

Quick Start Guide

Octet® BLI System

Octet® R8 BLI, Octet® BLI Discovery & Octet® Analysis Studio Software
A Beginner's Guide



1000146444



SARTORIUS

Contents

1	About this Document	6
1.1	Validity	6
1.2	Related Documents	6
1.3	Required Materials and Reagents	7
2	System Preparation	8
2.1	Loading the Biosensor Tray and Sample Plates	8
2.1.1	Preparing the Biosensor Tray	8
2.1.2	Loading the Biosensor Tray and Sample Plate	9
2.1.3	Rehydration	9
2.1.4	Required time for temperature equilibration	9
2.1.5	Microplate evaporation cover	9
2.2	Cleaning the Octet® R8 system	10
3	Assay 1 – Kinetics and Affinity Assay Loading Optimization	11
3.1	Assay Setup	11
3.2	Parameters	12
3.3	Duration	12
3.4	Materials Required	12
3.5	Loading Optimization – Plate Definition	13
3.6	Loading Optimization – Selecting Wells in the Sample Plate Map	14
3.7	Loading Optimization – Designating Well Types	15
3.8	Loading Optimization – Entering Sample Information	17
3.9	Loading Optimization – Assigning User-Specified Sample Concentrations	19
3.9.1	Assigning Sample Concentrations Using a Dilution Series	19
3.9.2	Selecting the Dilution Series option	19
3.9.3	Assigning a Concentration to the Human IgG Samples	21
3.10	Loading Optimization – Assay Definition	22
3.10.1	Creating different types of assay steps	23
3.10.2	Adding Assay 1 Parameters	24
3.11	Building an Assay	24
3.11.1	Starting the experiment	26
3.12	Change of Experiment Conditions	27
4	Assay 1 – Sample and Hydration Plate Preparation	28
4.1	Hydration Plate	28
4.2	Biotin Protein A	28
4.3	Human IgG	29
4.4	10X Kinetics Buffer	29
4.5	Run the Experiment	29
5	Assay 1 – Opening Experiment Files and Loading Optimization	30
5.1	Loading Optimization – Data Analysis	30
5.2	Pre-analysis Data Correction	30
5.3	Reference Sensor Subtraction	31
5.3.1	Reference Sample Correction	32
5.3.2	Data Correction	32
5.3.3	Step 1: Aligning the Y Axis	33
5.3.4	Step 2: Inter-step Correction	33
5.3.5	Step 3: Filtering	33

5.4	Analyzing Your Kinetic Data.....	34
5.4.1	Step 1: Choose the Step(s) to Analyze.....	34
5.4.2	Step 2: Choose a Binding Model.....	34
5.4.3	Step 3: Set Fitting Parameters.....	35
5.4.4	Step 4: Review the Data Fit Quality.....	36
5.5	Selecting the Ligand Concentration.....	38
5.5.1	Selecting the Analyte Concentration and Assay Parameters.....	40
6	Assay 2 – Kinetics and Affinity Determination.....	42
6.1	Assay Setup.....	42
6.2	Parameters.....	43
6.3	Duration.....	43
6.4	Materials Required.....	43
6.5	Kinetics and Affinity Determination – Plate Definition.....	44
6.6	Kinetics and Affinity Determination – Sample Information.....	44
6.7	Kinetics and Affinity Determination – Assigning User-Specified Sample Concentrations.....	44
6.8	Kinetics and Affinity Determination – Assay Definition.....	45
6.8.1	Starting the Experiment.....	45
7	Assay 2 – Sample and Hydration Plate Preparation.....	46
7.1	Hydration Plate.....	46
7.2	Biotin Protein A.....	46
7.3	Human IgG.....	46
7.4	10X Kinetics Buffer.....	47
7.5	Loading the Sample Plate.....	47
7.6	Run the Experiment.....	47
8	Assay 2 – Opening Experiment Files and Kinetics and Affinity Determination.....	48
8.1	Reference Sensor Subtraction.....	48
8.2	Reference Sample Subtraction.....	48
8.3	Subtracting Baseline Drift.....	49
8.4	Data Correction.....	49
8.5	Analyzing Your Kinetic Data.....	50
8.6	Review the Data Fit Quality.....	51
8.7	What to do Next?.....	52
9	Assay 3 – Quantitation Assay.....	53
9.1	Quantitation with Regeneration.....	53
9.2	Parameters.....	54
9.3	Duration.....	54
9.4	Materials Required.....	54
9.5	Quantitation with Regeneration – Plate Definition.....	55
9.6	Select and Designate Well Types.....	56
9.7	Entering Sample Information.....	57
9.8	Assigning User-Specified Sample Concentrations.....	57
9.9	Quantitation Assay – Replicate Groups.....	58
9.9.1	Assigning “Replicate Groups” in the “Sample Plate Map”.....	58
9.9.2	Assigning “Replicate Groups” in the “Replicate Group column” (alternative).....	59
9.10	Quantitation Assay – Managing Assay Parameter Settings.....	59
9.11	Quantitation Assay – Assigning Biosensors to Samples.....	60

10 Assay 3 – Sample and Hydration Plate Preparation	61
10.1 Hydration Plate	61
10.2 Human IgG	61
10.3 Unknowns	62
10.3.1 Regeneration Solution	62
10.3.2 Neutralization Solution	62
10.4 Loading the Sample Plate	63
10.5 Run the Experiment	63
11 Assay 3 – Opening Experiment Files and Quantitation with Regeneration	64
11.1 Setting Reference Wells and Changing Well Types	64
11.2 Quantitation Analysis	65
11.2.1 Step 1: Fit the Standard Curve	65
11.2.2 Step 2: Set Processing Parameters	65
11.2.3 Step 3: Setting Standard Sample Alerts	65
11.3 Viewing Analysis Results	66
11.4 What to do Next?	68
12 Frequently Asked Questions	69
12.1 Hydration Plate	69
12.2 Sample Plate	70
12.3 U- or V- shaped Plates	70
13 Trademark Information	70

1 About this Document

1.1 Validity

This document applies to the following versions of the device:

Device

Octet® R8

Octet® BLI Discovery

Octet® Analysis Studio

This document provides the basic steps in setting up and analyzing an Octet® BLI kinetics and affinity assay and an Octet® quantitation assay. The reagents in the Installation Kit are supplied in quantities that allow multiple repeats of the assays.

The scope of this quick start guide is to provide easy and fast access to general kinetics and affinity and quantitation experimental set-up conditions. Octet® BLI users should use this document to answer simple questions about the software and experimental design. For more in-depth inquiries, scientists should contact their local Octet® Field Applications Specialist (FAS).

1.2 Related Documents

- ▶ In addition to these instructions, please observe the following documents:
 - Operating instructions for Octet® BLI Discovery and Octet® BLI Analysis User Guides, supplied with the Octet® BLI system
 - Octet® BLI System: A Practical Guide
 - For more ideas and information visit "[A Compendium for Successful BLI and SPR Assays](#)"

1.3 Required Materials and Reagents

This document is designed to use reagents supplied with the Octet® BLI system when purchased. To perform the assays shown in this document users will require:

- Octet® Streptavidin (SA) Biosensor
 - Item No.: 18-5019 (1 Tray) | 18-5020 (5 Trays) | 18-5021 (20 Trays)
- Octet® Protein A (ProA) Biosensors
 - Item No.: 18-5010 (1 Tray) | 18-5012 (5 Trays) | 18-5013 (20 Trays)
- Octet® ProA Calibrator Set, Item No.: 18-1118
- Octet® Sample Diluent, Item No.: 18-1104
- Octet® Kinetics Buffer 10X, Item No.: 18-1105
- Octet® 10mM Glycine pH 1.7, Item No.: 18-1184
- Biotin Protein A at 250 µg/mL (600 µL)

Visit <https://shop.sartorius.com/> to purchase or replenish any additional materials or reagents, using the item numbers shown above.

2 System Preparation

2.1 Loading the Biosensor Tray and Sample Plates

An integral part of every Octet® BLI assay is the preparation and loading of the biosensor tray and the sample plates. Preparation of the sample plate for a kinetics and affinity assay and quantitation assay are discussed in their relevant sections.

For more information see the [instruction video](#).

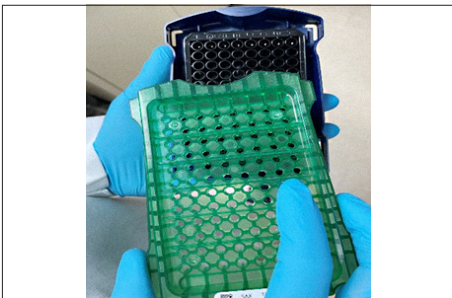
Loading of the biosensor tray and sample plate is required for every Octet® BLI assay and is performed as follows:

2.1.1 Preparing the Biosensor Tray

The biosensor tray consists of a hydration plate containing assay buffer and the required biosensors for the experiment.

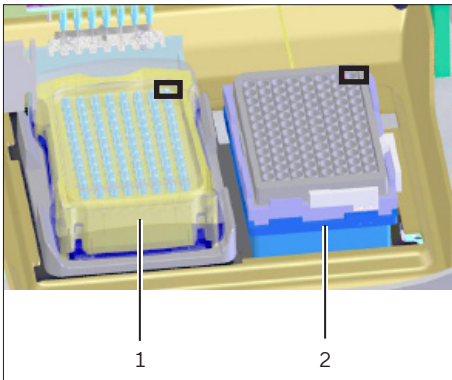
The assay buffer used for hydration purposes should match the assay buffer used for all sample dilutions and reagent preparations.

1. After preparing the method file, fill the appropriate number of wells of a standard black 96-well plate (hydration) with 200 μ L of assay buffer. There should be one well for each biosensor to be used.
2. Place the hydration plate in the blue bottom tray of a biosensor tray. The A1 corner should align to the top right of the blue tray.



3. Transfer the appropriate number of biosensors for the assay from their packaging to the green biosensor tray. Use of an Octet® AT transfer tool (Item No.: 18-5159) is recommended.
4. Place the green biosensor tray on top of the 96-well plate and blue bottom tray such that each of the biosensors is dipping into the wells with the assay buffer. The biosensor tray can then be loaded into the Octet® instrument.

2.1.2 Loading the Biosensor Tray and Sample Plate



1. Open the Octet® instrument door.
2. Place the biosensor tray (1) on the biosensor stage (left side) so that well A1 is located at the upper right corner.
3. Place the sample plate (2) on the sample stage (right side) so that well A1 is located at the upper right corner.
4. Ensure that the bottom of the sample plate and biosensor tray are flat on each stage.
5. Close the Octet® instrument door.
6. Allow the plate to equilibrate.

2.1.3 Rehydration

Before starting an experiment, ensure that the biosensors are properly rehydrated.

2.1.4 Required time for temperature equilibration

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For kinetics and affinity assays, we recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

2.1.5 Microplate evaporation cover

For kinetics and affinity assays, a microplate evaporation cover may also be used to prevent evaporation from samples during analysis and lengthen the experiment time (only applies to Octet® R8 instruments).

For more information see the [instruction video](#).

For more information, see “Microplate Evaporation Cover” on page 64 of the Octet® BLI Discovery User Guide.

2.2 Cleaning the Octet® R8 system

In general, Octet® BLI systems are essentially maintenance free but for best performance of the system it is recommended that cleaning of the system should be performed every two weeks or after any period of extended heavy usage. The following protocol should be used for cleaning the pick-up tips.

For more information see the [instruction video](#).



Fig. 1: Octet® R8 pick-up tips (door open)

1. Turn the instrument power off.
2. Open the instrument door by pulling up on the yellow handle.
3. Spray a Kimwipe® tissue with 70% isopropyl alcohol.
4. Wipe the sides of the pick-up tips with the wet Kimwipe® tissue (Do not worry about touching the lenses on the bottom, they are set back, and the wipe will not contact the lens).



5. Allow the pick-up tips to dry for at least one minute (with the instrument door open).
6. Close the instrument door by pulling down on the yellow handle.
7. Turn the instrument power on and allow the lamps to warm up prior to performing any assays.

3 Assay 1 – Kinetics and Affinity Assay Loading Optimization

In assay 1, the general assay setup uses Octet® Streptavidin (SA) Biosensors to capture varying levels of biotinylated Protein A and the association and dissociation of a single concentration of human IgG is determined.

For more information see the [instruction video](#).

After data analysis, which includes a check for non-specific binding (NSB) of analyte to the biosensor, a suitable biotinylated Protein A concentration is chosen for use in assay 2.

3.1 Assay Setup

The aim of this section is to create an Octet® BLI method containing a sample plate with the following samples and parameters.

A new black, flat bottom 96-well plate should be used for both the biosensor tray and sample plate.

It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types will be included in the assay.

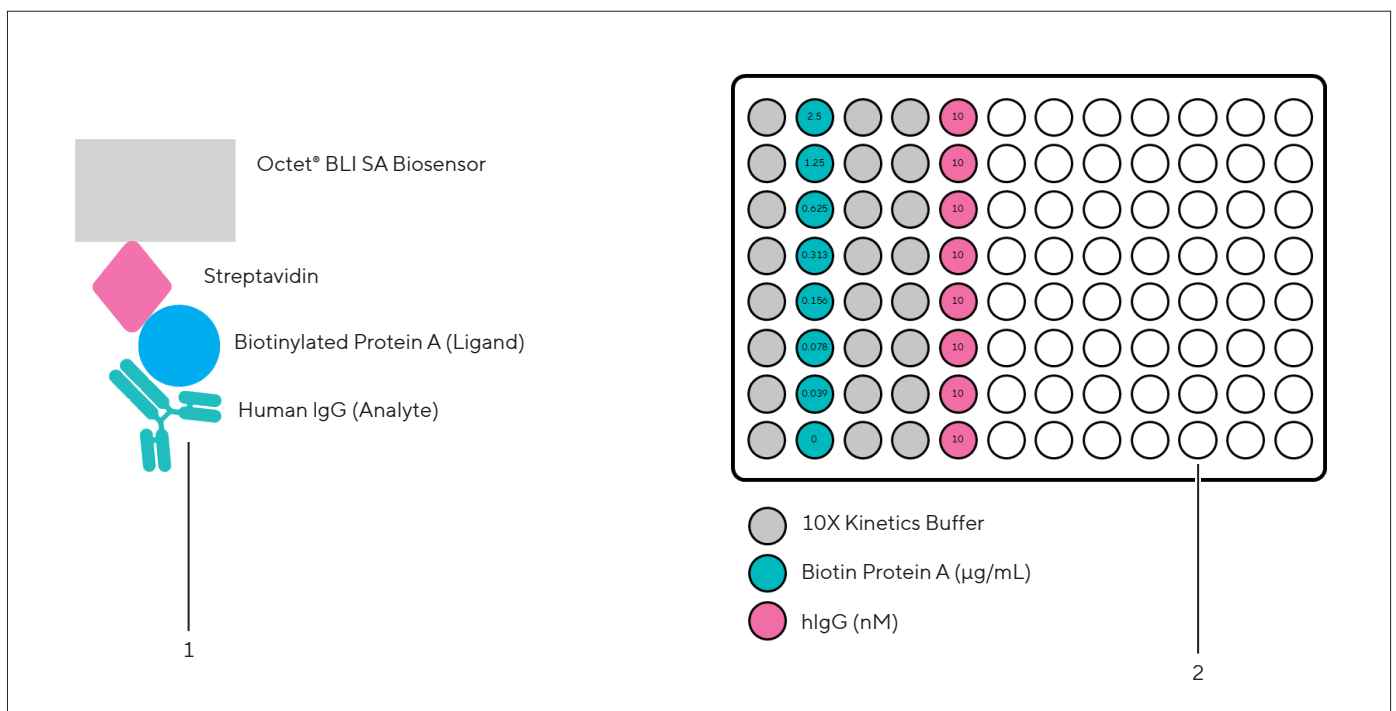


Fig. 2: Assay setup

Pos.	Name
1	General setup
2	Sample plate

3.2 Parameters

Step Number	Step Name	Time (S)	Shake Speed (RPM)	Column Position
1	Baseline	60	1000	1
2	Loading	300	1000	2
3	Baseline 2	30	1000	3
4	Baseline	60	1000	4
5	Association	600	1000	5
6	Dissociation	600	1000	4

3.3 Duration

Type	Duration
Sample Preparation Time	~10 minutes
Assay Duration	~40 minutes including biosensor hydration
Analysis Duration	~10 minutes

3.4 Materials Required

- Octet® ProA Calibrator Set 300 µg/mL Solution, Item No.: 18-1118
- Octet® Kinetics Buffer 10X, Item No.: 18-1105
- Biotin Protein A at 250 µg/mL
- Octet® Streptavidin (SA) Biosensor, Item No.: 18-5019 (1 Tray) | 18-5020 (5 Trays) | 18-5021 (20 Trays)

3.5 Loading Optimization – Plate Definition

It is highly recommended that new users read Chapter 9 – “Kinetics Experiments” of the “Octet® BLI Discovery User Guide” prior to performing the assay. A walkthrough version tailored to the needs of a new user is used here.

For more information see the [instruction video](#).

