# octet SYSTEM

User's Guide


fortéBIO™

P/N41-0000 rev 290806

# CONTENTS

# CHAPTER 1
# Welcome



Welcome to the FortéBio Octet System User Guide. This manual provides a brief overview of Bio-Layer Interferometry and explains how to:

- Operate the Octet instrument.
- Set up and run quantitation and kinetics experiments on the Octet instrument, perform data analysis, and generate reports.
- Maintain the Octet instrument.

For information on preparing samples for quantitation or kinetics experiments, please see the appropriate FortéBio Octet Biosensor product instructions. For information about the Origin® software that is included with the Octet System, please see the *Origin User's Manual*.

## 1.1 About This Manual

The following conventions are used in this manual.

| Convention | Example |
|---|---|
| Menu commands are bolded. | To start a new quantitation experiment, select **Experiment** →**Quantitation** →**New Quantitation Experiment** on the main menu. |
| Document names are italicized. | *Octet System User's Guide* |
| Numbered steps explain how to carry out a procedure.<br><br>A dash (—) precedes the description of the system response to a procedure step. | 1. To start the Octet software, click the  icon on the desktop.<br><br>— The main window appears. |

**NOTE**

A note presents pertinent details on a topic.

**CAUTION**

**CAUTION!** A caution note warns you that your actions may have nonreversible consequences or may cause loss of data.

**IMPORTANT**

Important information advises you of actions that are essential to the correct performance of the instrument or software.

## 1.2 Octet System Overview

The FortéBio Octet System includes the Octet instrument and Octet software, as well as the Origin® software, a graphing and analysis application for kinetic data analysis. The Octet System enables real-time quantitation or kinetic characterization of biomolecular interactions. Two system configurations are available, the Octet-QK for quantitation and kinetic applications or Octet-Q for quantitation only.

The Octet software controls the Octet instrument and enables you to:

- Define a quantitation or kinetic experiment and save the experiment for future use.
- Run the defined assay.
- View and save binding data to a user-specified location.
- Generate a report of quantitation or kinetic results in table and graph formats.
- Define custom assays in assay development mode.

### Bio-Layer Interferometry

The Octet System is based on proprietary Bio-Layer Interferometry (BLI), a label-free and highly sensitive biosensor technology. The tip of an Octet biosensor comprises an optical coating layer and a biocompatible matrix that is derivatized with a capture molecule (for example, anti-human IgG Fc) (Figure 1.1). The Octet biosensors are configured in a format that is compatible with a standard 96-well microplate.

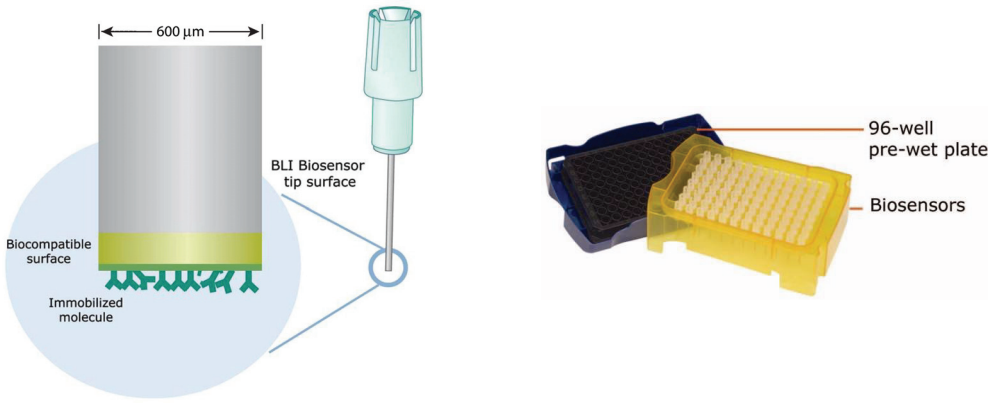



**Figure 1.1 A single Octet biosensor (left), plate of 96 biosensors (right)**

The Octet instrument passes white light through the biosensor and measures the interference patterns of light that is reflected back, a characteristic response profile of wavelength peaks and troughs (Figure 1.2).

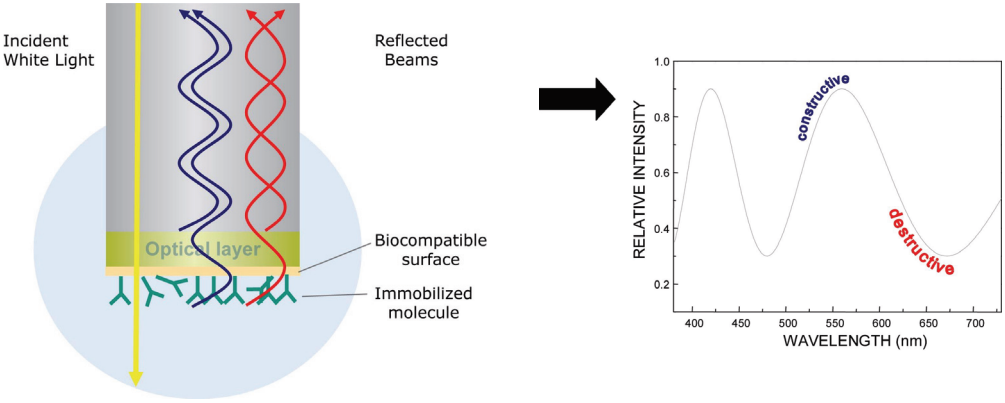



**Figure 1.2 Interference pattern of light that is reflected back through the biosensor**
The molecules bound to the tip of the optic fiber produce an interference pattern at the detector.

Any change in the number of molecules bound to the biosensor surface changes the optical path of the reflected light and causes a measurable shift in the interference pattern ($\Delta\lambda$ in nm) (Figure 1.3). Importantly, the response profile is not affected by unbound molecules, changes in the refractive index of the medium, particulate matter

in the medium, or changes in the flow rate. As a result, assays can even be conducted in cell culture media or crude lysates without interference.

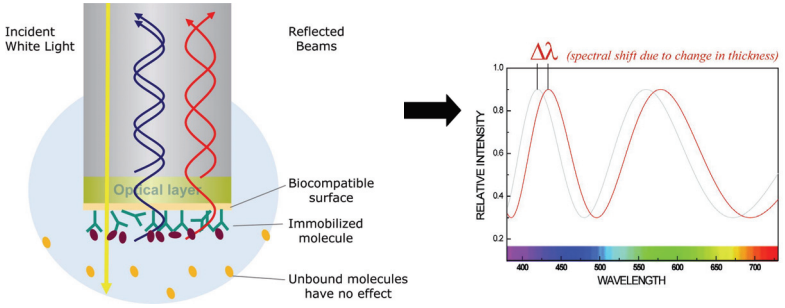



**Figure 1.3 Molecules that bind to the immobilized molecules on the biosensor change the optical path of the reflected light from the biosensor surface and shift the interference pattern of the light reflected through the biosensor.**

## Octet Binding Curves

### Quantitation Analysis

In a quantitation analysis, the raw binding curve represents the rate of increase of optical thickness as the sample binds to the Octet biosensor tip surface. As Figure 1.4 shows, the binding curve is concentration-dependent. The binding curves of standards of known concentration are used to generate a standard curve. Accurate quantitation is derived from the standard curve.

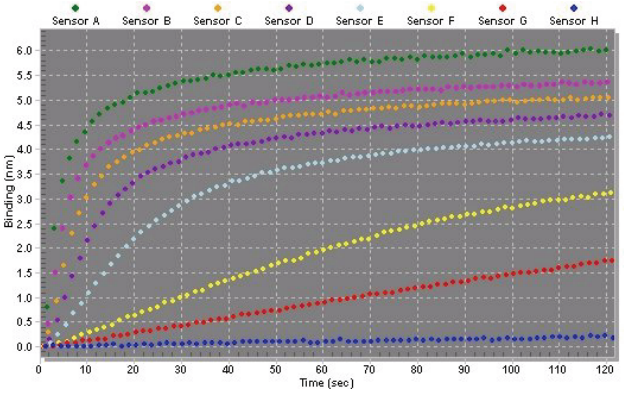



**Figure 1.4 Octet quantitation binding curves are concentration-dependent.**
The binding curve shows the interaction of the immobilized biosensor molecules with sample molecules in real time.

## Kinetic Analysis

Figure 1.5 shows a possible workflow for a kinetic analysis of protein-protein interactions using the FortéBio Streptavidin SBC Biosensor.

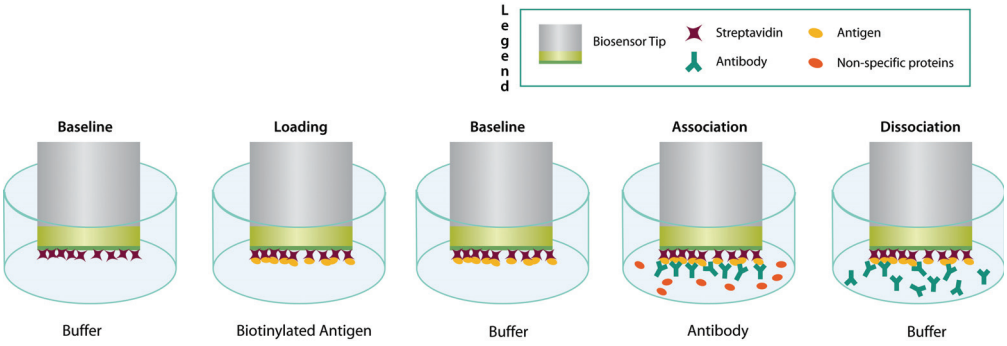



**Figure 1.5 Example Octet System kinetic assay workflow using the Streptavidin SBC Biosensor**

Kinetic assay steps may include:

- Establish a baseline in diluent.
- Load biotinylated antigen.
- Establish a baseline in diluent.
- Load antibody.
- Monitor dissociation in diluent.

An assay may include more or different steps, depending on your particular assay and whether some steps are performed *offline* (not in the Octet instrument).

In Figure 1.6, the binding curve illustrates the rate at which a protein (antibody or antigen) binds to ($k_a$ or *on rate*) and dissociates from ($k_d$ or *off rate*) the biotinylated capture molecule (antigen or antibody) immobilized on the Octet biosensor. The $k_{obs}$ is determined from the binding curve in Figure 1.6, then the $k_a$ is calculated from the $k_{obs}$ and the $k_d$ (see 5.13, page 57).

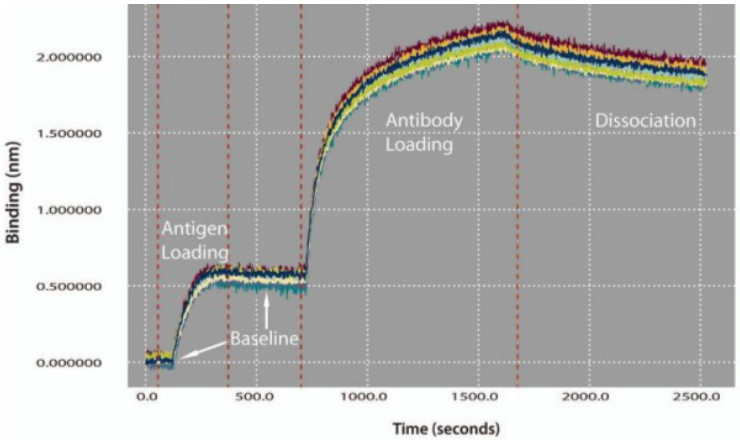



**Figure 1.6 Octet kinetic assay binding curves**

## 1.3 What's New In Octet Software 3.0

New features in the Octet Software Upgrade version 3.0 include:

- Protein A quantitation assay for use with the Octet Protein A Biosensor.
- Assay development mode that enables you to create custom assays with parameters (for example, min/max concentrations, sample flow rate, assay time, read time) optimized for your particular assay.
- *Activation* and *quenching* assay steps for use in online immobilization on the Octet Amine Reactive Biosensor.
- An Explorer-type windowpane that makes it easy to find data files, methods, or real-time binding charts.
- Optional flow (rpm) during instrument delay prior to starting an assay.

## 1.4 FortéBio Technical Support

You can contact FortéBio technical support at:

| | |
|---|---|
| Telephone: | 1-888-OCTET-QK (US only)<br>1-650-322-1360, Option B |
| E-mail: | support@fortebio.com |
| Address: | FortéBio, Inc.<br>1360 Willow Road, Suite 101<br>Menlo Park, CA 94025<br>USA |

# CHAPTER 2
# Getting Started



## 2.1 Octet System Specifications

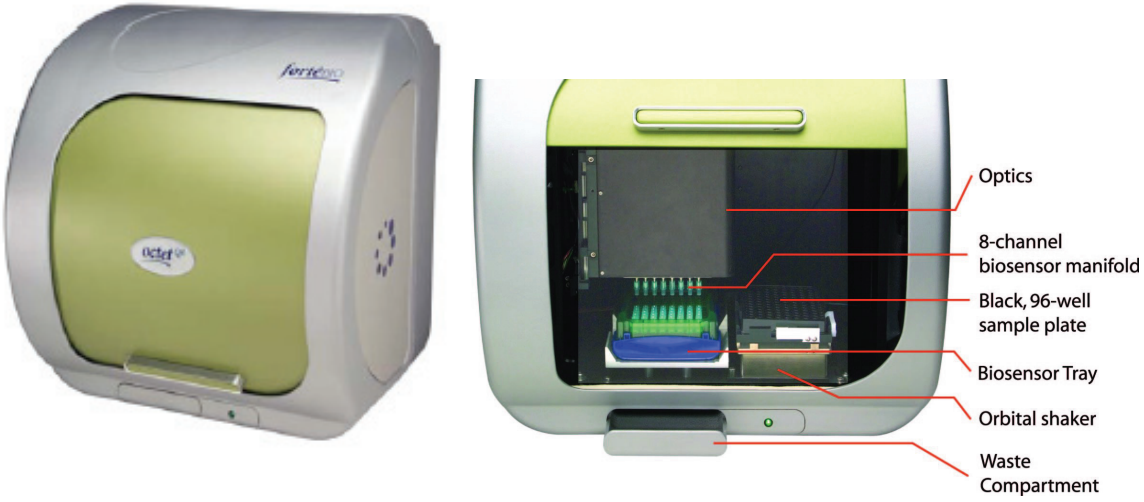



**Figure 2.1 Octet instrument, door closed (left) or open (right)**

| | |
|---|---|
| **Capabilities** | Protein quantitation.<br>Kinetic and affinity analyses ($k_{obs}$, $k_a$, $k_d$, $K_d$).<br>Binding specificity and cooperativity.<br>Kinetic screening. |
| **Sampling Format** | Recommended plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209).<br>SBS standard plate.<br>Single, test plate capacity.<br>Test volume: 200 µl, non-destructive and recoverable. |
| **Sample Types** | Purified samples, common culture media, crude lysates. |
| **Automation** | Up to eight biosensors in parallel.<br>Maximum of 96 tests unattended. |
| **Optics & Mechanics** | 8-channel biosensor manifold.<br>Optical interferometer.<br>Sample plate platform temperature range: From 2° C above ambient temperature to 40° C.<br>Orbital flow: Staticor, 0 rpm or 100-1500 rpm. |
| **Dimensions** | Height: 18.6 in (475 mm)<br>Depth: 17 in (430 mm)<br>Width: 20.8 in (530 mm)<br>Weight: 50 lbs (23 Kg) |
| **Electrical Requirements** | Mains: AC 100-240V, 2-0.9A, 50/60Hz, single phase.<br>Power consumption: 120W (240 peak). |
| **Data Handling & Storage** | Integrated software application for instrument control and data analysis.<br>PC operating system: Windows® XP<br>Interfacing: RS232, USB |

## 2.2 Starting the Octet System

1. Turn on the computer.
2. Turn on the Octet instrument using the power switch located on the rear panel (Figure 2.2).

**NOTE**

The instrument requires a minimum of one hour warm-up time. It is recommended that you leave the instrument on.

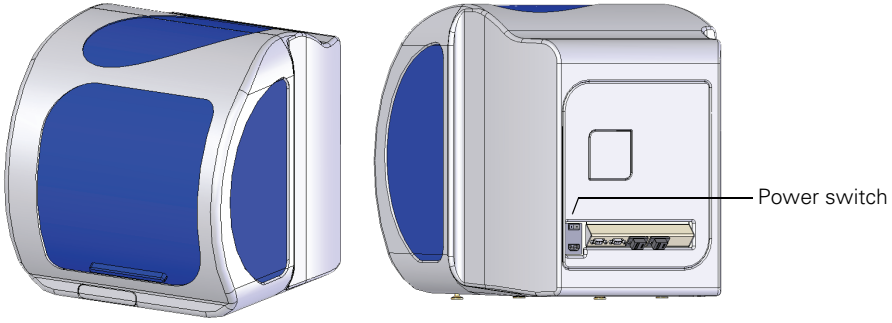



**Figure 2.2 Octet System, front view (left) and rear view (right)**

3. To start the Octet software, double-click the icon on the desk top. Alternatively, click the start button and select **All Programs → Octet Software** on the Windows® Start menu.

   — The Octet instrument initializes. The main window appears and displays the Experiment Wizard and the Instrument Status window (Figure 2.3).

   The Experiment Wizard enables you to start a new experiment or analyze experimental data.

   The Instrument Status window displays a log of instrument activity that can be saved to a text file (.txt).

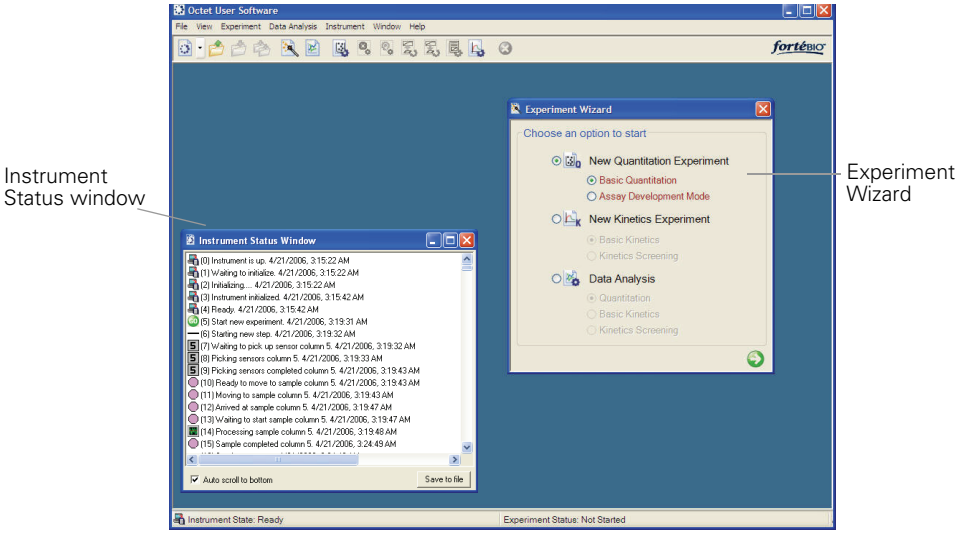



**Figure 2.3 Main window**
At startup, the main window displays the Experiment Wizard and Instrument Status window.

**NOTE**

If a problem occurs during operation of the instrument, it is recommended that you save the instrument log (click **Save to file** in the Instrument Status window) to enable FortéBio technical support to better assist you. Closing the Octet software resets the log.

## 2.3 Setting Analysis Options

Analysis options specify user preferences for quantitation and kinetic data analysis.

1. To view the analysis options, select **File → Options** on the main menu.
2. In the Options dialog box that appears, confirm the defaults or enter new settings (Figure 2.4).

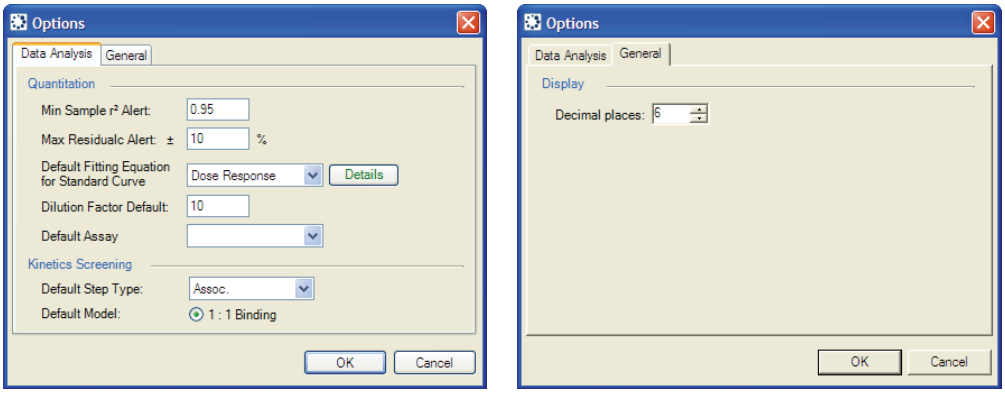



**Figure 2.4 Options dialog box, Data Analysis tab (left), General tab (right)**

| Option | Description |
|---|---|
| **Data Analysis Tab** | |
| **Quantitation** | |
| Min Sample $r^2$ Alert | The threshold $r^2$ value for a sample binding curve or a standard binding curve. If the $r^2$ value of a standard or sample binding curve is less than the threshold value, the standard or sample row is highlighted in the Standard Curve tab (Figure 3.14, page 29) or Concentration Results tab (Figure 3.16, page 32). |
| Max Residual Alert | Specifies a threshold residual value for standards. If a calculated standard concentration deviates $\pm$ 10% or greater from the expected concentration, the standard row is highlighted in the Standard Curve tab (Figure 3.14, page 29). |
| Default Fitting Equation for Standard Curve | Specifies the default model that is used to fit a standard curve. Click **Details** to view more information about the model. |
| Dilution Factor Default | Specifies a dilution factor for the unknowns. |
| Default Assay | Make a selection from the drop-down list to specify the default quantitation assay. |
| **Kinetics Screening** | |
| Default Step Type | If no step type is selected during kinetic experiment setup, the default step type is assigned when you double-click a sample column in the Assay Definition tab. |
| **General Tab** | |
| Decimal Places | Specifies the number of decimal places (from two to nine) for the computed data. **Note:** Six decimal places are recommended for the Protein A assay. |

## 2.4 Setting the Plate Temperature

The default plate temperature is 30° C. The current temperature is displayed in the status bar at the bottom of the main window. If you change the sample platform temperature, allow sufficient time for the sample plate to equilibrate to the new temperature before you begin an experiment (~5 min for a plate at room temperature or ~15 min for a plate at 4° C).

