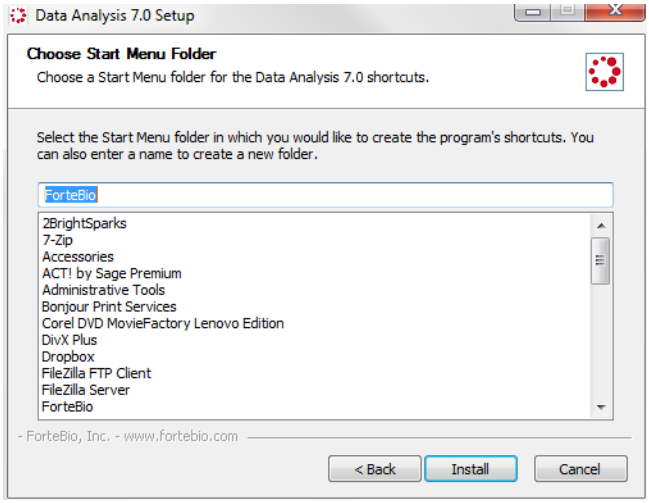


Figure B-8: *Choose Start Menu Folder Dialog Box*

The default Start Menu folder is **ForteBio**.

6. Click **Install**.

   The installation wizard takes a few seconds to install (Figure B-9).

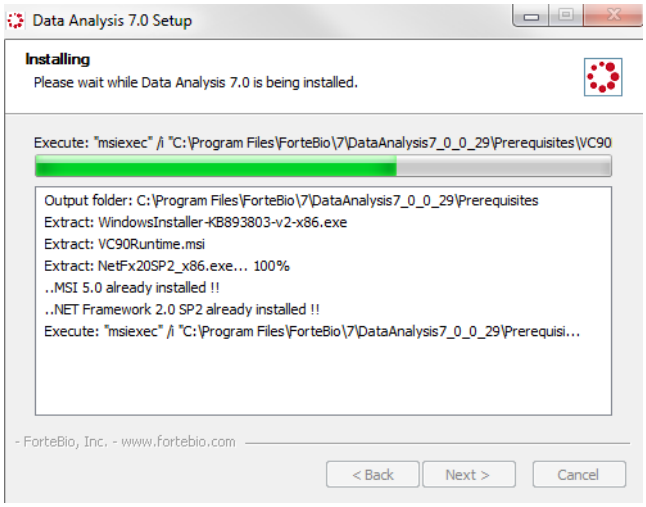


Figure B-9: *Installation Progress*

The installation wizard displays the Completing the Data Analysis 7.0 Setup Wizard dialog box (Figure B-10).

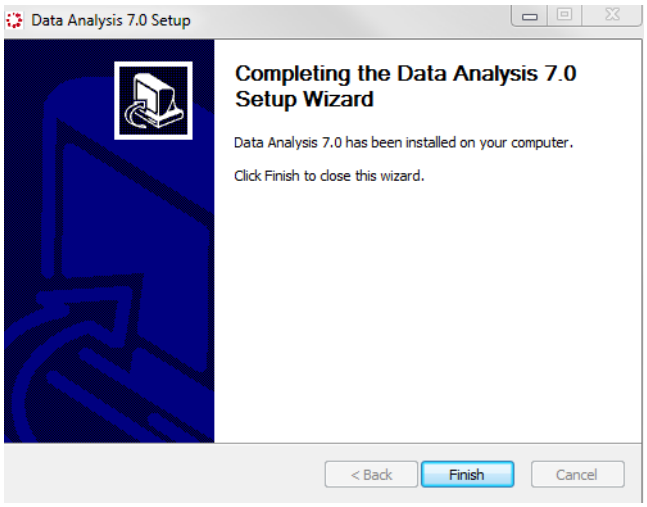


Figure B-10: *Completing the Data Analysis 7.0 Setup*

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

INSTALLING THE FORTEBIO GXP SERVER MODULE

The ForteBio GxP Server module can be installed and run from the following locations:

- A local host computer where the ForteBio Data Acquisition or Data Analysis 7.0 21 CFR Part 11 software is installed
- A remote host computer networked to a machine where the ForteBio Data Acquisition or Data Analysis 7.0 21 CFR Part 11 software is installed

Upon launching the Octet System Data Acquisition or Data Analysis 7.0 CFR 11 software, you are required to select the GxP Server module host location. If the GxP Server module is installed in multiple locations, you can select any host server. The user session event record will be saved only to the host location selected, making it possible to have records for the same user in multiple locations.

NOTE: For administrators only. *To ensure that all records are saved to one location, ForteBio recommends that administrators install a single copy of the ForteBio GxP Server module on the network that can then be accessed by all users.*

To install the ForteBio GxP Server software:

1. Navigate to the window listing the files located on the installation CD.
2. Double-click **ForteBio GxP Server 7.0.exe** to launch the installer.
3. If prompted with the *Do you want the following program from an unknown publisher to make changes to this computer?* message, reply **Yes**.

   The installation wizard should display (Figure B-11).

Figure B-11: *ForteBio GxP Server 7.0 Software Setup Wizard*

4. Click **Next** to display the Choose Install Location dialog box (Figure B-12).

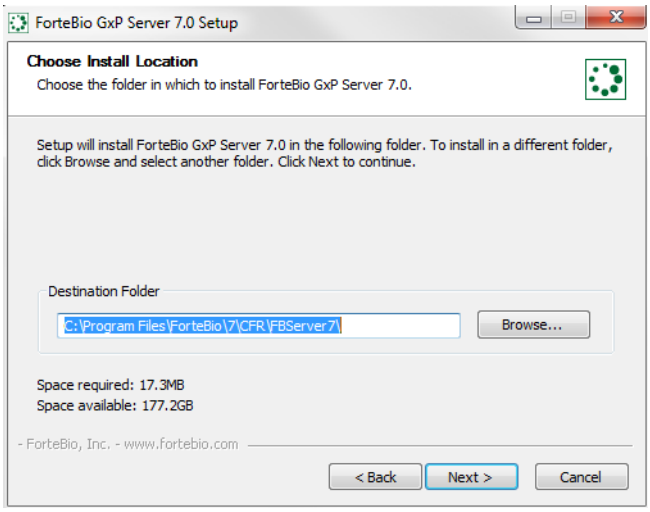


Figure B-12: *Choose Install Location*

The default location for the software on the local machine is **C:\Program Files\ForteBio\DataAnalysis7**.

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

   The Choose Start Menu Folder dialog box displays (Figure B-13).

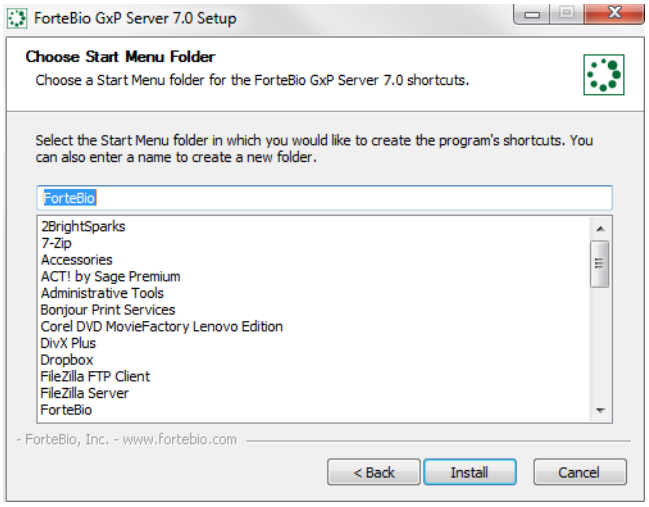


Figure B-13: *Choose Start Menu Folder Dialog Box*

The default Start Menu folder is **ForteBio**.

6. Click **Install**.

   The installation wizard takes a few seconds to install (Figure B-14).