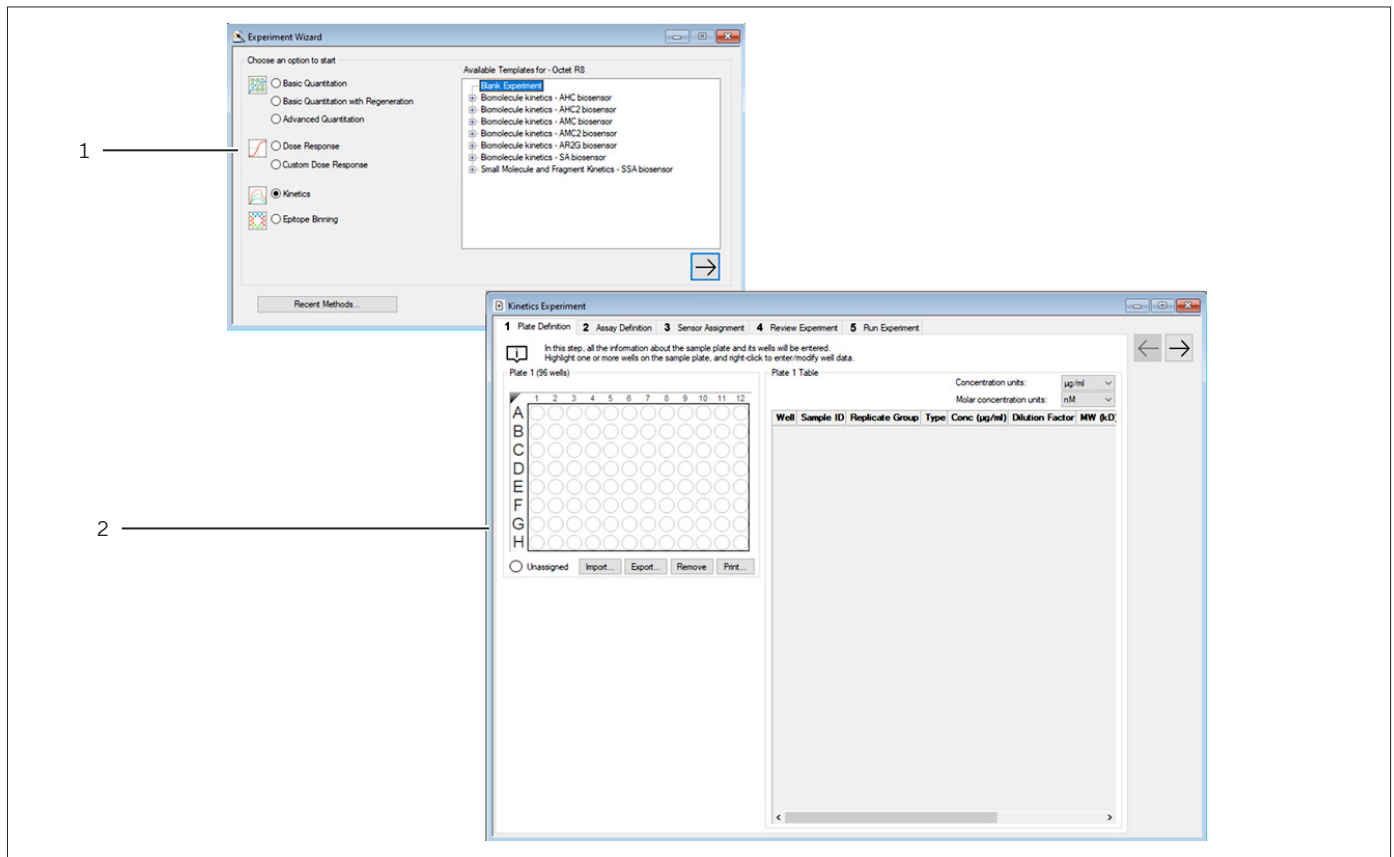


Fig. 3: Experiment wizard and kinetics and blank experiment window

Pos.	Name
------	------

1	Experiment Wizard
2	Kinetics and Blank Experiment

1. Launch the Octet® BLI Discovery software by double clicking on the Octet® BLI Discovery desktop icon.
2. Ensure the instrument is at the correct temperature for the assay. Click the “Experiment Menu” from the toolbar and select “Set Plate Temperature”. Ensure the current temperature is set to 30 °C. If not, set the temperature to 30 °C and click “OK”. This ensures the instrument is at the correct temperature prior to performing the assay.
3. From the Experiment Menu, select “New Experiment Wizard” or alternatively press “CTRL + N” or click the New Experiment Wizard toolbar button.
4. Select “Kinetics and Blank Experiment” and press “Enter” or click the “forward arrow”. The Basic Kinetics Experiment window will appear.

3.6 Loading Optimization – Selecting Wells in the Sample Plate Map

There are several ways to select wells in the Sample Plate Map (See Chapter 9 – Selecting Wells in the Sample Plate Map of the Octet® BLI Discovery User Guide). The easiest method is to click a column header or select adjacent column headers by click-hold-drag.

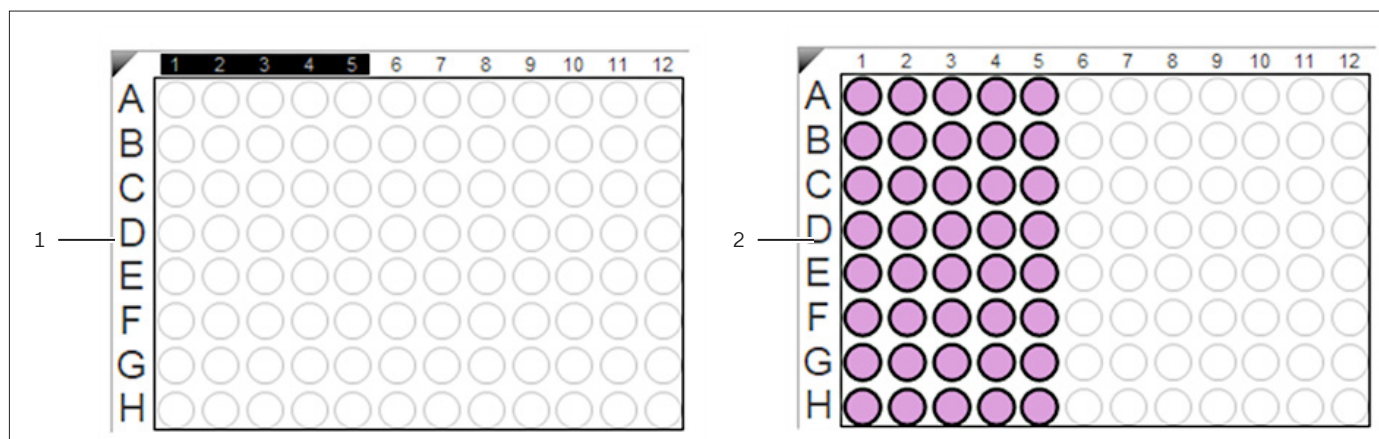


Fig. 4: Selecting columns and wells

Pos.	Name
1	Sample plate with selected columns
2	Sample plate with wells in columns

1. For assay 1, hold the Ctrl key and select columns 1, 2, 3, 4 and 5, which will select all wells in columns 1 – 5.

3.7 Loading Optimization – Designating Well Types

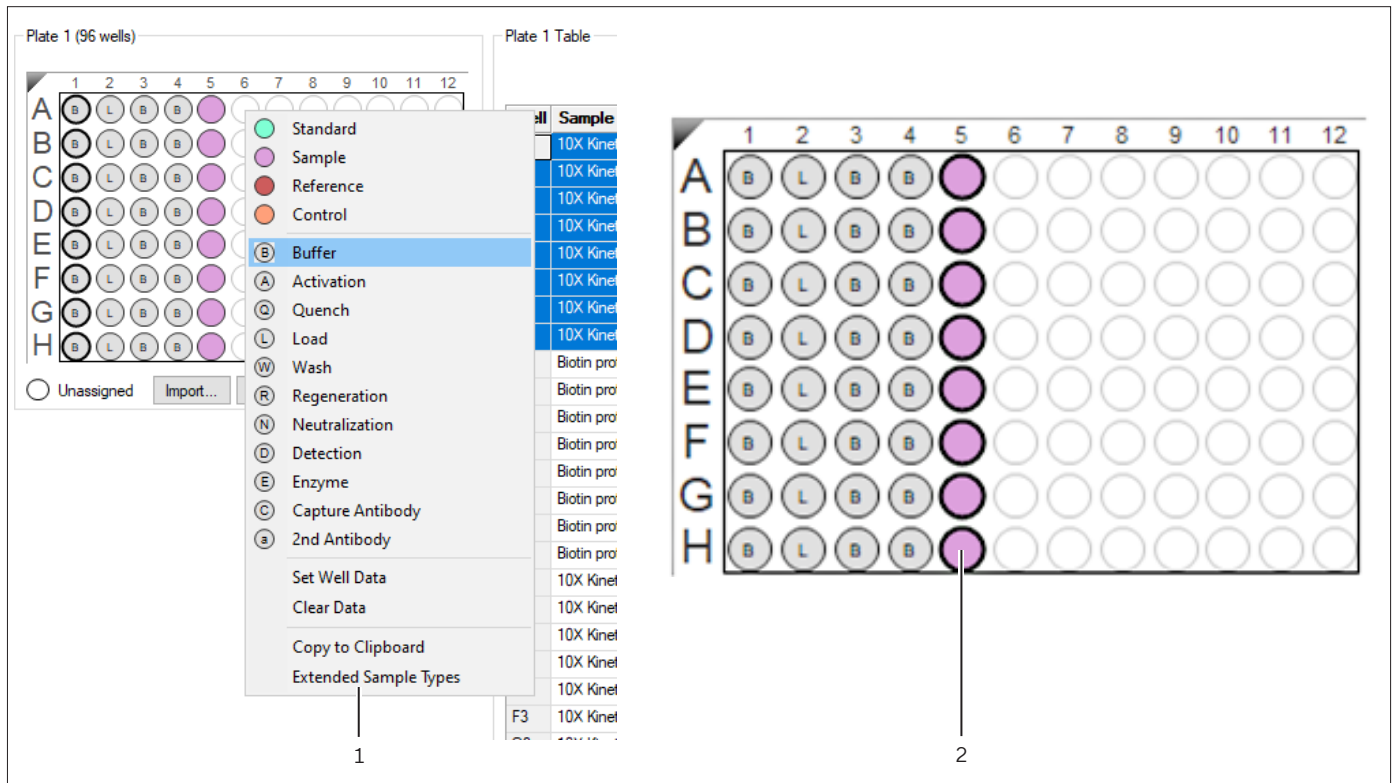


Fig. 5: Designating a well type in the plate definition window

Pos.	Name
1	Designating a well type
2	Assay 1 plate definition.

- In the Sample Plate Map, select the wells required by clicking the column header (Column 1), right-click and select the correct sample type. For more information see the [instruction video](#).
- Assign the following well types to each column:

Column Number	Well Type
1	Buffer
2	Load
3	Buffer
4	Buffer
5	Sample

- If required, to remove a well designation, select the well(s) and click "Remove" or, right-click the well(s) and select "Clear Data".
- The final Sample Plate Map should look like the assay 1 plate definition.

Alternative Method of Designating the Plate

1. An alternative method of designating the plate is to Highlight columns 1, 3 and 4, right click on an active well and select Buffer, highlight column 2, right click and select load, highlight column 5, right click and select sample.

3.8 Loading Optimization – Entering Sample Information

You must specify sample (analyte) concentration or the molecular weight and concentration for the software to calculate sample concentration. Otherwise, the Octet® BLI Analysis Studio software cannot determine a k_a or K_D value. If the sample concentration is not specified, only k_d and k_{obs} are calculated.

For more information see the [instruction video](#).

There are several ways to enter sample information (See Chapter 9 – Entering Sample Information in the Octet® BLI Discovery User Guide).

The easiest method is to highlight the desired cells in the Plate Table:

Plate 1 (96 wells)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							
B	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							
C	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							
D	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							
E	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							
F	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							
G	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							
H	Ⓟ	Ⓟ	Ⓟ	Ⓟ	Ⓟ							

Plate 1 Table

Well	Sample ID	Replicate Group	Type	Conc
A1			Ⓟ Buffer	
B1			Ⓟ Buffer	
C1			Ⓟ Buffer	
D1			Ⓟ Buffer	
E1			Ⓟ Buffer	
F1			Ⓟ Buffer	
G1			Ⓟ Buffer	
H1			Ⓟ Buffer	
A2			Ⓟ Load	
B2			Ⓟ Load	
C2			Ⓟ Load	
D2			Ⓟ Load	
E2			Ⓟ Load	

Table View 3

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)
A1	10X Kinetics Buffer		Ⓟ Buffer	
B1			Ⓟ Buffer	
C1			Ⓟ Buffer	
D1			Ⓟ Buffer	
E1			Ⓟ Buffer	
F1			Ⓟ Buffer	
G1			Ⓟ Buffer	
H1			Ⓟ Buffer	
A2			Ⓟ Load	
B2			Ⓟ Load	

Table View 4

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)
A1	10X Kinetics		Ⓟ Buffer	
B1	10X Kinetics		Ⓟ Buffer	
C1	10X Kinetics		Ⓟ Buffer	
D1	10X Kinetics		Ⓟ Buffer	
E1	10X Kinetics		Ⓟ Buffer	
F1	10X Kinetics		Ⓟ Buffer	
G1	10X Kinetics		Ⓟ Buffer	
H1	10X Kinetics		Ⓟ Buffer	
A2			Ⓟ Load	
B2			Ⓟ Load	

Fig. 6: Entering sample information

Pos.	Name
------	------

- | | |
|---|---|
| 1 | Selecting a single well or column on the sample plate |
| 2 | Associated cells are highlighted in the table |
| 3 | Entering sample information |
| 4 | Text is copied to any highlighted cell |

1. Select a single well or column on the sample plate to highlight the associated cell in the table.
2. Directly enter the text into a single cell.
3. To copy the text each highlighted cell press “Enter”.
4. Assign the following Sample ID to each well:

Wells	Sample ID
A1 - H1	10X Kinetics Buffer
A2 - H2	Biotin protein A
A3 - H3	10X Kinetics Buffer
A4 - H4	10X Kinetics Buffer
A5 - H5	Human IgG

3.9 Loading Optimization – Assigning User-Specified Sample Concentrations

3.9.1 Assigning Sample Concentrations Using a Dilution Series

A 1 in 2 dilution series of biotin protein A is used in assay 1 and the ligand concentration should be entered at the plate definition stage. The simplest method is to select the desired cell in the concentration column and enter the number manually, but the most practical method is by using the “Set Well Data” software feature.

For more information see the [instruction video](#).

1. In the “Sample Plate Map (or table)”, select the wells A2 – G2 (H2 is not selected as a zero concentration will be added), right-click and select “Set Well Data”.
2. The “Set Well Data” dialog box will appear.

3.9.2 Selecting the Dilution Series option

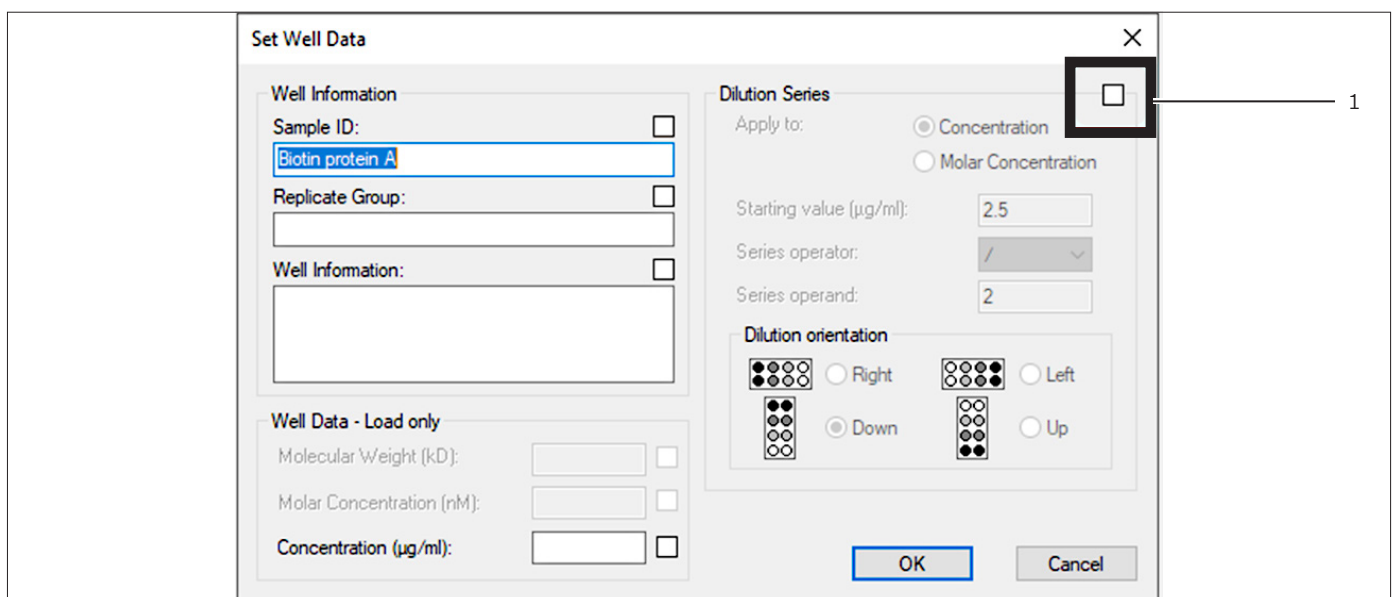


Fig. 7: Sample plate map – assigning sample concentrations by value

Pos.	Name
1	Dilution Series Option

1. Select the Dilution Series option and enter the starting concentration value (2.5 µg/mL). The “Series Operator” and “Series Operand” should be left as / and 2, respectively and the “Dilution Orientation” should be left as “Down”.
2. Click “OK”.

The Sample Plate Table then displays the biotin protein A concentration series.

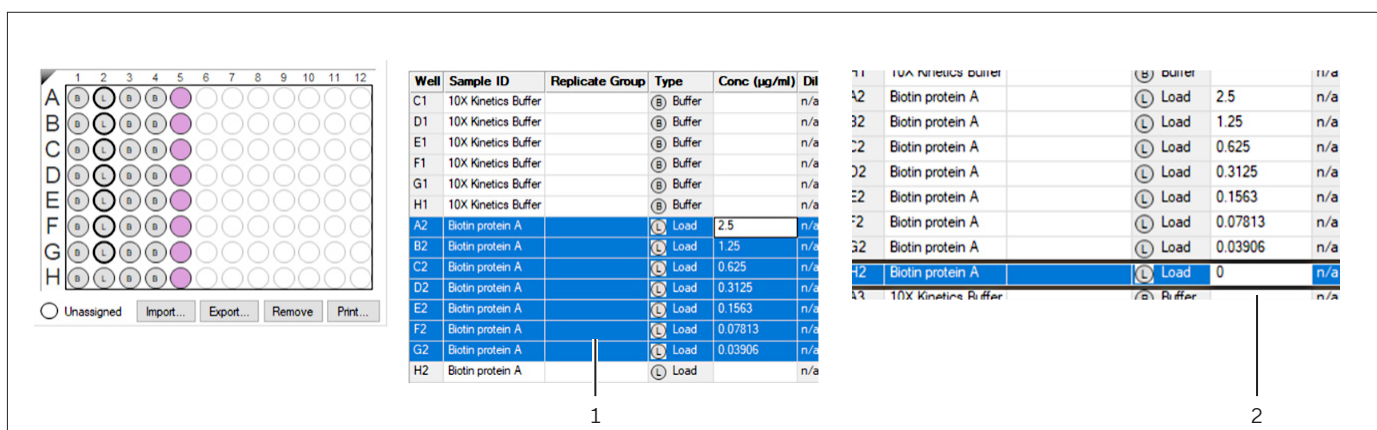


Fig. 8: Sample plate table

Pos.	Name
1	Biotin protein A concentration series
2	Zero value for the concentration series, entered manually

1. In the Sample Table Select cell H2.
2. Insert 0 for the concentration.

3.9.3 Assigning a Concentration to the Human IgG Samples

The final step of defining the plate setup is to assign a concentration to the Human IgG samples. As with entering sample information (see chapter 3.8, page 17), single concentrations can be inserted using a single value.