**NOTE**

If you close the Octet software, the sample platform temperature is reset to 30° C when you start the software.

**To change the plate temperature:**

1. Select **Experiment → Set Plate Temperature** on the main menu.
2. Enter the temperature of interest in the Temperature Settings box that appears (Figure 2.5).

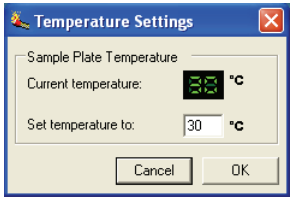



**Figure 2.5 Temperature Settings box**

# CHAPTER 3
# Quantitation Analysis



A quantitation experiment enables you to determine sample concentration using a reference set of standards. To set up a quantitation experiment in the Octet software, you:

- Specify the well locations of the active sensors and the associated samples.
- Select the type of quantitation assay.
- Designate the sample type (unknown, control, or standard).
- Specify the standard concentrations.

You can save the experiment method (.fmf) as well as the standard curve (.fsc) that is generated during a session. After the experiment is run, you can export a results report (.doc).

## 3.1 Setting Up a Quantitation Experiment

For more details on how to prepare the sensors, see the appropriate product instructions.

**NOTE**

Before you begin an experiment, check the plate temperature (displayed in status bar). Confirm that the temperature is appropriate for your experiment or set a new temperature. (For more details on setting the temperature, see page 12.) If you close the Octet software, the sample platform temperature is reset to 30° C.

### Specifying the Sensor & Sample Configuration

At startup, the Octet software displays the Experiment Wizard. If the wizard is not displayed, click the Experiment Wizard button or select **Experiment →New Experiment Wizard** on the main menu.

1. In the Experiment Wizard, select the **New Quantitation Experiment** and **Basic Quantitation** options. (Figure 3.1).

   Alternatively, select **Experiment → Quantitation → New Quantitation Experiment** on the main menu.

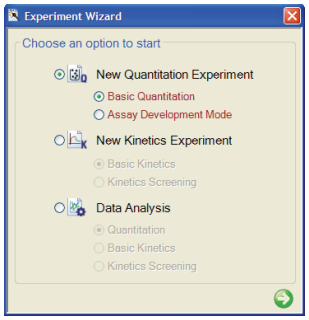



**Figure 3.1 Experiment Wizard**

2. Click the arrow.
   — The Quantitation Experiment Setup window appears (Figure 3.2).

**NOTE**

More than one Quantitation Experiment Setup window can be open at the same time.

Figure 3.2 shows how to specify the active sensor and sample locations.

**To specify the active sensor locations, do one of the following:**

- Click **Fill Plate** to select all 12 columns in the plate (eight sensors per column).
- Draw a box around the wells of interest in one or more columns in the sensor plate map.
- Click the wells of interest in a column of the sensor plate map.

**To delete one or more sensor locations:**

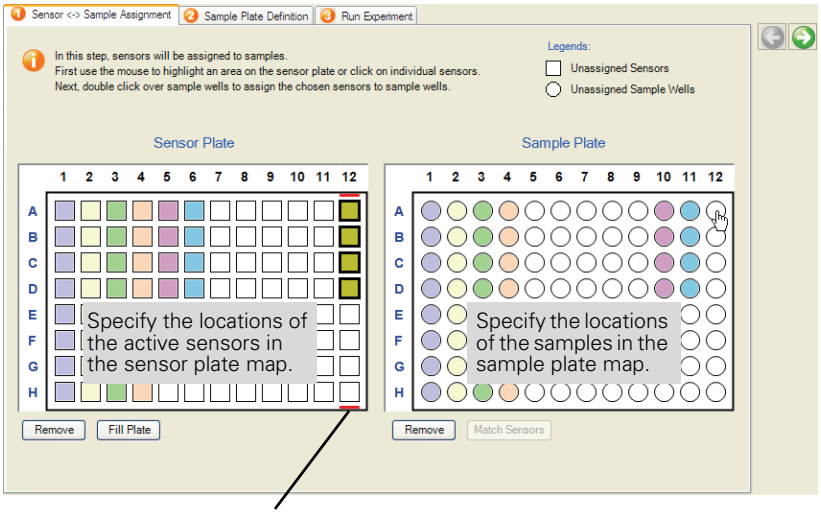
- Select the wells in the sensor plate map and click **Remove**.
  - The sensor locations are removed. Any matching sample locations are also removed.

**To specify the sample locations, do either of the following:**

- Click **Match Sensors**.
  - The well locations in the sample plate that match the active sensor locations are selected.
- In the sample plate map, double-click a column.
  - The wells that match the active sensor locations are selected in the column.

**To delete one or more sample locations:**

- Select the wells in the sample plate map and click **Remove**.
  - The sample locations as well as the corresponding sensor locations are removed.




The red bars at the top and bottom of a column indicate the active sensor column. Double-click a column in the sample plate map to direct the active sensor to that column in the sample plate.

Matching sensors and samples can be located in different columns. However, the sensors and samples must occupy corresponding wells in the columns. The paired sensor and sample locations are identified by matching colors.

**Figure 3.2 Quantitation Experiment Setup window, Sensor↔Sample Assignment tab.**

## Assigning the Sample Wells

Use the sample plate map to:

- Define the sample wells as standards, controls, or unknowns.
- Enter the concentration information for the standards.
- Enter additional sample information (optional).

**NOTE**

Well designations can also be entered after data acquisition. However, it may be less convenient since the information is entered one well at a time and data analysis does not proceed automatically at the end of data acquisition.

1. After you specify the sensor and sample locations, click the Sample Plate Definition tab (Figure 3.3). Alternatively, click the arrow to advance to the tab.
2. Confirm the default assay type or select a new type.

1. Select a quantitation assay.
2. To view the assay parameters for the selected assay, click the Details button.

   **Note:** To view all information in the Assay Parameters table, scroll up/down, and if necessary, put the cursor over a table cell.

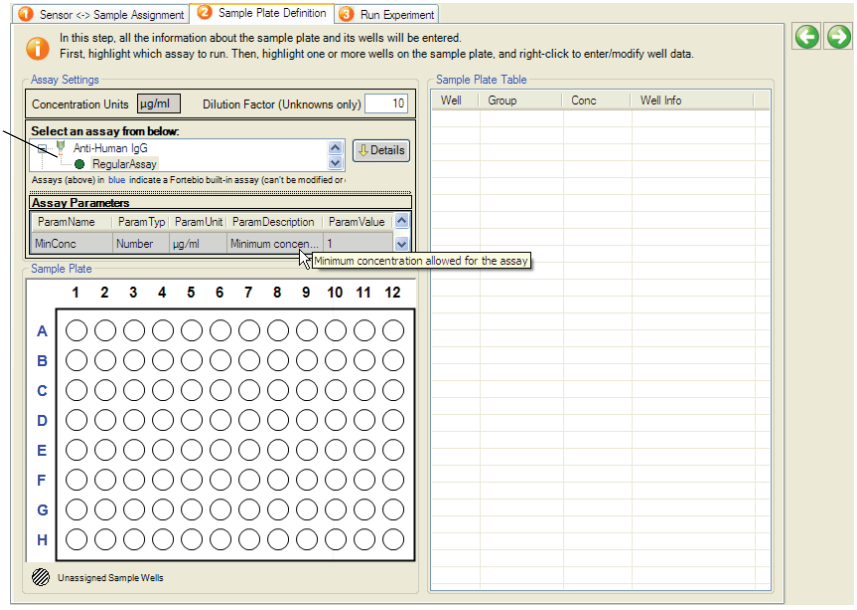



**Figure 3.3 Quantitation Experiment Setup window, Sample Plate Definition tab**
Select the assay type and assign well definitions (standards, controls, unknowns) in this tab.

## Designating Standards

Figure 3.4 and Figure 3.5 show the steps to designate standards and assign concentrations.

**To designate standards:**

1. Select the wells that you want to define as standards.
2. Right-click the selection and choose **Set Group →Standards** on the shortcut menu.
   - The standard wells are marked Sta and the Sample Plate table is automatically updated.
3. To remove a well designation, select the well(s) of interest. RIght-click the selection and choose **Clear Data** on the shortcut menu.

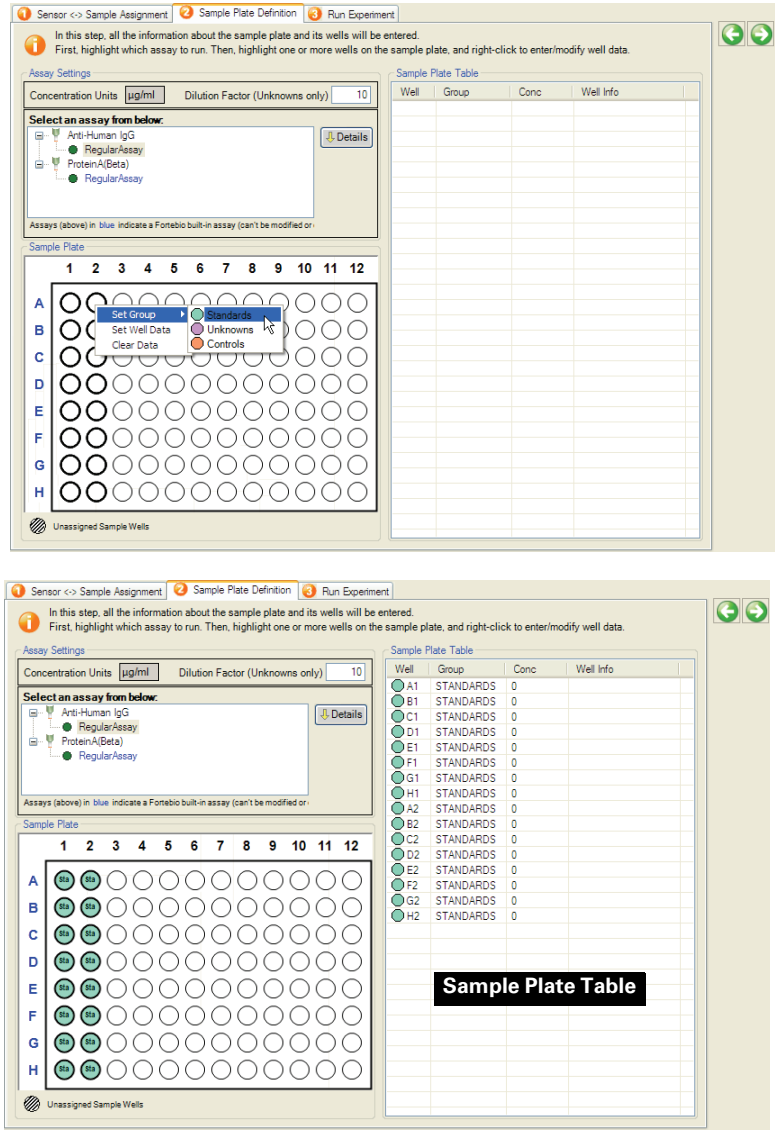
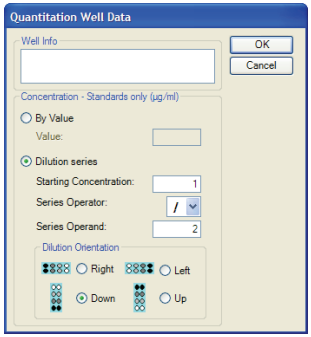
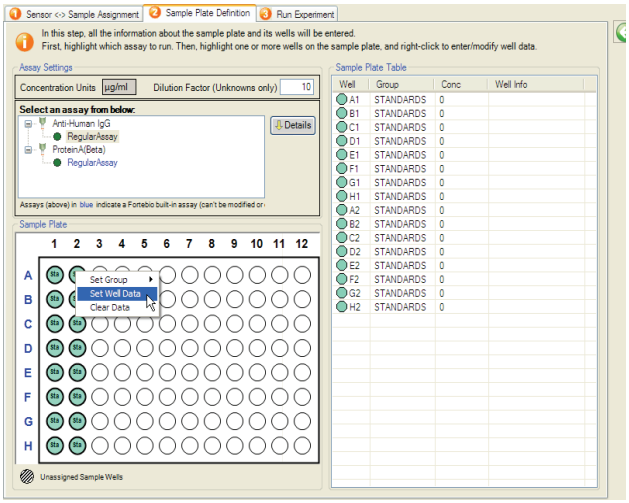



**Figure 3.4 Designating standards**

**To begin assigning standard concentrations, first open the Quantitation Well Data box:**

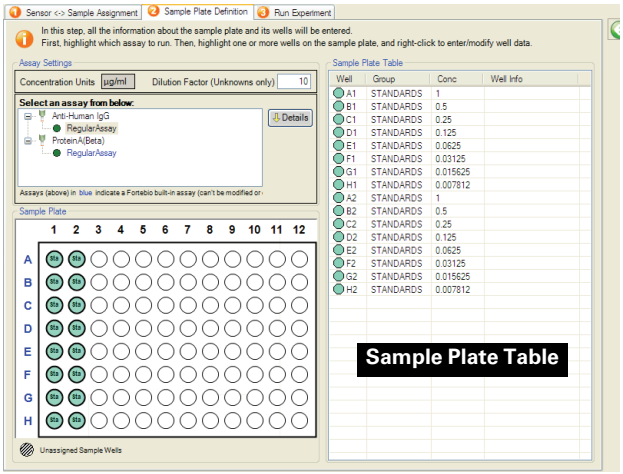
1. Select the standard wells of interest.
2. Right-click the selection and choose **Set Well Data** on the shortcut menu.
   - The Quantitation Well Data box appears.




**To assign concentrations using a dilution series:**

1. Confirm that the wells of interest are selected in the sample plate map.
2. In the Quantitation Well Data box, select the **Dilution Series** option.
3. Enter the starting concentration.
4. Select a series operator and enter an operand.
5. Select the appropriate dilution orientation.
   o = highest concentration
   ● = lowest concentration
6. Click **OK**.




**To assign a user-specified concentration value to a selected standard(s), do either of the following:**

- Select the well(s) of interest and open the Quantitation Well Data box. Select the **By Value** option, enter the concentration in the Value box, and click **OK**.
  OR
- Enter the values in the Sample Plate table.
  **Note:** Edit commands are available in the table.

**To annotate wells, do either of the following:**

- Select the well(s) of interest and open the Quantitation Well Data box. Enter the information in the Well Info box, and click **OK**.
  OR
- Enter well information in the Sample Plate table.

**Figure 3.5 Assigning standard concentrations and annotating wells**

## NOTE

If you use a saved standard curve for the analysis, the sample plate does not need to include standards. To analyze the data, start a data analysis session and import the standard curve. For more details on conducting a data analysis session, see page 33.

### Designating Controls & Unknowns

Figure 3.6 and Figure 3.7 show the steps to designate unknowns or controls and enter notes about them.

**To designate controls or unknowns:**

1. Select the wells of interest (draw a box around the column(s) or row(s), or click a well).
2. To designate controls, right-click the selection, and choose **Set Group → Controls** on the shortcut menu.
   - The control wells are marked (Con) and the Sample Plate table is automatically updated.
3. To designate unknowns, right-click the selection, and choose **Set Group → Unknowns** on the shortcut menu that appears.
   - The unknown wells are marked (Unk) and the Sample Plate table is automatically updated.
4. To remove a well designation, select the well(s) of interest. RIght-click the selection and choose **Clear Data** on the shortcut menu.
5. If you are working with diluted unknowns, enter the dilution factor in the Dilution Factor box.
   - The dilution factor is applied to all unknowns.

   **Note:** Only one dilution factor can be applied.

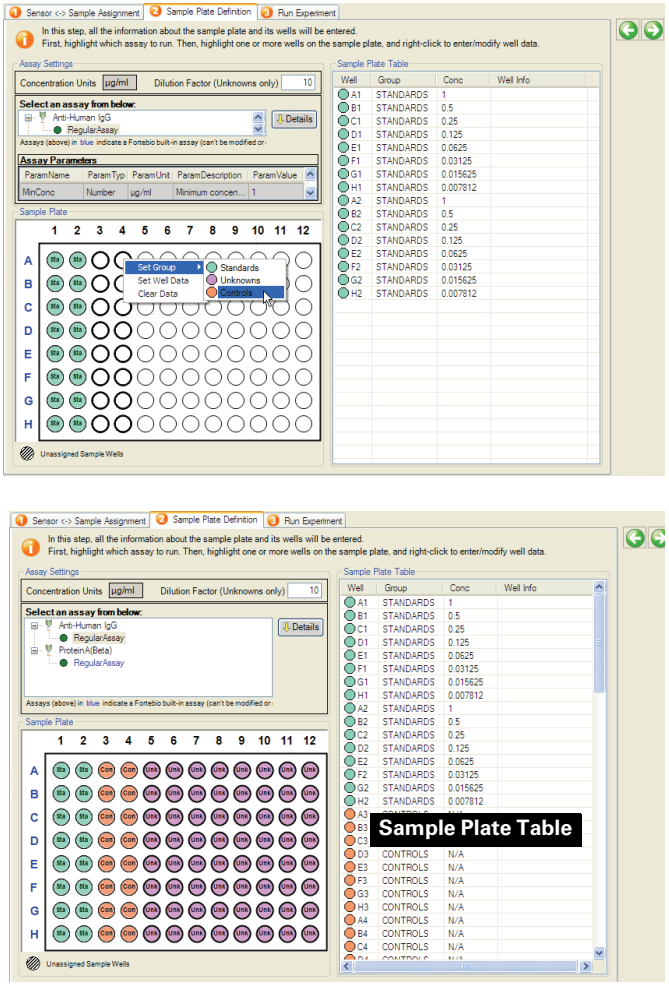



**Figure 3.6 Designating controls or unknowns**

**To annotate wells:**

1. Select the wells of interest.
2. Right-click the selection and choose **Set Well Data** on the shortcut menu.
   - The Quantitation Well Data dialog box appears.
3. Enter information in the Well Info box and click **OK**.
   - The information appears in the sample plate table.

**To edit standard concentration or well information in the Sample Plate table:**

1. In the Sample Plate table, double-click the cell that you want to edit.
2. Enter the new information. Alternatively, right-click the cell to view a shortcut menu of edit commands.

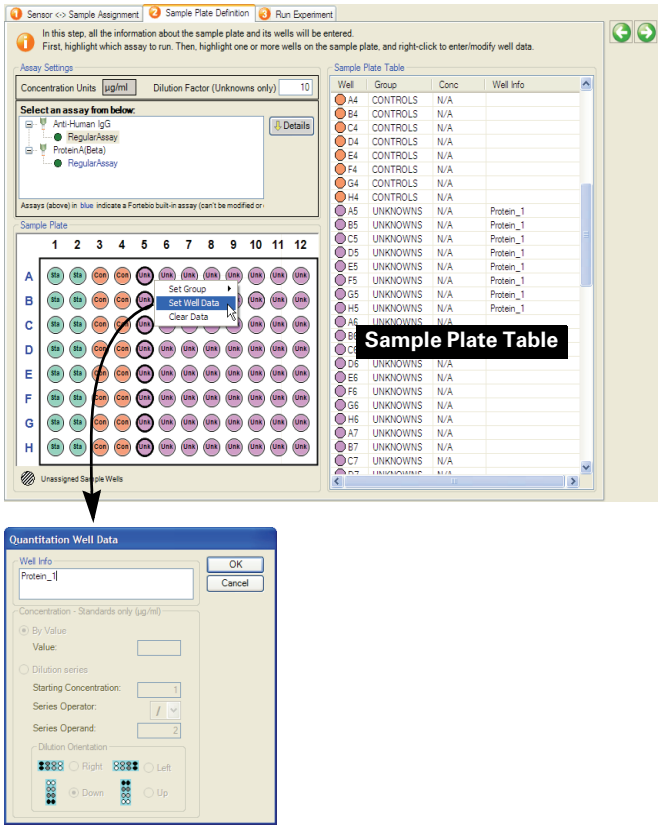



**Figure 3.7 Entering information about controls or unknowns**

## 3.2 Saving a Quantitation Experiment

The software automatically saves the experiment information that you specify (sensor and sample locations, well definitions (unknown, control, or standard), standard concentrations, and well information) to an experiment method (.fmf) at the start of the run. If you set up an experiment, but do not start the run, you can manually save the experiment method.

1. To manually save an experiment, click the Save Method File button . Alternatively, select **File → Save Method File** on the main menu.

   If there is more than one open experiment and you want to save them all, click the Save All Methods Files button .

2. In the Save dialog box that appears, 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** on the main menu and enter a new name for the experiment.