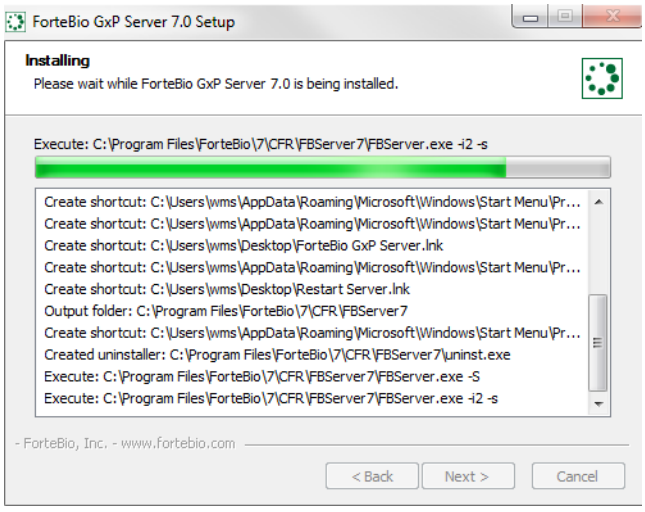


Figure B-14: *Installation Progress*

The installation wizard displays the Completing the ForteBio GxP Server 7.0 Setup Wizard dialog box (Figure B-15).

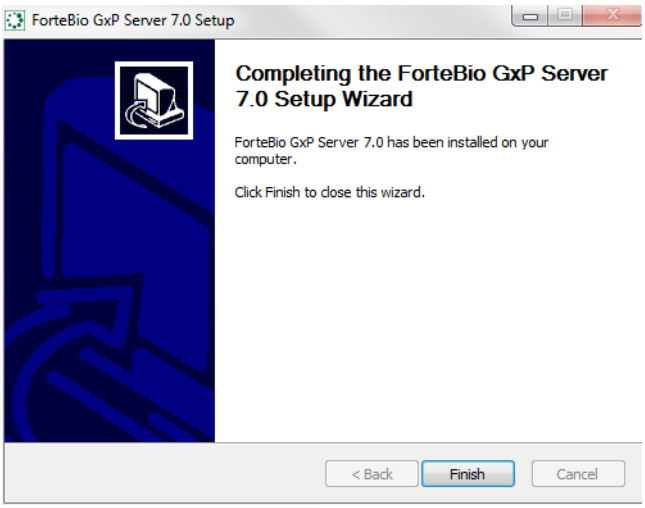


Figure B-15: *Completing the ForteBio GxP Server Software 7.0 Setup*

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

ADMINISTRATOR ACCOUNT SETUP

To set up the administrator account:

1. Launch the Data Acquisition or Data Analysis software by double-clicking on the desktop icon:

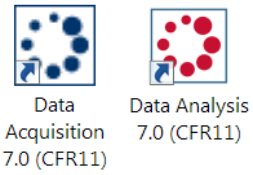


Figure B-16: *Data Acquisition and Data Analysis Desktop Icons*

The **Login** dialog box will display:

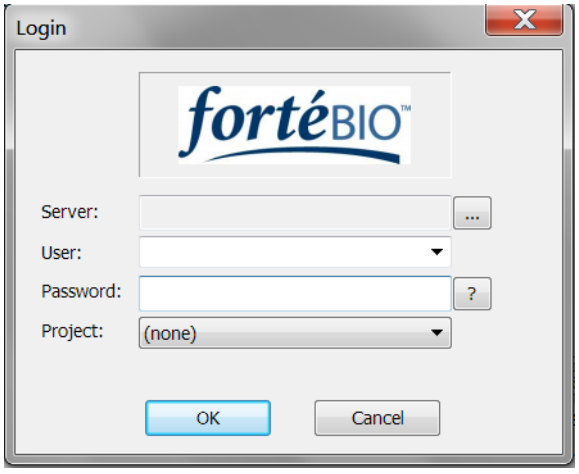


Figure B-17: *Login Dialog Box*

2. Select a **Server** location by clicking **...** (**Browse**).

   The **Authentication Server** dialog box will display:

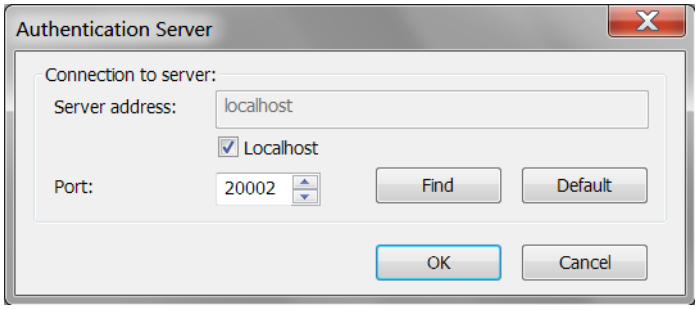


Figure B-18: *Authentication Server Dialog Box*

Click **Default** to recall the default server settings of localhost and Port 2002.

- ***Local host*—**If the local computer is to be used as the GxP Server module host, select the **Localhost** check box. Change the Port number if needed.
- ***Remote host on same subnet*—**If the GxP Server module is hosted on the same subnet, deselect the **Localhost** check box and click **Find**. A list of potential GxP Server module addresses will be listed. Choose the desired location from the list and click **OK**.

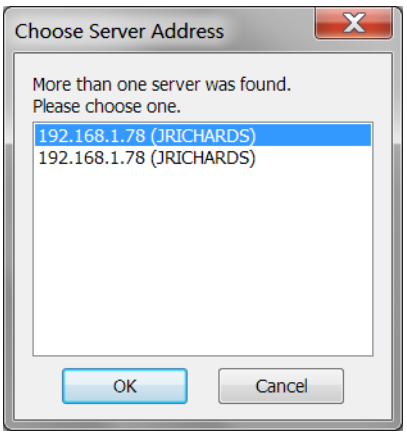


Figure B-19: *GxP Server Address Search Results*

- ***Remote host on another subnet*—**If the GxP Server module is hosted on a different subnet, deselect the **Localhost** check box. Enter the IP address of the computer hosting the GxP Server module.

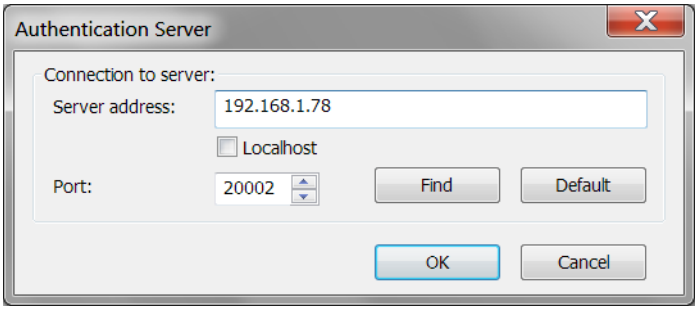


Figure B-20: *Manual Entry of Remote Host Address*

When the GxP Server module host location has been selected or entered, click **OK** to save changes and exit the **Authentication Server** dialog box. The GxP Server module location will now be listed as the **Server** in the **Login** box.