Pos.	Name	Sample	Molar Conc (nM)
A5	Human IgG	Sample	10
B5	Human IgG	Sample	10
C5	Human IgG	Sample	10
D5	Human IgG	Sample	10
E5	Human IgG	Sample	10
F5	Human IgG	Sample	10
G5	Human IgG	Sample	10
H5	Human IgG	Sample	10

Fig. 9: Initial layout of the assay definition tab

Pos.	Name
------	------

- | | |
|---|--|
| 1 | Molar concentration column in the sample table |
| 2 | Entered text is copied to any highlighted cell in the sample table |

1. Select the Molar Conc (nM) column cells for A5 – H5.
2. Insert 10 and then click “Enter”.

3.10 Loading Optimization – Assay Definition

Like plate definition (see chapter “3.5 Loading Optimization – Plate Definition”, page 13), Assay Definition follows two simple steps:

- Define the step types
- Build the assay by assigning a step type to a column(s) in the sample plate

Initially the plate layout and a single Baseline step are shown and additional step types must be added to the “Step Data List”.

For more information see the [instruction video](#).

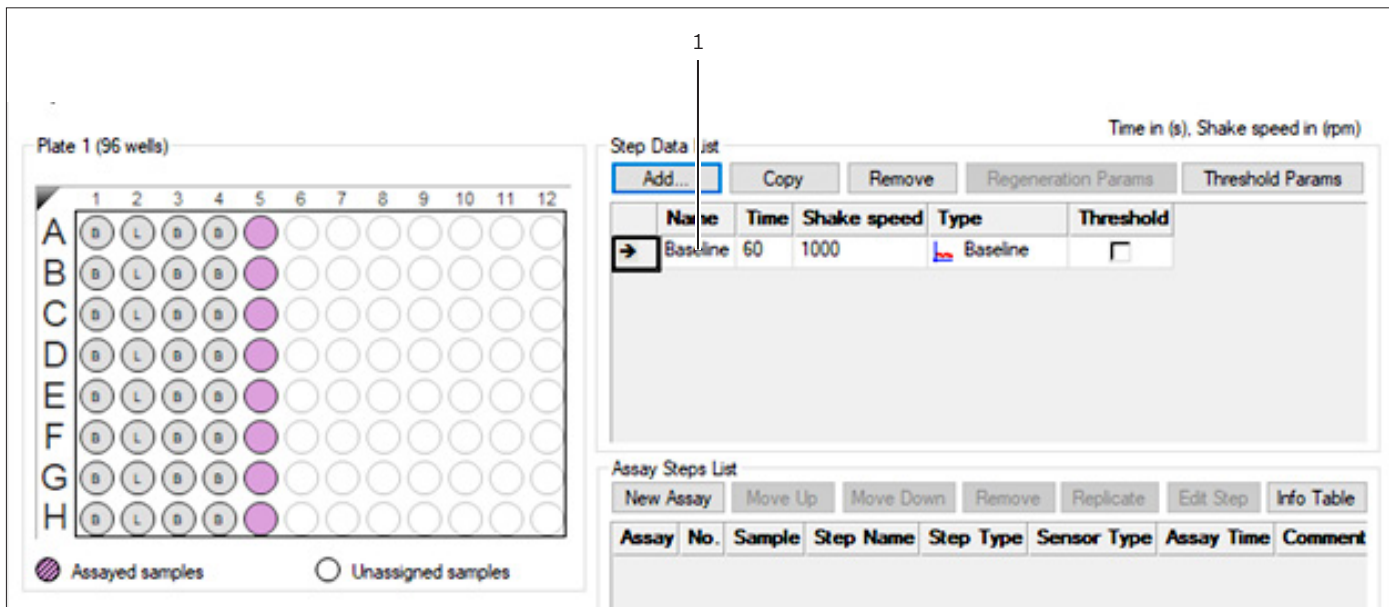


Fig. 10: Assay definition

Pos.	Name
1	Baseline step

3.10.1 Creating different types of assay steps

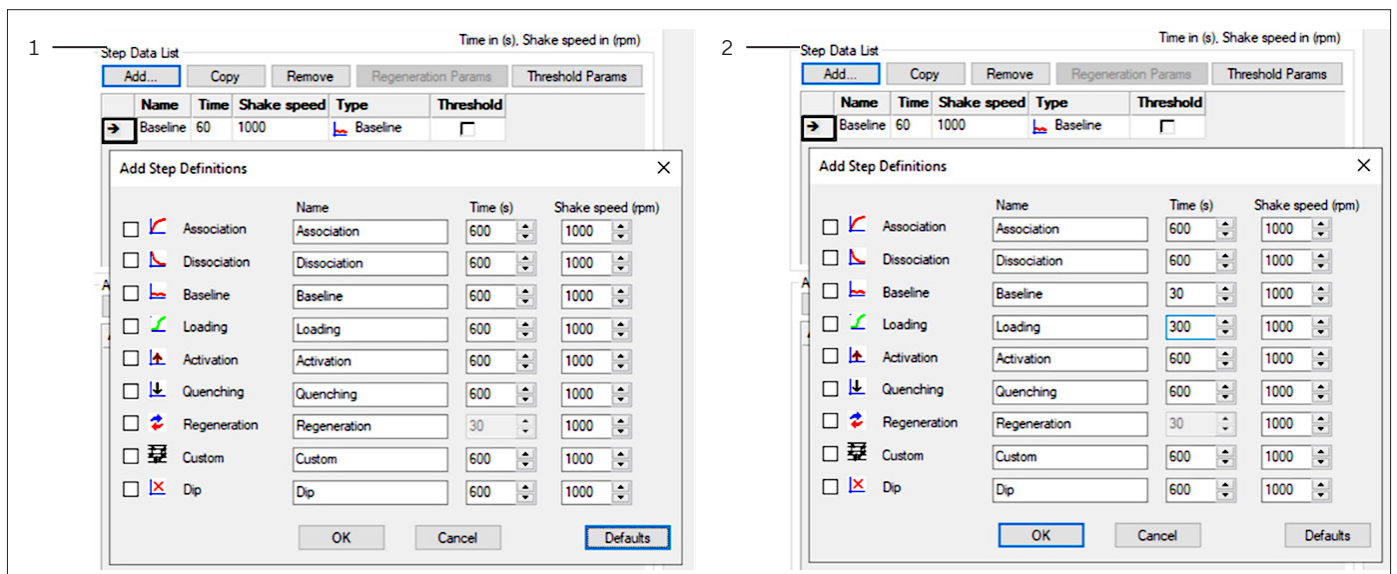


Fig. 11: Add step Definitions window to add multiple assay parameters in a single step

Pos.	Name
------	------

1	Assay Step Definition dialog box
2	Entered Parameters

1. Click "Add".
2. In Assay Step Definition dialog box, specify the step information:
 - Choose a step type.
 - Set the step time and shake speed
 - Time range: 2 to 48,000 seconds
 - Shake speed: Off 0 rpm or On (100 to 1,500 rpm)
3. Click OK to save the newly defined step. The new step type appears in the "Step Data List".
4. Create all assay step types in a single addition or repeat the previous steps for each step type to create until all the desired steps are added.

3.10.2 Adding Assay 1 Parameters

The following step types and parameters are required:

Step Number	Step Name	Time (S)	Shake Speed (RPM)
1	Baseline	60	1000
2	Loading	300	1000
3	Baseline 2	30	1000
4	Baseline	60	1000
5	Association	600	1000
6	Dissociation	600	1000

1. To add all parameters in a single step – Click “Add”.
2. The “Add Step Definitions” window appears.
3. “Select” Association, Dissociation, Baseline and Loading
4. Update the time settings in the “Add Step Definition” to match those in the table above:
 - Association (600 s)
 - Dissociation (600 s)
 - Baseline (30 s)
 - Loading (300 s)
5. Ensure the Shake Speed (rpm) is set to 1000.
6. Click “OK”.
7. The software recognizes that the step name “Baseline” already exists and asks if you want to use “Baseline2” instead. Accept this.
8. Click “OK”.
9. The added parameters will appear in the “Step Data List”.

3.11 Building an Assay

After creating the different step types that the assay will use, step types are assigned to columns in the Sample Plate. For more information see the [instruction video](#).

1. Select a step type in the “Step Data List”.
2. In the Sample Plate Map, double-click the column that is associated with the selected step type.

To build the assay shown here:

Step Number	Step Name	Time (S)	Shake Speed (RPM)	Column Position
1	Baseline	60	1000	1
2	Loading	300	1000	2
3	Baseline 2	30	1000	3
4	Baseline	60	1000	4
5	Association	600	1000	5
6	Dissociation	600	1000	4

1. Select “Baseline” in the “Step Data List”.
2. In the Sample Plate Map, double-click column 1 that is associated with the selected step type.
3. Select “Loading” in the “Step Data List”.
4. In the Sample Plate Map, double-click column 2 that is associated with the selected step type.
5. Repeat the previous steps to define each step in the assay.

As each step is added, the total Experiment and Assay Time are updated.

The screenshot displays the software interface for configuring an assay. On the left is a 96-well plate map labeled 'Plate 1 (96 wells)' with columns 1-12 and rows A-H. Column 1 is shaded grey, and column 5 has a purple circle in each well. A legend below the plate shows a purple circle for 'Assayed samples' and a white circle for 'Unassigned samples'. On the right is the 'Step Data List' table with buttons for 'Add...', 'Copy', 'Remove', 'Regeneration Params', and 'Threshold Params'. The table lists steps: Baseline (60s), Association (600s), Dissociation (600s), Loading (300s), and Baseline2 (30s). The 'Dissociation' row is selected with a mouse cursor. Below this is the 'Assay Steps List' table with buttons for 'New Assay', 'Move Up', 'Move Down', 'Remove', 'Replicate', 'Edit Step', and 'Info Table'. It shows one step: Assay No. 1, Sample 1, Step Name Baseline, Step Type Baseline, Sensor Type SA (Streptavidin), and Assay Time 0:01:30. A vertical line connects the 'Assay No.' column of the 'Assay Steps List' to the 'Step Data List'.

Fig. 12: Assigning a step type to a column in the sample plate

Pos.	Name
1	Baseline step

3.11.1 Starting the experiment

If required, the final step of assay definition is to change the default sensor type to a user specified sensor type (for example here, SAX (High Precision Streptavidin) to SA (Streptavidin)).

For more information see the [instruction video](#).

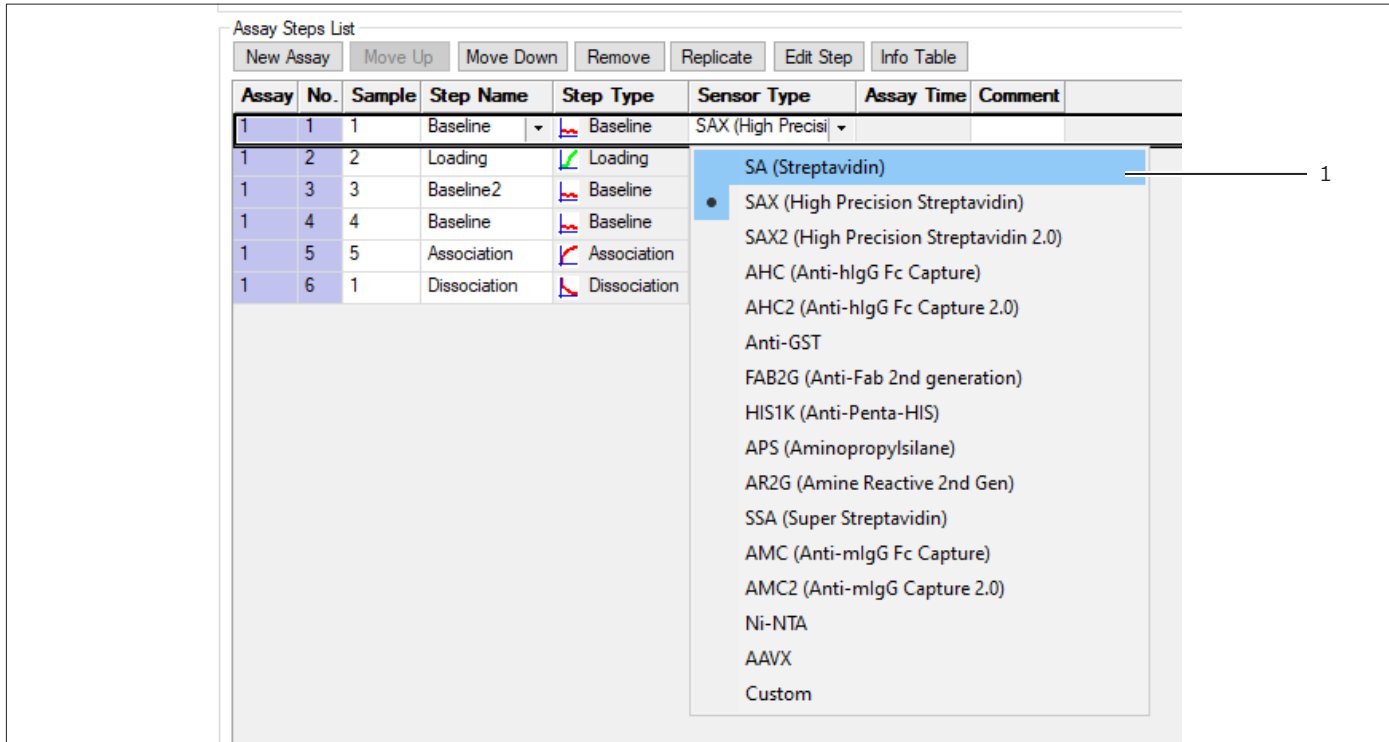


Fig. 13: Selecting an assay sensor type

Pos.	Name
1	Selection of SA (Streptavidin)

- Click the drop-down arrow in Sensor Type and click SA.
- Click through to the "Sensor Assignment" tab. Biosensors are automatically positioned in column 1.
 - Where required, biosensor position can be changed by highlighting the desired column(s) and clicking "Remove".
 - After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container.
 - To return the biosensors to the tray, click the "Replace sensors in tray after use" check box.

3. Here, the biosensors will not be reused so untick the “Replace sensors in tray after use” check box.
4. Click through to the “Review Experiment” tab. Using the assay step arrow, click through the assay to ensure the correct step sequence has been input (Columns 1, 2, 3, 4, 5, 4).
 - If incorrect, return to the Assay Definition tab and correct the column associated with the assay step. If correct, click through to the “Run Experiment” tab.
 - In the Kinetics data repository section, click the three dots and select a suitable location for saving the assay. It is recommended to make a separate folder for assays performed during the getting started guide (for example C:\OctetData\Getting Started Guide).
 - Then in the experiment run name, enter Assay 1 - Loading Optimization.

3.12 Change of Experiment Conditions

In the Run Settings and Advance Settings boxes you can change some of the experiment conditions if necessary, but the default settings are usually the best choice. See page 197 of Octet® BLI Discovery User Guide for further information.