### Opening an Experiment

1. Click the Open Method File button . Alternatively, select **File → Open → Experiment Method File** on the main menu.
2. In the dialog box that appears, navigate to the experiment (.fmf) of interest, and click **Open**.

**NOTE**

When you open a method, reselect the assay type. (This information is not saved.)

## 3.3 Performing a Quantitation Experiment

You are ready to start the experiment after you specify the experiment or open a saved experiment method (.fmf).

**IMPORTANT**

Make sure that the Octet biosensors are properly prewetted before starting the experiment. For details on how to prepare the biosensors, see the appropriate product instructions.

### Loading the Sensor Tray & Sample Plate

1. Open the Octet instrument door (lift the handle up).
2. Place the sensor tray on the sensor stage (left side) so that position A1 is located at the upper right corner (Figure 3.8).
3. Place the sample plate on the sample stage (right side) so that position A1 is located at the upper right corner.

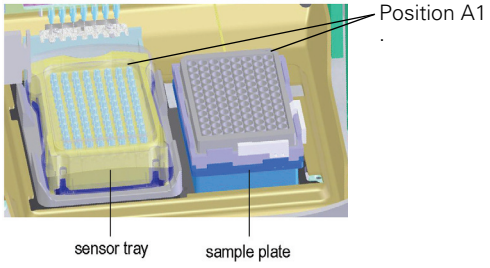



**Figure 3.8 Sensor stage (left) and sample stage (right)**

**IMPORTANT**

Make sure that the bottom of the sample plate and sensor tray are flat on each stage.

4. Close the Octet door and allow the plate to equilibrate.

   The time required for temperature equilibration differs, depending on the temperature that your application requires. The Anti-Human IgG (Fc specific) assay requires five minutes; the Protein A assay requires ten minutes.

### Locking the Octet Software

You can lock the Octet software to prevent access by another user during an experiment.

1. Select **File –Lock application** on the main menu.
2. In the dialog box that appears, enter your user name and a password.
3. Click **Lock**.
4. To unlock the application before the end of the experiment, enter the password in the dialog box and click **Unlock**.

**NOTE**

The lock and password expire at the end of the experiment.

## Starting the Experiment

**To start an experiment:**

1. Click the Run Experiment tab or click the arrow to advance to the tab.
2. In the Run Experiment tab, confirm the defaults or enter new settings. For details on the settings, see the table below.
   **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.

   When the experiment starts, the Runtime Binding Chart window appears (Figure 3.11) and displays the binding data in real time as well as the experiment progress.
4. To stop an experiment in progress, click the button.
   - The experiment is aborted. The data for the active sensor is lost and the sensor is ejected into the waste tray.

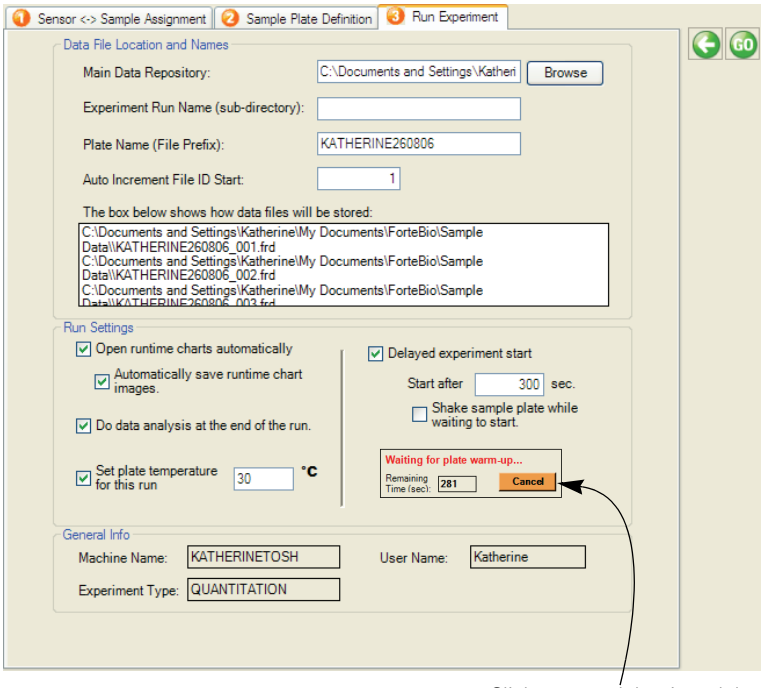



**Figure 3.9 Starting an experiment**

### CAUTION

**CAUTION!** Do not open the Octet instrument door when an experiment is in progress. If the door is opened when the instrument is operating, the data from the active sensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the sensors and starting from the beginning.

| Item in the Run Experiment Tab | Description |
|---|---|
| **Data File Location and Names** | |
| Main Data Repository | The location where 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 sensor. |

| Item in the Run Experiment Tab | Description |
|---|---|
| Plate Name | The prefix for the data files (.frd). |
| Auto Increment File ID Start | Each file is saved with a number after the file name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd. |
| **Run Settings** | |
| Open runtime charts automatically. | Choose this option to display the binding chart for the current sensor during data acquisition. |
| Automatically save runtime chart images. | Choose this option to save the binding charts (.jpg). The binding data (.frd) is saved regardless of whether chart images are saved. |
| Do data analysis at the end of the run. | Choose this option to automatically proceed with data analysis after the data are acquired. Note: If this option is not chosen, you will need to start a data analysis session (for more details, see *Viewing the Data Files & Calculated Results*, page 26). |
| Set plate temperature for this run. | Choose this option to specify a plate temperature. Enter the temperature in the box. If this option is not chosen, the plate temperature is set to the default 30° C.<br>Note: If the actual plate temperature is not equal to the set plate temperature, a warning appears. The software gives you the option to i) wait until the set temperature is reached before proceeding with the run, ii) continue without waiting until the set temperature is reached, or iii) cancel the run. |
| Delayed experiment start. | Choose this option to specify a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click GO. |
| Shake sample plate while waiting to start | If the experiment has a delayed start time, choose this option to shake the plate until the experiment starts. |
| **General Info** | |
| Machine Name | The name of the computer that controls the Octet instrument and acquires the data. |
| Experiment Type | The type of experiment (quantitation, basic kinetics, kinetics screening) |
| User Name | The user logon name. |

### Viewing the Runtime Binding Chart

If you chose **Open runtime charts automatically** in the Run Experiment tab (Figure 3.9), the runtime binding charts are automatically displayed when data acquisition starts. The chart window also displays the run status and the elapsed time for the current sample column. If the charts are not automatically displayed, select **View → Enable Runtime Binding Charts** on the main menu. The binding chart is automatically saved (.jpg) at the end of data acquisition.

## CAUTION

**CAUTION!** It is recommended that you do not close the chart window until all of the samples are analyzed. Otherwise, the charts will not be saved. If you do not want to display the chart, minimize the chart window. The software saves the binding chart as displayed at the end of the experiment. This means, if you modify the chart, for example, hide the data for a particular sensor, the chart will not include the hidden data.

- To close the chart window for the current assay, click the Stop/Start Opening Runtime Binding Charts button .
  - — The chart window closes and the chart for the current assay is **not saved**.
- Click the button again to open the chart window for the next assay.
- Double-click anywhere in the window to display two chart windows simultaneously.
- To zoom in on an area in the chart, select an area with the mouse and click. To reset the zoom, press the **z** key.

To view the binding chart for a particular assay, click the assay name.

To hide the sensor data in the chart, remove the check mark next to the sensor.

**Note:** The saved chart includes the sensor data as displayed at the end of the run. If you modify the chart, press the **z** key to return the chart to the original view.

If you are satisfied with the binding data before the assay step is finished, click **Skip Assay Step** to begin the next assay step.

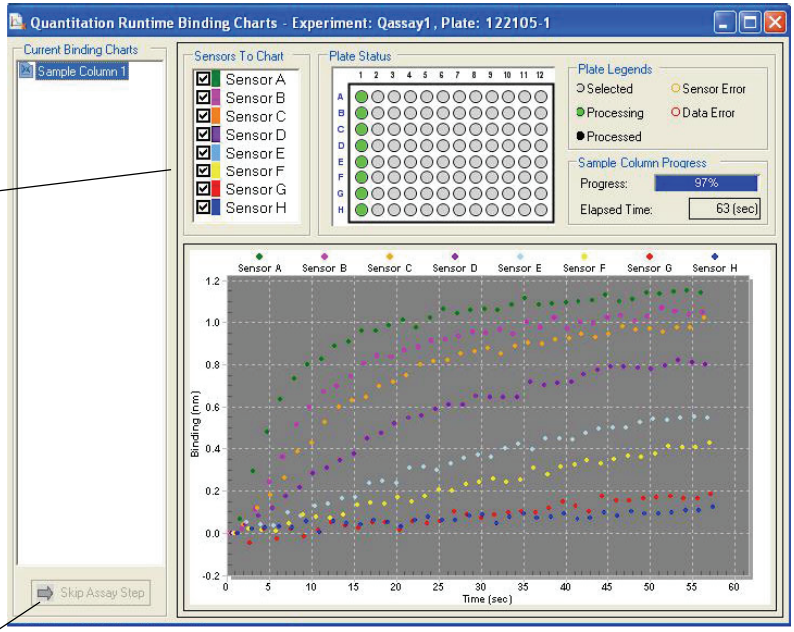



**Figure 3.10 Runtime binding chart window**
Double-click anywhere in the window to display two charts windows simultaneously.

## 3.4 Viewing the Data Files & Calculated Results

If you chose the option **Do data analysis at the end of the run** (Figure 3.9), the Quantitation Data Analysis window (Figure 3.12) appears when data acquisition is completed and displays the data files in the experiment.

If you did not choose this option, click the New Quantitation Data Analysis button . Alternatively, select **Data Analysis →New Quantitation Data Analysis** on the main menu. Figure 3.12 shows how to select data files for analysis.

**NOTE**

To view quantitation data at any time, click the button to open another Quantitation Data Analysis window and select the data of interest from the Explorer pane. More than one data analysis window can be open at a time.

The Quantitation Data Analysis window has three tabs:

- Data File Selection tab (see page 27)
- Standard Curve tab (see page 29)
- Concentration Results tab (see page 32)

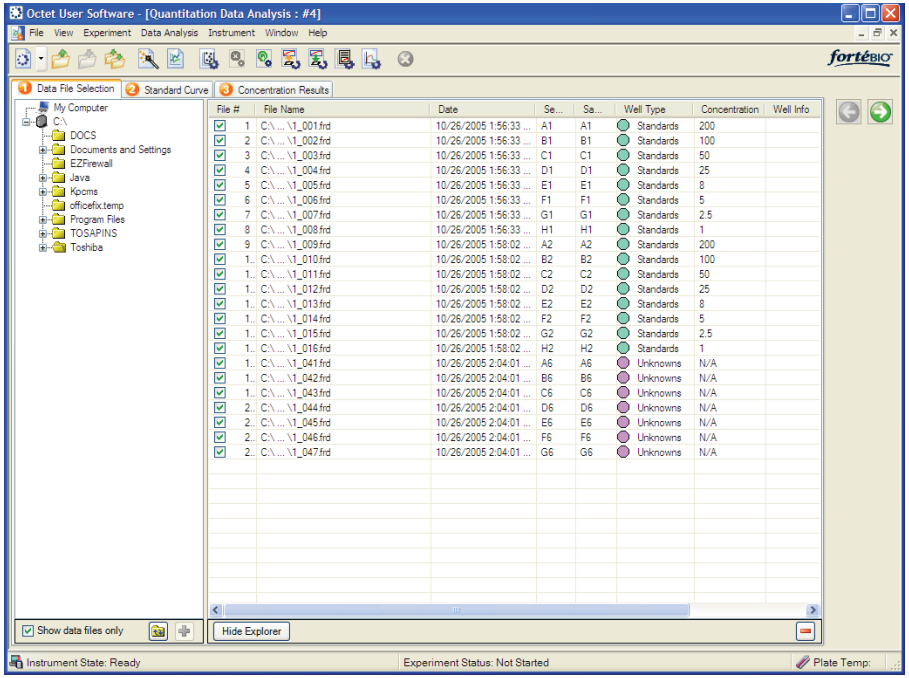


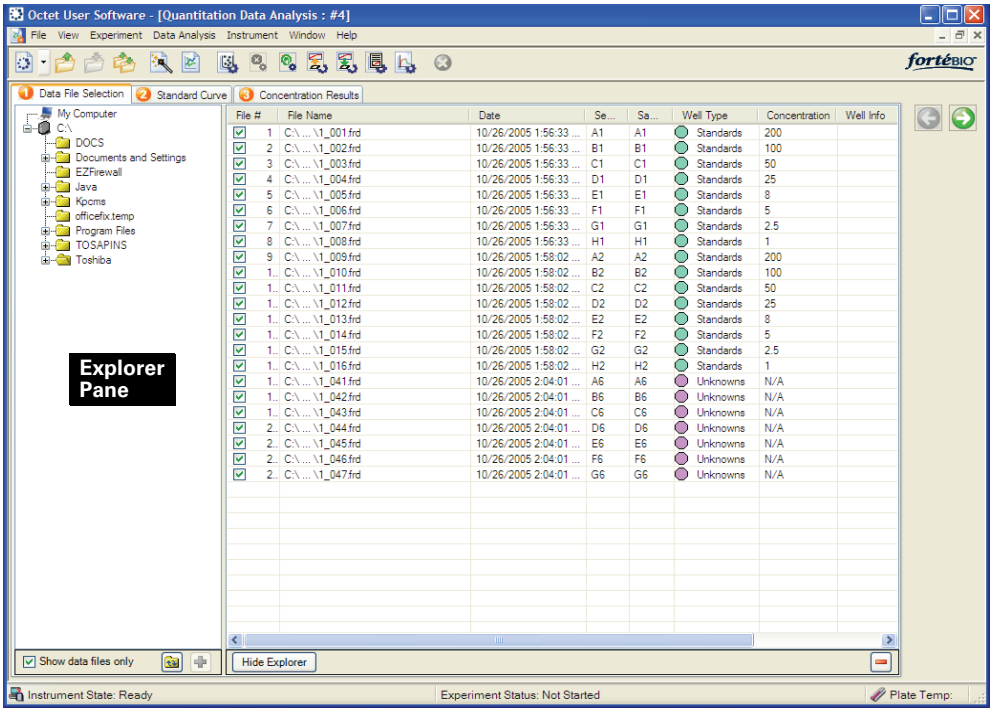

**Figure 3.11 Quantitation Data Analysis window**

## Data File Selection Tab

The Data File Selection tab shows the data files (.frd) in the experiment.

Figure 3.12 shows how to add or remove files from an analysis.

Figure 3.13 shows how to edit the well designation, well information, or standard concentration.




**To add all files (.frd) in a directory to the analysis, do either of the following in the Explorer pane:**

- Double-click the directory of interest.
- Select the directory interest and click the ➕ button.

**To add user-specified files to the analysis:**

1. Open the directory of interest.
2. Select the files of interest and click the ➕ button.
   To select adjacent files, press and hold the **Shift** key while you click the first and last file in the selection. To select non-adjacent files, press and hold the **Ctrl** key while you click the files.

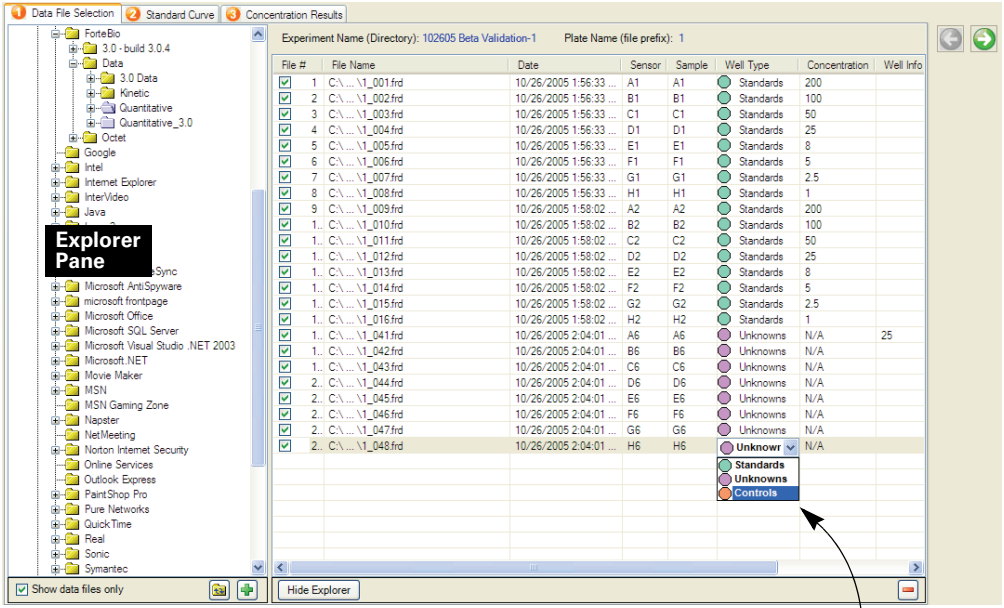
**To remove one or more files from the analysis, do one of the following:**

- Remove the check mark next to the file name that you want to exclude.
  - The file is removed from the analysis, but the row remains in the table.
- Select the row(s) that you want to exclude and click the ➖ button or the **Delete** key.
  - The row is removed from the table.

**To restore a file to the analysis:**

1. Put a check mark next to the row.
2. Navigate to the file in Explorer and click the ➕ button.

**Figure 3.12 Adding or removing data files (.frd) from an analysis**




**To change a well designation:**

1. In the row of interest, click the cell in the Well Type column.
2. Make a selection from the drop-down list of well types (standards, unknowns, controls).

**To edit a standard concentration or well information:**

1. In the row of interest, click the field (Well Type or Concentration) that you want to edit.
2. Enter the new information. Alternatively, right-click the cell to view a shortcut menu of edit commands.

**Figure 3.13 Editing the well designation, standard concentration, or well information**

| Item in the Data File Selection Tab | Click To... |
|---|---|
| Show data files only | Display only Octet data files (.frd) in the Explorer pane. |
|  | Refresh the Explorer pane. |
|  | Add a file (.frd) selected in the Explorer pane to the list of files for analysis. |
| Hide Explorer | Hide the Explorer pane. |
|  | Removes the selected file from the list of files for analysis. |

### Standard Curve Tab

- To generate the standard curve, click the Standard Curve tab in the Quantitation Data Analysis window (Figure 3.14). Alternatively click the arrow to advance to the tab.
  - The software computes the calculated concentration, and a residual and $r^2$ value for each standard, and plots the standard curve.

In the Standard Curve tab you can:

- View the standard curve and the calculated standard concentrations, calibrator $r^2$, and residual values for the standards.
- Remove a standard(s) from the analysis and recalculate the standard curve.
- Import a standard curve.
- Change the standard curve model.

**NOTE**

For more information on how to customize the sensor data display and export the data from the Standard Curve tab, see Appendix B, page 83.

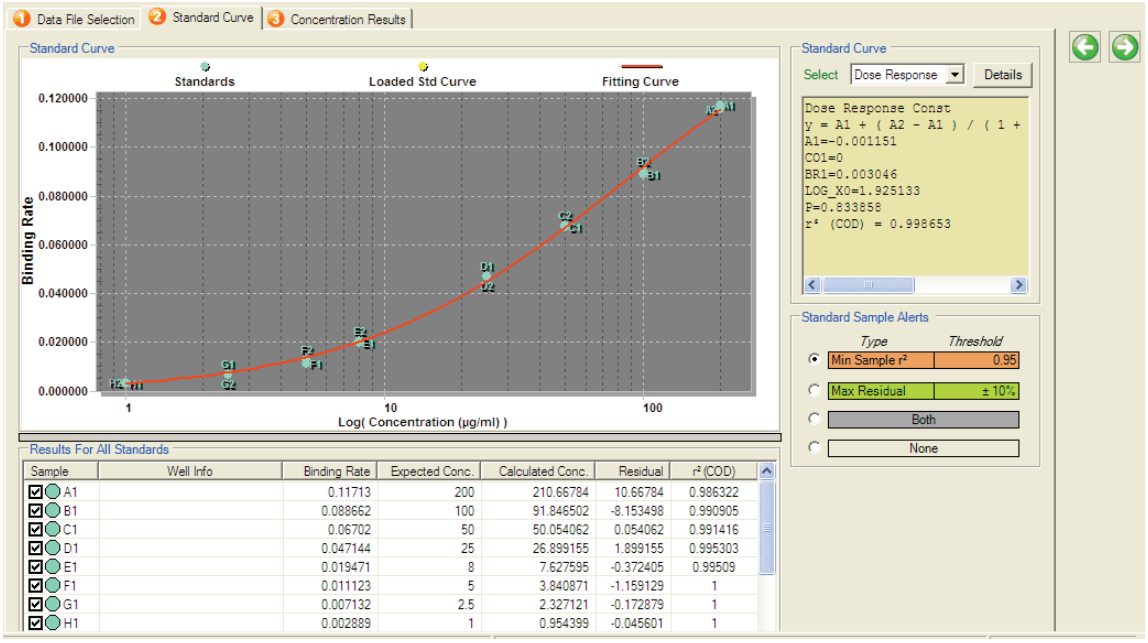



**Figure 3.14 Quantitation Data Analysis window, Standard Curve tab**

| Item in the Standard Curve Tab | Description |
|---|---|
| **Results For All Standards** | |
| Sample | The well location in the sample plate. |
| Well Info | User-specified notes about a standard. |
| Binding Rate | The rate (Δ nm/ Δ time) of sample binding to the sensor computed by the software. |
| Expected Conc | The user-specified standard concentration that was entered during sample plate definition. |
| Calculated Conc | The standard concentration computed from the standard curve. |
| Residual | Residual = Expected standard concentration - Calculated standard concentration. |
| $r^2$ (COD) | The $r^2$ of the curve fit used to determine the binding rate |
| **Standard Curve** | |
| Select | A drop-down list of available curve fitting models. Select the Dose Response model for the Anti-Human IgG quantitation assay. Select the Linear Point to Point model for the Protein A quantitation assay. |
| Details | Click to view the equation and information on the parameters of the selected curve-fitting model. |
| **Standard Sample Alerts** | |
| Min Sample $r^2$ | Choose this option to apply a threshold $r^2$ value to the standard binding curves. If a the binding curve of a standard does not meet the $r^2$ threshold value, the standard is highlighted in orange color.<br>Note: The $r^2$ threshold value is set in the Options dialog box (select **File** → **Options** on the main menu.) |
| Max Residual | Choose this option to apply a threshold residual range to the standards. The default max residual threshold is ±10%. This means a standard is highlighted in green color if the difference between the expected concentration and the calculated concentration is greater than greater than 110% of the expected concentration or less than 90% of the expected concentration.<br>Note: The Max Residual threshold value is set in the Options dialog box (select **File** →**Options** on the main menu.) |
| Both | Choose this option to apply both the Min Sample $r^2$ and the Max Residual threshold to the data. |
| None | If this option is chosen, no thresholds are applied to the standard data. |

### Removing a Standard

You can remove one or more standards from an analysis (for example, a standard that does not meet the sample $r^2$ or residual threshold) and recompute the standard curve.