NOTE: *Once the GxP Server module host location is selected, this location will be used as the default selection for the administrator account. It does not need to be re-selected each time a new session is initiated.*

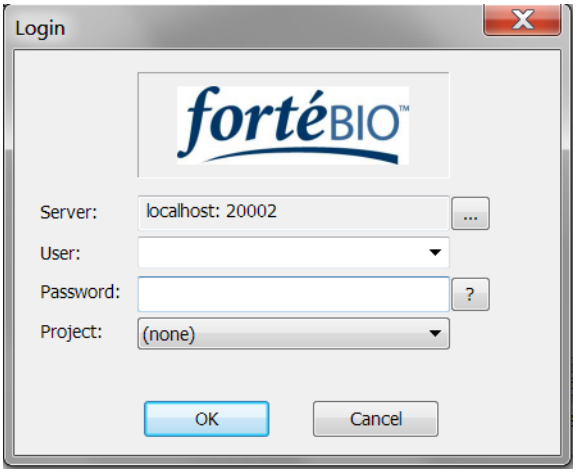


Figure B-21: *Login Dialog Box—DisplayingGxP Server Location*

3. From the **User** drop-down list, select **Administrator**.
4. Leave the Password blank and the **Project** set to **(none)** and click **OK.**

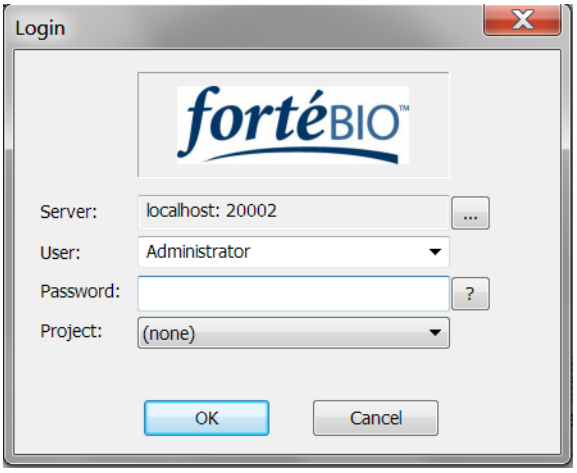


Figure B-22: *Administrator User Selection*

The **Change Password** dialog box will display (Figure B-23).

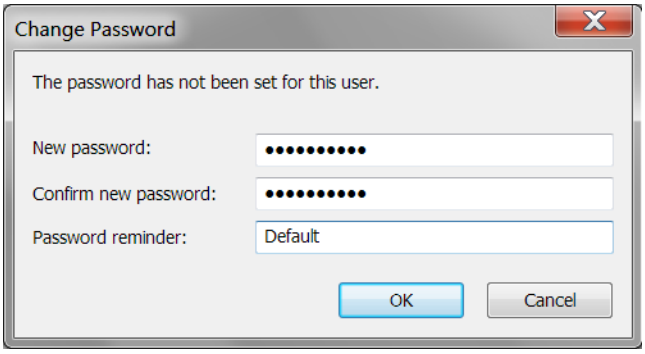


Figure B-23: *Change Password Dialog Box*

5. Enter a **New password** and **Password reminder** (optional) and click **OK**.

   The Data Acquisition or Data Analysis software will now launch and initiate an administrator user session which will allow access to administration options.

STARTING AN ADMINISTRATOR USER SESSION

Administrators initiate new user sessions the same way non-administrative users do.

To start an administrator user session:

1. Launch the Data Acquisition or Data Analysis software by double-clicking on the desktop icon:

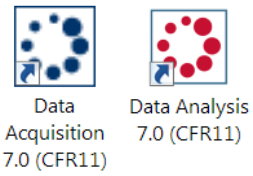


Figure B-24: *Data Acquisition and Data Analysis Desktop Icons*

   The **Login** dialog box will display:

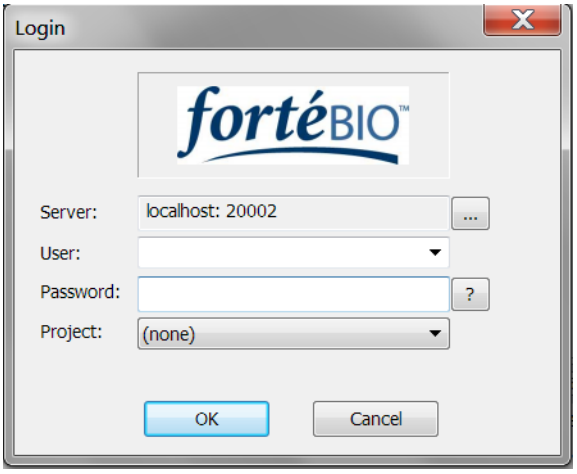


Figure B-25: *Login Dialog Box*

2. Confirm that the **Server** location is correct. If not, please see "Administrator Account Setup" on page 374.
3. Select **Administrator** from the **User** drop-down list.

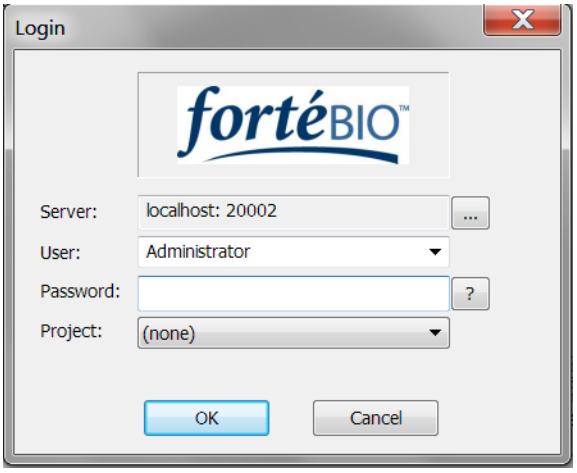


Figure B-26: *Administrator User Name Selection*

4. Enter your password in the **Password** text box. Click **?** for a password reminder if needed.

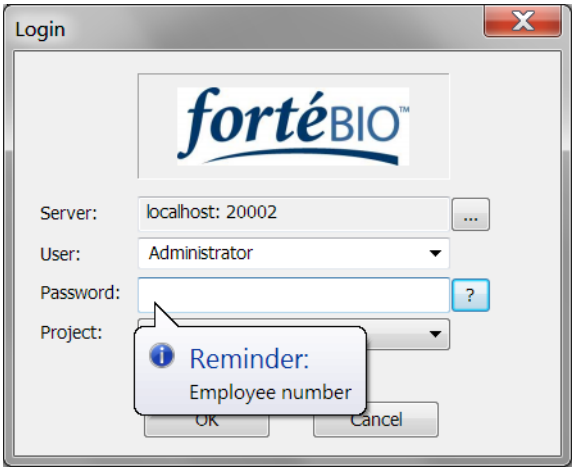


Figure B-27: *Password Reminder*

5. Select a project from the **Project** drop-down list, if required.

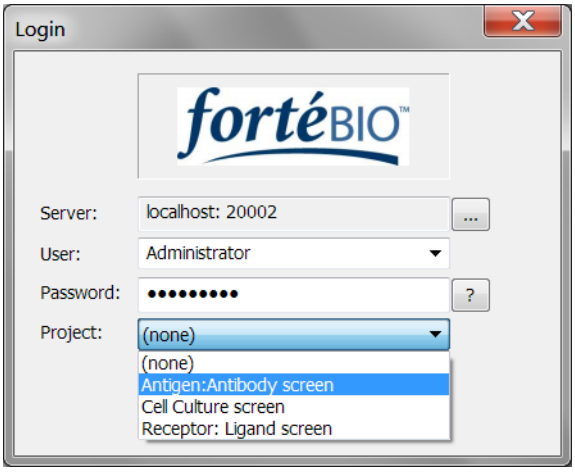


Figure B-28: *Project Selection*

6. Click **OK.**

   The Data Acquisition or Data Analysis software will now launch and start the administrator session. During the session, the administrator account and project selected at login display in the software status bar:

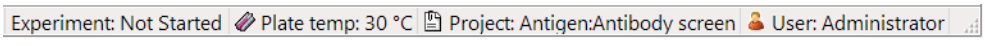


Figure B-29: *Status Bar*

NOTE: *Administrator and user sessions are automatically locked after a period of inactivity set using the* ***UserIdleMin*** *constant. Please see "Administrator Constants" on page 392 for more information. The* ***Login*** *dialog box will display and a message indicating the session has been locked will be shown. You can choose to log back into the session or log off at this time. Administrator and user sessions will not be locked during experimental data acquisition.*

ACCESSING ADMINISTRATOR OPTIONS

The 21 CFR Part 11 software Server Administration options allow administrators to mange users, groups, projects and constants and view associated events.

These options can be accessed in the Data Acquisition and Data Analysis software or by launching the ForteBio GxP Server module directly.