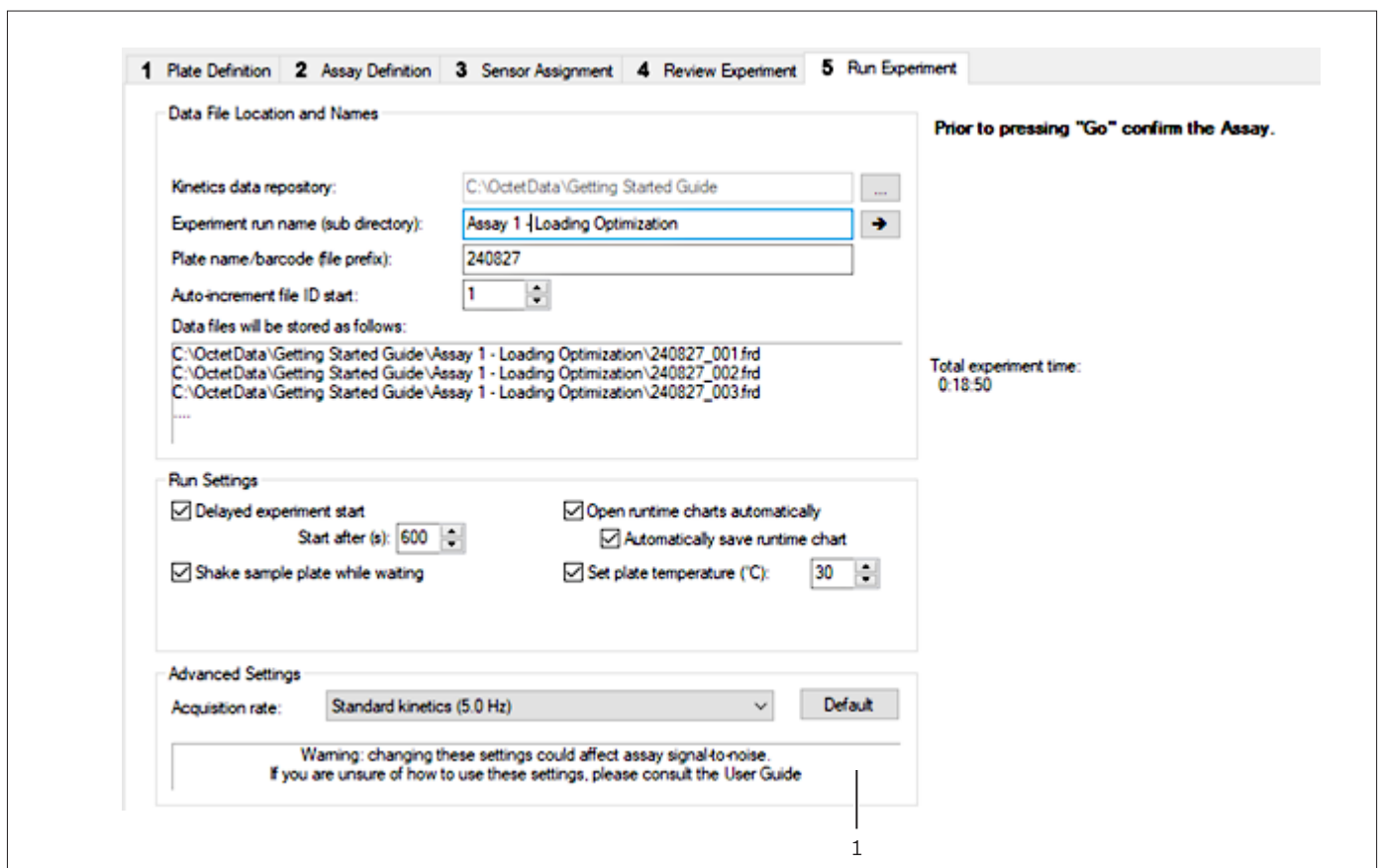


Fig. 14: Run Experiment Window – Octet® R8

Pos.	Name
1	Run Experiment Window

4 Assay 1 – Sample and Hydration Plate Preparation

4.1 Hydration Plate

1. Add 200 μL of 10X Kinetics Buffer to sample plate positions A1 – H1.
2. Transfer eight (8) Octet® BLI SA Biosensors to an empty biosensor tray.
3. Prepare and load the hydration plate (see chapter “2.1 Loading the Biosensor Tray and Sample Plates”, page 8).

4.2 Biotin Protein A

Biotin Protein A is supplied at a concentration of 250 $\mu\text{g}/\text{mL}$. A top concentration of 2.5 $\mu\text{g}/\text{mL}$ with enough volume to perform a 1 in 2 dilution step is required.

1. In an Eppendorf tube, add 10 μL of 250 $\mu\text{g}/\text{mL}$ biotin Protein A stock to 990 μL 10X Kinetics Buffer and mix thoroughly.
2. Prepare the biotin Protein A concentration series from the 2.5 $\mu\text{g}/\text{mL}$ sample:
 - In an Eppendorf tube mix 250 μL of 2.5 $\mu\text{g}/\text{mL}$ biotin Protein A with 250 μL of 10X Kinetics Buffer to make 1.25 $\mu\text{g}/\text{mL}$ biotin Protein A.
3. Continue the dilution series to obtain biotin Protein A solutions with:
 - 0.625 $\mu\text{g}/\text{mL}$
 - 0.313 $\mu\text{g}/\text{mL}$
 - 0.156 $\mu\text{g}/\text{mL}$
 - 0.078 $\mu\text{g}/\text{mL}$
 - 0.039 $\mu\text{g}/\text{mL}$
4. Add 200 μL of each biotinylated Protein A concentration to the correct position on the sample plate shown in the table below and add 200 μL of 10X Kinetics Buffer to position H2.

Sample Plate Position	Biotinylated Protein A Final Concentration ($\mu\text{g}/\text{mL}$)
A2	2.5
B2	1.25
C2	0.625
D2	0.313
E2	0.156
F2	0.078
G2	0.039
H2	0

5. Place any remaining 2.5 $\mu\text{g}/\text{mL}$ biotinylated protein solution on ice for use in Assay 2.

4.3 Human IgG

The Octet® ProA Calibrator Set contains a human IgG solution supplied at a concentration of 300 µg/mL (2 µM).

1. A top concentration of 10 nM with enough volume for eight (8) wells of 200 µL is required. Prepare the following dilution:

Human IgG Starting Concentration (nM)	Human IgG Final Concentration (nM)	Human IgG (µL)	10X Kinetics Buffer (µL)
2000	10	15	2985

2. Mix Thoroughly and add 200 µL of human IgG (10 nM) to positions E1 – H1 on the sample plate.
3. Place any remaining 10 nM human IgG solution on ice for use in assay 2.

4.4 10X Kinetics Buffer

1. Add 200 µL of 10X Kinetics Buffer to the following positions on the sample plate:
 - A1 – H1
 - A3 – H3
 - A4 – H4
2. Load the sample plate and biosensor tray (without lid) as shown in chapter “2.1 Loading the Biosensor Tray and Sample Plates”, page 8).
3. Ensure the instrument door is closed.

4.5 Run the Experiment

1. In the Octet® BLI Discovery Software ensure the instrument temperature matches the assay.
2. Select the Run Experiment tab and to start the experiment, click “GO”.

5 Assay 1 – Opening Experiment Files and Loading Optimization

5.1 Loading Optimization – Data Analysis

It is highly recommended that new users read Chapters 2, 3, 4 and 5 of the “Octet® Analysis Studio User Guide” prior to analyzing the data from Assay 1. A walkthrough version tailored to the needs of a new user is described here.

For more information see the [instruction video](#).

5.2 Pre-analysis Data Correction

To view assay 1 data in the Experiment Builder, open the data folder by double-clicking the magenta-colored data folder in Experiment Explorer.

Before analysis, the Preprocess Data Screen lets you subtract any non-specific binding or baseline drift data and make other data corrections if necessary.

1. Click the Preprocess Data tab to view the “Preprocess Data” screen.

5.3 Reference Sensor Subtraction

As shown in the Reference Sensor tab, biosensor H1 (pink line), which had no biotin Protein A loaded, shows no non-specific binding of the human IgG to the SA Biosensor.

Nevertheless, it is good practice to subtract non-specific binding in experiments where reference biosensors were used for analyte binding with no ligand present.

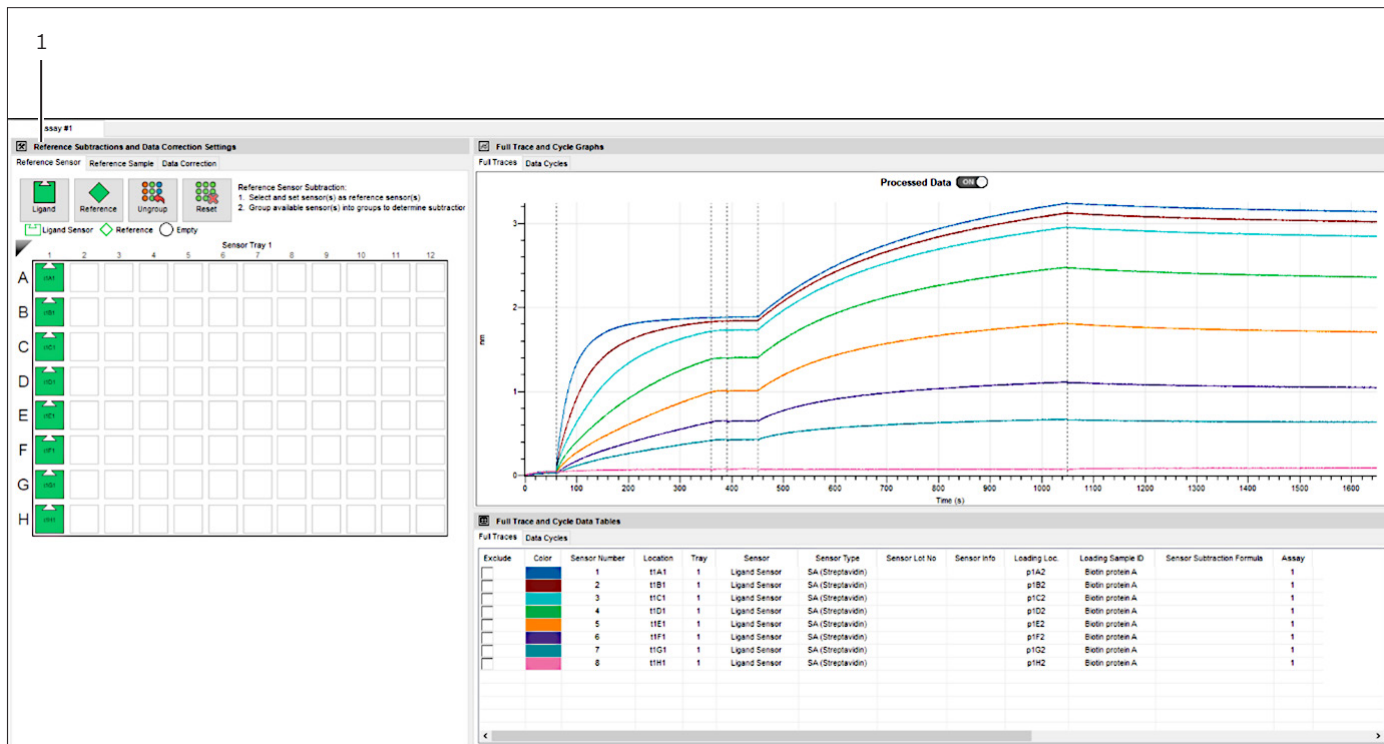
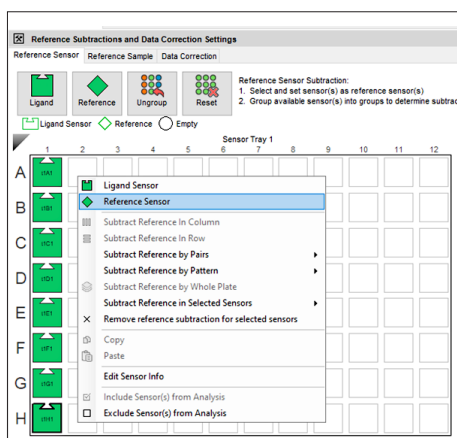
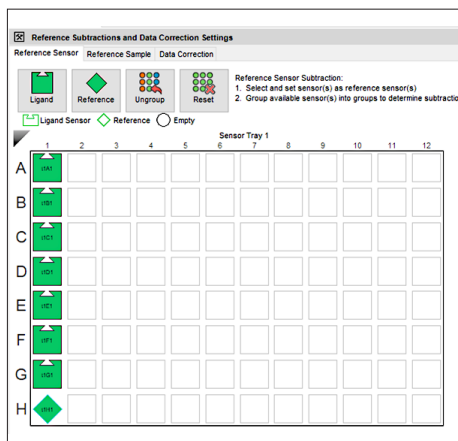


Fig. 15: Preprocess data screen

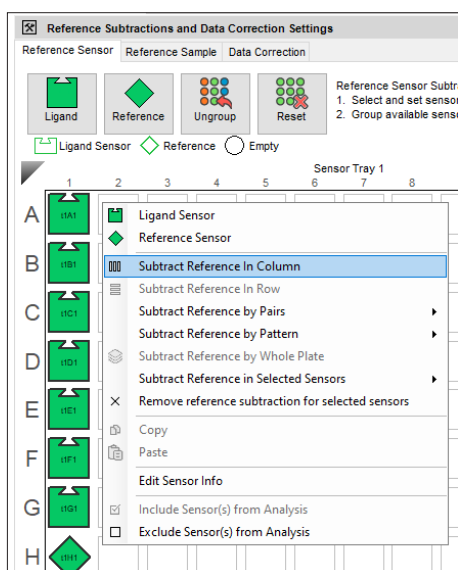
Pos.	Name
1	Reference Sensor tab



1. Select well H1 and then Right Click anywhere on the sample plate and select Reference Sensor.



- The selected wells appear as a diamond, to indicate it is set as reference well.



- To subtract the reference well – select the biosensor containing column, right click anywhere on the highlighted biosensors and select “Subtract Reference in Column”.

5.3.1 Reference Sample Correction

- To subtract any baseline drift in your experiment using reference sample wells that contained ligand but no analyte, click the “Reference Sample” tab.
- In assay 1, no buffer reference sample was used so modification to the Reference Sample tab is not required.

5.3.2 Data Correction

The Data Correction tab has options for correcting measurement artifacts before you perform curve fitting.

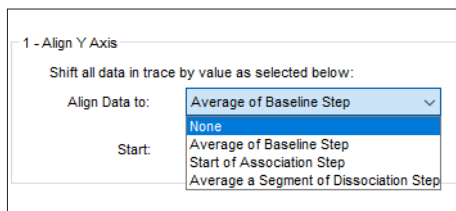
For more information see the [instruction video](#).

- Click the “Data Correction” tab.

5.3.3 Step 1: Aligning the Y Axis

To fit curves correctly, they need to be aligned to a common reference point on both the X and Y axes. Curves are aligned to the X-axis automatically during the assay as biosensors move in parallel.

The Align Y Axis function lets you apply a Y-axis adjustment to align the start of the association phase to zero. Most kinetic fitting models require a zero baseline for fitting, so this step is almost always a preparatory step in kinetic evaluation



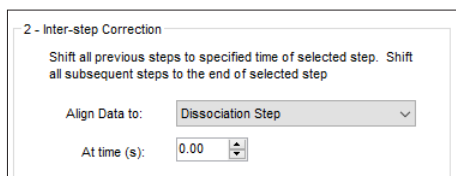
1. Click the “Align Data” to box to select “Average of Baseline Step”.

Selection	Description
Average of Baseline Step	Use this option to adjust the baseline step so start of the association is as close as possible to zero on the y-axis, prior to fitting the kinetic data. The last 5s of the baseline should be used when selecting to Y Align to the Average of Baseline Step.

5.3.4 Step 2: Inter-step Correction

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

Inter-step correction should only be used for experiments where the baseline and dissociation steps were done in the same well. It is not recommend using the inter-step correction with very fast kinetics as some kinetic information can be lost.



1. Click the “Align Data to” box and select “Dissociation Step”.

Selection	Description
Dissociation Step	Aligns the end of the association step on the Y-axis with the beginning of the adjacent dissociation step and the end of the baseline step to the beginning of the adjacent association step. If the default time used in the inter-step correction does not fix the bulk shifts or signal jumps between the association and dissociation steps, then modify the timing to remove these artifacts before fitting. Increasing the time to between 1 and 3 seconds is commonly used.

5.3.5 Step 3: Filtering

Savitzky-Golay filtering removes high-frequency noise from the data. For the purpose of assay 1 and 2 the box should be unchecked.

5.4 Analyzing Your Kinetic Data

For more information see the [instruction video](#).

1. Click the “Kinetic Analysis” tab.

5.4.1 Step 1: Choose the Step(s) to Analyze

Analyzing, association and dissociation generates k_{obs} , k_a , k_d , and K_D . This option is best if you are doing Local (individual) fitting with Full or Partial fit steps, or if you’ll be doing Global (group) fitting.

1. In the Step to Analyze “box in the Fitting Parameters window, select – “Association and Dissociation”.

5.4.2 Step 2: Choose a Binding Model

It is recommended to use the 1:1 model unless the interaction is known to be more complex. You should choose an appropriate kinetic binding model for an interaction based on knowledge of the interaction and the molecules involved, valency and predicted stoichiometry of binding or estimated binding constants.

1. In the Binding Model box in the Fit Parameters window, click the drop-down menu and select the model “1:1”.

5.4.3 Step 3: Set Fitting Parameters

Where multiple concentrations of a ligand, here biotinylated Protein A, are assessed, local fitting is the most suitable fitting parameter as kinetic parameters are derived individually for each interaction of analyte with each ligand concentration.