1. Remove the check mark next to the sample name.

2. To generate the new standard curve, click the Recalculate button . Alternatively, select **Data →Analysis Recalculate** on the menu bar.

### Setting Thresholds

1. Select **File →Options** on the main menu.
   — The Options dialog box appears (Figure 3.15).
2. In the Data Analysis tab, enter a new value for the Min Sample $r^2$ Alert or the Max Residual Alert and click **OK**.

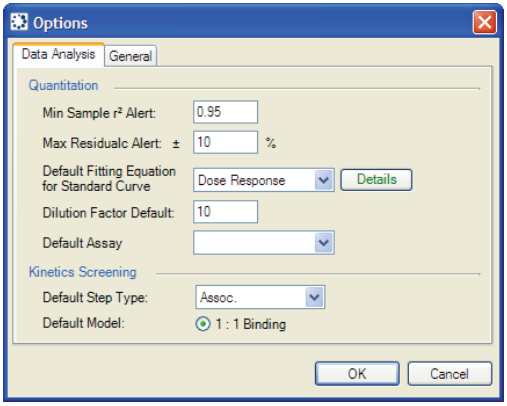



**Figure 3.15 Options dialog box, Data Analysis tab**

### Saving a Standard Curve

1. Click the Export Quantitation Standard Curve button . Alternatively, select **Data Analysis →Export Quantitation Standard Curve** on the menu bar
2. In the dialog box that appears, specify the directory and file name (.fsc), and click **Save**.

## Importing a Standard Curve

You can use a saved standard curve to calculate concentrations. You can also import a standard curve to compare two standard curves.

1. Click the Import Quantitation Standard Curve button . Alternatively, select **Data →Analysis Load Quantitation Standard Curve** on the menu bar.
2. In the dialog box that appears, specify the directory and file name (.fsc), and click **Open**.
3. Click the Recalculate button .
   — The imported standard curve is displayed in yellow.

## Calculating Concentration Results

- To compute the calculated concentrations for the unknowns and controls, click the Concentration Results tab in the Quantitation Data Analysis window (Figure 3.16). Alternatively click the arrow to advance to the tab.

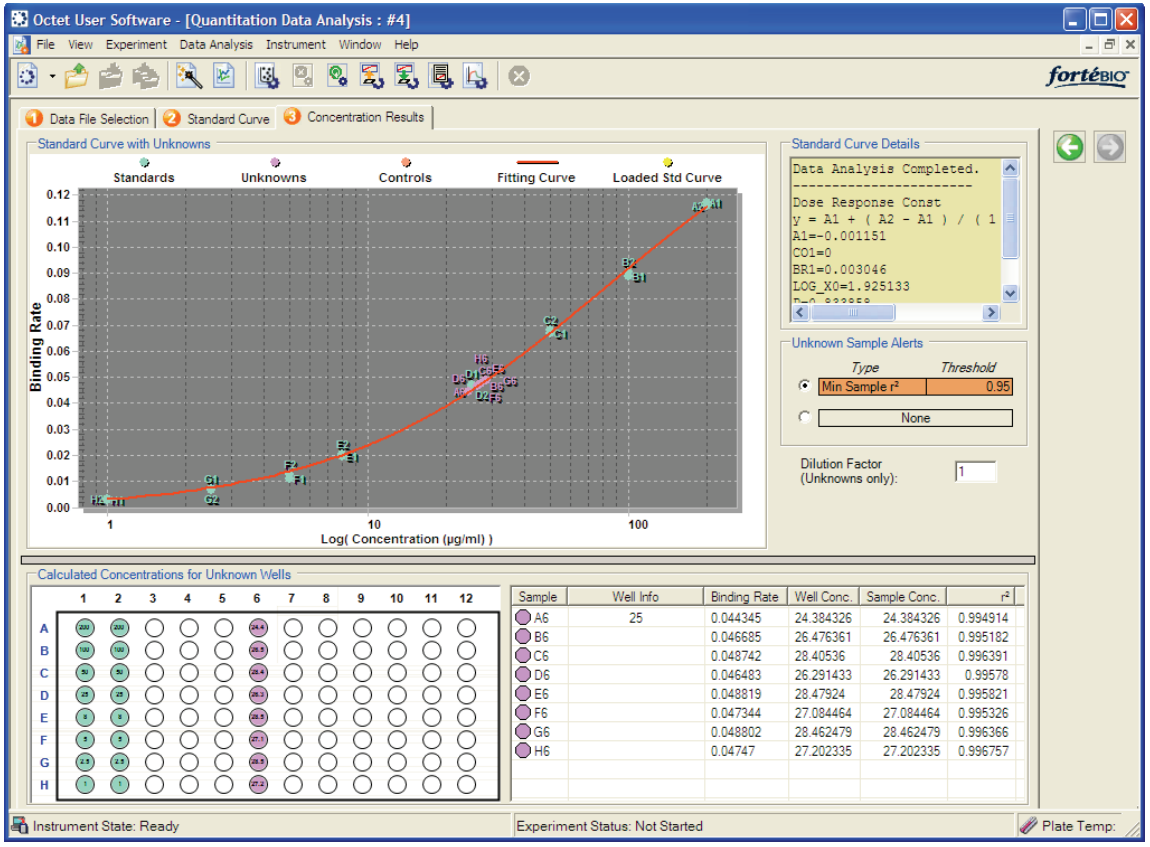



**Figure 3.16 Quantitation Data Analysis window, Concentration Results tab**

| Item in the Concentration Results Tab | Description |
|---|---|
| Standard Curve Details | The standard curve parameters for the curve fitting model that was selected in the Standard Curve tab. |
| **Unknown Sample Alerts** | |
| Min Sample $r^2$ | Choose this option to highlight a sample if $r^2 < 0.95$. |

| Item in the Concentration Results Tab | Description |
|---|---|
| None | If this option is chosen, no thresholds are applied to the data. |
| Dilution Factor (Unknowns only) | The factor by which all unknowns were diluted. For example, if the dilution factor = 10, the unknowns are diluted 1:10 with diluent. |
| **Calculated Concentrations for Unknown Wells** | |
| Sample | The well location in the sample plate. |
| Well Info | User-specified notes about a sample. |
| Binding Rate | The rate (Δ nm/ Δ time) of sample binding to the sensor computed by the software. |
| Well Conc. | Concentration of the sample without taking dilution into consideration. |
| Sample Conc. | Concentration of the sample taking into account the specified dilution factor. |

## 3.5 Starting a Data Analysis Session

A data analysis session enables you to:

- View data.
- Analyze data if you did not choose the **Do data analysis at the end of the run** option in the Run Experiment tab (Figure 3.9) during experiment setup.
- Reanalyze data.

Data analysis proceeds automatically after data acquisition if you selected **Do data analysis at the end of the run** in the Run Experiment tab (Figure 3.9). If you did not choose this option, you must start a data analysis session to:

- Select the files (.frd) for analysis.
- Create or import a standard curve.
- Calculate concentration results.

1. To start a data analysis session, do one of the following:
   - Click the **New Quantitation Analysis** button  .
   - Select **Data Analysis →New Quantitation Data Analysis** on the main menu.
   - Click the Experiment Wizard button  . Choose the **Data Analysis** and **Quantitation** options in the wizard, and click the  arrow.

   — The Quantitation Data Analysis window appears (Figure 3.17).
2. To select the files (.frd) for analysis, double-click a directory or individual files of interest in the Explorer pane (Figure 3.17).

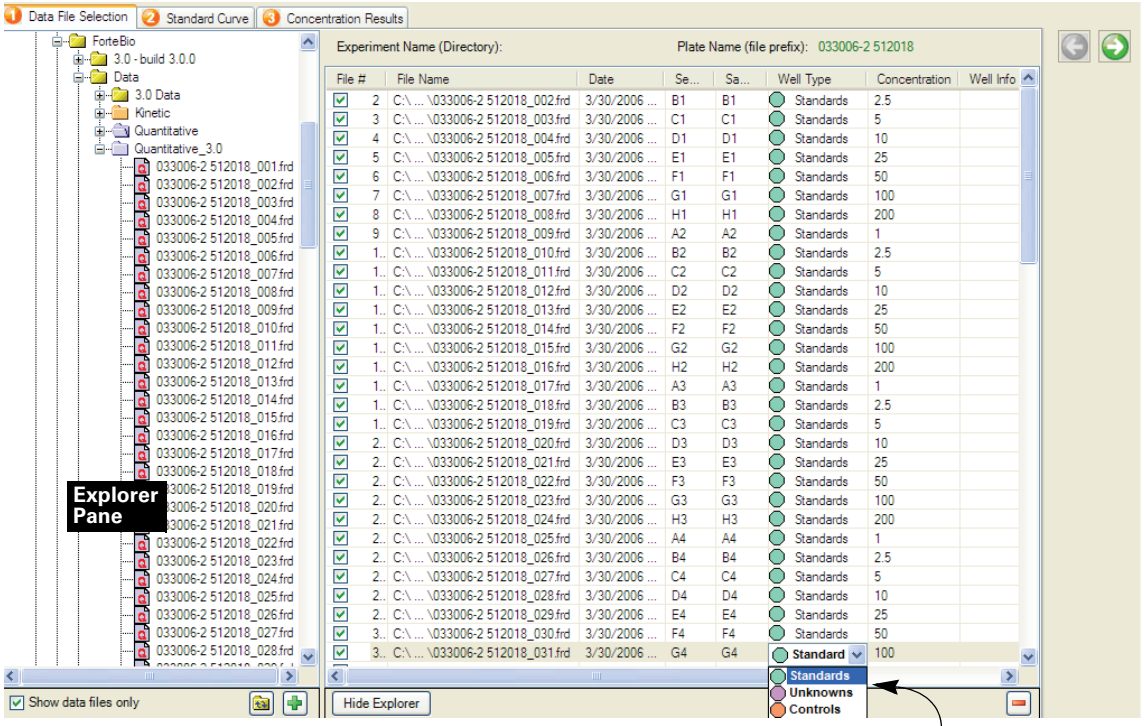
**NOTE**

If the well definitions, standard concentrations, or well information were not specified during experiment setup, you can do so in the Data File Selection tab. For more details, see Figure 3.13, page 28.

3. To generate the standard curve, click the Standard Curve tab.
4. To calculate concentration results, click the Concentration Results tab.

**To select data for quantitation analysis:**

1. In the Explorer pane, double-click the directory of interest.
2. To remove a file from the analysis, click the row to remove the check mark.
3. To remove a file from the analysis and delete the row from the list, select the row and click the button.




**To edit the:**

- Well type - Click at the right in the Well Type field and make a selection from the drop-down list of well types.
- Concentration or well information - Click the field in the table and enter the new value or information.

**Figure 3.17 Steps to select and edit data in the Quantitation Data Analysis window**

## 3.6 Generating a Report

1. Click the Data Analysis Report button  . Alternatively, select **File →Save Quantitation Data Analysis Report.**
2. In the dialog box that appears, specify a directory and name for the report (.doc), and click **Save**.
   — The report is displayed.

# CHAPTER 4
# Working in Assay Development Mode



The Octet System provides several predefined quantitation assays. When you work in assay development mode, you can define a quantitation assay that specifies custom values for the following parameters:

| User-Specifiable Parameter | Description |
|---|---|
| Min Concentration | The minimum protein concentration (µg/ml) allowed in the assay. |
| Max Concentration | The maximum protein concentration (µg/ml allowed in the assay. |
| Assay Time | The length of time (seconds) that the binding data is acquired. |
| Flow Rate | The orbital flow rate (rotations per minute) of the sample platform. |
| Data Analysis Read Time | The amount of binding data (seconds) that is analyzed. |

## 4.1 Viewing the Assays in the System

1. Open the Experiment Wizard (click the  button).
2. Select **New Quantitation Experiment** and **Assay Development Mode**. Alternatively, select **Experiment Quantitation →Assay Development Mode** on the main menu.
   — The Assay Development Mode dialog box appears (Figure 4.1).
3. To view details about an assay, select the assay in the file tree. If the details do not appear, click **Details**.

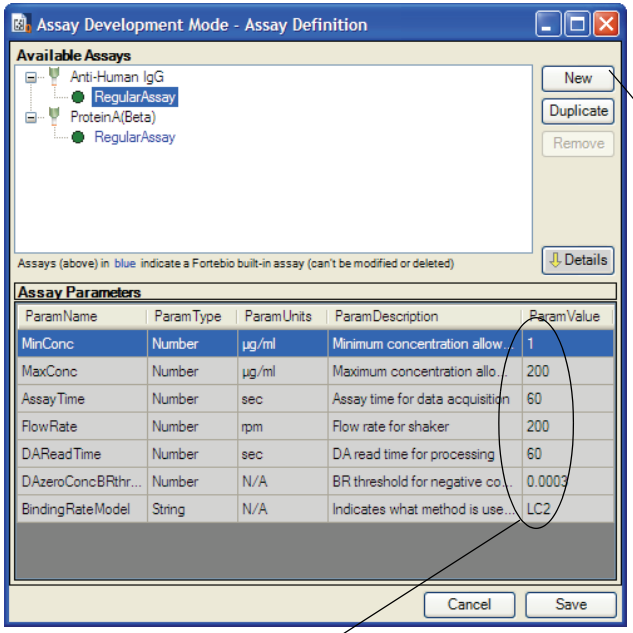
## 4.2 Defining a New Assay

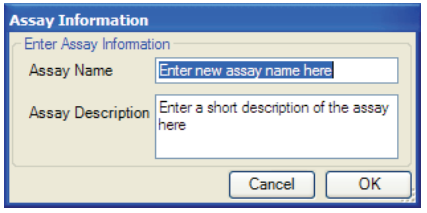
**NOTE**

You cannot modify or delete a factory installed assay.

**To define a new assay:**

1. In the Experiment Wizard, select New Quantitation Experiment and Assay Development Mode.
   - The Assay Development Mode - Assay Definition box appears.
2. Click **New**. Alternatively select an assay that you want to edit, and click **Duplicate**.
   - The Assay Information box appears.


3. Enter the assay name and information about the assay.
4. Click **OK**.
   - The new assay appears in the Available Assays list.
5. To change a parameter value, double-click the value that you want to edit and enter the new value.
6. Click **Save**.

**To delete a user-defined assay from the system:**

1. In the Available Assays list, select that assay that you want to remove.
2. Click **Remove** and **Save**.

**Figure 4.1 How to define a new assay or delete an assay from the system**

# CHAPTER 5
# Basic Kinetics Analysis



A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction.

## 5.1 Setting Up a Basic Kinetics Experiment

To set up a kinetics experiment in the Octet software, you specify the:

- Well locations of the sensors and samples.
- Assay steps (baseline, loading, association, dissociation, activation, and quenching).

The experiment method (.fmf) that you specify is automatically saved when the experiment is run.

For more details on how to prepare the sensors, see the appropriate product instructions.

**NOTE**

Before you begin an experiment, check the sample plate temperature (displayed in status bar). Confirm that the temperature is appropriate for your experiment or set a new temperature. (For more details on setting the temperature, see page 12.) If you close the Octet software, the sample platform temperature is reset to 30° C.

### Specifying the Sensor & Sample Configuration

At startup, the Octet software displays the Experiment Wizard. If the wizard is not displayed, click the Experiment Wizard button  or select **Experiment →New Experiment Wizard** on the main menu.

1. In the Experiment Wizard, choose the **New Kinetics Experiment** and **Basic Kinetics** options (Figure 5.1).

   Alternatively, select **Experiment → Kinetics → New Kinetics Experiment** on the main menu.


**Figure 5.1 Experiment Wizard**

2. Click the  arrow in the wizard.

   — The Kinetics Setup window appears (Figure 5.2).

   Figure 5.2 shows how to designate the locations of the sensors and samples.

**NOTE**

More than one Kinetics Setup window can be open at the same time.

**To specify the active sensor locations, do one of the following:**

- Click **Fill Plate** to select the entire plate.
- Draw a box around one or more column(s).
- Click a single location.
- Click **Import**. In the dialog box that appears, select a tab-delimited text file (.txt) that specifies sensor locations and sensor loading information (assay time, flow rate, and type of assay step).

**To specify the sample locations, do either of the following:**

- Click **Match Sensors**.
  - All well locations that match the active sensor locations are selected.
- In the sample plate, draw a box around the well(s) of interest
- Click **Import**. In the dialog box that appears, select a tab-delimited text file (.txt) that specifies sample locations and sample information (sample ID, molar concentration, sample or buffer information).

**To delete a sensor or sample location(s):**

- Select the column(s) or well(s) that you want to delete and click **Remove**.

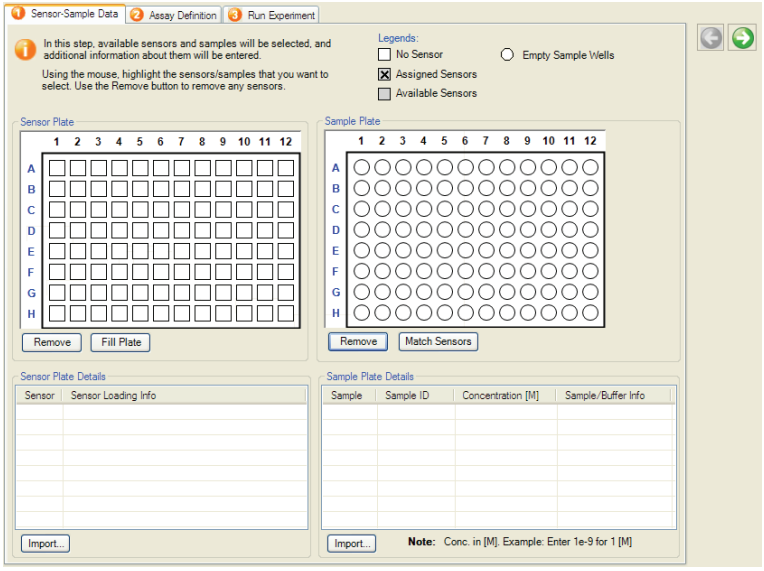


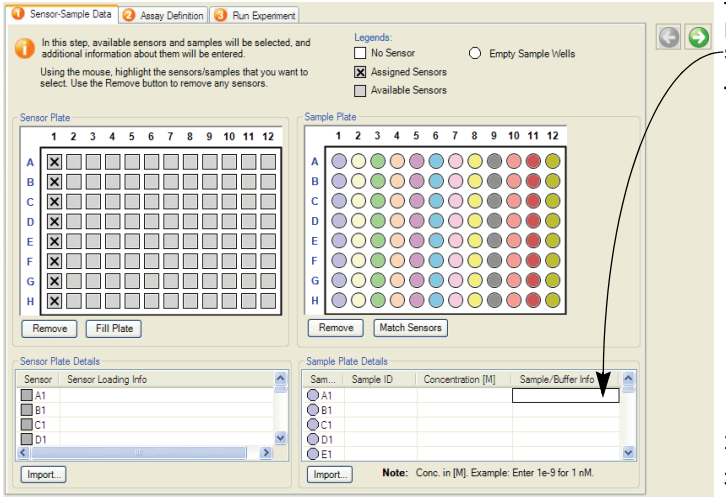

**Figure 5.2 Specifying the active sensor and sample locations**

## Entering Sensor or Sample Information

You can enter information about sensor loading, sample ID, sample concentration, or sample buffer. Entering the information is optional. The information can be entered manually or by importing a tab-delimited text file (.txt) (Figure 5.3).

**IMPORTANT**

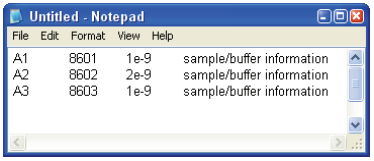
If you do not enter sample concentrations, only $k_{obs}$ and $k_d$ will be calculated. $k_a$ and $K_D$ cannot be calculated. If you forget to enter sample information here, you can also enter it during analysis.