- ***Data Acquisition and Data Analysis software***Click **Security** > **Server Administration**:

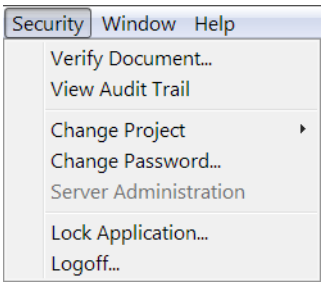


Figure B-30: *Security Menu*

- ***ForteBio GxP Server module on network location—***Double-click on the **FBServerConfig.exe** file in the **FBServer7** folder from the installed location:

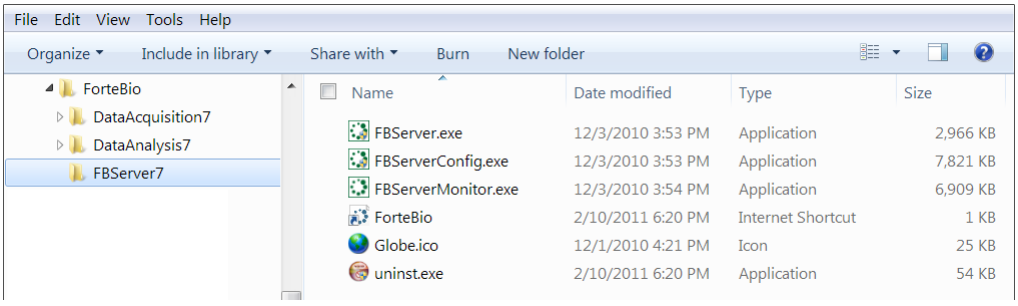


Figure B-31: *Accessing the GxP Server on the Network*

- ***ForteBio GxP Server module on a local host computer*** - Double-click the ForteBio GxP Server desktop icon:

Figure B-32: *Security Menu*

NOTE: *When accessing the ForteBio GxP Server module directly, additional tools are also provided to test server functionality. Please see "Accessing the GxP Server Module Directly" on page 396 for more information.*

The **ForteBio GxP Server Administration** window will display:

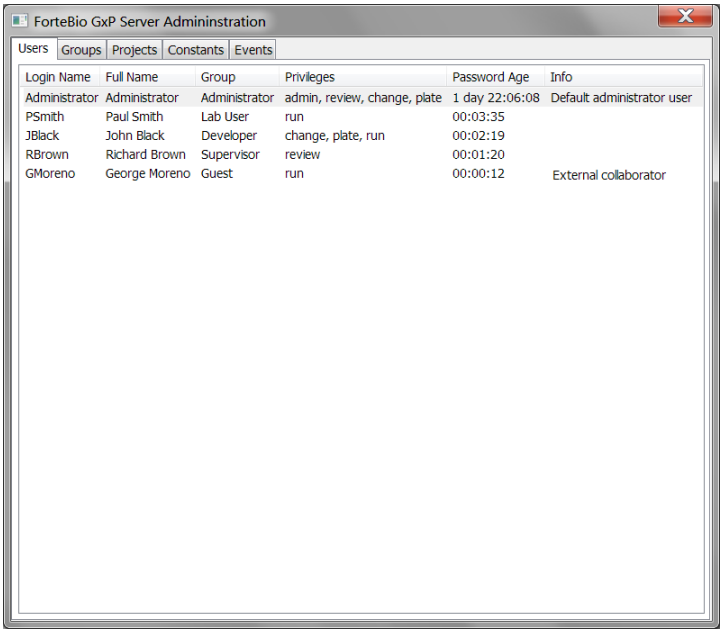


Figure B-33: *GxP Server Administration Window*

Administrator Tabs

Five tabs are available in the **ForteBio GxP Server Administration** window that contain the following options:

- **Users Tab**—Allows user and password management and individual privileges selection
- **Groups Tab**—Allows user group management and group privileges selection
- **Projects Tab**—Allows project management and setup
- **Constants Tab**—Allows setup of password requirements, cached server credentials and screen lock due to inactivity.
- **Events Tab**—Displays event logs for individual user accounts, projects or machines

To view the information contained on a tab, just click on the tab.

Tab View

Each tab displays a list of administrator entries and associated setting information that can be sorted by clicking on any of the column headers:

Login Name	Full Name	Group	Privileges	Password Age	Info
GMoreno	George Moreno	Guest	run	00:11:23	External collaborator
RBrown	Richard Brown	Supervisor	review	00:12:32	
JBlack	John Black	Developer	change, plate, run	00:13:30	
PSmith	Paul Smith	Lab User	run	00:14:46	
Administrator	Administrator	Administrator	admin, review, change, plate	1 day 22:17:19	Default administrator user

Figure B-34: *Tab Contents Sorted by Password Age*

Tab Menu

Right-clicking on an entry or on a blank area in the tab will display the **Tab** menu. **Tab** menu options vary depending on the tab selected.

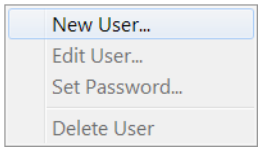


Figure B-35: *Tab Menu*

User Account Administration

The **Users Tab** allows administrators to add and delete user accounts as well as set and change individual user account privileges and passwords.

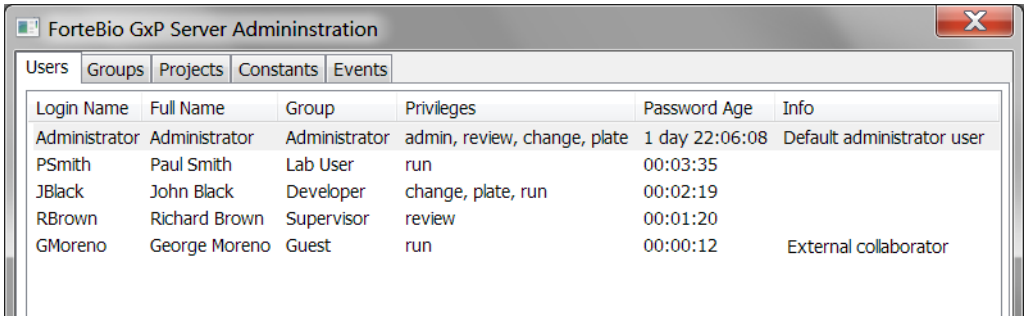


Figure B-36: *Users Tab*

Creating a New User Account

To create a new user account:

1. Right-click anywhere in the **Users Tab** and select **New User** from the **Tab** menu, or double-click in a blank area.

   The **New User** dialog box will display:

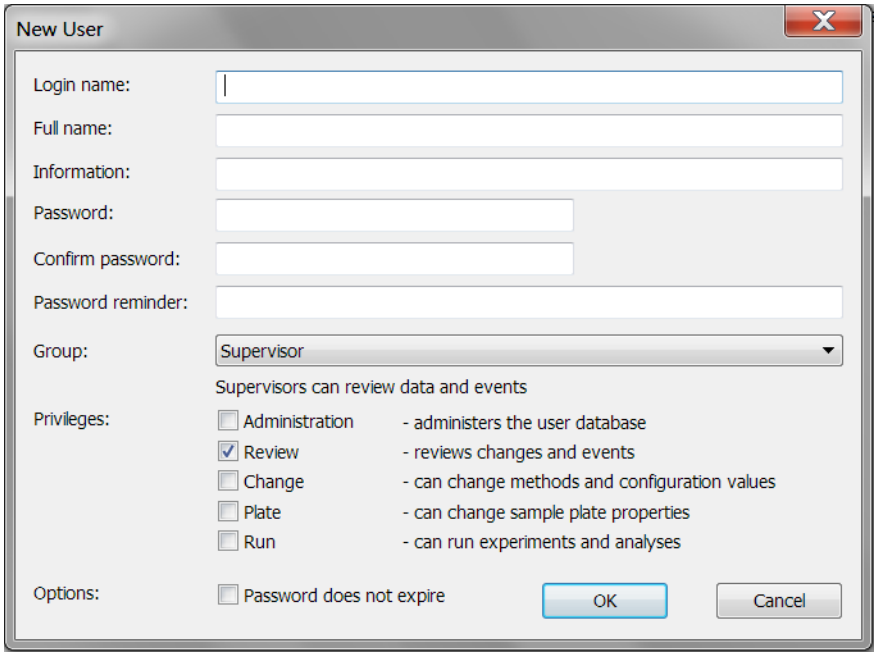


Figure B-37: *New User Dialog Box*

2. ***Assign Account Details.*** Enter the user's **Login name**, **Full name**, **Information** (optional), **Password**, and **Password reminder** (optional).
3. ***Assign a User Group.*** Select a user group from the **Group** drop down list. The following default group selections are available:
   - **Administrators**—can add, delete and change user accounts and groups
   - **Supervisors**—can review data and events
   - **Developers**—can create, run, save and export data
   - **Lab Users**—can only run experiments
   - **Guests**—have no explicit privileges, these must be assigned by the administrator

If other user groups have been created by an administrator, they will also be available for selection in the **Group** drop down box. For more information, please see "Creating a New User Group" on page 389.