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

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

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

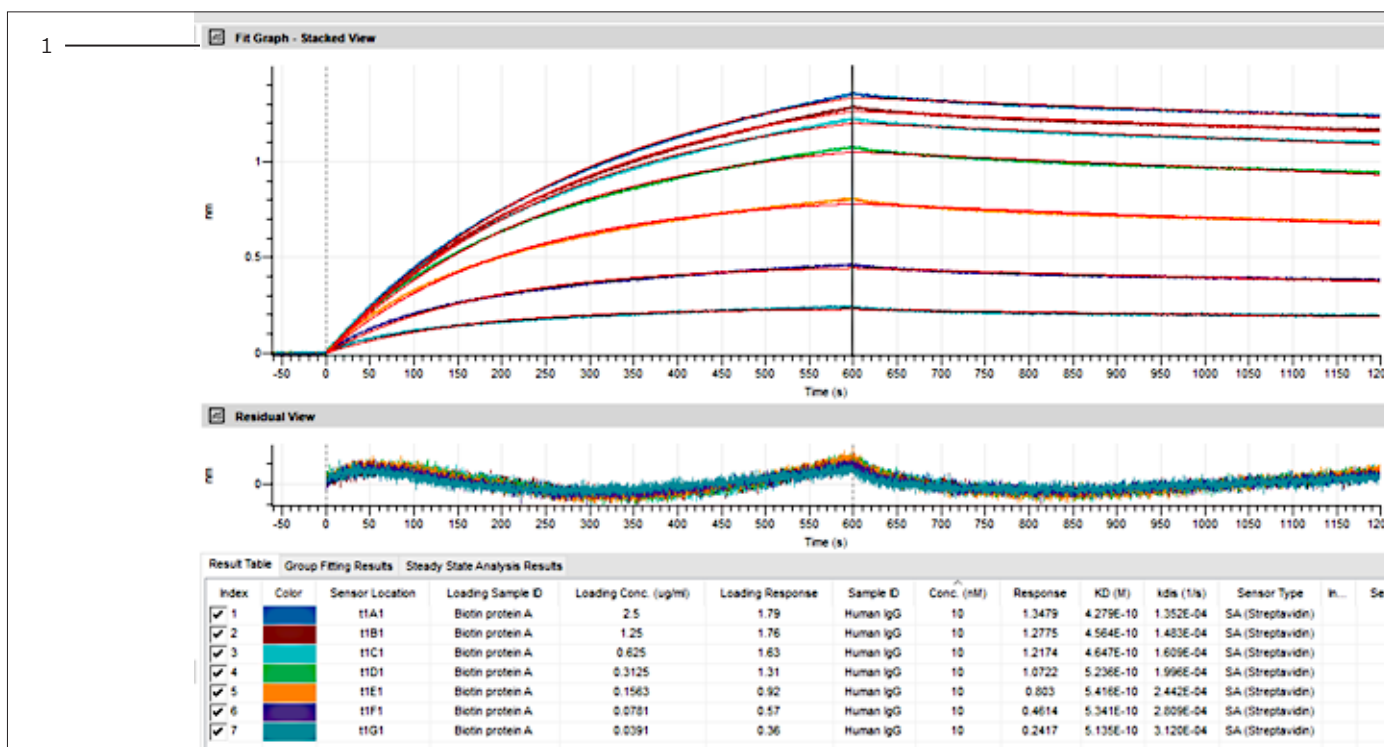


Fig. 16: Fit Graph and Residual View

Pos.	Name
1	Fit Graph window

1. In the Fitting box in the Fitting Parameters window, select “Local (Individual)”:
2. Select Fit Steps – “Full (assoc and dissoc)”:

5.4.4 Step 4: Review the Data Fit Quality

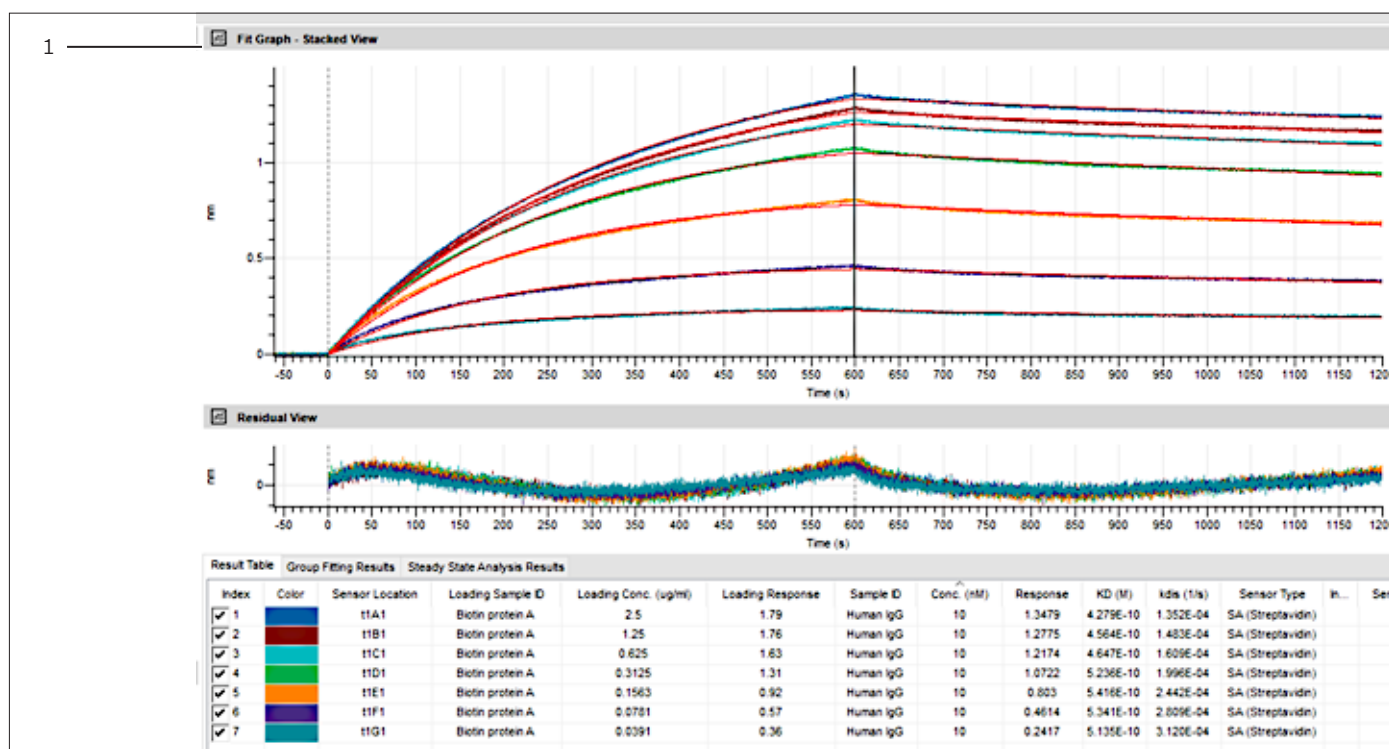


Fig. 17: Fit Graph and Residual View

Pos.	Name
1	Fit Graph window

Upon completion of curve fitting, it's best to evaluate both the quality of the fit and the reliability of calculated binding and affinity constants.

For more information see the [instruction video](#).

To assess how well the fitted curves adhere to the experimental data, there are a few general guidelines:

- Visually inspect the data and determine if the fit lines (red lines) conform well to the data traces. If the fit lines are far from the actual data, this indicates the fit is not ideal.
- As shown, the red fit lines overlay the data well, indicating a good fit:
- Confirm that the apparent kinetic constants and affinity are consistent with expectations based on literature or previous experimental knowledge of the interaction. The aim of assay 1 is to determine the optimal loading concentration of biotin Protein A, therefore visual analysis of the traces is important. Where traces are at or close to equilibrium and the binding model fits well, a good estimate of k_{ar} , k_d and K_D can be made to guide selection of analyte concentrations and association and dissociation step times, as well as loading concentration, in the next assay.

- Residuals are calculated in the Analysis Results table and plotted in the Residual Graph and can be examined to validate the fit. Residual values should not be greater than $\pm 10\%$ of the maximum response of the fitted curve. The shape of the residual plot corresponds to differences between the fit curve and the experimental data and should show signals that are equally distributed above and below the mean. Higher values indicate inaccurate fitting.
- Here, as shown, the maximum response of the fitted curve is 1.3479 nm and therefore, residuals over 0.13479 would indicate a poor fit of the model to the data. All residuals are less than ± 0.03 nm, therefore, the 1:1 model fit is a suitable fit to this data.
- The R² value indicates how well the fit and the experimental data correlate. In general, R² values above 0.95 are considered as a good fit for large molecule kinetics.

5.5 Selecting the Ligand Concentration

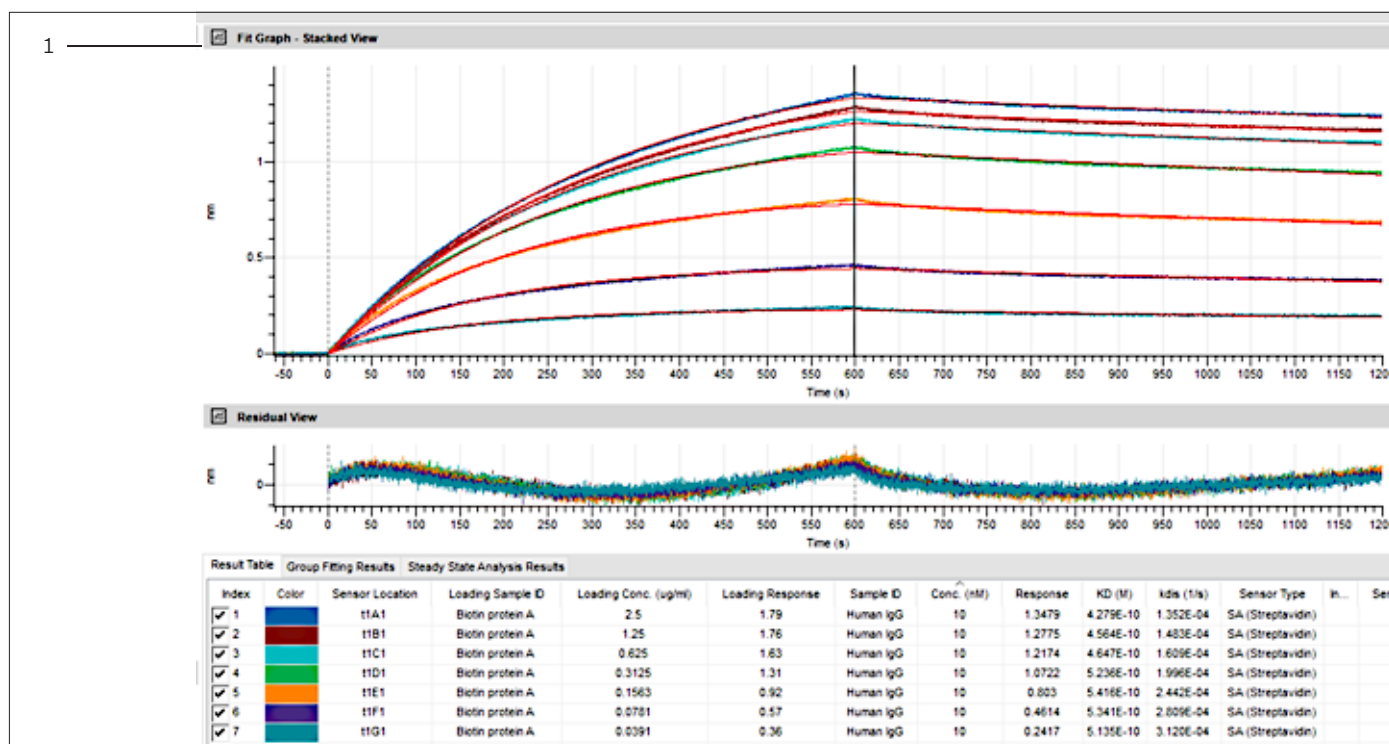


Fig. 18: Fit Graph and Residual View

Pos.	Name
1	Fit Graph window

The aim of assay 1 is to use the data to allow the user to select appropriate conditions for assay 2, where full kinetics and affinity for the interaction can be determined.

For more information see the [instruction video](#).

An important factor in designing a kinetics and affinity assay is to ensure that an appropriate loading concentration is chosen, which ensures that:

- The response from analyte binding to the ligand is easily detectable and allows space for titration of the analyte to lower concentrations.
- Heterogeneity caused by overloading of the biosensor is avoided.
- Diffusion limitation caused by using high loading levels and low analyte concentrations is avoided.

In general, the lowest loading concentration that will meet the needs of the assay should be chosen as an excess of ligand bound to the sensor can lead to data artifacts due to crowding, steric hindrance, and possible aggregation on the biosensor surface. Over-saturation of the sensor may also promote weaker, non-specific interactions at higher analyte concentrations, or analyte rebinding. Together, these artifacts may significantly impact observed binding kinetics.

In general, choose the lowest loading concentration that gives a reasonable analyte response. In this kinetic assay an analyte response of approximately 0.8 nm is desirable.

Therefore, 0.156 $\mu\text{g}/\text{mL}$ of biotinylated Protein A would be a suitable concentration for assay 2 as the final human IgG response is 0.803 nm.

For this user guide, 0.156 $\mu\text{g}/\text{mL}$ will be used for assay 2 though users should be driven by the data results of their own assay 1 data.

5.5.1 Selecting the Analyte Concentration and Assay Parameters

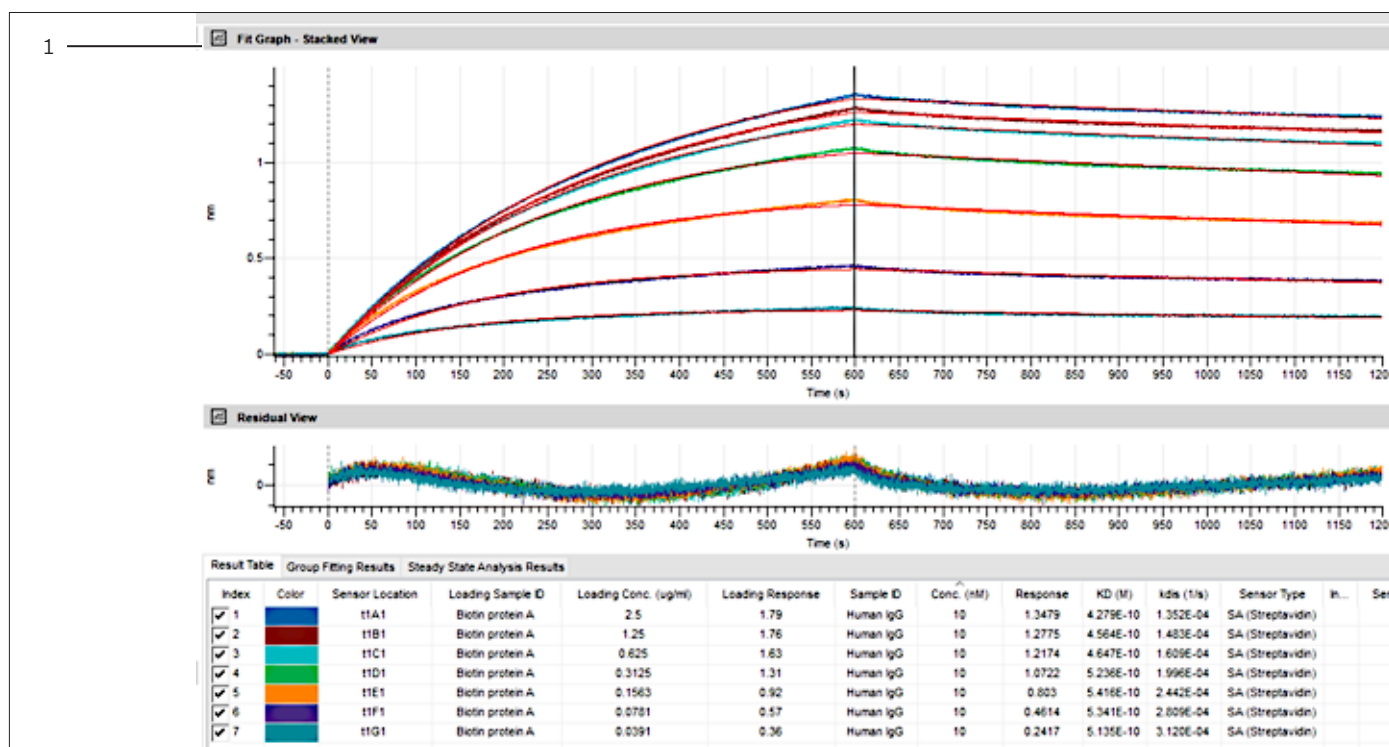


Fig. 19: Fit Graph and Residual View

Pos.	Name
1	Fit Graph window

It is highly recommended that new users read “A Compendium for Successful BLI and SPR Assays” for a complete review on analyte concentration series and dissociation times.

One of the most critical parameters in determining accurate kinetics and affinity is the selection of an appropriate analyte concentration series for fitting to an appropriate kinetic or steady state model. Unfortunately, this crucial step is often overlooked, leading to the use of unsuitable analyte concentration series.

In general, the analyte dilution series measured should ideally range from a concentration of about 0.1–10 fold of the expected K_D , using a 2-fold or 3-fold dilution series.

As shown a K_D of ~0.5 nM was determined using a single analyte concentration and a top analyte concentration of 5 nM would be appropriate. Due to additional factors that affect the observed sensorgram (including but not limited to the association time required to generate sufficient curvature and the dissociation rate) a top analyte concentration of 10 nM is suggested for use in assay 2.

In general, a dissociation time that provides at least a 5% decrease in signal from the end of the association should be used, therefore the dissociation time of 600 s is used in assay 2 to allow for a sufficient decrease in the observed dissociation response and subsequent determination of an accurate dissociation value.

Where necessary, the required dissociation time to achieve at least a 5% decrease in signal can be calculated using the following equation:

$$\textit{Minimum Dissociation Time} = \frac{-\ln(0.95)}{kd}$$

6 Assay 2 – Kinetics and Affinity Determination

In assay 2, the general assay setup uses Octet® Streptavidin (SA) Biosensors to capture biotinylated Protein A at a concentration determined during Assay 1 – loading optimization (see chapter “3 Assay 1 – Kinetics and Affinity Assay Loading Optimization”, page 11).

The biosensors are then used to determine the kinetics and affinity of an analyte concentration series of human IgG binding to protein A. After data analysis, the kinetics and affinity of the interaction will be known

6.1 Assay Setup

The aim of this section is to create an Octet® BLI method containing a sample plate with the following samples and parameters.

It is important to use the same flat bottom 96-well plate for both the biosensor tray and sample plate as used in Assay 1.

It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types will be included in the assay.