**To enter information manually:**
Double-click a field in the sensor plate or sample plate table, and enter the information.

**To Import a tab-delimited text file:**

1. First format the information of interest in a tab-delimited text file (as shown below). Enter information separated by tabs. The sample wells can be specified by column or row (for example, A1, A2, A3, or A1,B1, C1).




2. Click **Import**.
3. In the dialog box that appears, select the .txt of interest, and click **Open**.

**Figure 5.3 Entering sensor or sample information**

## Types of Assay Steps

The Octet software provides predefined assay steps and you can create your own custom assay step (Figure 5.5).

| Assay Step Type | Select This Step When... |
|---|---|
| Baseline | Establishing the sensor baseline in diluent. **Note:** This step may be performed offline (not in the Octet instrument). |
| Loading | Binding the first protein of interest to the sensor. **Note:** This step may be performed offline (not in the Octet instrument). |
| New Loading | Defining a custom loading step. |
| Association (Assoc.) | Binding the second protein of interest during kinetic analysis. |
| Dissociation (Dissoc.) | Monitoring the dissociation of the protein complex. |
| Activation | Chemically activating the sensor surface to prepare for protein loading. |
| Quenching | Blocking unreacted surface immobilization sites on the sensor. |

## Defining a Kinetic Assay

**To define a kinetic assay:**

1. Click the Assay Definition tab or click the arrow to advance to the tab.
2. Select the step type of interest in the Step Data Setup list.
   **Note:** You can create a custom step with a user-specified assay time or flow rate for inclusion in an assay (see Figure 5.5).
3. Double-click the column in the sample plate map that is associated with the selected step.
   - The step is added to the Assay Steps List.
4. Repeat step 2 and step 3 to define all of the steps in the assay.
5. Click a column in the sensor plate map that represents the assay. (The first column is highlighted by default.)
6. To define another assay, repeat step 2 to step 5.

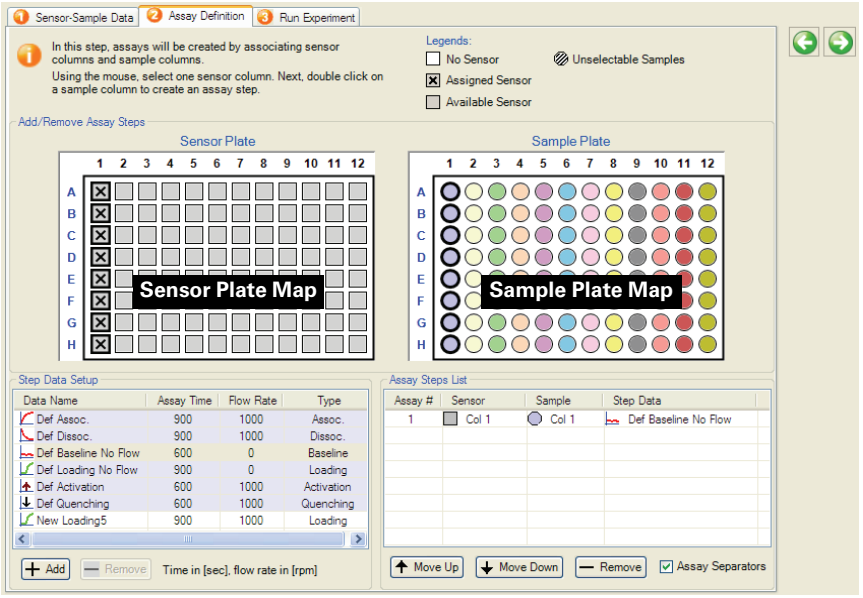



**Figure 5.4 Defining a kinetic assay**

## NOTE

If you select a sensor and sample column during assay setup that do not have the same number of active wells, a message appears and requests confirmation to continue. If a sample column has fewer wells than the number of sensors, some sensors will not be incubated in the sample solution. If a sample column has more wells than the sensor column, some of the sample wells will not receive a sensor.

## Creating a Custom Step

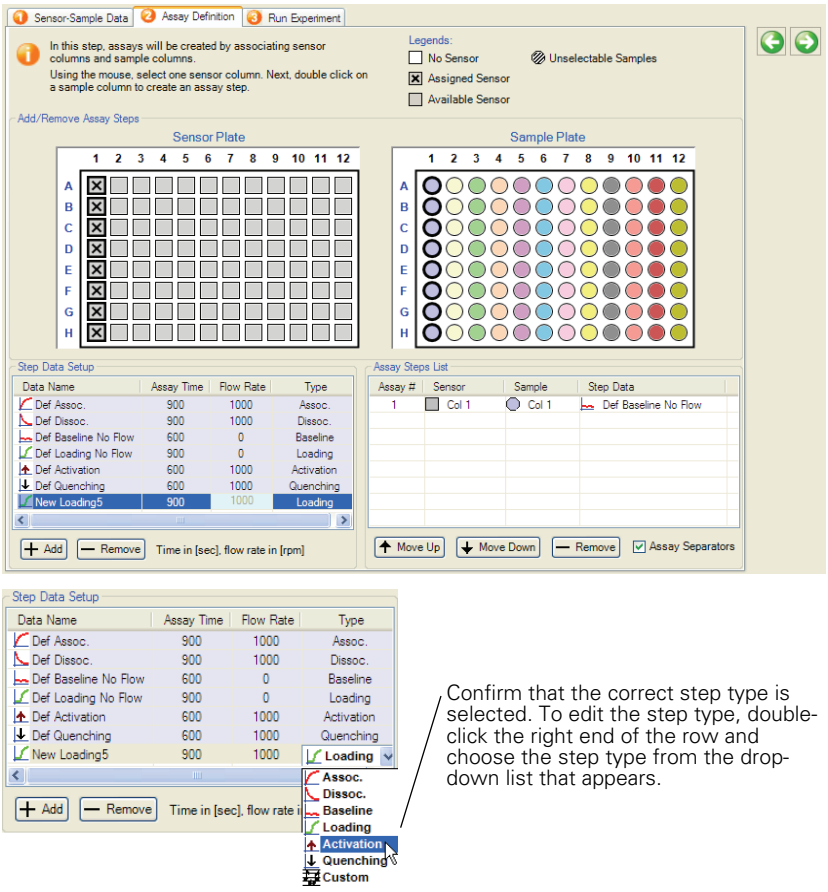
**To create a custom step:**

1. Click the **+ Add** button.
   - A new step is added to the Step Data Setup pane.
2. To edit the step (data name, assay time, flow rate, or type), double-click the row.
   Assay time range: 2 - 48,000 seconds
   Flow rate range: 0 or 60 - 1500 rpm

**To remove a custom step:**

1. Select the step that you want to remove.
2. Click the **- Remove** button. Alternatively, press the **Delete** key.

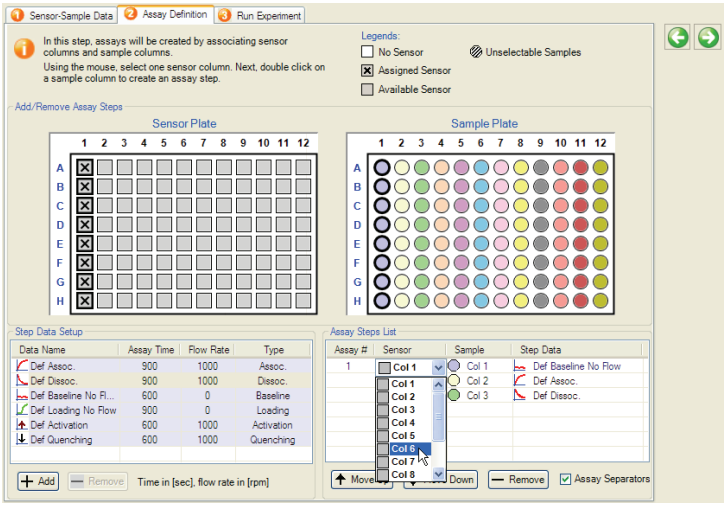
**Note:** A factory installed step cannot be edited or removed.




Confirm that the correct step type is selected. To edit the step type, double-click the right end of the row and choose the step type from the drop-down list that appears.

**Figure 5.5 Creating a custom step**

## Editing an Assay



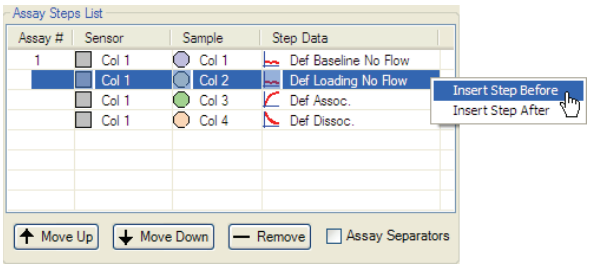

**To edit a step in the assay:**

1. Select the row of interest.
2. Click Sensor, Sample, or Step Data, and make a selection from the drop-down list that appears.

**To reorder or remove a step:**

1. Select the step.
2. Click the ↑ **Move Up**, ↓**Move Down**, or — **Remove** button.

**Note:** You can reorder the steps in an assay, but you cannot move a step between assays.




**To insert a step:**

1. Right-click a row, and select **Insert Step Before** or **Insert Step After** from the shortcut menu that appears.
   - The default step type is added to the list.

   **Note:** The default step is a user-specified option. For more details, see *Setting Analysis Options*, page 10.
2. To edit the step (sensor column, sample column, or step data type), click the row and make a selection from the drop-down list that appears.

**Figure 5.6 Editing a kinetics assay**

### Saving a Kinetics Experiment

The software automatically saves the experiment information that you specify (sensor and sample locations, sensor and sample information, and assay steps) to an experiment method (.fmf) at the start of the run. If you set up an experiment, but do not start the run, you can manually save the experiment method.

1. To manually save an experiment, click the Save Method File button  . Alternatively, select **File → Save Method File** on the main menu.

   If there is more than one open experiment and you want to save them all, click the Save All Methods Files button  .

2. In the Save dialog box that appears, 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** on the main menu and enter a new name for the experiment.

### Opening an Experiment Method

1. Click the Open Method File button  . Alternatively, select **File → Open → Experiment Method File** on the main menu.
2. In the dialog box that appears, navigate to the directory and .fmf of interest, and click **Open**.

**NOTE**

When you open a method, reselect the assay type. (This information is not saved.)

## 5.2 Performing a Basic Kinetics Experiment

You are ready to start the experiment after you specify the assay or open a saved experiment method (.fmf).

**IMPORTANT**

Make sure that the Octet biosensors are properly prewetted before starting the experiment. For details on how to prepare the biosensors, see the appropriate product instructions.

### Loading the Sensor Tray & Sample Plate

1. Open the Octet instrument door (lift the handle up).
2. Place the sensor tray on the sensor stage (left side) so that position A1 is located at the upper right corner (Figure 5.7).
3. Place the sample plate on the sample stage (right side) so that position A1 is located at the upper right corner.

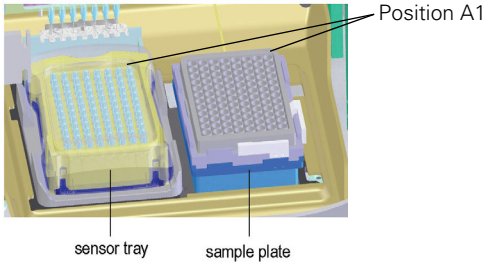



**Figure 5.7 Sensor stage (left) and sample stage (right)**

4. Close the Octet door and allow the plate to equilibrate for five minutes. (The time required for temperature equilibration may be different, depending on the temperature that your application requires.)

**IMPORTANT**

Make sure that the bottom of the sample plate and sensor tray are flat on each stage. Otherwise the orbital movement of the stage may result in sample loss.

### Locking the Octet Software

You can lock the Octet software to prevent access by another user while an experiment is in progress.

1. Select **File –Lock application** on the main menu.
2. In the dialog box that appears, enter your user name and a password.
3. Click **Lock**.
4. To unlock the application before the end of the experiment, enter the password in the dialog box and click **Unlock**.

**NOTE**

The lock and password expire at the end of the experiment.

## Starting the Experiment

**To start an experiment:**

1. Click the Run Experiment tab or click the arrow to advance to the tab,
2. Confirm the default settings or enter new settings.
3. Enter a name for the Experiment Run subdirectory.
4. To start the experiment, click GO.
   - If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

   When the experiment starts, the Runtime Binding Chart window appears (Figure 5.9) and displays the binding data in real time as well as the experiment progress.
5. To stop an experiment in progress, click the button.
   - The experiment is aborted, the data for the active sensors is lost, and the sensors in the manifold are ejected into the waste tray.

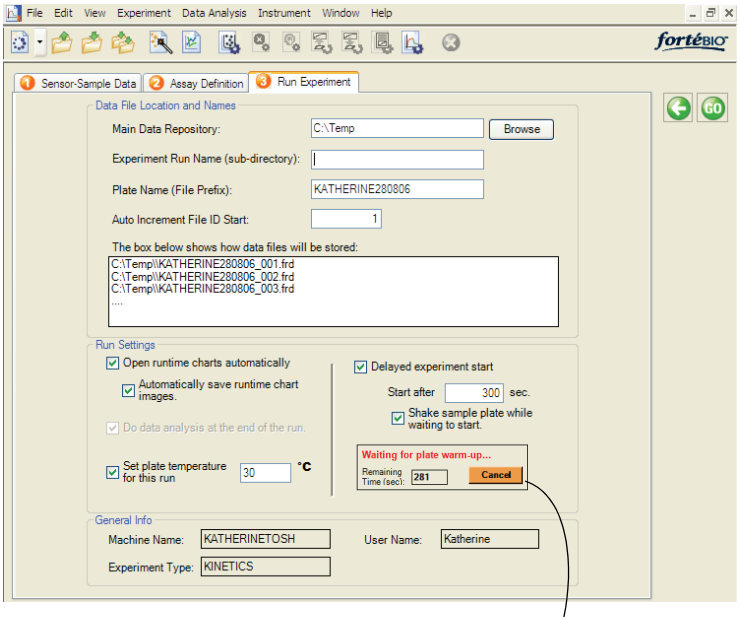



**Figure 5.8 Running a kinetics experiment**

**CAUTION**

**CAUTION!** Do not open the door when an experiment is in progress. If the door is opened when the Octet instrument is operating, the experiment is aborted, the data for the active sensors is lost. The sensors in the manifold are ejected into the waste tray.

| Item in the Run Experiment Tab | Description |
|---|---|
| **Data File Location and Names** | |

| Item in the Run Experiment Tab | Description |
|---|---|
| Main Data Repository | The location where the subdirectory will be created. The subdirectory contains the .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 sensor that includes the data from all steps that the sensor performs. |
| Plate Name | The prefix for the data files (.frd). |
| Auto Increment File ID Start | Each file is saved with a number after the file name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd. |
| **Run Settings** | |
| Open runtime charts automatically. | Choose this option to display the binding chart for the current sensor during data acquisition. |
| Automatically save runtime chart images. | Choose this option to save the binding charts (.jpg). The binding data (.frd) is saved regardless of whether chart images are saved. |
| Set plate temperature for this run. | Choose this option to specify a plate temperature. Enter the temperature in the box. If this option is not chosen, the plate temperature is set to the default 30° C.<br>Note: If the actual plate temperature is not equal to the set plate temperature, a warning appears. The software gives you the option to: i) wait until the set temperature is reached before proceeding with the run, ii) continue without waiting until the set temperature is reached, or iii) cancel the run. |
| Delayed experiment start. | Choose this option to specify a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click GO. |
| **General Info** | |
| Machine Name | The name of the computer that controls the Octet instrument and acquires the data using the Octet software. |
| Experiment Type | The type of experiment (quantitation, basic kinetics, or kinetics screening) |
| User Name | The user logon name. |

### Viewing the Runtime Binding Chart

If you chose **Open runtime charts automatically** in the Run Experiment tab (Figure 5.8), the runtime binding charts are automatically displayed when data acquisition starts. The chart window also displays the run status and the elapsed time for the current sample column. If the charts are not automatically displayed, select **View → Enable Runtime Binding Charts** on the main menu. The binding chart is automatically saved (.jpg) at the end of data acquisition.

**CAUTION**

**CAUTION!** It is recommended that you do not close the chart window until all of the samples are analyzed. Otherwise, the charts will not be saved. If you do not want to display the chart, minimize the chart window. The software saves the binding chart as displayed at the end of the experiment. This means, if you modify the chart, for example, hide the data for a particular sensor, the chart will not include the hidden data.

- To close the chart window for the current assay, click the Stop/Start Opening Runtime Binding Charts button .
  - — The chart window closes and the chart for the current assay is **not saved**.
- Click the button again to open the chart window for the next assay.
- Double-click anywhere in the window to display two chart windows simultaneously.
- To zoom in on an area in the chart, select an area with the mouse and click. To reset the zoom, press the **z** key.

To view the binding chart for a particular assay, click the assay name.

To hide the sensor data in the chart, remove the check mark next to the sensor.

**Note:** The saved chart includes the sensor data as displayed at the end of the run. If you modify the chart, press the **z** key to return the chart to the original view.

If you are satisfied with the binding data before the assay step is finished, click **Skip Assay Step** to begin the next assay step.

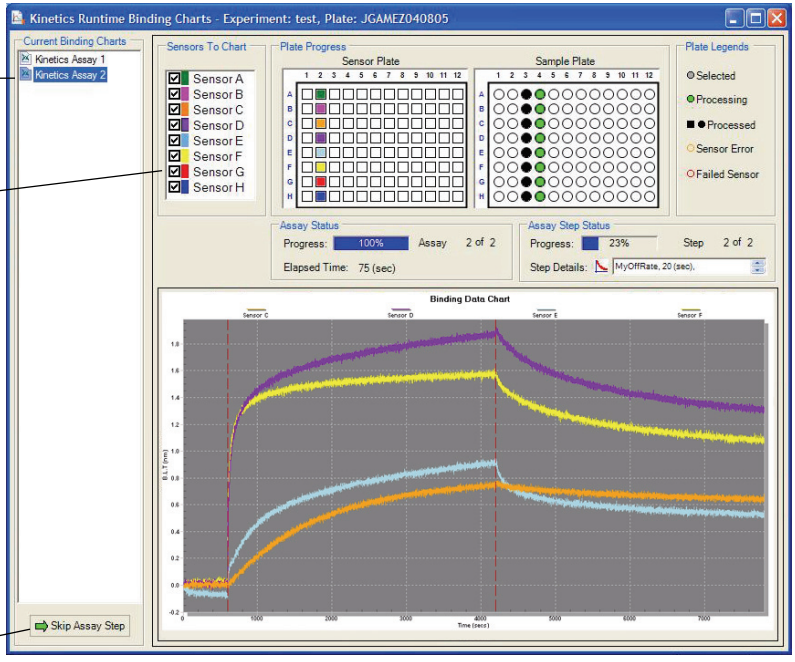



**Figure 5.9 Runtime binding chart window**
Double-click anywhere in the window to display two charts windows simultaneously.

## 5.3 Analyzing Basic Kinetics Data

The raw data files include the data from all of the assay steps that a sensor performs. When selecting files for analysis, you can include data from one or more assays conducted on the same plate. You can also include data files from different plates or experiments.

### Selecting Data

**NOTE**

You can select as many files as you need for an analysis. However, the Data Visualization tab displays up to eight data files (eight assays) without crowding.

1. Do one of the following to start a kinetics data analysis session:
   - Click the New Kinetics Data Analysis button  .
   - Select **Data Analysis →New Kinetics Data Analysis** on the main menu.
   - Select Data Analysis Basic Kinetics in the Experiment Wizard.

   — The Kinetics Data Analysis window appears (Figure 5.10).

   Figure 5.10 shows how to select files for analysis.

   Figure 5.11 shows how to edit the step type.

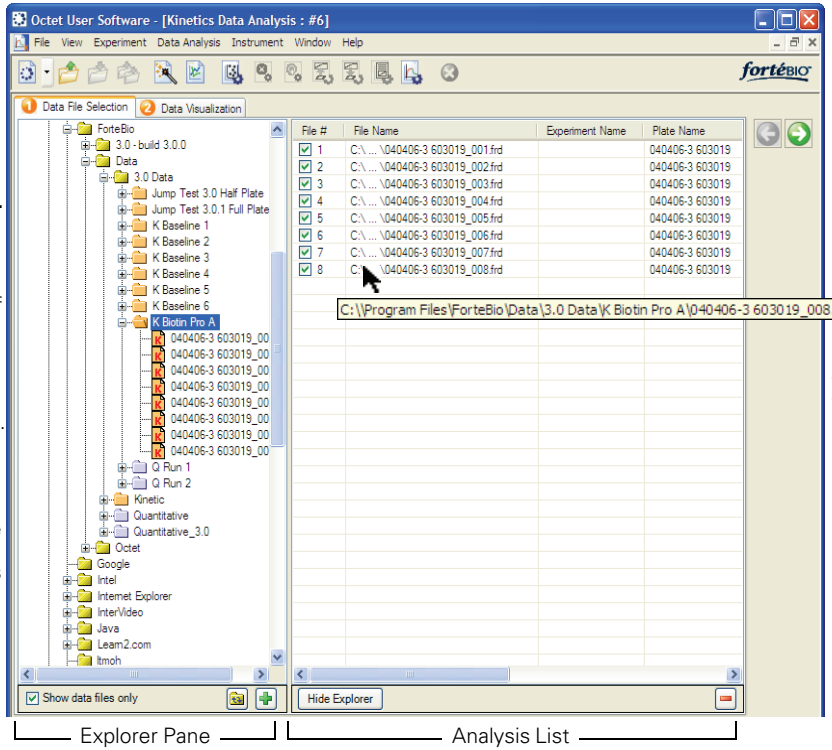
**To remove a file from the analysis, do either of the following:**

- Remove the check mark next to the file.
  - The file remains in the list, but is excluded from the analysis.
- Select the file(s), then click the button.
  - The file is removed from the list.