4. ***Assign Privileges.*** Each user account can be assigned specific privileges. The privileges displayed initially will be those defined in the user group selected in the previous step. Privileges for the default user groups are shown in Table B-1. If needed, change user account privileges by selecting or deselecting the check boxes next to each privileges.
   - **Administration**—Can administer the user database
   - **Review**—Can review changes and events
   - **Change**—Can change methods and configuration values
   - **Plate**—Can change sample plate properties
   - **Run**—Can run experiments and analyses

Table B-1: *Default User Group Privileges*

Privilege	*Administrator*	*Supervisor*	*Developer*	*Lab User*	*Guest*
Administration	✓				
Review	✓	✓			
Change	✓		✓		
Plate	✓		✓		
Run			✓	✓	

5. ***Options***—Select the **Password does not expire** check box if desired. This check box is deselected by default. Deselecting this option will let user account passwords expire at the **set PasswordTTL** constant. For more information on setting constants please see "Administrator Constants" on page 392.
6. Click **OK** to save changes and exit.

Viewing and Changing User Account Settings

To view and change user account settings:

1. Right-click on the user account and select **Edit User** from the **Tab** menu, or double-click on the user account.

   The **Edit User** dialog box will display:

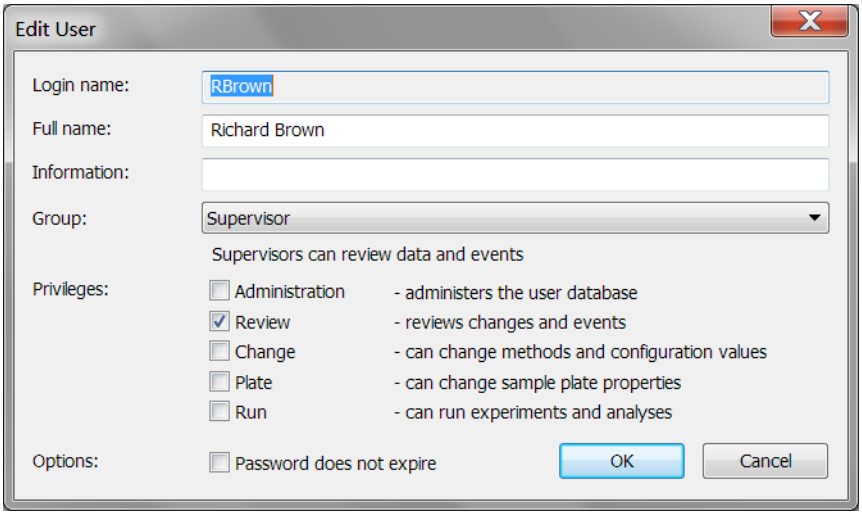


Figure B-38: *Edit User Dialog Box*

2. If needed, modify the user account settings. For more details on individual settings, please refer to “Creating a New User Account” on page 384.
3. Click **OK** to save changes and exit.

Deleting a User Account

To delete a user account:

1. Right-click on the user account and select **Delete User** from the **Tab** menu.
2. Click **OK** in the dialog box displayed.

Changing User Account Passwords

To change user account passwords:

1. Right-click on the user account and select **Set Password** from the **Tab** menu.

   The **Change Password** dialog box will display:

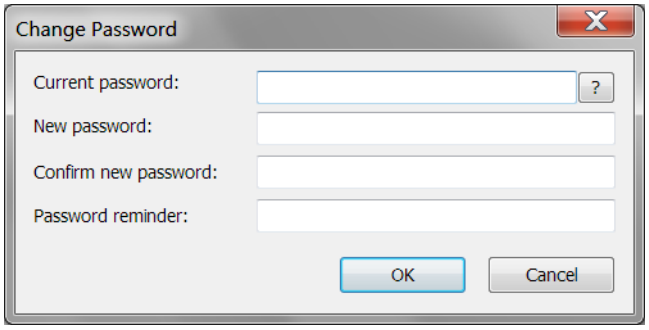


Figure B-39: *Change Password Dialog Box*

2. Enter the **Current password** for the user account. Click **?** for a password reminder.
3. Enter the **New Password** and **Password reminder** (optional).
4. Click **OK** to save changes and exit.

Changing the Administrator Password

1. Initiate a new administrator user session with the existing password.
2. When the software launches, select **Change Password...** from the **Security** menu.

   The **Change Password** dialog box will display:

NOTE: *The **Change Password** dialog box can also be accessed by right-clicking on the administrator account in the **Users Tab** and selecting **Set Password** from the **Tab** menu.*

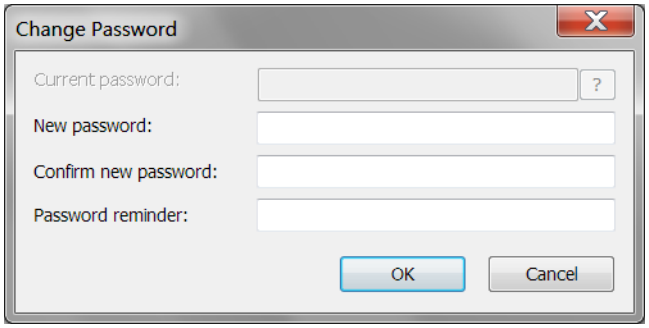


Figure B-40: *Administrator Change Password Dialog Box*

3. Enter the **Current password** for your user account. Click **?** for a password reminder.
4. Enter the **New Password** and **Password reminder** (optional).
5. Click **OK** to save changes and exit.

Group Administration

The **Groups Tab** allows administrators to add and delete user groups as well as set and change group privileges.

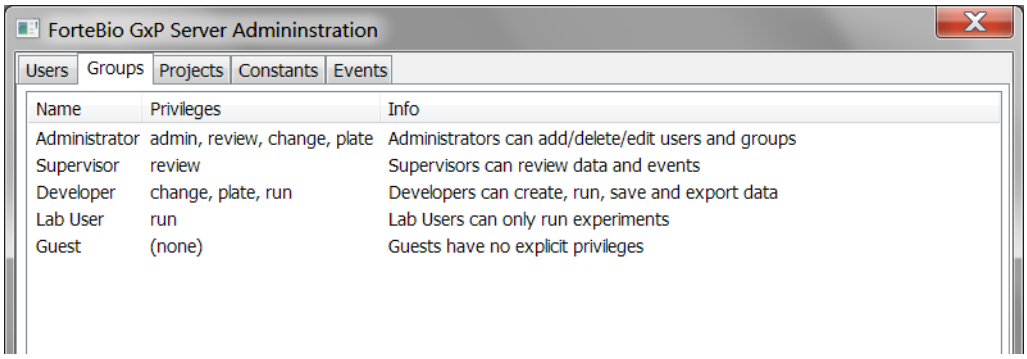


Figure B-41: *Groups Tab*

When a user account is assigned to a user group, the privileges defined in the group are also applied to the individual user account. The following default user groups are available and the privileges assigned to each are shown Table B-2:

- **Administrators** - Can add, delete and change user accounts and groups
- **Supervisors** - Can review data and events
- **Developers** - Can create, run, save and export data
- **Lab Users** - Can only run experiments

- **Guests** - Have no explicit privileges, these must be assigned by the administrator

Table B-2: *Default user group privileges.*

Privilege	*Administrator*	*Supervisor*	*Developer*	*Lab User*	*Guest*
Administration	✓				
Review	✓	✓			
Change	✓		✓		
Plate	✓		✓		
Run			✓	✓	

Creating a New User Group

1. Right-click anywhere in the **Groups Tab** and select **New Group** from the **Tab** menu or double-click in a blank area.