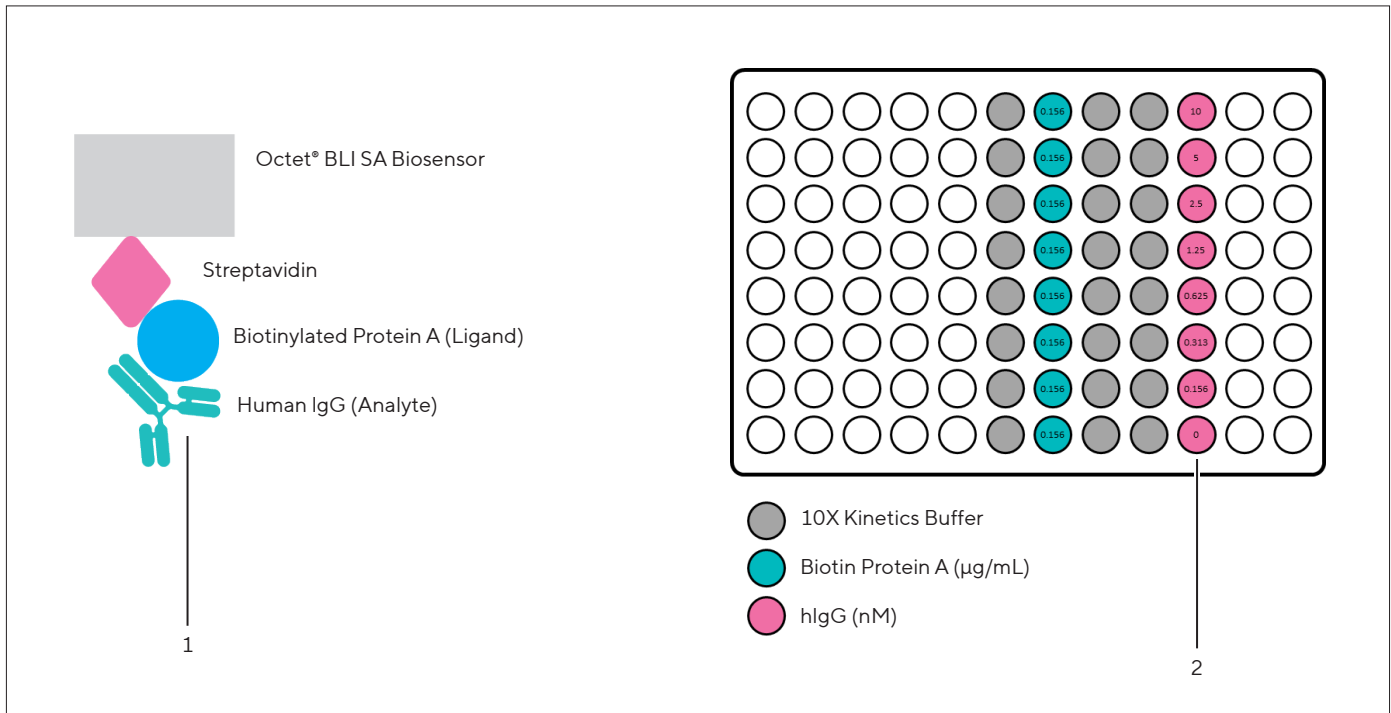


Fig. 20: General setup and sample plate

Pos.	Name
1	General setup
2	Sample plate

6.2 Parameters

Step Number	Step Name	Time (S)	Shake Speed (RPM)	Column Position
1	Baseline	60	1000	6
2	Loading	300	1000	7
3	Baseline 2	30	1000	8
4	Baseline	60	1000	9
5	Association	600	1000	10
6	Dissociation	600	1000	9

6.3 Duration

Type	Duration
Sample Preparation Time	~10 minutes
Assay Duration	~40 minutes including biosensor hydration
Analysis Duration	~10 minutes

6.4 Materials Required

- 2.5 µg/mL Biotinylated protein A solution prepared in assay 1
- 10 nM human IgG solution prepared in assay 1
- Octet® Kinetics Buffer 10X: Item No.: 18-1105
- Octet® Streptavidin (SA) Biosensor: Item No.: 18-5019 (1 Tray) | 18-5020 (5 Trays) | 18-5021 (20 Trays)

6.5 Kinetics and Affinity Determination – Plate Definition

- Following the same protocol as used in assay 1 (see chapter “3.5 Loading Optimization – Plate Definition”, page 13), assign the following Sample ID to each well as shown in the table below:

Wells	Sample ID
A6 – H6	10X Kinetics Buffer
A7 – H7	Biotin Protein A
A8 – H8	10X Kinetics Buffer
A9- H9	10X Kinetics Buffer
A10 – H10	Human IgG

6.6 Kinetics and Affinity Determination – Sample Information

- Following the same protocol as used in assay 1 (see chapter “3.8 Loading Optimization – Entering Sample Information”, page 17), enter the sample information as shown in the table above.
- Enter the biotin Protein A concentration as 0.156 µg/mL.

6.7 Kinetics and Affinity Determination – Assigning User-Specified Sample Concentrations

- Following the same protocol as used in Assay 1 (see chapter “3.9 Loading Optimization – Assigning User-Specified Sample Concentrations”, page 19), enter the human IgG concentration as shown in the table below:

Well	Human IgG Concentration (nM)
A10	10
B10	5
C10	2.5
D10	1.25
E10	0.625
F10	0.3125
G10	0.1563
H10	0

6.8 Kinetics and Affinity Determination – Assay Definition

- Following the same protocol as used in Assay 1 (see chapter “3.10 Loading Optimization – Assay Definition”, page 22), enter the assay step definitions as shown in the table below:

Step Number	Step Name	Time (S)	Shake Speed (RPM)	Column Position
1	Baseline	60	1000	6
2	Loading	300	1000	7
3	Baseline 2	30	1000	8
4	Baseline	60	1000	9
5	Association	600	1000	10
6	Dissociation	600	1000	9

- Assign the column positions following the same protocol as used in Assay 1 (see chapter “3.11 Building an Assay”, page 24).

6.8.1 Starting the Experiment

If required, the final step of assay definition is to change the default sensor type to a user specified sensor type (see chapter “3.11.1 Starting the experiment”, page 26).

- Click the drop-down arrow in Sensor Type and click SA.
- Ensure sensors are assigned to column 2 (A2 – H2) in the “Sensor Assignment” tab and then click the “Review Experiment” tab. Using the assay step arrow, click through the assay to ensure the correct step sequence has been input (Columns 6, 7, 8, 9, 10, 9).
- If incorrect, return to the Assay Definition tab and correct the column associated with the assay step. If correct, click through to the “Run Experiment” tab.
- In the Kinetics data repository section, click the three dots and select a suitable location for saving the assay.
 - It is recommended to make a separate folder for assays performed during the getting started guide (e.g., C:\OctetData\Getting Started Guide).
 - Then in the experiment run name, enter Assay 2 – Kinetics and Affinity Determination.

7 Assay 2 – Sample and Hydration Plate Preparation

7.1 Hydration Plate

1. Add 200 μL of 10X Kinetics Buffer to sample plate positions A2 – H2. Transfer eight (8) Octet® BLI SA Biosensors to an empty biosensor tray.
2. Prepare and load the hydration plate as shown in chapter “2.1 Loading the Biosensor Tray and Sample Plates”, page 8.

7.2 Biotin Protein A

1. Prepare a final biotin Protein A concentration of 0.156 $\mu\text{g}/\text{mL}$ using the 2.5 $\mu\text{g}/\text{mL}$ solution prepared in Assay 1 (see chapter “4.2 Biotin Protein A”, page 28).

Biotinylated Protein A Starting Concentration ($\mu\text{g}/\text{mL}$)	Biotinylated Protein A Final Concentration ($\mu\text{g}/\text{mL}$)	Biotinylated Protein A (μL)	10X Kinetics Buffer (μL)
2.5	0.156	125	1875

2. Mix thoroughly and add 200 μL of biotinylated Protein A to the positions A7 – H7 on the sample plate.

7.3 Human IgG

1. Prepare a 1 in 2 serial dilution of human IgG using the 10 nM solution prepared in Assay 1 (see chapter “4.3 Human IgG”, page 29).
2. In an Eppendorf tube mix 250 μL of 10 nM human IgG with 250 μL of 10X Kinetics Buffer to make 5 nM human IgG.
3. Continue the dilution series to obtain human IgG solutions with:
 - 2.5 nM
 - 1.25 nM
 - 0.625 nM
 - 0.3125 nM
 - 0.1563 nM

4. Add 200 μ L of each human IgG concentration to the correct position on the sample plate shown in the table below and add 200 μ L of 10X Kinetics Buffer to position H10.

Sample Plate Position	Biotinylated Protein A Final Concentration (μ g/mL)
A10	10
B10	5
C10	2.5
D10	1.25
E10	0.625
F10	0.3125
G10	0.1563
H10	0

7.4 10X Kinetics Buffer

1. Add 200 μ L of 10X Kinetics Buffer to positions A6 – H6, A8 – H8, and A9 – H9 on the sample plate.

7.5 Loading the Sample Plate

1. Load the sample plate as shown in chapter “2.1 Loading the Biosensor Tray and Sample Plates”, page 8.

7.6 Run the Experiment

1. In the Octet® BLI Discovery Software. Select the “Run Experiment” tab and to start the experiment, click “GO”.

8 Assay 2 – Opening Experiment Files and Kinetics and Affinity Determination

To view assay 2 data in the Experiment Builder, open the data folder by double-clicking the magenta-colored data folder in Experiment Explorer.

Before analysis the Preprocess Data Screen lets you subtract any non-specific binding or baseline drift data and make other data corrections if necessary. Click the Preprocess Data tab to view the “Preprocess Data” screen.

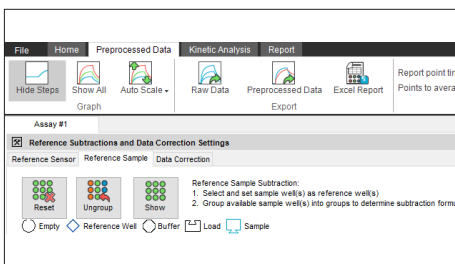
For more information see the [instruction video](#).

8.1 Reference Sensor Subtraction

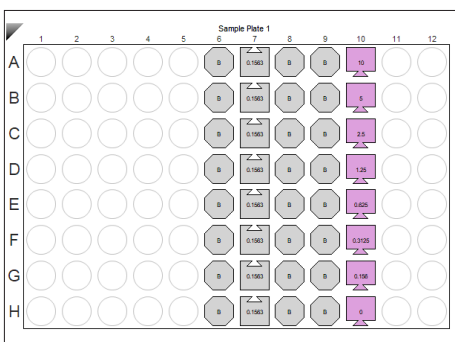
No reference sensors are used in assay 2.

8.2 Reference Sample Subtraction

1. To subtract any baseline drift in your experiment using reference sample wells that contained ligand but no analyte, click the “Reference Sample” tab in the Reference Subtractions and Data Corrections window.

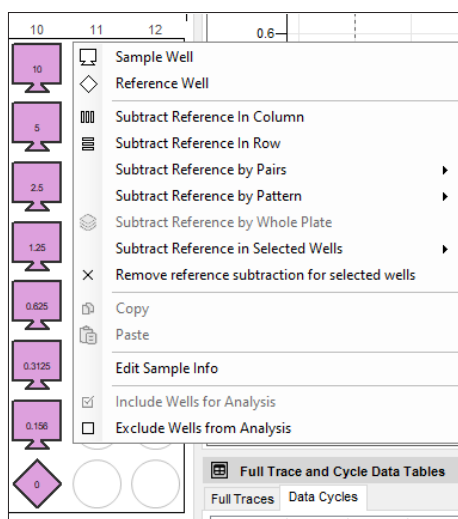
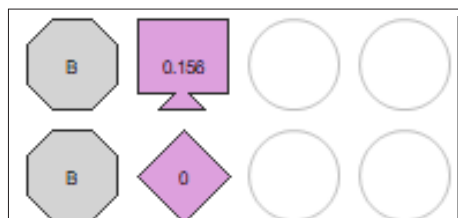
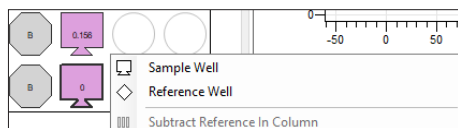


2. Sample analyte concentrations display in the sample/load wells on the sample plate.



8.3 Subtracting Baseline Drift

Assay 2 uses an analyte concentration of 0.156 – 10 nM and well H10 is the reference sample well containing only buffer.



1. Select well H10 and then Right Click anywhere on the sample plate and select Reference Well.
2. The selected wells appear as a diamond, to indicate it is set as reference well.
3. To subtract the reference well – select the analyte containing column, right click anywhere on the highlighted samples and select “Subtract Reference in Column”.

8.4 Data Correction

1. Click the “Data Correction” tab and follow the same steps as used in assay 1 to correct the data (see chapter “5.3.3 Step 1: Aligning the Y Axis”, page 33 and chapter “5.3.4 Step 2: Inter-step Correction”, page 33).

8.5 Analyzing Your Kinetic Data

Unlike assay 1 where a local fitting of the data was performed, in assay 2 a global fit of the data is performed as the most accurate kinetic and affinity constants are obtained when performing a global fit using several analyte concentrations.

For more information see the [instruction video](#).

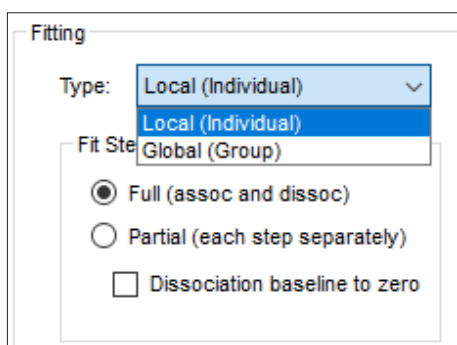
When performing global fits of data R_{max} should remain unlinked by biosensor when separate biosensors are used for each individual analyte concentration.

When R_{max} is linked, the theoretical maximum response is calculated assuming equal binding capacity between biosensors. Different biosensors have slight variability in surface capacity. R_{max} can be linked if the same biosensor is used for every analyte concentration in the series.

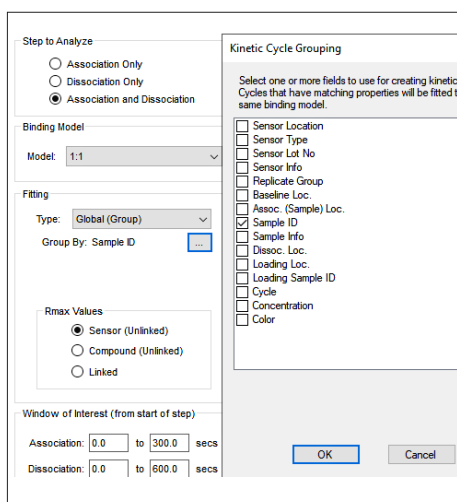
This strategy is typically used in small molecule analysis, where dissociation is rapid and complete and enables re-use of the same biosensor for a new sample concentration.

In standard large molecule kinetics assays where each sample is run on a new or regenerated biosensor, R_{max} should be unlinked to enable calculation of separate R_{max} for each sample.

1. In the Fitting box in the Fitting Parameters window, select “Full (assoc and dissoc)” and select “Global (Group)” as the type.



2. In the group by box select “Sample ID” and in the R_{max} value box select “Sensor (Unlinked)”.
3. Data in the Analysis Results table and the Analysis Graphs updates after the type and fit steps are applied.



8.6 Review the Data Fit Quality

Protein A has the potential to bind up to five molecules of IgG. Therefore, a perfect 1:1 data fit should not be expected but low ligand load levels help ensure the best 1:1 fit possible.

For more information see the [instruction video](#).

After curve fitting, it's best to evaluate both the quality of the fit and the reliability of calculated binding and affinity constants. To assess how well the fitted curves adhere to the experimental data, a few general guidelines should be followed.

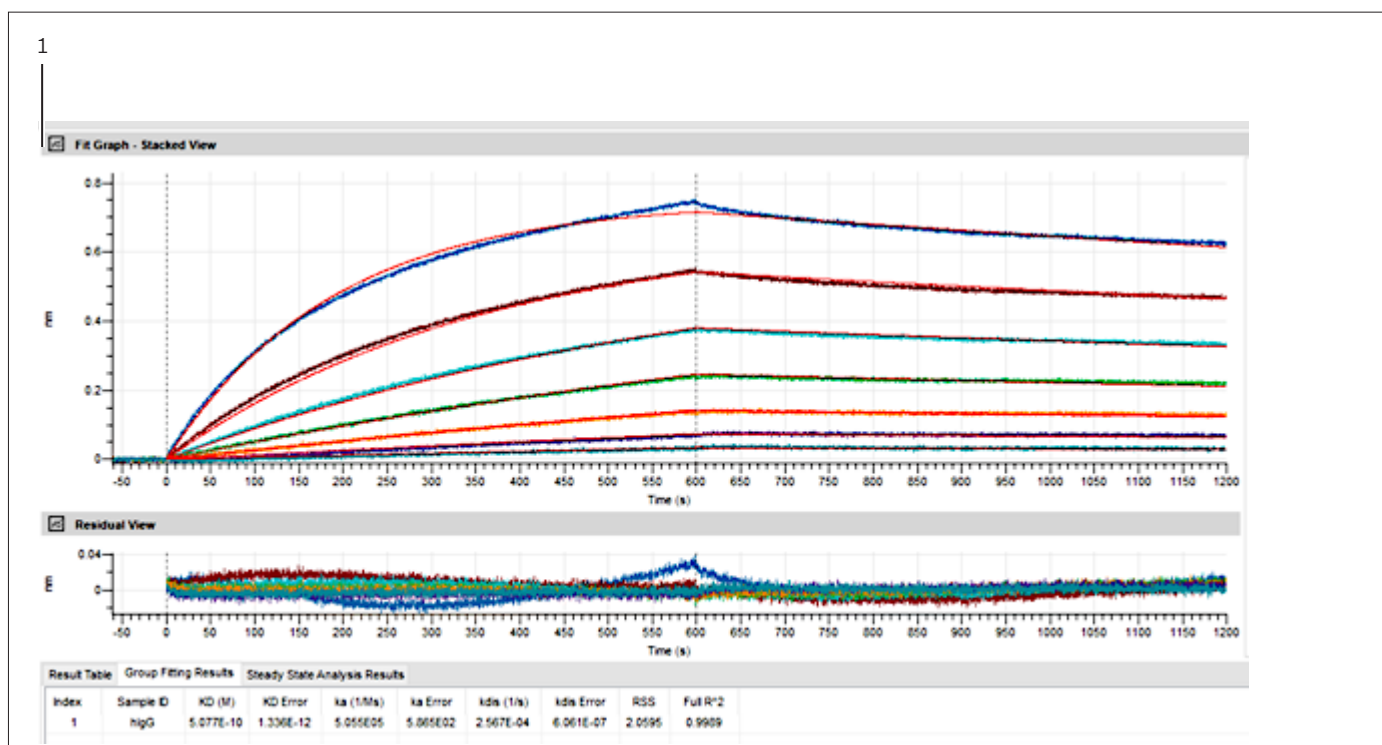


Fig. 21: Fit Graph and Residual View for assay 2

Pos.	Name
------	------

1	Fit Graph
---	-----------

1. Visually inspect the data and determine if the fit lines (red lines) conform well to the data traces. If the fit lines are far from the actual data, this indicates the fit is not ideal. For assay 2 the red fit lines overlay on the data well, indicating a good fit.
2. Confirm that the apparent kinetic constants and affinity are consistent with expectations based on literature or previous experimental knowledge of the interaction. The aim of assay 2 was to determine the kinetics and affinity of an analyte concentration series of human IgG binding to protein A. In general, the following parameters should be observed:

k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
$\sim 5e^5$	$\sim 2e^{-4}$	$\sim 0.2 - 1$

3. Residuals are calculated in the Analysis Results table and plotted in the Residual Graph and can be examined to validate the fit. Residual values should not be greater than $\pm 10\%$ of the maximum response of the fitted curve. The shape of the residual plot corresponds to differences between the fit curve and the experimental data and should show signals that are equally distributed above and below the mean.
4. Although the R_{max} values are unlinked due to different biosensors being used to assess each analyte concentration, the R_{max} values should be within 20% of each other.
5. Errors for the determined parameters should be $\leq 1\%$ for accurate determination of affinity from this type of experiment. This value can be determined by dividing the parameters error by the parameter and multiplying by 100.
6. The R^2 value indicates how well the fit and the experimental data correlate. In general, R^2 values above 0.95 are considered as a good fit for large molecule kinetics.