**To select files for analysis, do either of the following:**

- Double-click the directory or individual files of interest.
- Drag and drop files from the desktop or a Windows folder.

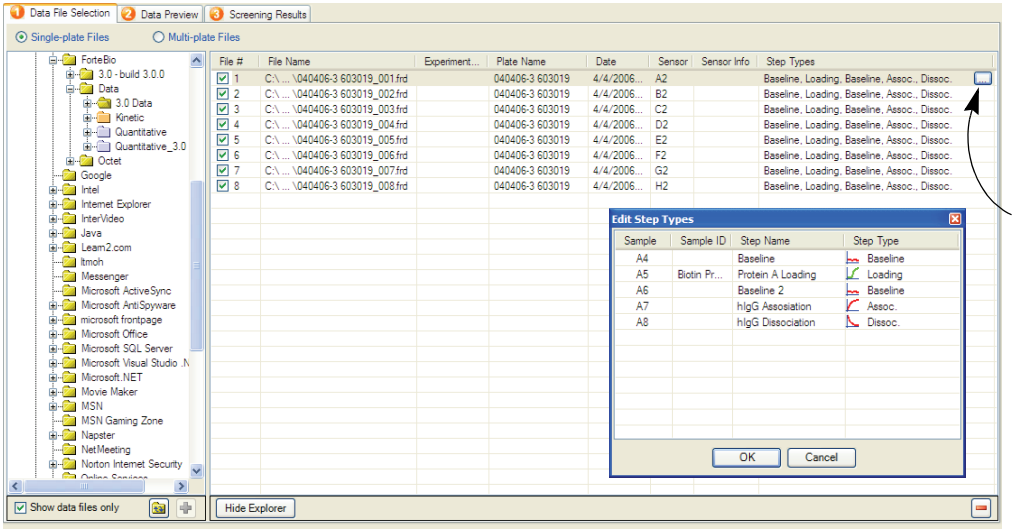
**Note:** In the Explorer pane, kinetic directories and files are orange and quantitation directories and files are blue.




Position the cursor over a file to display the complete path name.

**Figure 5.10 Kinetics Data Analysis window, Data File Selection tab**

| Item in the Data File Selection Tab | Description |
|---|---|
| Show data files only | Choose this option to display only data files (.frd) when you browse a directory in the Explorer pane. |
|  | Click to refresh the Explorer pane. |
|  | Click to add the selected directory or file(s) to the analysis list. |
|  | Click to remove the selected file(s) from the analysis list. |
| Hide Explorer | Click to hide the Explorer pane. |




**To edit the step type:**

1. Click the right end of the row that you want to edit.
2. Choose a step type in the dialog box that appears.

**Figure 5.11 Editing the step type in the Data File Selection tab**

## Viewing Data

- To view the sensor data, click the Data Visualization tab. Alternatively, click the arrow to advance to the tab.
  - The binding charts for all sensors are displayed (thumbnails of individual sensor data at the left) (Figure 5.12).

  In this tab you can view all or particular assay steps, choose the dissociation model, specify a quantity of data (seconds) for analysis that is less than the data collection time, remove data from the analysis.

To remove data from the analysis, remove the check mark in the thumbnail. Alternatively, click the sensor graph in the binding chart. To return the data to the analysis, place a check mark in the thumbnail.

Place the cursor over a thumbnail or a graph in the binding chart to display sensor data details.

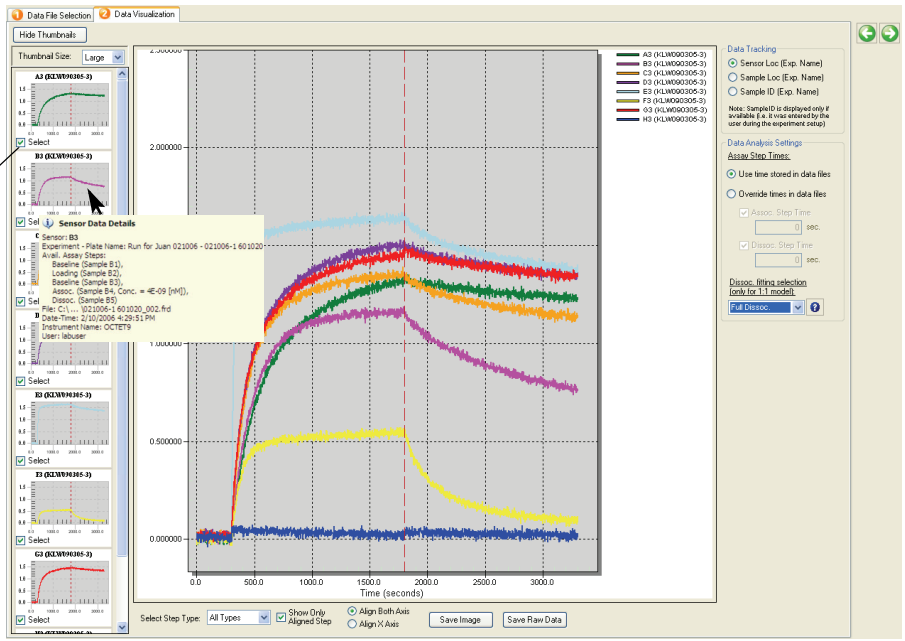



**Figure 5.12 Kinetics Data Analysis window, Data Visualization tab**

| Item in the Data Visualization Tab | Description |
|---|---|
| Hide/Show Thumbnails | Click to hide/display the thumbnail window pane. |
| Thumbnail Size | Choose Large or Small from the drop-down list to specify the size of the thumbnail display. |
| Select Step Type | Choose the assay step type(s) for display from this drop-down list. |
| Show Only Aligned Step | Choose this option to display only the step type chosen in the Select Step Type drop-down list. If this option is not chosen, the aligned step and all subsequent steps are displayed. |

| Item in the Data Visualization Tab | Description |
|---|---|
| Align Both Axis | Choose this option to align the x- and y-axes of the runtime binding charts at 0,0. |
| Align X Axis | Choose this option to align only the x-axis of the runtime binding chart. |
| Save Image | Opens a Save dialog box that enables you to export the image as displayed on the screen. This includes any alignment of the axes or steps. |
| Save Raw Data | Opens a Save dialog box that enables you to save the binding data to a text file (.txt) of the image as displayed on the screen. This includes any alignment of the axes or steps. |
| **Data Tracking** | |
| Sensor Loc (Exp Name) | Choose this option to use the sensor well location as the tracking ID in the Origin results worksheet (Figure 5.13). |
| Sample Loc (Exp Name) | Choose this option to use the sample well location as the tracking ID in the Origin results worksheet. |
| Sample ID (Exp Name) | Choose this option to use the sample ID entered during experiment setup as the tracking ID in the Origin results worksheet. |
| **Data Analysis Settings** | |
| Use time stored in data files | Choose this option to analyze all of the data collected. |
| Override times in data files | Choose this option to specify a quantity of data for analysis (seconds) that is less than the duration of data collection. The data for analysis will be acquired from the start of the experiment up to the specified time. |
| Assoc. Step Time | To analyze a user-specified quantity of association step data, place a check mark here and enter the amount of data (seconds) that you want to analyze. |
| Dissoc. Step Time | To analyze a user-specified quantity of dissociation step data, place a check mark here and enter the quantity of data (seconds) that you want to analyze. |
| Dissoc. fitting selection (only for 1:1 model) | Choose a dissociation fitting model from the drop-down list. |
|  | Click to display information about the selected dissociation model. |

**NOTE**

For more information on how to customize the sensor data display and export the data, see Appendix B, page 83.

### Starting the Analysis

1. In the Data Visualization tab, click the  button.
   — The Origin software opens and displays a worksheet with the binding data from the selected sensors. The ForteBio data analysis options are displayed.

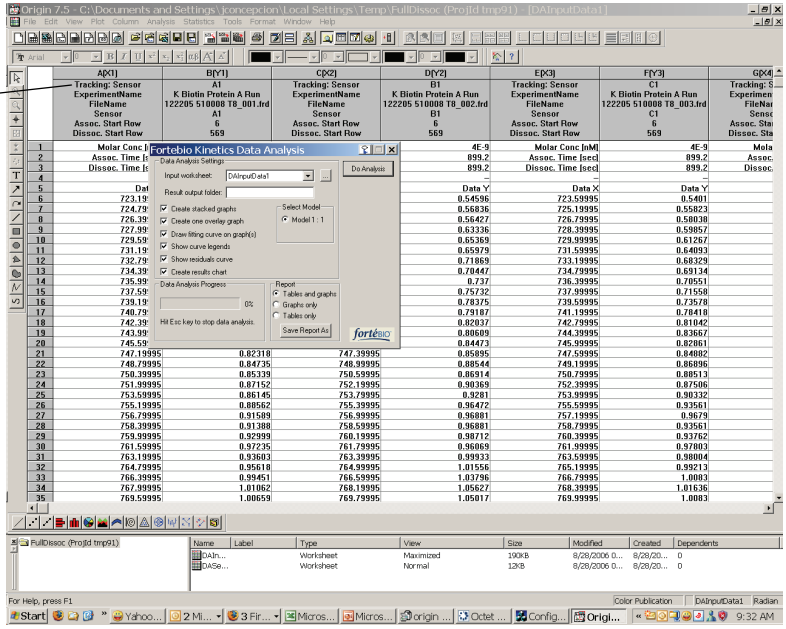
   Figure 5.13 shows the steps to analyze basic kinetics data.
2. To generate an analysis report (.doc), click **Save Report As** (Figure 5.13).
3. To save the analysis to an Origin project, select **File →Save Project** on the Origin main menu (Figure 5.13).

**NOTE**

If you save the results to an Origin project (.opj), you can work with the data in the Origin application without opening the Octet software.

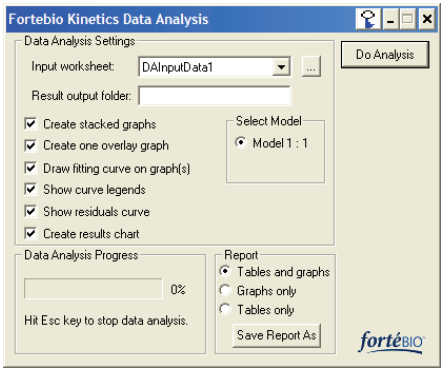
To save the analysis to an Origin project (.opj), choose **File →Save As**.

Tracking by sensor well location.




**To analyze basic kinetic data:**

1. Specify a directory for the results. Click the ... button to browse for a directory.
2. Confirm the default analysis settings or remove the check mark from the options that you do not want to include. (For details on the analysis options, see page 58).
3. Click **Do Analysis**.
   - The overlay graph (Figure 5.14), results chart (Figure 5.15), and results table (Figure 5.16) are created.




**Note:** A single exponential is used to fit both the association and dissociation rates ($k_a$ and $k_d$). The model provides a single $k_{obs}$ and $k_d$. You must know the protein concentration to compute $k_a$ and $K_D$:

Association model: $y = y_0 + A \times (1 - \exp[K_{obs}(t-t_0)])$
Dissociation model: $y = y_0 + A \times \exp[k_d(t-t_0)]$

**To save analysis results:**

- To generate a report of the analysis results (.doc), click **Save Report As**.
- To generate an Origin project (.opj), select **File →Save Project** on the Origin main menu.
  Note: If you save the results to an Origin project, you can work with the data in the Origin application without opening the Octet software.

**Figure 5.13 Origin software main window and FortéBio kinetics data analysis settings**

| FortéBio Kinetics Data Analysis Options | Description |
|---|---|
| Do Analysis | Click to analyze the current kinetic data. |
| Input worksheet | Name of the active data worksheet for the Origin software analysis. |
| Result output folder | Specifies the directory for the analysis results. |
| Create stacked graphs | Choose this option to display the binding data from each sensor in a separate graph (Figure 5.14). |
| Create one overlay graph | Choose this option to display all of the sensor binding data in one graph. |
| Draw fitting curve on graph(s) | Choose this option to display the fitting curve in a graph. |
| Show curve legends | Choose this option to display the figure legend. |
| Show residuals curve | Choose this option to generate the residual graph (Figure 5.14). |
| Create results chart | Choose this option to generate a results chart (Figure 5.15). |
| Report | |
| Tables and graphs | Choose this option to include both the binding charts and table of kinetic analysis results in the report. |
| Graphs only | Choose this option to include only the binding charts in the report. |
| Tables only | Choose this option to include only the results table in the report (Figure 5.16). |
| Save Report As | Opens a Save As dialog box so that you can save the analysis results to a report (.doc). |

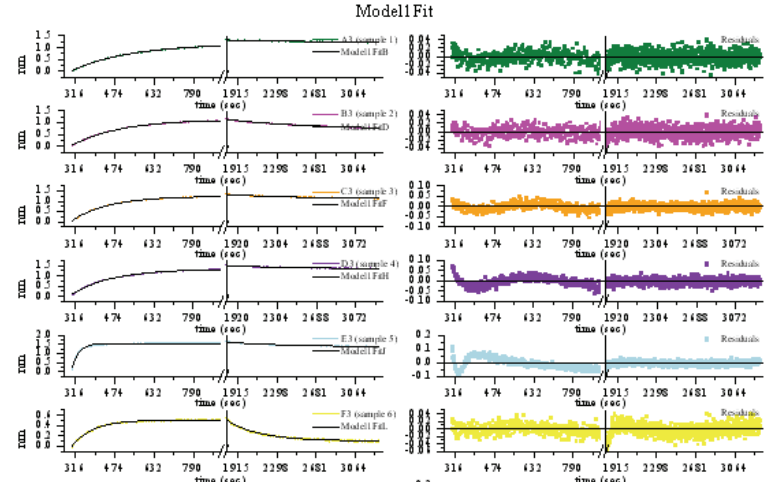
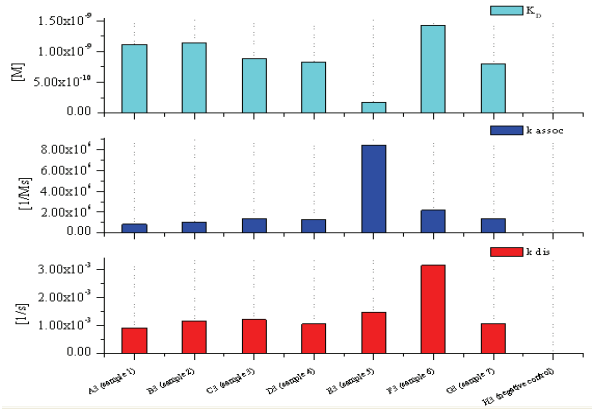



**Figure 5.14 Sensor binding data (left) and residuals (right) in stacked format**




The result chart shows the $K_D$, $k_a$, and $k_{dis}$ for each sensor.

**Figure 5.15 Basic kinetics analysis, results chart**

| TrackingID | A3 (sample 1) | B3 (sample 2) | C3 (sample 3) | D3 (sample 4) | E3 (sample 5) | F3 (sample 6) | G3 (sample 7) | H3 (negative control) |
|---|---|---|---|---|---|---|---|---|
| Sensor | A3 | B3 | C3 | D3 | E3 | F3 | G3 | H3 |
| FileName | sample_001.frd | sample_002.frd | sample_003.frd | sample_004.frd | sample_005.frd | sample_006.frd | sample_007.frd | sample_008.frd |
| MolarConc [M] | 4E-9 | 4E-9 | 4E-9 | 4E-9 | 4E-9 | 4E-9 | 4E-9 | 0 |
| **kobserved [1/s]** | **4.22E-3** | **5.19E-3** | **6.67E-3** | **6.16E-3** | **3.52E-2** | **1.20E-2** | **6.45E-3** | ----- |
| kobsErr | 3.63E-5 | 3.36E-5 | 4.97E-5 | 6.03E-5 | 6.77E-4 | 1.41E-4 | 6.80E-5 | ----- |
| **kdis [1/s]** | **9.19E-4** | **1.15E-3** | **1.21E-3** | **1.05E-3** | **1.49E-3** | **3.15E-3** | **1.07E-3** | ----- |
| kdisErr | 8.55E-5 | 2.12E-5 | 4.16E-5 | 5.55E-5 | 3.65E-5 | 3.37E-5 | 6.48E-5 | ----- |
| **kassoc [1/Ms]** | **8.24E5** | **1.01E6** | **1.36E6** | **1.28E6** | **8.43E6** | **2.20E6** | **1.34E6** | -- |
| **$K_D$ [M]** | **1.11E-9** | **1.13E-9** | **8.90E-10** | **8.27E-10** | **1.76E-10** | **1.43E-9** | **7.99E-10** | -- |
| kobsRsq | 0.99778 | 0.99831 | 0.99699 | 0.99524 | 0.96715 | 0.9889 | 0.99423 | ----- |
| kobsChisq | 1.8656E-4 | 1.34531E-4 | 2.82316E-4 | 4.80641E-4 | 0.00144 | 1.50654E-4 | 5.27853E-4 | ----- |
| kdisRsq | 0.81032 | 0.98646 | 0.9503 | 0.91209 | 0.96397 | 0.98226 | 0.88451 | ----- |
| kdisChisq | 1.56012E-4 | 1.44825E-4 | 1.52303E-4 | 1.58693E-4 | 1.62414E-4 | 1.9066E-4 | 1.62148E-4 | ----- |

* Low calculation confidence.

**Figure 5.16 Basic kinetic analysis, results table**

## 5.4 Generating a Report

- To generate a report of the analysis results (.doc), click **Save Report As** in the Kinetics Data Analysis dialog box (Figure 5.13, page 57).
- To generate an Origin project (.opj), select **File →Save Project** on the Origin main menu.

**NOTE**

If you save the results to an Origin project, you can work with the data in the Origin application without opening the Octet software.

# CHAPTER 6
# Kinetics Screening



Kinetics screening enables you to analyze sensor-sample binding or dissociation interactions for many samples in parallel. For example, you can use kinetics screening to look for the presence of a binding protein or compare sample *off rates*, how fast a bound protein dissociates from the Octet sensor.

In a kinetics screening experiment, samples are prepared offline (not in the Octet instrument), then a single-step assay (one sensor per sample) that monitors association or dissociation is performed in the Octet instrument.

## 6.1 Setting Up a Kinetics Screening Experiment

To set up a kinetics experiment in the Octet software, specify the:

- Well locations of the sensors and samples.
- Assay step (association or dissociation).

The experiment method (.fmf) that you specify is automatically saved when the experiment is run.

For more details on how to prepare the sensors, see the appropriate product instructions.

**NOTE**

Before you begin an experiment, check the sample plate temperature (displayed in status bar). Confirm that the temperature is appropriate for your experiment or set a new temperature. (For more details on setting the temperature, see page 12.) If you close the Octet software, the sample platform temperature is reset to 30° C.

### Specifying the Sensor & Sample Configuration

1. In the Experiment Wizard, choose the **New Kinetics Experiment** and **Kinetics Screening** options (Figure 6.1). If the Experiment Wizard is not displayed, click the Experiment Wizard button .

   Alternatively, select **Experiment → Kinetics → New Kinetics Screening Experiment** on the main menu.


**Figure 6.1 Experiment Wizard**

2. Click the arrow in the wizard.

   — The Kinetics Screening Setup window appears (Figure 6.2).

   Figure 6.2 shows how to designate the locations of the sensors and samples.

**NOTE**

More than one Kinetics Screening Setup window can be open at the same time.

**To specify the active sensor locations, do one of the following:**

- Click **Fill Plate** to select the entire plate.
- Draw a box around one or more column(s).
- Click **Import**. In the dialog box that appears, select a tab-delimited text file (.txt) that specifies sensor locations and sensor loading information (assay time, flow rate, and type of assay step).
- Click a single well.

**To delete a sensor location:**

- Select a column or well in the sensor plate map and click **Remove**.

**To specify the sample locations, do one of the following:**

- Click **Match Sensors**.
  - All well locations that match the active sensor locations are selected.
- In the sample plate, draw a box around the well(s) of interest
- Click **Import**. In the dialog box that appears, select a tab-delimited text file (.txt) that specifies sample locations and sample information (sample ID, molar concentration, sample or buffer information).

**To delete a sample location(s):**

- Select the column(s) or well(s) that you want to delete and click **Remove**.