   The **New Group** dialog box will display:

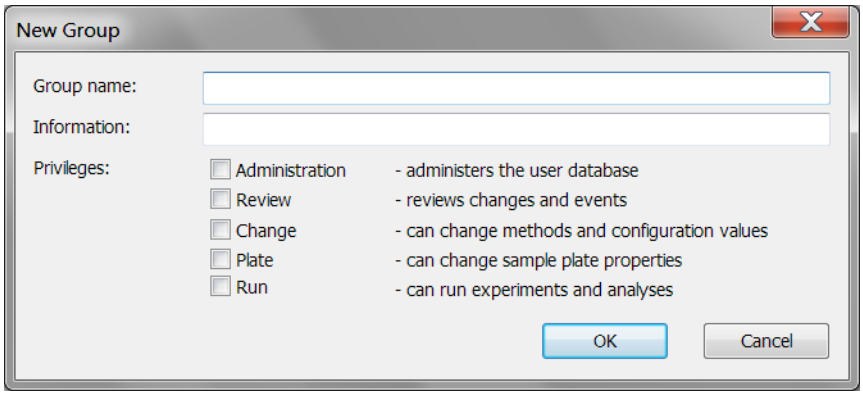


Figure B-42: *New Group Dialog Box*

2. Enter the **Group name** and **Information** (optional).
3. ***Privileges*** - Each group can be assigned specific privileges. Add group privileges by selecting or deselecting the check boxes next to each privilege:
   - **Administration** - Can administer the user database
   - **Review** - Can review changes and events
   - **Change** - Can change methods and configuration values
   - **Plate** - Can change sample plate properties
   - **Run** - Can run experiments and analyses
4. Click **OK** to save changes and exit.

Viewing and Changing Group Settings

1. Right-click on the group and select **Edit Group** from the **Tab** menu or double click on the group.

   The **Edit Group** dialog box will display:

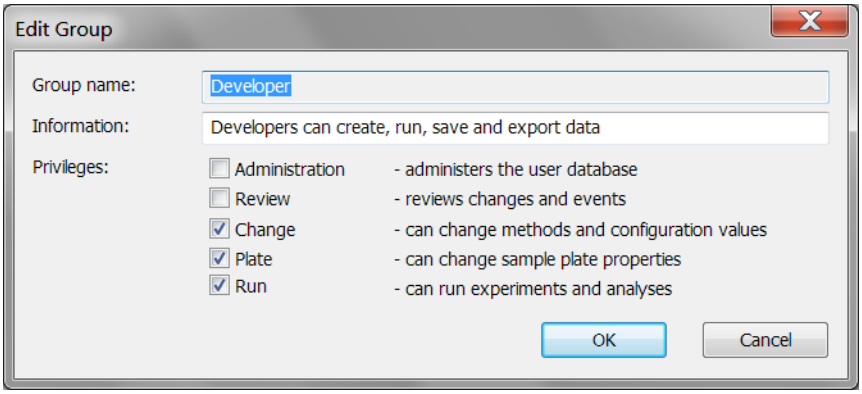


Figure B-43: *Edit Group Dialog Box*

2. If needed, modify the group settings. For more details on individual settings, please refer to “Creating a New User Group” on page 389.
3. Click **OK** to save changes and exit.

Deleting a User Group

1. Right-click on the group and select **Delete Group** from the **Tab** menu.
2. Click **OK** in the dialog box displayed.

Project Administration

The **Projects Tab** allows administrators to add and delete user projects. Projects are selected when a new user session is initiated in the Data Acquisition or Data Analysis software, allowing all user, system and software events for a particular project to be monitored.

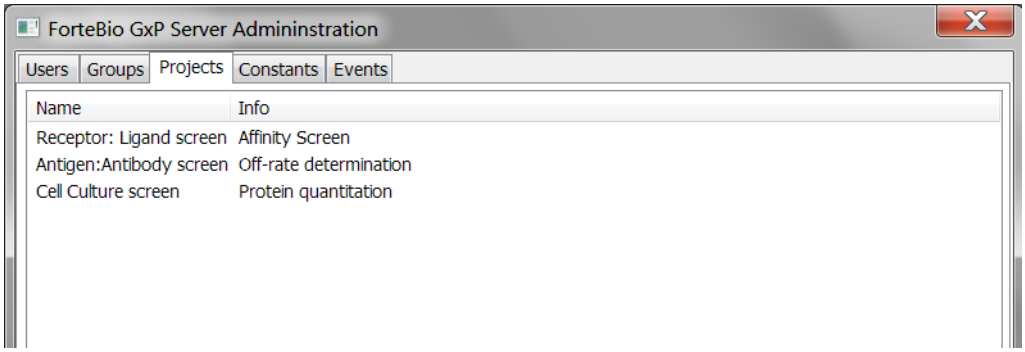


Figure B-44: *Projects Tab*

Creating a New Project

1. Right-click anywhere in the **Projects Tab** and select **New Project** from the **Tab** menu, or double-click in a blank area.

   The **New Project** dialog box will display.

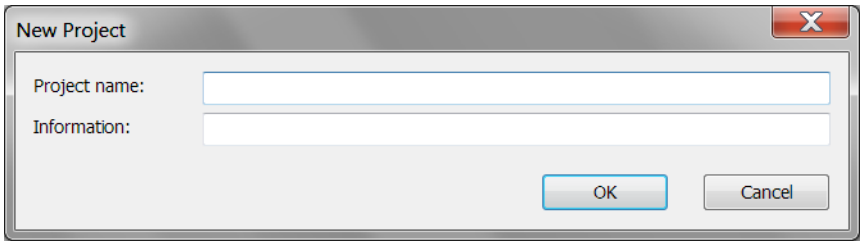


Figure B-45: *New Project Dialog Box*

2. Enter the **Project name** and **Information** (optional).
3. Click **OK** to save changes and exit.

Viewing and Changing Project Settings

1. Right-click on the project and select **Edit Project** from the **Tab** menu, or double-click on the project.

   The **Edit Project** dialog box will display:

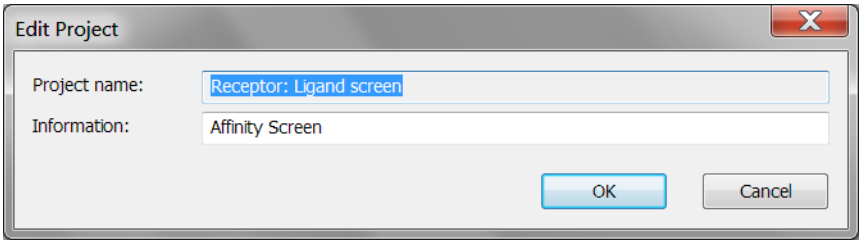


Figure B-46: *Edit Project Dialog Box*

2. If needed, modify the project settings.
3. Click **OK** to save changes and exit.

Deleting a Project

1. Right-click on the project and select **Delete Project** from the **Tab** menu.
2. Click **OK** in the dialog box displayed.

Administrator Constants

The **Constants Tab** allows administrators to set GxP Server module constant settings.