8.7 What to do Next?

Utilizing the principles learnt in assays 1 and 2 it is possible to start exploring Octet® BLI kinetics and affinity determination. Reusing the assay parameters used in Assay 2 some possible parameters that can be assessed include:

- How precise are the data if triplicates of the same samples are performed? (See Adding an Assay Through Replication in the Octet® BLI Discovery User Guide)
- What happens to the quality of the 1:1 fit if the ligand concentration is decreased to analyte response below 0.3 nM?
- What is the effect of assay temperature?
- What is the effect of sample plate shake speed?
- What is the effect of using different buffers for sample preparation. For example, 1X KB, PBS or HBS?

For more ideas and information visit the [“Octet® BLI Resource Library”](#).

9 Assay 3 – Quantitation Assay

In assay 3, the general assay setup uses Octet® Protein A (ProA) Biosensors to capture various known concentrations of human IgG to generate a standard curve and samples with “unknown” concentrations. To minimize biosensor requirements, a regeneration step is included. After data analysis, these unknown sample concentrations are then compared against the standard curve and their true concentrations determined.

For more information see the [instruction video](#).

Key stages in using the software are described in detail during assay 1 and assay 2, therefore it is recommended to complete assays 1 and 2 before performing assay 3.

9.1 Quantitation with Regeneration

The aim of this section is to create an Octet® BLI method containing a sample plate with the following samples and parameters.

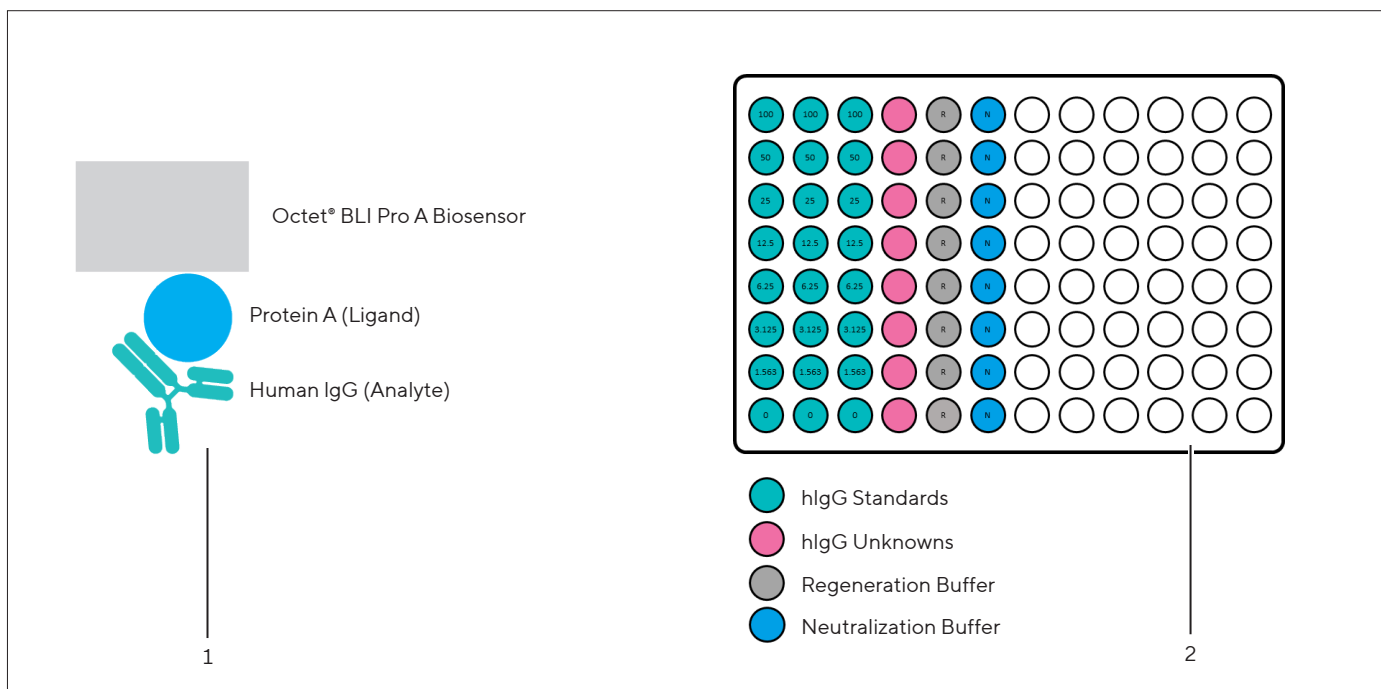


Fig. 22: General setup and sample plate

Pos.	Name
1	General setup
2	Sample plate

9.2 Parameters

Sensors are preconditioned three times prior to use and three regeneration cycles are performed between assay steps.

It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types will be included in the assay.

Step Name	Time (S)	Shake Speed (RPM)	Column Position
Quantitation	120	400	1, 2, 3 and 4
Regeneration	5	400	5
Neutralization	5	400	6

9.3 Duration

Type	Duration
Sample Preparation Time	~10 minutes
Assay Duration	~30 minutes including biosensor hydration
Analysis Duration	~10 minutes

9.4 Materials Required

- Octet® ProA Calibrator Set, Item No.: 18-1118
- Octet® Protein A (ProA) Biosensors, Item No.: 18-5010 (1 Tray) | 18-5012 (5 Trays) | 18-5013 (20 Trays)
- Regeneration Buffer: Octet® 10mM Glycine pH 1.7, Item No.: 18-1184
- Neutralization Buffer: Octet® Sample Diluent, Item No.: 18-1104
- 1X PBS

9.5 Quantitation with Regeneration – Plate Definition

It is highly recommended that new users read Chapter 6 – “Quantitation Experiments” of the “Octet® BLI Discovery User Guide” prior to performing the assay. A walkthrough version tailored to the needs of a new user is used here.

For more information see the [instruction video](#).

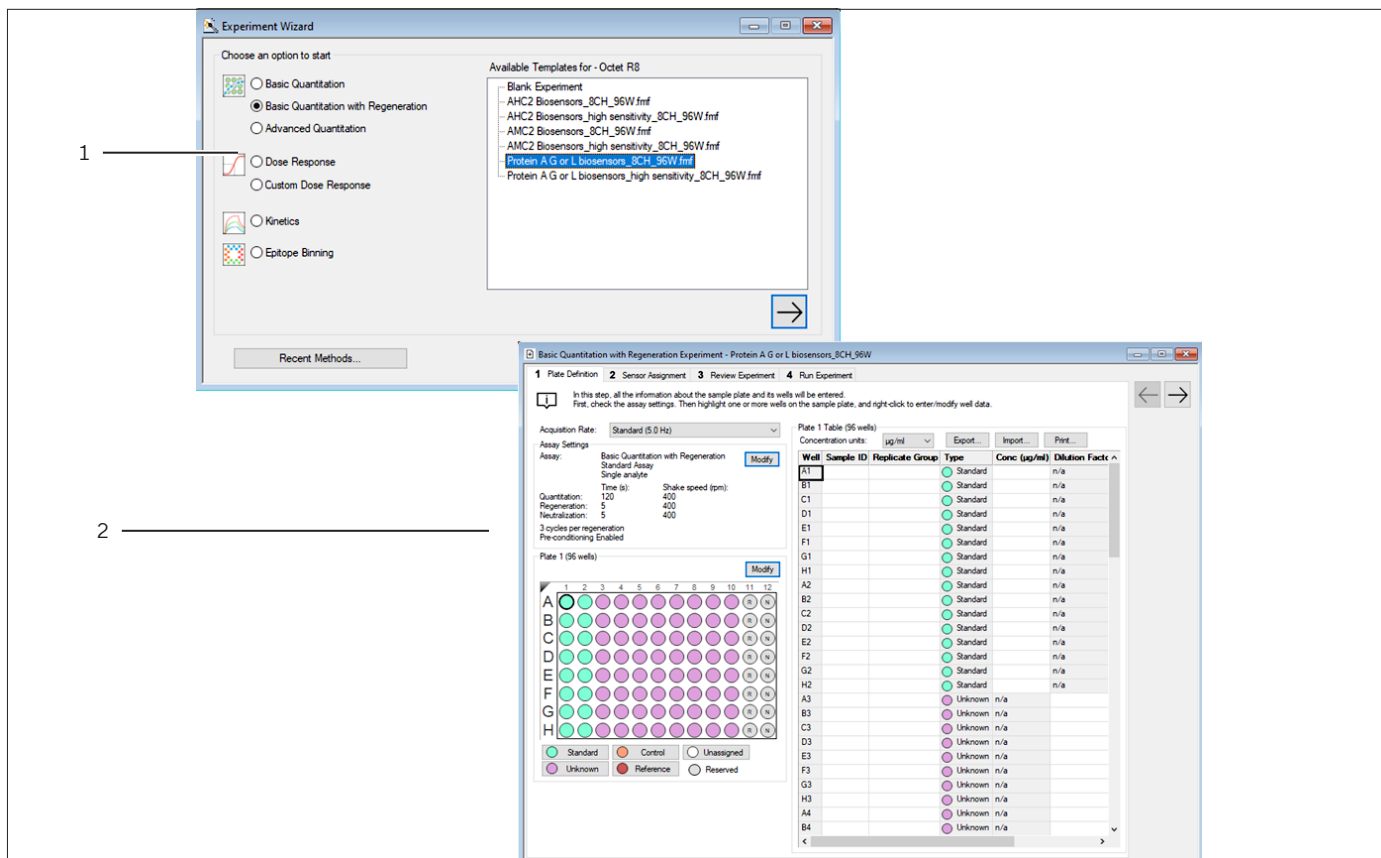


Fig. 23: Experiment Wizard and Basic Quantitation with Regeneration window

Pos.	Name
1	Experiment Wizard
2	Basic Quantitation with Regeneration” and “Protein A G” or “L Biosensors_8CH_96W

1. From the “Experiment Menu”, select “New Experiment Wizard” or alternatively press “CTRL + N” or click the New Experiment Wizard toolbar button.
2. Select “Basic Quantitation with Regeneration” and “Protein A G” or “L Biosensors_8CH_96W”.
3. Press “Enter” or click the “forward arrow”. The Basic Quantitation Experiment window will appear.

9.6 Select and Designate Well Types

In the Sample Plate Map, select the wells required by clicking the column header, right-click and select the correct sample type. The “Standard, Control, Unassigned, Unknown”, and “Reference” buttons can also be used to assign designations.

In a Basic Quantitation with Regeneration, the Sample Plate Map includes gray wells. These wells are reserved by the system and specify the location of particular sample types. Reserved samples cannot be removed from the sample plate, but you can change their column location to any empty column. To change the location of a reserved column right-click a column header in the “Sample Plate Map” and select “Regeneration or Neutralization”.

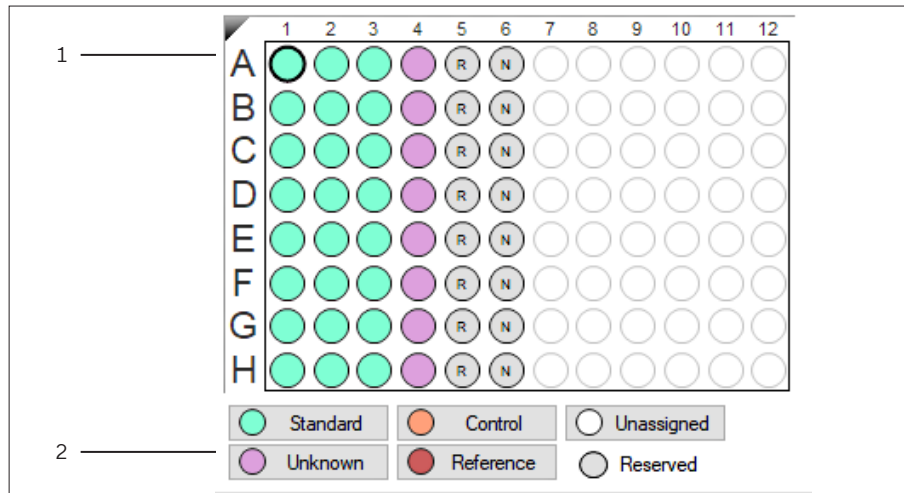


Fig. 24: Assay 3 plate definition

Pos.	Name
1	Sample plate map
2	Standard, Control, Unassigned, Unknown, and Reference buttons

1. Assign the following well types to each column:

Column Number (s)	Well Type
1, 2 and 3	Standards
4	Unknowns
5	Regeneration
6	Neutralization

9.7 Entering Sample Information

For more information see the [instruction video](#).

1. Following the same protocol as used in Assay 1 (see chapter “3.8 Loading Optimization – Entering Sample Information”, page 17), enter the sample information as shown in the table below:

Wells	Sample ID
A1 - H1	Human IgG
A2 - H2	Human IgG
A3 - H3	Human IgG
A4 - H4	Unknown 1, Unknown 2, ... Unknown 7
A5 - H5	Regeneration Buffer
A6 - H6	Neutralization Buffer

9.8 Assigning User-Specified Sample Concentrations

A 1 in 2 dilution series human IgG is used in assay 3 and the concentration should be entered at the plate definition stage. The simplest method is to select the desired cell in the concentration column and enter the number manually, but the most practical method is by using the “Set Well Data” software feature.

For more information see the [instruction video](#).

1. Following the same protocol as used in assay 1 (see chapter “3.9 Loading Optimization – Assigning User-Specified Sample Concentrations”, page 19), enter the human IgG concentration as shown in the table below:

Wells	Human IgG Concentration ($\mu\text{g/mL}$)
A1, A2 and A3	100
B1, B2 and B3	50
C1, C2 and C3	25
D1, D2 and D3	12.5
E1, E2 and E3	6.25
F1, F2 and F3	3.125
G1, G2 and G3	1.563
H1, H2 and H3	0

9.9 Quantitation Assay – Replicate Groups

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

For more information see the [instruction video](#).

Replicate Group information can also be entered in the Results table during data analysis.

1

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	Human IgG	100	Standard	100	n/a	
B1	Human IgG	50	Standard	50	n/a	
C1	Human IgG	25	Standard	25	n/a	
D1	Human IgG	12.5	Standard	12.5	n/a	
E1	Human IgG	6.25	Standard	6.25	n/a	
F1	Human IgG	3.125	Standard	3.125	n/a	
G1	Human IgG	1.563	Standard	1.563	n/a	
H1	Human IgG	0	Standard	0	n/a	
A2	Human IgG	100	Standard	100	n/a	
B2	Human IgG	50	Standard	50	n/a	
C2	Human IgG	25	Standard	25	n/a	
D2	Human IgG	12.5	Standard	12.5	n/a	
E2	Human IgG	6.25	Standard	6.25	n/a	
F2	Human IgG	3.125	Standard	3.125	n/a	
G2	Human IgG	1.563	Standard	1.563	n/a	
H2	Human IgG	0	Standard	0	n/a	
A3	Human IgG	100	Standard	100	n/a	
B3	Human IgG	50	Standard	50	n/a	
C3	Human IgG	25	Standard	25	n/a	
D3	Human IgG	12.5	Standard	12.5	n/a	
E3	Human IgG	6.25	Standard	6.25	n/a	
F3	Human IgG	3.125	Standard	3.125	n/a	
G3	Human IgG	1.563	Standard	1.563	n/a	
H3	Human IgG	0	Standard	0	n/a	
A4	Unknown 1	Unknown 1	Unknown	n/a		
B4	Unknown 2	Unknown 2	Unknown	n/a		
C4	Unknown 3	Unknown 3	Unknown	n/a		
D4	Unknown 4	Unknown 4	Unknown	n/a		
E4	Unknown 5	Unknown 5	Unknown	n/a		
F4	Unknown 6	Unknown 6	Unknown	n/a		
G4	Unknown 7	Unknown 7	Unknown	n/a		
H4	Unknown 8	Unknown 8	Unknown	n/a		

Fig. 25: Adding replicate group from the sample plate map

Pos.	Name
1	Replicate Group column

9.9.1 Assigning “Replicate Groups” in the “Sample Plate Map”

1. Select the samples to group, e.g., A1, A2 and A3, right-click and select “Set Well Data”.
2. In the “Set Well Data” dialog box, enter a name in the “Replicate Group” box and click “OK”. E.g., A1, A2, A3 can be called 100.
3. Repeat the previous steps to assign new samples to the existing Replicate Group, or to designate another set of samples to a new “Replicate Group”.

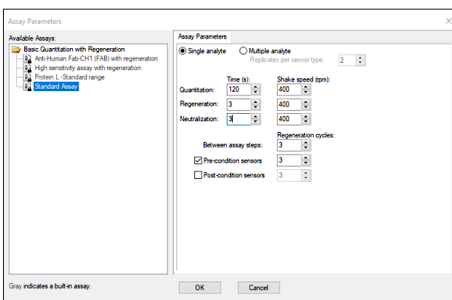
9.9.2 Assigning “Replicate Groups” in the “Replicate Group column” (alternative)

1. A quicker method of assigning replicate groups for the standards is to copy and paste the concentration values from the Conc. Column to the Replicate Group column. For the unknown samples, copy the Sample ID to the Replicate Group column.

9.10 Quantitation Assay – Managing Assay Parameter Settings

For more information see the [instruction video](#).

1. To view the user-modifiable settings for an assay, click “Modify” in the “Assay Settings” box.
2. The “Assay Parameters” box appears.