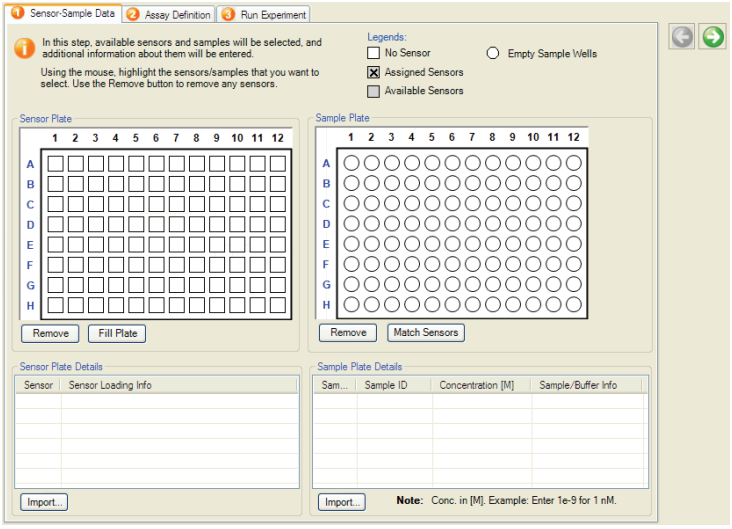
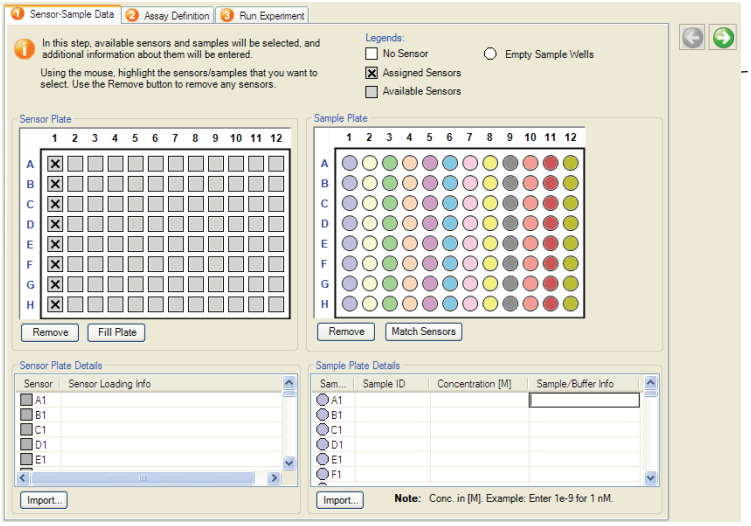



**Figure 6.2 Specifying sensor and sample locations**

## Entering Sensor & Sample Information

You can enter information about sensor loading, sample ID, sample concentration, or sample buffer. Entering the information is optional. The information can entered manually or by importing a tab-delimited text file (.txt) (Figure 6.3).

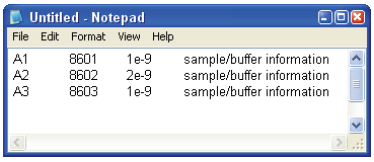



**To enter information manually:**
Double-click a field in the sensor plate or sample plate table, and enter the information.

**To Import a tab-delimited text file:**

1. First format the information of interest in a tab-delimited text file (as shown below).

   Enter information separated by tabs. The sample wells can be specified by column or row (for example, A1, A2, A3, or A1,B1, C1).




2. Click **Import**.
3. In the dialog box that appears, select the .txt of interest, and click **Open**.

**Figure 6.3 Entering sensor or sample information**

## Defining the Kinetics Screening Assay

- Click the Assay Definition tab or click the  arrow to advance to the tab.

  Figure 6.4 shows how to define a single-step (association or dissociation) kinetics screening assay.

**NOTE**

Since a kinetics screening experiment is a single-step assay, only the association or dissociation step is performed in the Octet instrument. The other steps are performed offline.

### Types of Assay Steps

The Octet software provides predefined assay steps. Figure 6.5 shows how to create your own custom assay step.

| Assay Step Type | Use This Step When... |
|---|---|
| Assoc. | Binding the second protein of interest during kinetic analysis. |
| Dissoc. | Monitoring the dissociation of the protein complex. |
| Baseline | Establishing the sensor baseline in diluent. **Note:** This step may be performed offline (not in the Octet instrument). |
| Loading | Binding the first protein of interest to the sensor. **Note:** This step may be performed offline (not in the Octet instrument). |
| Activation | Chemically activating the sensor surface to prepare for protein loading. |
| Quenching | Blocking unreacted surface immobilization sites on the sensor. |
| New Loading | Defining a custom loading step. |

**To define a single-step kinetics screening assay:**

1. Select a step type from the Select Step drop-down list.
   **Note:** You can create a custom step with a user-specified assay time or flow rate for inclusion in an assay (see Figure 6.5).
2. Click **Create**.
   - The selected step type is assigned to all of the samples.

**Note:** The preceding assay steps are performed offline (outside the Octet instrument).

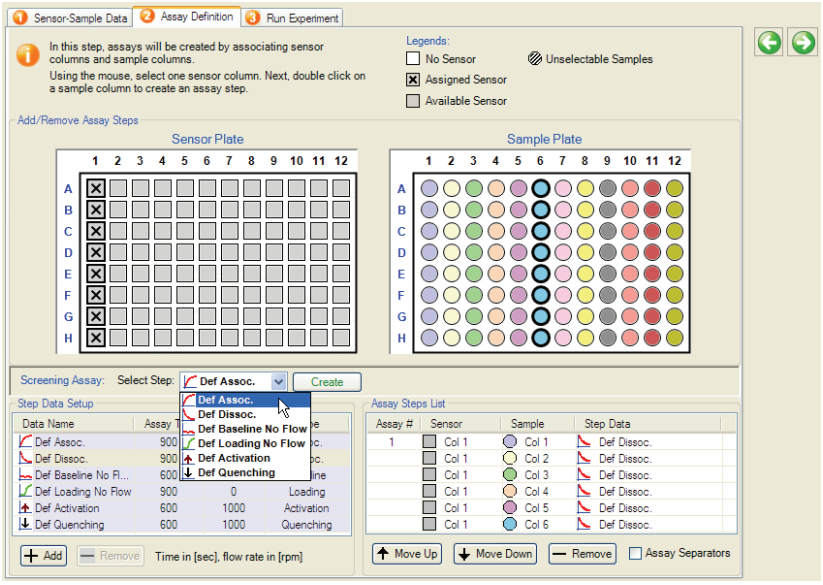



**Figure 6.4 How to define a single-step kinetics screening assay**

## NOTE

During assay setup, if you select a sensor and sample column that do not have the same number of active wells, a message appears and requests confirmation to continue. If a sample column has fewer wells than the number of sensors, some sensors will not be incubated in the sample solution. If a sample column has more wells than the sensor column, some of the sample wells will not receive a sensor.

## Creating a Custom Step

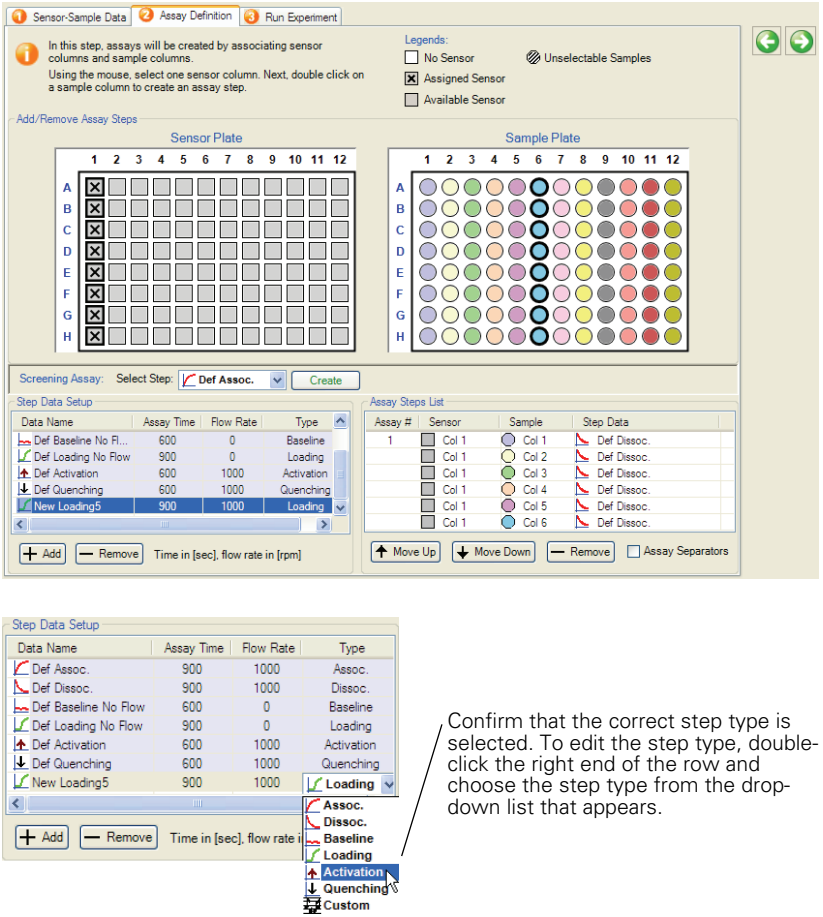
**To create a custom step:**

1. Click the **+ Add** button.
   - A new step is added to the Step Data Setup pane.
2. To edit the step (data name, assay time, flow rate, or type), double-click the row.
   Assay time range: 2 - 48,000 seconds
   Flow rate range: 0 or 60 - 1500 rpm

**To remove a custom step:**

1. Select the step that you want to remove.
2. Click the **- Remove** button. Alternatively, press the **Delete** key.

**Note:** A factory installed step cannot be edited or removed.




Confirm that the correct step type is selected. To edit the step type, double-click the right end of the row and choose the step type from the drop-down list that appears.

**Figure 6.5 How to create a custom step**

### Saving a Kinetics Screening Experiment

The software automatically saves the experiment information that you specify (sensor and sample locations, sensor and sample information, and assay steps) to an experiment method (.fmf) at the start of the run. If you set up an experiment, but do not start the run, you can manually save the experiment method.

1. To manually save an experiment, click the Save Method File button  . Alternatively, select **File → Save Method File** on the main menu.

   If there is more than one open experiment and you want to save them all, click the Save All Methods Files button  .

2. In the Save dialog box that appears, 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** on the main menu and enter a new name for the experiment.

### Opening an Experiment Method

1. Click the Open Method File button  . Alternatively, select **File → Open → Experiment Method File** on the main menu.
2. In the dialog box that appears, navigate to the directory and .fmf of interest, and click **Open**.

**NOTE**

When you open a method, reselect the assay type. (This information is not saved.)

## 6.2 Performing a Kinetics Screening Experiment

You are ready to begin a kinetics screening experiment after you specify the assay or open a saved kinetics screening experiment method (.fmf).

**IMPORTANT**

Make sure that the Octet biosensors are properly prewetted before starting the experiment. For details on how to prepare the biosensors, see the appropriate product instructions.

### Loading the Sensor Tray & Sample Plate

1. Open the Octet instrument door (lift the handle up).
2. Place the sensor tray on the sensor stage (left side) so that position A1 is located at the upper right corner (Figure 6.6).
3. Place the sample plate on the sample stage (right side) so that position A1 is located at the upper right corner.

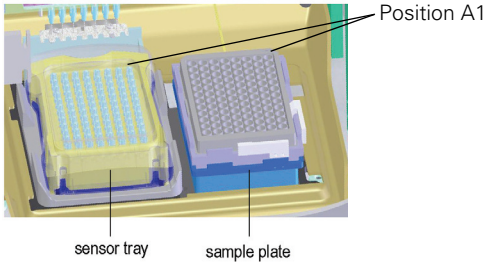



**Figure 6.6 Sensor stage (left) and sample stage (right)**

4. Close the Octet door and allow the plate to equilibrate for five minutes. (The time required for temperature equilibration may be different, depending on the temperature that your application requires.)

**IMPORTANT**

Make sure that the bottom of the sample plate and sensor tray are flat on each stage. Otherwise the orbital movement of the stage may result in sample loss.

### Locking the Octet Software

You can lock the Octet software to prevent access by another user while an experiment is in progress.

1. Select **File –Lock application** on the main menu.
2. In the dialog box that appears, enter your user name and a password.
3. Click **Lock**.
   — The lock and password expire at the end of the experiment.
4. If you want to unlock the application before the end of the experiment, enter the password in the dialog box and click **Unlock**.

## Starting the Experiment

**To start an experiment:**

1. Click the Run Experiment tab or click the arrow to advance to the tab,
2. Confirm the default settings or enter new settings.
3. Enter a name for the Experiment Run subdirectory.
4. To start the experiment, click 60.
   - If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

   When the experiment starts, the Runtime Binding Chart window appears and displays the binding data in real time as well as the experiment progress.
5. To stop an experiment in progress, click the button.
   - The experiment is aborted, the data for the active sensor is lost, and the sensor is ejected into the waste tray.

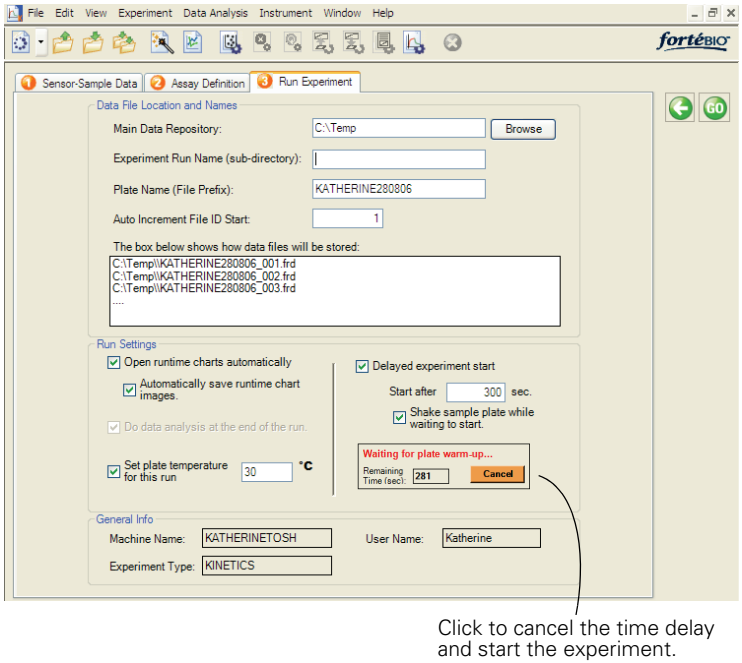



**Figure 6.7 Running a kinetics screening experiment**

### CAUTION

**CAUTION!** Do not open the door when an experiment is in progress. If the door is opened when the Octet instrument is operating, the experiment is aborted and the data from the active sensor is lost.

| Item in the Run Experiment Tab | Description |
|---|---|
| **Data File Location and Names** | |
| Main Data Repository | The location where the subdirectory will be created. The subdirectory contains the .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 sensor that includes the data from all steps that the sensor performs. |

| Item in the Run Experiment Tab | Description |
|---|---|
| Plate Name | The prefix for the data files (.frd). |
| Auto Increment File ID Start | Each file is saved with a number after the file name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd. |
| **Run Settings** | |
| Open runtime charts automatically. | Choose this option to display the binding chart for the current sensor during data acquisition. |
| Automatically save runtime chart images. | Choose this option to save the binding charts (.JPG). The binding data (.frd) is saved regardless of whether chart images are saved. |
| Set plate temperature for this run. | Choose this option to specify a plate temperature. Enter the temperature in the box. If this option is not chosen, the plate temperature is set to the default 30° C.<br>Note: If the actual plate temperature is not equal to the set plate temperature, a warning appears. The software gives you the option to: i) wait until the set temperature is reached before proceeding with the run, ii) continue without waiting until the set temperature is reached, or iii) cancel the run. |
| Delayed experiment start. | Choose this option to specify a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click GO. |
| **General Info** | |
| Machine Name | The name of the computer that controls the Octet instrument and acquires the data using the Octet software. |
| Experiment Type | The type of experiment (quantitation, basic kinetics, or kinetics screening) |
| User Name | The user logon name. |

### Viewing the Runtime Binding Chart

If you chose **Open runtime charts automatically** in the Run Experiment tab (Figure 6.7), the runtime binding charts are automatically displayed when data acquisition starts. If the charts are not automatically displayed, select **View → Enable Runtime Binding Charts** on the main menu.

The chart window also displays the run status and the elapsed time for the current sample column.

**NOTE**

Do not close the chart window until all of the samples are analyzed. Otherwise, the charts will not be saved. If you do not want to display the charts, minimize the chart window.

- To close the chart window for the current assay, click the Stop/Start Opening Runtime Binding Charts button  .
  - — The chart window closes and the chart for the current assay is **not saved**.
- Click the  button again to open the chart window for the next assay.

## 6.3 Analyzing Kinetics Screening Data

The raw data files include the data from all of the assay steps that a sensor performs. When selecting files for analysis, you can include data from one or more assays conducted on the same plate.

### Selecting Data

1. Do one of the following to start a kinetics screening data analysis session:
   - Select **Data Analysis →New Kinetics Screening Data Analysis** on the main menu.
   - Select **Data Analysis** and **Kinetics Screening** in the Experiment Wizard. (To display the Experiment Wizard, click the button.)

   — The Kinetics Data Analysis window appears (Figure 6.8).

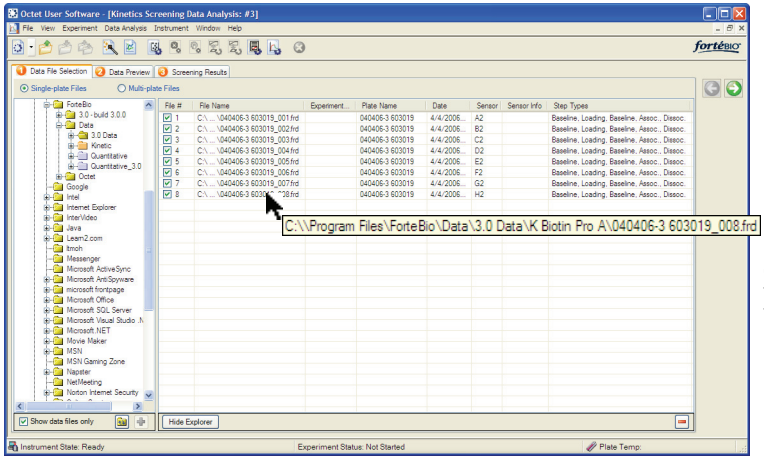
Figure 6.8 shows how to select files for analysis.

Figure 6.9 shows how to edit the step type.

**To select files for analysis:**

- Double-click the directory or individual files of interest.
- You can select files from the same or different plates.

Note: In the Explorer pane, kinetic directories and files are orange and quantitation directories and files are blue.




Position the cursor over a file to display the complete path name.

**To remove a file from the analysis, do either of the following:**

- Remove the check mark next to the file.
  - The file remains in the list, but is excluded from the analysis.
- Select the file(s), then click the button.
  - The file is removed from the list.

**Figure 6.8 Selecting files for kinetics screening analysis in the Data File Selection tab**

| Item in the Data File Selection Tab | Description |
|---|---|
| Show data files only | Choose this option to show only Octet data files in the Explorer pane. |
|  | Click to refresh the Explorer pane. |
|  | Click to add the selected directory or file(s) to the analysis list. |
|  | Click to remove the selected file(s) from the analysis list. |
| Hide Explorer | Click to hide the Explorer pane. |

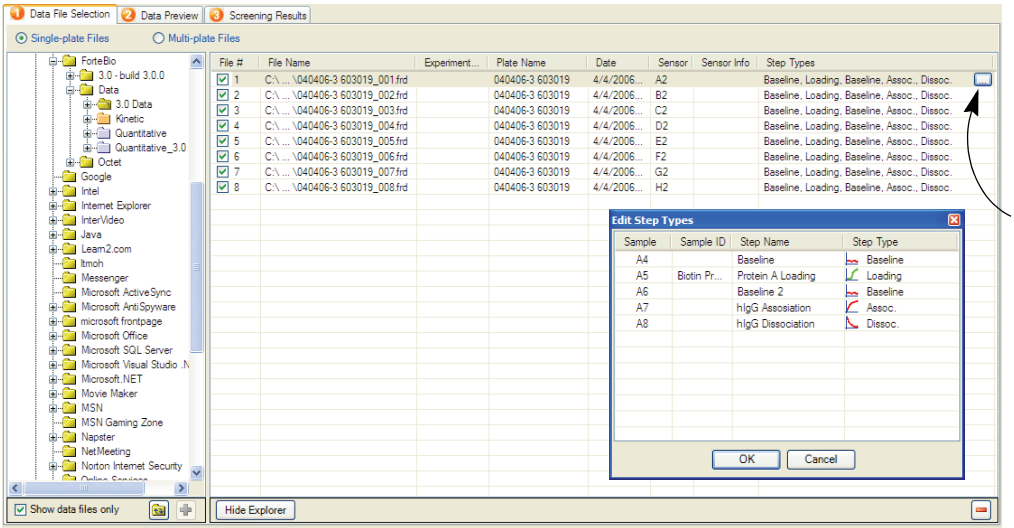



**Figure 6.9 Editing the step type in the Data File Selection tab**

## Viewing Data

- To view the sensor data, click the Data Preview tab. Alternatively, click the  arrow to advance to the tab.
  - — The binding charts for all sensors are displayed (thumbnails of individual sensor data at the left) (Figure 6.10).

  In this tab you can view all or particular assay steps, choose the dissociation model, specify a quantity of data (seconds) for analysis that is less than the data collection time, remove data from the analysis.