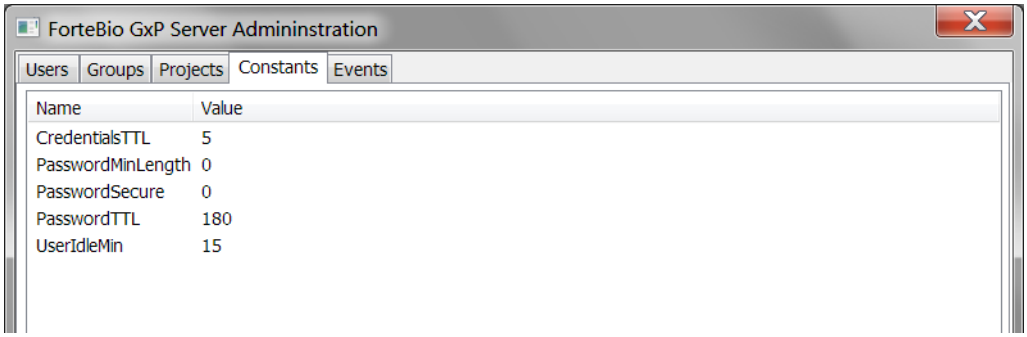


Figure B-47: *Constants Tab*

Available administrator constants and their associated value ranges are shown in Table B-3.

Table B-3: Administrator Constants

Constant	Description	Default Value	Value Range
CredentialsTTL	The number of days that the server settings are stored in the cache. This allows the software to operate in case the server is temporarily down.	5	Minimum=0, no max value
PasswordMin-Length	Minimum number of characters that a password must contain.	0	Minimum=0, no max value
PasswordSecure	Level of password complexity. Setting the constant to 0 has no password restrictions. Setting the constant to 1 requires passwords to contain at least one alpha, one numeric, and one punctuation character.	0	0-1
PasswordTTL	Amount of time that a password is allowed to remain unchanged.	180	Minimum=0, no max value
UserIdleMin	Idle time allowed during a user session after which the session is automatically closed.	15	Minimum=0, no max value

Creating a New Constant

1. Right-click anywhere in the **Constants Tab** and select **New Constant** from the **Tab** menu or double-click in a blank area.

   The **New Constant** dialog box will display:

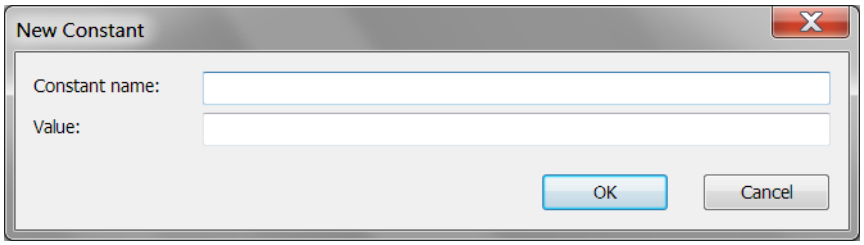


Figure B-48: New Constant Dialog Box

2. Enter the **Constant name** and **Value**. Please refer to Table B-3 for a list of available constants and value ranges.

3. Click **OK** to save changes and exit.

Viewing and Changing Constants

1. Right-click on the constant and select **Edit Constant** from the **Tab** menu or double-click on the constant.

   The **Edit Constant** dialog box will display:

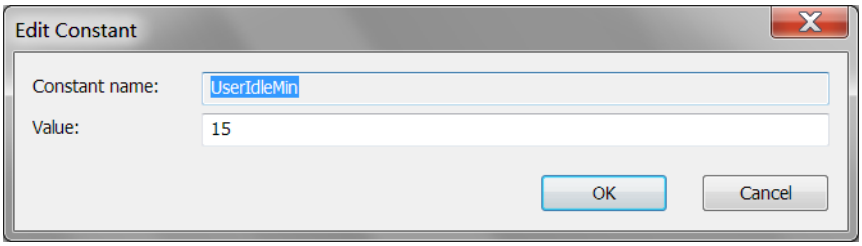


Figure B-49: *Edit Constant Dialog Box*

2. If needed, modify the constant settings. For more information on available constants and their values, please see Table B-3.
3. Click **OK** to save changes and exit.

Deleting a Constant

1. Right-click on the constant and select **Delete Constant** from the **Tab** menu.
2. Click **OK** in the dialog box displayed.

Event Log

The **Events Tab** allows administrators to view all the user, system and software event information recorded by the GxP Server module.

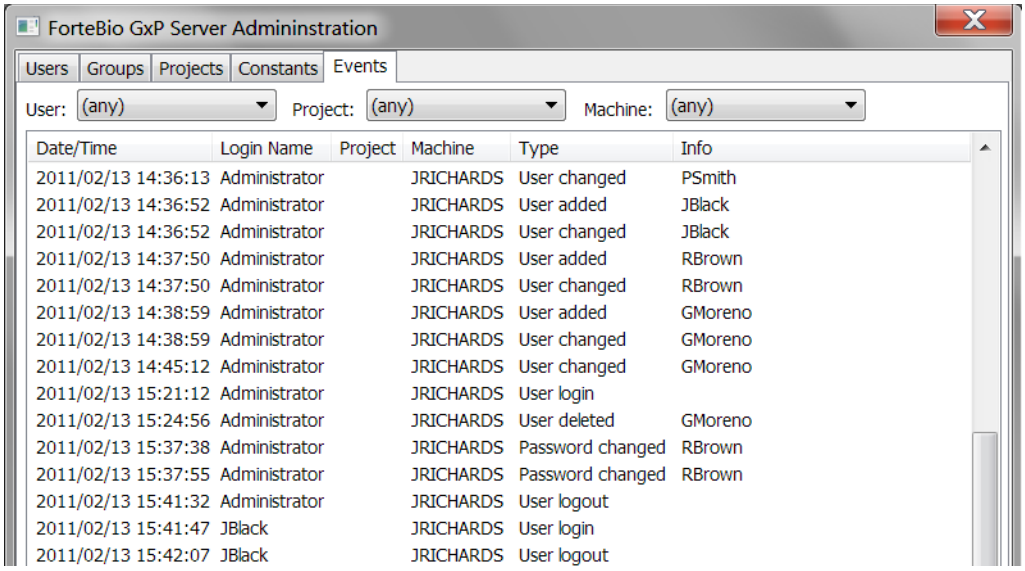


Figure B-50: *Events Tab*

Events are tracked for individual user accounts, projects and machines. By default, a historical log of all events recorded on the active GxP Server module will display.

To view events for a specific user account, project or computer, click on the **User**, **Project** or **Machine** drop-down list and select an entry:

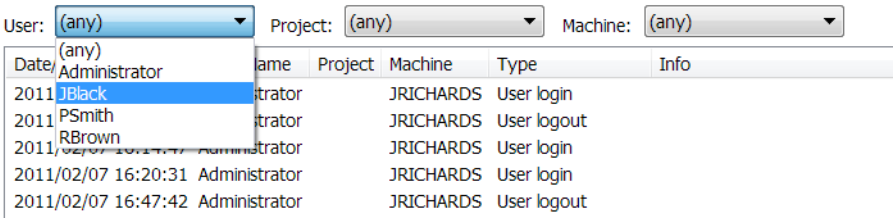


Figure B-51: *Selecting Events by User Name*

NOTE: *Selections can be made in either one or all of the **User**, **Project** or **Machine** drop-down lists.*

The list will then only display events for the entries selected:

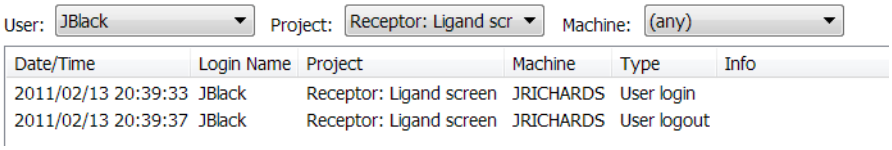


Figure B-52: *Events Displayed for User Name*

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

- **(any)** - Displays all user, project or machine events
- **(none)** - Displays all user and machine events not associated with a specific project (**Project** list only)

ACCESSING THE GXP SERVER MODULE DIRECTLY

The GxP Server module can be accessed by administrators directly without having to initiate an administrator user session. Direct access provides administrators with server testing options as well as access to all administrative functions discussed earlier in this section.