3. Select the type of Analyte. Here, Single analyte is selected as one biosensor is used per sample well.
4. Adjust the Time and Shake speed (rpm) of each step to the parameters shown in the table below:

Step Name	Time (S)	Shake Speed (RPM)
Quantitation	120	400
Regeneration	3	400
Neutralization	3	400

5. Adjust the Between assay steps to the parameters shown in the table below:

	Regeneration Cycles
Between Assay Steps	3
Pre-condition Sensors	3
Post-condition Sensors	Unticked

6. Click “OK” to continue.

9.11 Quantitation Assay – Assigning Biosensors to Samples

After the sample plate is defined, biosensors must be assigned to the samples. Click through to the “Sensor Assignment” tab. Biosensors are automatically positioned in column 1.

For more information see the [instruction video](#).

1. Click through the “Sensor Assignment” tab to the “Review Experiment” tab. Using the assay step arrow, click through the assay to ensure the correct step sequence has been input.
2. If incorrect, return to the Assay Definition tab and correct the column associated with the assay step. If correct, click through to the “Run Experiment” tab.
3. In the Quantitation data repository section, click the three dots and select a suitable location for saving the assay.
 - It is recommended to make a separate folder for assays performed during the getting started guide (e.g., C:\OctetData\Getting Started Guide).
 - Then in the experiment run name, enter Assay 3 – Quantitation with Regeneration.

10 Assay 3 – Sample and Hydration Plate Preparation

10.1 Hydration Plate

1. Add 200 μL of 1X PBS to sample plate positions A1 – H1.
2. Transfer eight (8) Octet® BLI® Protein A (ProA) Biosensors to an empty biosensor tray.
3. Prepare and load the hydration plate as shown in chapter “2.1 Loading the Biosensor Tray and Sample Plates”, page 8.

10.2 Human IgG

The Octet® ProA Calibrator Set contains a human IgG solution supplied at a concentration of 100 $\mu\text{g}/\text{mL}$.

Prepare a 1 in 2 serial dilution of human IgG using the 100 $\mu\text{g}/\text{mL}$ solution and Sample Diluent.

1. Prepare a 1 in 2 serial dilution of human IgG using the 100 $\mu\text{g}/\text{mL}$ solution and Sample Diluent.
2. In a 2 mL Eppendorf tube mix 800 μL of 100 $\mu\text{g}/\text{mL}$ human IgG with 800 μL of Sample Diluent to make 50 $\mu\text{g}/\text{mL}$ human IgG.
3. Continue the dilution series to obtain human IgG solutions with:
 - 25 $\mu\text{g}/\text{mL}$
 - 12.5 $\mu\text{g}/\text{mL}$
 - 6.25 $\mu\text{g}/\text{mL}$
 - 3.125 $\mu\text{g}/\text{mL}$
 - 1.563 $\mu\text{g}/\text{mL}$
4. Mix thoroughly and add 200 μL of human IgG to the positions on the sample plate and add 200 μL of Sample Diluent to position H1, H2 and H3.

Sample Plate Position	Human IgG Final Concentration ($\mu\text{g}/\text{mL}$)
A1, A2 and A3	100
B1, B2 and B3	50
C1, C2 and C3	25
D1, D2 and D3	12.5
E1, E2 and E3	6.25
F1, F2 and F3	3.125
G1, G2 and G3	1.563

10.3 Unknowns

The Octet® ProA Calibrator Set contains a human IgG solution supplied at a concentration of 100 µg/mL.

1. Prepare an initial dilution to 90 µg/mL of human IgG using the 100 µg/mL solution and Sample Diluent.

Human IgG Starting Concentration (µg/mL)	Human IgG Final Concentration (µg/mL)	Human IgG (µL)	Sample Diluent (µL)
100	90	1800	200

2. Prepare a 1 in 2 serial dilution of human IgG using the 90 µg/mL solution and Sample Diluent.
3. In a 2 mL Eppendorf tube mix 800 µL of 90 µg/mL human IgG with 800 µL of Sample Diluent to make 45 µg/mL human IgG.
4. Continue the dilution series to obtain the human IgG solutions with:
 - 22.5 µg/mL
 - 11.25 µg/mL
 - 5.625 µg/mL
 - 2.813 µg/mL
 - 1.406 µg/mL
5. Mix thoroughly and add 200 µL of human IgG to the positions on the sample plate and add 200 µL of Sample Diluent to position H4.

Sample Plate Position	Human IgG Final Concentration (µg/mL)
A4	90
B4	45
C4	22.5
D4	11.25
E4	5.625
F4	2.813
G4	1.406
H4	0

10.3.1 Regeneration Solution

1. Add 200 µL of regeneration buffer (Octet® 10mM Glycine pH 1.7) to positions A5 – H5 on the sample plate.

10.3.2 Neutralization Solution

1. Add 200 µL of neutralization buffer (Octet® Sample Diluent) to positions A6 – H6 on the sample plate.

10.4 Loading the Sample Plate

1. Load the sample plate as shown in chapter “2.1 Loading the Biosensor Tray and Sample Plates”, page 8.

10.5 Run the Experiment

1. In the Octet® BLI Discovery Software. Select the “Run Experiment” tab and to start the experiment, click “GO”.

11 Assay 3 – Opening Experiment Files and Quantitation with Regeneration

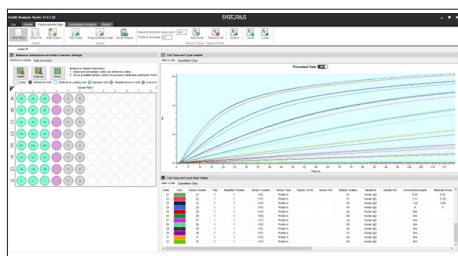
It is highly recommended that new users read Chapters 9 and 10 of the “Octet® Analysis Studio User Guide” prior to analyzing the data from Assay 3. A walkthrough version tailored to the needs of a new user is used here.

For more information see the [instruction video](#).

To view Assay 3 data in the Experiment Builder, open the data folder by double-clicking the teal-colored data folder in Experiment Explorer.

Before analysis the Preprocess Data Screen lets you subtract any non-specific binding or baseline drift data and make other data corrections if necessary.

1. Click the Preprocess Data tab to view the “Preprocess Data” screen.



11.1 Setting Reference Wells and Changing Well Types

Well types can be changed if they were not designated in Octet® BLI Discovery software.

1. In the sample plate, select the well(s) H1, H2, H3 and H4, right-click, and select “Reference” as the new well type from the menu.
2. Right click anywhere on the sample plate and select “Subtract Reference in Column”.

11.2 Quantitation Analysis

The Quantitation Analysis Screen lets you determine sample concentration using a reference set of standards. Once you've made your reference subtraction and data corrections in the Preprocess Data screen, click the "Quantitation Analysis" tab to start your analysis.

For more information see the [instruction video](#).

11.2.1 Step 1: Fit the Standard Curve

1. Select "4PL (Unweighted)" in the Standard Curve Equation box. This applies a symmetrical dose response curve. No points are weighted during the curve fitting.
2. Uncheck all options in the Standard Curve Grouping Options dialog to use for creating standard curves.

11.2.2 Step 2: Set Processing Parameters

In the Processing Parameters box, select "Initial Slope". This is the curve-fitting equation that models the binding data. "Initial Slope" is chosen because it calculates the initial slope of the acquired quantitation data (nm/second) and is the most appropriate equation for a basic quantitation with regeneration experiment.

1. Leave the "Read Time, Zero Concentration Threshold" and "Low Concentration Threshold" with the predetermined values.

Standard Curve Settings

Standard Curve Equation
 Dose Response - 4PL (Unweighted) v
 Standard Curve Grouping Options
 Group By: None

Processing Parameters
 Binding Rate Equation:
 Initial Slope v
 Read Time: 119.80 secs
 Zero Conc. Threshold: 0.0001 BR
 Low Conc. Threshold: 0.0030 BR

Advanced Settings

Standard Sample Alerts

Type	Threshold
<input type="checkbox"/> Min Sample r^2	0.9800
<input type="checkbox"/> Max Residual (%)	10.00
<input checked="" type="checkbox"/> High Conc. CV	10.00
<input checked="" type="checkbox"/> High Dilution CV	10.00
<input type="checkbox"/> Sample Alert	

11.2.3 Step 3: Setting Standard Sample Alerts

Although optional, it is recommended that Sample Alerts are used to highlight any issues with standards used in Assay 3.

1. Ensure the values match those in the picture below:

Standard Sample Alerts

Type	Threshold
<input type="checkbox"/> Min Sample r^2	0.9800
<input type="checkbox"/> Max Residual (%)	10.00
<input checked="" type="checkbox"/> High Conc. CV	10.00
<input checked="" type="checkbox"/> High Dilution CV	10.00
<input type="checkbox"/> Sample Alert	

11.3 Viewing Analysis Results

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

For more information see the [instruction video](#).

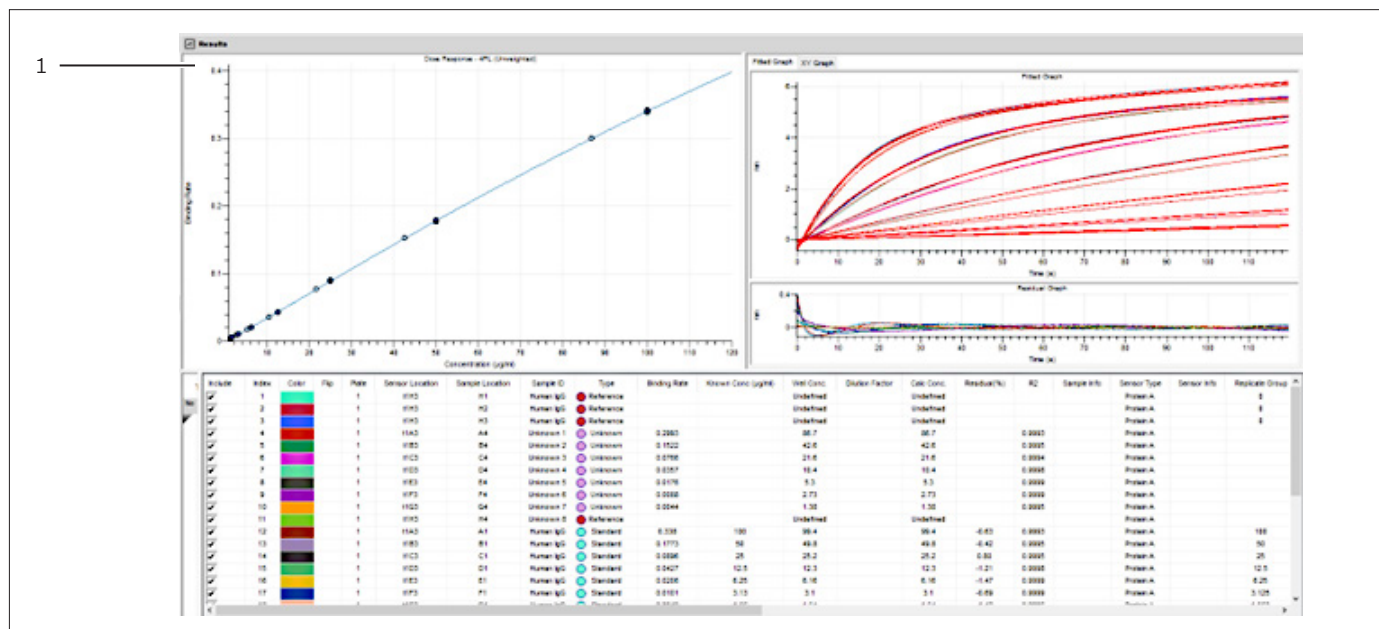


Fig. 26: Results panel

Pos.	Name
1	Results panel

To assess how well the fitted curves adhere to the experimental data, in the following are a few general guidelines:

1. Visually inspect the data and determine if the fit lines (red lines) conform well to the data traces. If the fit lines are far from the actual data, this indicates the fit is not ideal.
2. The R2 value indicates how well the fit and the experimental data correlate. In general, R2 values for Standard and Unknown Sample above 0.98 are considered as a good fit (note, this is the Min Sample R2 value in the “Standard Sample Alerts”).
3. Replicate groups were assigned during preparation of the method for Assay 3. Concentration CV (Conc CV) indicates how well the known concentration and calculated concentration agree and values below 10% are considered as a good agreement (note, this is the High Concentration CV value in the “Standard Sample Alerts”).
4. Examine RSS values. RSS is a measure of error between the experimental data and the fitted line. A smaller RSS indicates a better fit.

5. Although in general, “unknown” protein concentrations are determined by comparison to a standard curve prepared using ‘known concentrations. For the purpose of assay 3 the concentration of the prepared “unknown” samples is known and therefore, the percent error between determined and expected results can be determined using the following equation:

$$\text{Percent Error} = \frac{\text{True Value} - \text{Determined Value}}{\text{True Value}}$$

6. The determined concentration should be within $\pm 10\%$ of the prepared concentration.

Human IgG Final Concentration ($\mu\text{g/mL}$)	Determined Concentration ($\mu\text{g/mL}$)	Error (%)
90	86.7	3.67
45	42.6	5.33
22.5	21.6	4.00
11.25	10.4	7.56
5.625	5.3	5.78
2.813	2.73	2.95
1.406	1.38	1.85
0	0	0

Standard absorbance-based measurement of protein concentration is a reflection of how much material is present but does not indicate the amount of biologically-active molecules that can interact specifically with the ligand of interest.

Octet® quantitation assays can be used to assess the amount of properly folded protein by comparing the observed response to a known standard. This provides the correct concentration of active analyte being used, and aids in accurate measurement of kinetics and affinity.

11.4 What to do Next?

Utilizing the principles learnt in Assay 3 it is possible to start exploring Octet® BLI quantitation assays. Reusing the assay parameters used in Assay 2 some possible parameters that can be assessed include:

- What happens if the low concentration threshold is set to 0.3?
- What happens if Req is used as the binding rate instead of initial slope?
- Try using the sample alerts to check the replicate variation.
- What is the effect of assay temperature?
- What is the effect of sample plate shake speed?
- What is the effect of using different buffers for sample preparation. For example, PBS or HBS?

For more ideas and information visit the [“Octet® BLI Resource Library”](#).

12 Frequently Asked Questions

12.1 Hydration Plate

**Do I have to use black, flat-bottom plate as the biosensor hydration plate?
Can I use a clear (transparent), white, or other plates as the hydration plate?**

Black plates are required to reduce the background reflected light to a minimum during biosensor pick-up and sample data acquisition. The black hydration plate is an integral part of the Octet® BLI system optics and must stay underneath the biosensors (at the sensor tray location) in the instrument during the entire experiment.

The hydration plate is important for proper determination of the integration time of each biosensor during data collection. The Octet® BLI instrument starts a BLI assay from the point of biosensor pick-up (hydration plate location), before dipping the biosensors into the first solution on the sample plate. After the biosensors are loaded, the instrument optimizes the amount of light collected by adjusting integration times. When the optics head moves over to the sample plate and dips the biosensors into sample wells, the same integration time setting is used to collect the Octet® BLI signals. The integration time is conceptually similar to the exposure time (shutter speed) of a camera; the longer the integration time, the more light a spectrometer will receive. The goal of optimizing the integration time is to ensure there is adequate (but not too much) light. Additionally, the reflected light from the hydration plate and the sample plate must be as similar as possible. Although BLI does not measure the amount of light, but rather the change of the reflection pattern, the exposure level must be within the right range in order to capture signals for calculating the change of the reflection pattern. Either over-exposing (which results in a CCD signal saturation error) or under-exposing (which results in a CCD signal too low error) is problematic. The optimization of integration times can be considered as an automatic internal adjustment of the Octet® BLI optics, ensuring a consistent amount of light is analyzed each time a biosensor is used, as the light source ages and its intensity diminishes.

Clear, white or other non-black plates cannot be used as hydration plates because they have too much background reflection compared to the recommended black plates and will cause a spectrometer CCD signal error in data collection. Too much background light reflection will lead to shorter than usual integration times. Therefore, insufficient light will reach the spectrometer, resulting in “CCD signal too low” errors.

Having too little or no fluid in the wells of a black hydration plate can also cause “CCD signal too low” errors, as insufficient hydration volume will result in unusually low integration times. Therefore, a minimum 200 µL/well of hydration volume must be used.

Please note: Clear or white plates cannot be used. Black plates with clear bottoms cannot be used either.

12.2 Sample Plate

Do I have to use black, flat-bottom plate as the Sample Plate? Can I use a clear (transparent), white, or other plates as the hydration plate?

The black plates are required to reduce background reflected light to a minimum during biosensor pick-up and sample data acquisition. For Octet® BLI, only a small portion of light is reflected back for the interference change measurement, while most incident light passes through. The light that passes through will bounce back from the plate bottom and walls, resulting in signal noise collected by the spectrometer.

Using black flat-bottom plates allows for the absorption of the background light, minimizing signal noise.

Clear, white or other non-black plates cannot be used as sample plates. Too much background reflection occurs with these plates and results in overloading the spectrometer's CCD sensor which reduces assay sensitivity and causes CCD signal saturation errors.

12.3 U- or V- shaped Plates

Can I use U- or V- shaped Plates as Hydration or Sample Plates for my Experiments?

Black, flat bottom 96-well plates must be used for both biosensor hydration and sample plates in ALL Octet® BLI systems. U- and V-shape plates (regardless if they are black or not) cannot be used as sample plates as they cannot produce uniform reflection signals.

The uneven background reflection will cause problems with high background noises in the binding traces.

Only use black, flat-bottom plates for Octet® BLI assays.

13 Trademark Information

Kimwipe® is a registered trademark of Kimberly-Clark Worldwide, Inc.

Sartorius BioAnalytical Instruments Inc.
47661 Fremont Blvd.
Fremont, CA, 94538
USA

www.sartorius.com

The information and figures contained in these instructions correspond to the version date specified below.

Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.

Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

Copyright notice:

These instructions, including all components, are protected by copyright.

Any use beyond the limits of the copyright law is not permitted without our approval.

This applies in particular to reprinting, translation and editing irrespective of the type of media used.

Last updated:

07 | 2025

© 2025

Sartorius BioAnalytical Instruments Inc.
47661 Fremont Blvd.
Fremont, CA, 94538, USA

EL | Publication No.: WIQ6007-e250702