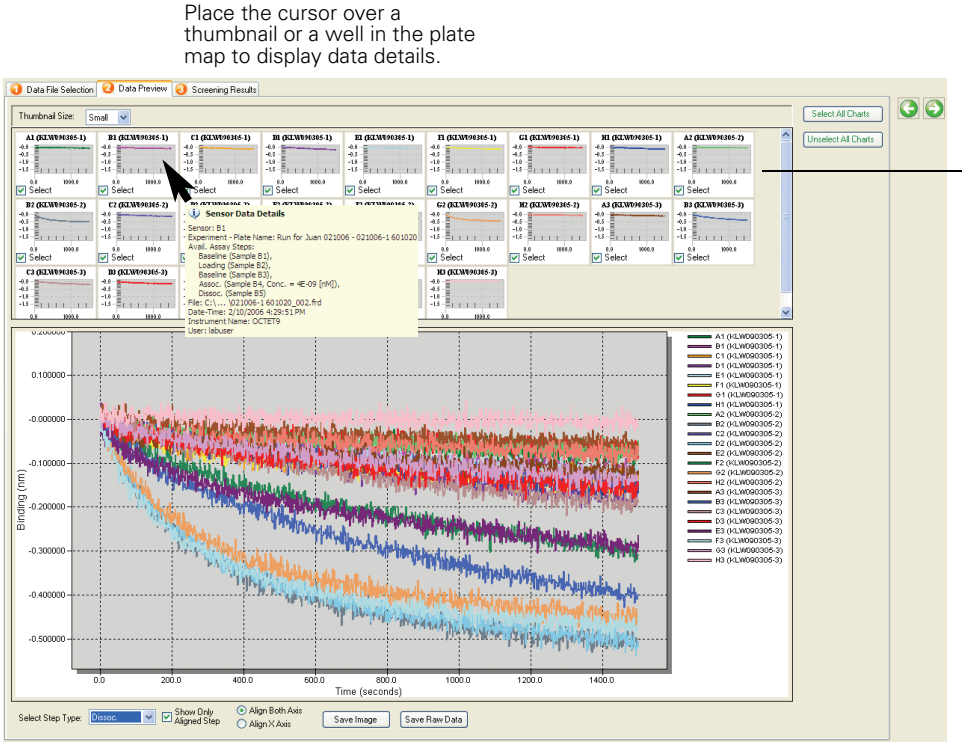



**Figure 6.10 Kinetics Screening Data Analysis window, Data Preview tab**
**Note:** A plate map is displayed if the data are from the same plate.

| Item in the Data Preview Tab | Description |
|---|---|
| Hide/Show Thumbnails | Click to hide/display the thumbnail window pane. |
| Thumbnail Size | Choose Large or Small from the drop-down list to select the size of the thumbnail display. |
| Select All Chart | Click to display all of the data selected in the Data File Selection tab. |
| Unselect All Charts | Click to hide all of the data selected in the Data File Selection tab. |
| Select Step Type | Select All Types from the drop-down list to display the binding data for all steps in an assay. Alternatively, to display binding data starting at a particular step type, select the step type of interest. For example, if you select Assoc., only the association and dissociation steps are displayed. |
| Show Only Aligned Step | Choose this option to display only the step type chosen in the Select Step Type drop-down list. |
| Align Both Axes | Choose this option to align the binding charts at 0,0. |
| Align X Axis | Choose this option to align only the x-axis. |

| Item in the Data Preview Tab | Description |
|---|---|
| Save Image | Opens a Save dialog box that enables you to export the image as displayed on the screen. This includes any alignment of the axes or steps. |
| Save Raw Data | Opens a Save dialog box that enables you to save the binding data to a text file (.txt) of the image as displayed on the screen. This includes any alignment of the axes or steps. |

**NOTE**

For more information on how to customize the sensor data display and export the data, see Appendix B, page 83.

### Viewing Kinetic Screening Results

- Click Screening Results tab or click the button to advance to the tab.
  - The Octet software computes the kinetic constant ($k_d$ or $k_{obs}$), depending on the step type selected (Figure 6.11).

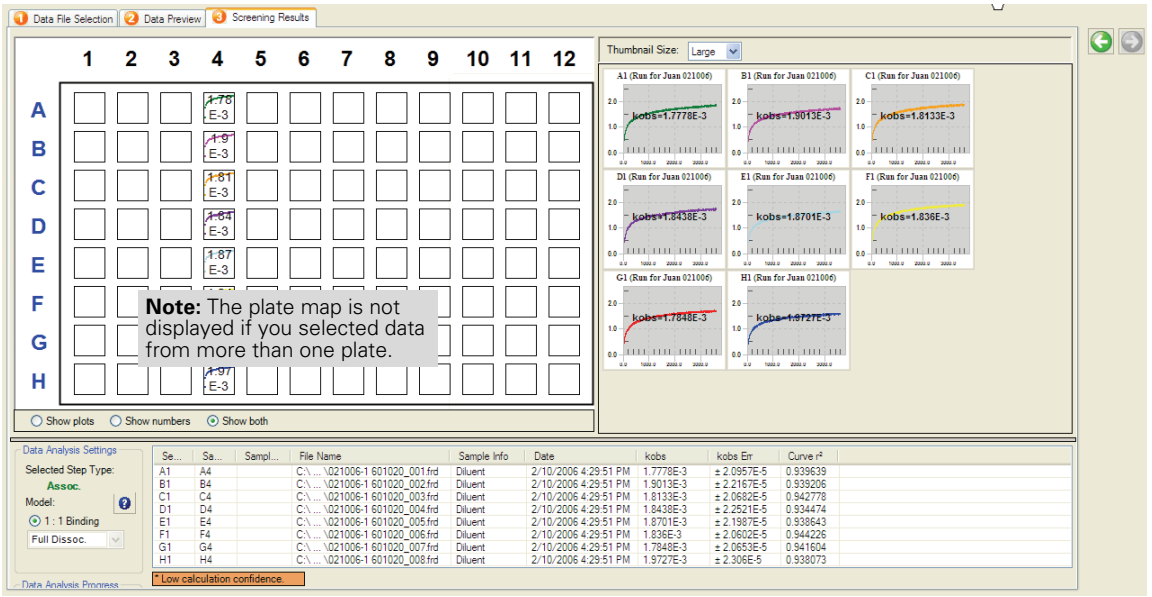



**Figure 6.11 Screening Results tab**

| Item in the Screening Results Tab | Description |
|---|---|
| Show Plots | Choose this option to display only the graphical plots of the binding data. |
| Show Numbers | Choose this option to display only the calculated $k_d$ or $k_{obs}$. |
| Show both | Choose this option to display both the graphical plot of the binding data and the computed kinetic constant. |
| Thumbnail Size | Choose large or small from the drop-down list to specify the thumbnail size. |
| Partial Dissoc. | The model does not assume fully reversible binding. Not all of the bound protein may dissociate as time approaches infinity. |
| Full Dissoc. | The model assumes the protein binding interaction is fully reversible and all of the bound protein will dissociate as time approaches infinity. |
|  | Click to display information about the selected dissociation model. |
| Low calculation confidence | A data file highlighted in orange color indicates one or more of the following:<br>• The error for $k_d$ or $k_{obs}$ is larger than 50% of the parameter value.<br>• The error for $k_d$ or $k_{obs}$ could not be calculated.<br>• The computed $k_d$ or $k_{obs}$ is a negative value. |

## 6.4 Generating a Report

1. Click the Data Analysis Report button  . Alternatively, select **File →Save Kinetics Screening Data Analysis Report.**
2. In the dialog box that appears, specify a directory and name for the report (.doc), and click **Save**.

   — The report is displayed.

# CHAPTER 7
# Maintenance



## 7.1 Cleaning the Instrument

If you use the Octet instrument regularly, clean the interior horizontal surfaces daily with a Kimwipe tissue moistened with a 30-60% isopropyl alcohol. Otherwise, clean once a week or as needed.

1. Turn off the power to the instrument and open the door.
2. Wipe the sensor and sample platform.
3. Carefully wipe the eight sensor pickup tips.
4. Allow the surfaces to dry for at lease one minute with the door open.

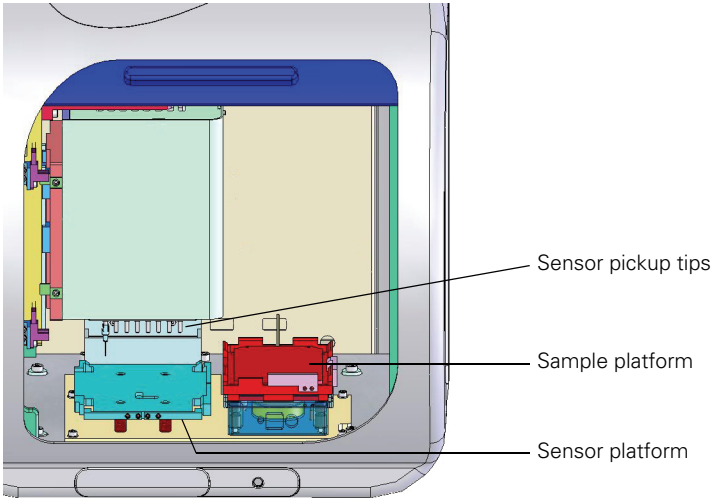



**Figure 7.1 Octet instrument**

## 7.2 Emptying the Waste Container

Empty the waste container after every run of a 96-sensor tray.

1. Press the container to open it.
2. Pull the container out and completely remove it from the instrument.
3. Remove the container insert with the sensor tips and dispose of both in a biohazard container suitable for sharp objects.

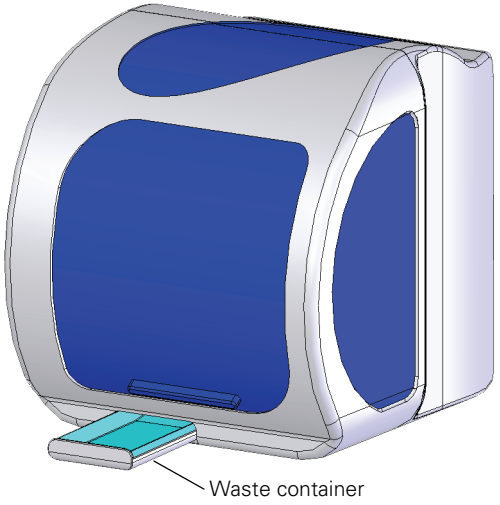



**Figure 7.2 Waste container for the Octet instrument**

# APPENDIX A
# Menu Commands & Toolbar Buttons

**Figure A.1 Toolbar**

**Table A.2** Menu commands and toolbar buttons

| Menu Command | Toolbar Button | Function |
|---|---|---|
| File →Open → Experiment Method File |  | Opens a dialog box that enables you to select an experiment method file (.fmf). |
| File →Save Method File |  | Opens a dialog box that enables you to save an experiment method file (.fmf). |
| File →Save All Method Files |  | |
| File →Save Method File As | | Opens a dialog box that enables you to save an experiment method file to a new name without overwriting the original file. |
| File →Lock Application | | Opens a dialog box that enables you to lock the software to prevent access by another user while an experiment is in progress. For more details, see page 22. |
| File →Save Quantitation Data Analysis Report | | Opens a dialog box that enables you to save the report. |
| File →Save Kinetics Screening Data Analysis Report | | Opens a dialog box that enables you to save the report. |
| File →Options | | Opens the Options dialog box (see page 10). |
| File →Exit | | Closes the application after prompting you to save any changes. |
| View →Enable Runtime Binding Charts | | Choose this option to automatically display the runtime binding charts during data acquisition. |
| View →Instrument Status | | Choose this option to display the Instrument Status window (Figure 2.3, page 10). |
| Experiment →New Experiment Wizard |  | Opens the Experiment Wizard. |

**Table A.2** Menu commands and toolbar buttons

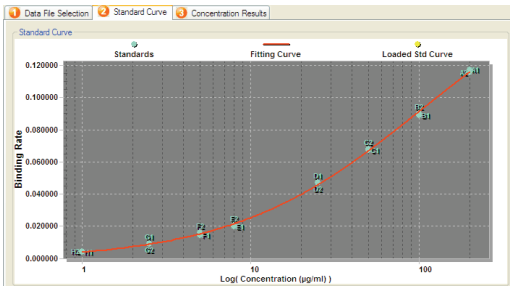
| Menu Command | Toolbar Button | Function |
|---|---|---|
| Experiment → Quantitation →New Quantitation Experiment |  Click the button and make a selection from the drop-down list to choose another experiment . | Opens the Quantitation Experiment Setup window (Figure 3.2, page 15). |
| Experiment → Quantitation →Assay Development Mode | | Opens the Assay Definition dialog box that enables you to define a new assay, edit an assay, or remove an assay from the system. **Note:** Factory installed predefined assays cannot be removed or edited. |
| Experiment Kinetics →New Kinetics Experiment | | Opens the Kinetics Setup window (Figure 5.2, page 41). |
| Experiment →Kinetics →New Kinetics Screening Experiment | | Opens the Kinetics Screening Setup window (Figure 6.2, page 63). |
| Experiment →Stop |  | Click to abort an experiment. Note: Data from the active sensor is lost. |
| Experiment →Set Plate Temperature | | Open the Temperature Settings dialog box that enables you to specify the sample platform temperature (from 2° C above ambient temperature to 42° C). |
| Data Analysis →New Quantitation Data Analysis |  | Opens the Quantitation Data Analysis window (Figure 3.12, page 27). |
| Data Analysis →New Kinetics Data Analysis |  | Opens the Kinetics Data Analysis window (Figure 5.10, page 52). |
| Data Analysis →New Kinetics Screening Data Analysis | | Opens the Kinetics Screening Data Analysis window (Figure 6.8, page 71). |
| Data Analysis →Stop Data Analysis |  | Stops the data analysis. |
| Data Analysis → Recalculate |  | Recomputes the sample concentrations. |
| Data Analysis →Load Quantitation Standard Curve |  | Opens a dialog box that enables you to select a standard curve (.fsc) for import during quantitation data analysis. |

**Table A.2** Menu commands and toolbar buttons

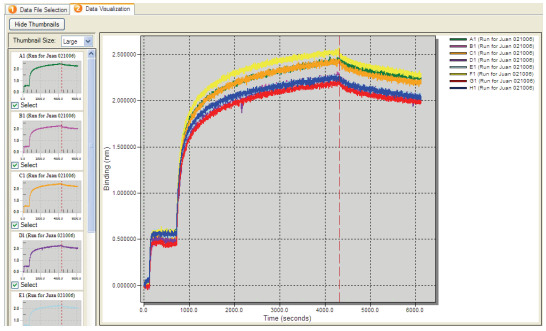
| Menu Command | Toolbar Button | Function |
|---|---|---|
| Data Analysis → Export Quantitation Standard Curve |  | Opens a dialog box that enables you to save a standard curve (.fsc). |
| Instrument →Reset | | Resets the instrument and the log in the Instrument Status window. |
| Instrument →Stop Shaker | | Stops the sample plate shaker. |
| Window →Close | | Closes the active window after prompting you to save any changes. |
| Window →Close All | | Closes all windows after prompting you to save any changes. |
| Window →Arrange Cascade | | Organizes all windows in a cascade arrangement. |
| Window →Arrange Tile Horizontal | | Tiles the windows horizontally. |
| Window →Arrange Tile Vertical | | Tiles the windows vertically. |
| Help →Octet User Software Help | | Opens the online Octet System User's Guide. |
| Help →About Octet User Software | | Displays the Octet software properties. |

# APPENDIX B
# Working With the Data Display

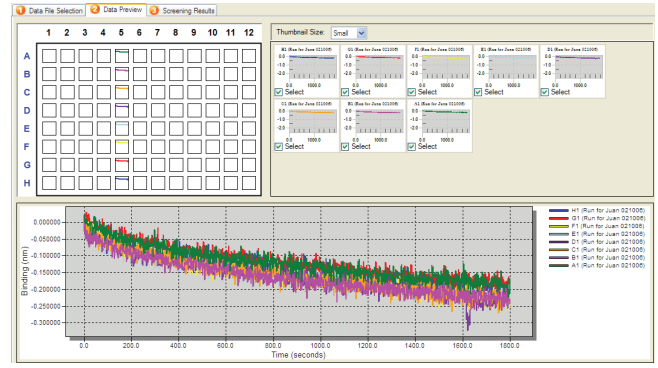
You can customize the data display in the Standard Curve tab, Data Visualization tab, or the Data Preview tab (Figure B.1).




Standard Curve tab in the Quantitation Data Analysis window




Data Visualization tab in the Kinetics Data Analysis window




Data Preview tab in the Kinetics Screening Data Analysis window

**Figure B.1 Standard curve data (top), basic kinetics data (middle), kinetics screening data (bottom)**

- To display a shortcut menu of options for displaying, annotating, or exporting the data, right-click the chart.

**NOTE**

Not all shortcut menu options are available in the Standard Curve tab.

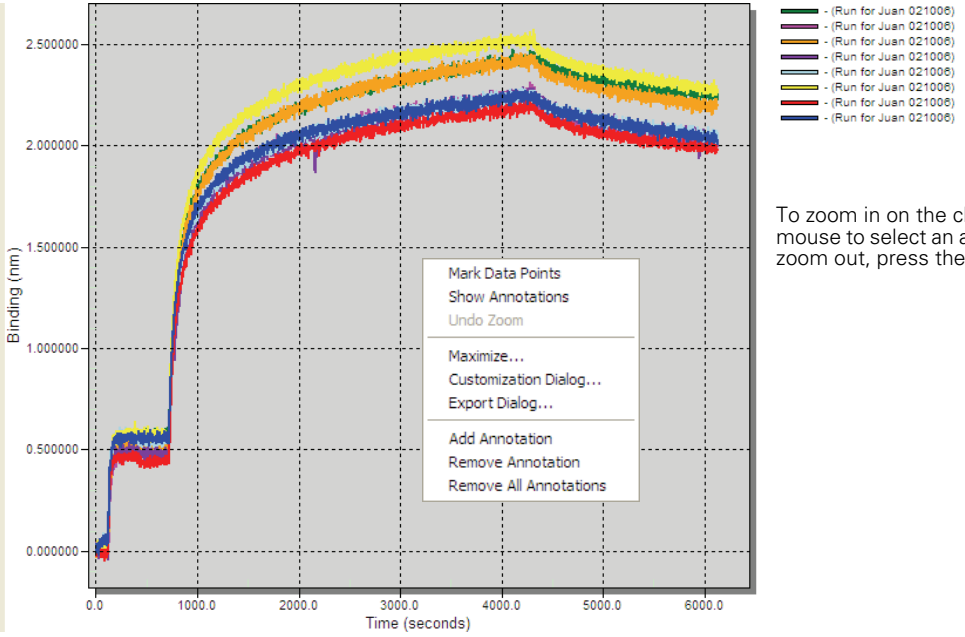



**Figure B.2 Binding data, basic kinetics experiment**

| Item in the Shortcut Menu | Description |
|---|---|
| Mark Data Points | Displays the data points on the runtime binding charts. |
| Show Annotations | Display user-entered notes on the chart. |
| Maximize | Enlarges the chart display. |
| Customization Dialog | Opens the Customization dialog box (Figure B.3). Note: You can also double-click the runtime binding chart to display the Customization dialog box. |
| Export Dialog | Opens a dialog box that enables you to export the runtime binding chart to a graphic image file. |

| Item in the Shortcut Menu | Description |
|---|---|
| Add Annotation | Opens a dialog box that enables you to enter notes. The annotation is added to the chart at the location of the cursor when you selected **Add Annotation**. |
| Remove Annotation | To delete an annotation, right-click the annotation and select **Remove Annotation**. |
| Remove All Annotations | Removes all annotations from the runtime binding chart. |

- To display the Customization dialog box, double-click the data chart (Figure B.3).

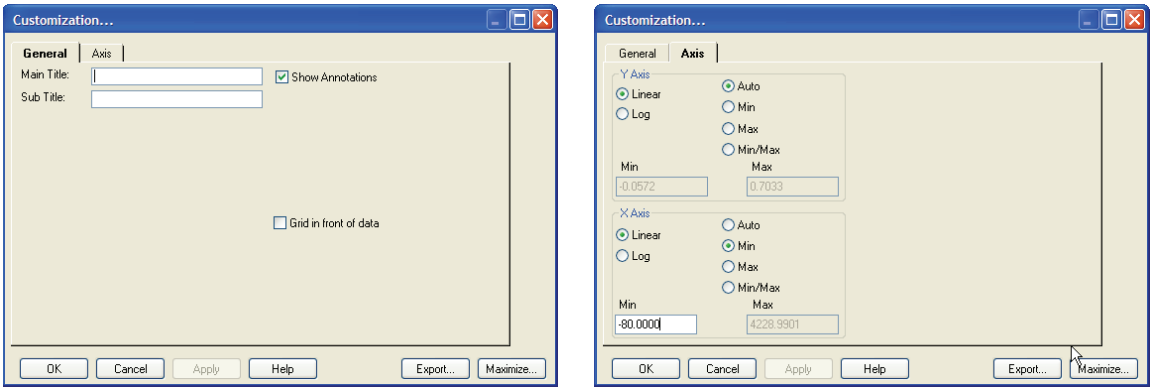



**Figure B.3 Customization dialog box, General tab (left) and Axis tab (right)**

| Component in the Customization Tab | Description |
|---|---|
| **General Tab** | |
| Main Title | Enter a title for the binding chart and click **Apply** to display the title. |
| Sub Title | Enter subtitle to add to the binding chart and click **Apply** to display the subtitle. |
| Show Annotations | Displays user-entered annotations on the runtime binding chart. |
| Grid in front of data | Displays the chart grid lines in front of the graphical data. |
| Export | Click to display a dialog box that enables you to save the binding chart to a graphic file or print the chart. |
| Maximize | Maximizes the display of the binding chart. |
| **Axis Tab** | |
| Linear | Choose this option to display a linear scale x- or y-axis. |
| Log | Choose this option to display a log scale x- or y-axis. |

| Component in the Customization Tab | Description |
|---|---|
| Auto | Choose this option to automatically optimize the x- and y-axis display. |
| Min | Choose this to option to specify a minimum for the x- or y-axis. |
| Max | Choose this option to specify a maximum for the x- or y-axis. |
| Min/Max | Choose this option to specify a minimum and maximum for the x- or y-axis. |

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