To access the GxP Server module directly:

- ***If the GxP Server module is installed on a network location*** - Double-click on the **FBServerConfig.exe** file in the **FBServer7** folder from the installed location:

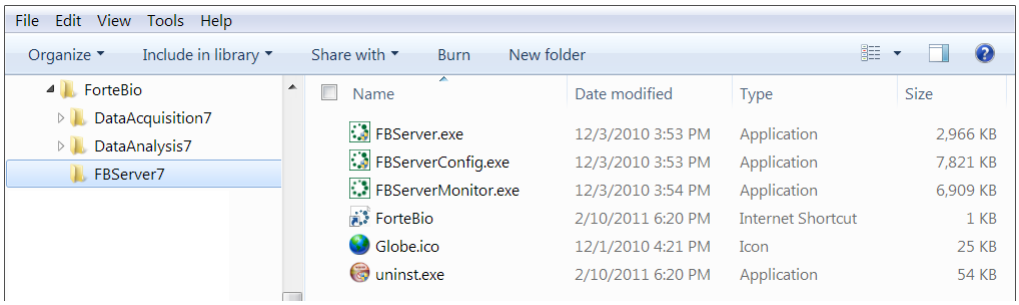


Figure B-53: *Accessing the GxP Server on the Network*

- ***If the GxP Server module is installed on a local host computer*** - Double-click the ForteBio GxP Server desktop icon:

Figure B-54: *ForteBio GxP Server Desktop Icon*

The **ForteBio GxP Server Configuration** window will display:

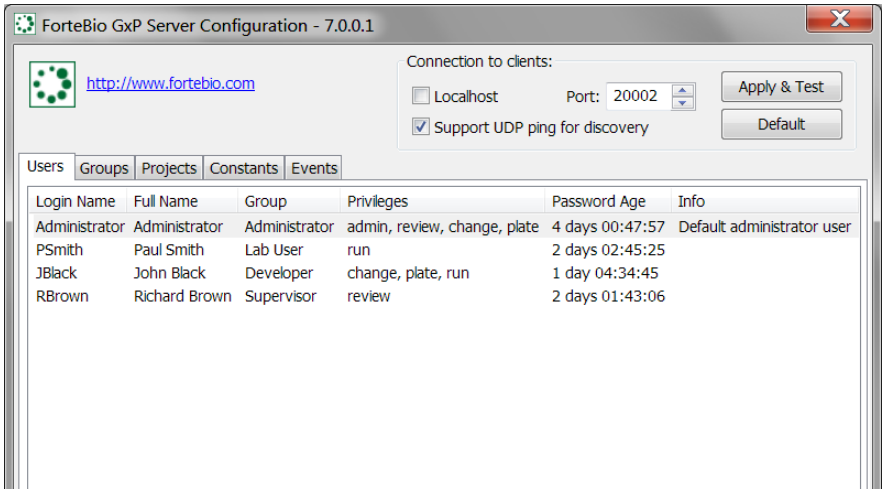


Figure B-55: *GxP Server Configuration Window*

Use of the **User**, **Groups**, **Projects**, **Constants** and **Events** tabs are described in "Accessing Administrator Options" on page 381.

Server Testing

The GxP Server module can be tested to ensure it is accessible and functioning properly.

1. In the **Connections to Clients** box, make changes to the server settings if needed.

Figure B-56: *Connections to Clients Box*

2. Click **Apply & Test**. If the GxP Server module is found and functioning properly, the following message will display:

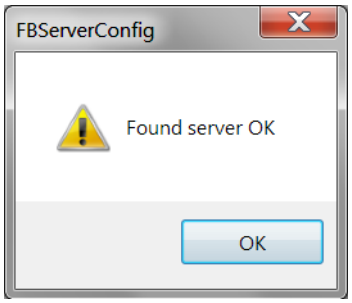


Figure B-57: *Server Found*

To return to the originally configured GxP Server module settings, click **Default** at any time.

RESTARTING THE GXP SERVER MODULE

If the host location of the GxP Server module cannot be found during user login or if users are unable to login with valid credentials, the GxP Server module may be offline and need to be restarted.

NOTE: *ForteBio recommends contacting your IT department to confirm whether or not network or firewall settings may have been changed. This may also be preventing access to the GxP Server module.*

- ***If the GxP Server module is installed on a network location*** - Double-click on the **FBServer.exe** file in the FBServer7 folder from the installed location:

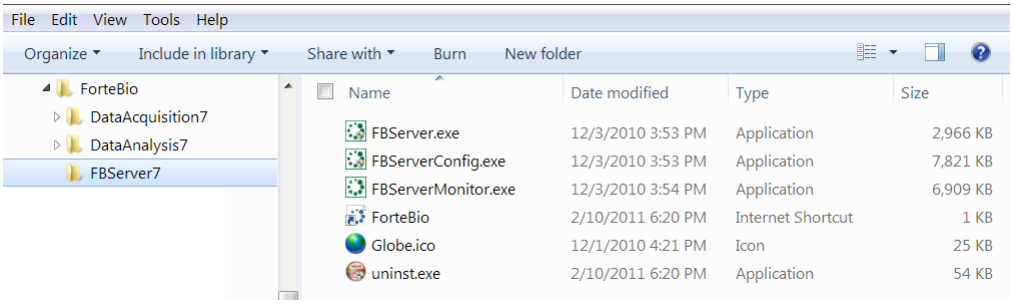


Figure B-58: *Accessing the GxP Server on the Network*

- ***If the GxP Server module is installed on a local computer*** - Double-click the Restart Server desktop icon:

Figure B-59: *Restart Server Desktop Icon*

The **Restart Server** window will display momentarily as the GxP Server module restarts:

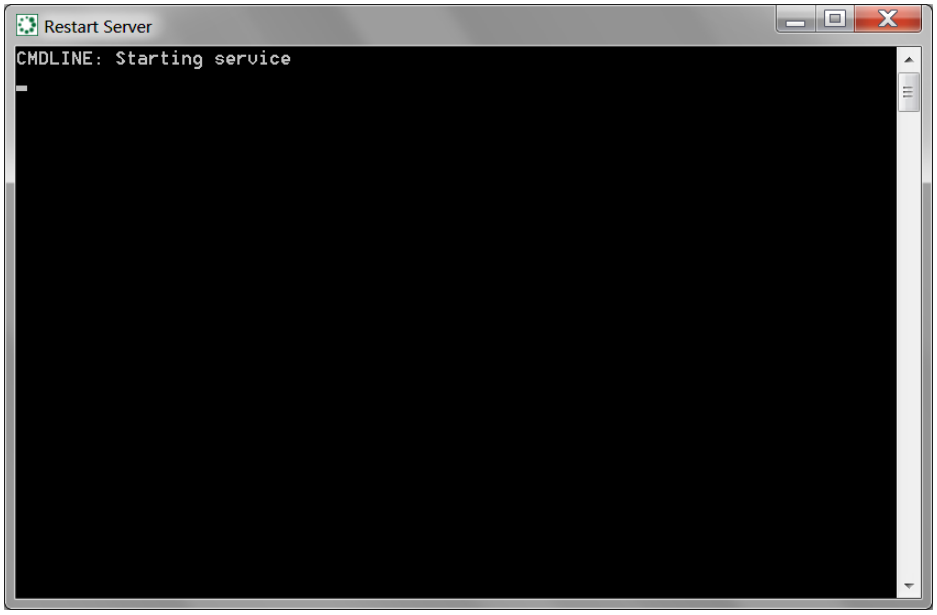


Figure B-60: *Restart Server Window*

Index

Symbols

Numerics

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

Index

Symbols

Numerics

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

Index

Symbols

Numerics

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W