BLItz

User Guide

fortéBIO
A Division of **Pall Life Sciences**

PALL Life Sciences

BLItz System User Guide
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INTRODUCTION

Welcome to the *Pall ForteBio BLItz User Guide*. This guide explains how to:

- Configure, install, and operate your BLItz® system and BLItz Pro™ software.
- Set up and run quantitation and kinetics experiments on the BLItz instrument.

This guide contains information on materials required for using the BLItz system, installation procedure, setting up and running experiments, and performing data analysis.

The guide provides a set of specific instructions in "Working with Experiments" on page 39 based on the Starter kit included with your BLItz system.

NOTE: *If you are a new user, we highly recommend that you first perform the tests outlined in the BLItz Demo Guide to familiarize yourself with the BLItz system operations before embarking on your own research.*

For further information on any topic, contact technical support using information provided in "BLItz Support" on page 4.

We wish you the best in your research!

BLITZ SYSTEM PACKAGE

The BLItz system enables real-time quantitation of solution-based analytes or kinetic characterization of molecular interactions.

The BLItz system package consists of the BLItz instrument, the BLItz Pro software CD, power cord, USB cable, *BLItz Quickstart Guide*, *BLItz Demo Guide*, and a demo kit. (Figure 1-1).

Figure 1-1: *BLItz System Package Contents*

IMPORTANT: *Immediately store the sample diluents, human IgG, mouse IgG, and hydrochloric acid reagents at 2–8°C.*

Table 1-1 lists the BLItz system demo kit contents.

Contents	*Description*
Reagents	• One (1) tray of 32 Protein A biosensors • 200 µL of 10 mg/mL human IgG stock • 100 µL of 10 mg/mL mouse IgG in carrier protein containing buffer • 50 mL of sample diluent
Accessories	• 500 µL of 0.5M hydrochloric acid for deep cleaning drop holder • 20 black polypropylene conical bottom microcentrifuge tubes (0.5mL) • 96-well, black, flat-bottom polypropylene (Greiner Bio-One, #655209) for hydrating biosensors • One (1) drop holder • Swab, pack of 20, to wipe drop holder

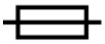
Materials (Required—Not Provided)	• Pipettors, any color:
	  • 2–20 µL
	  • 20–200 µL
	  • 100–1000 µL
	• Appropriate pipette tips, any color
	• Lint-free lab wipes
	• Gloves
	• Laptop or desktop computer—Ensure that your computer meets the following minimum specifications:
	  • 32-bit or 64-bit Windows XP and Windows 7
	  • Monitor resolution of at least 1,024 x 768
	  • 1 USB 2.0 port
	  • 2.5 GHz Dual Core CPU
	  • 2 GB RAM

Table 1-1: *BLItz Demo Kit Contents*

For information about BLItz system specifications and basic functions, see "BLItz System Specifications" on page 5. For information about installing and configuring the BLItz Pro software to use with the BLItz system, see "BLItz Installation and Usage" on page 13.

CONVENTIONS USED IN THIS GUIDE

Table 1-2: *BLItz Symbols and Instrument Labels*

Symbol	*Definition*
	NOTE: *A note presents pertinent details on a topic; for example, general information about tips or alternate options.*
	IMPORTANT: *An important message for instances where the experiment or procedure will not work if not properly followed.*
	WARNING: *A warning warns you that your actions could cause irreversible consequences or damage.*
	Electrical hazard
	Heat/hot
	Fuse

BLITZ SUPPORT

Pall ForteBio wants to ensure that you are completely satisfied with the BLItz system and software, and will address any concerns in a timely manner—it's our way of supporting your research. We are also extremely interested in your product feedback and application needs, and would be happy to discuss either with you.

For technical questions or to speak with one of our support staff, email technical support via our online form which also lets you attach files. You may also contact technical support at:

Pall ForteBio LLC
1360 Willow Road, Suite 201
Menlo Park, CA 94025
USA
Tel: +1-888-628-3875
Fax: +1-650-322-1370
E-mail: fortebio_support@pall.com
http://www.blitzmenow.com/email_support.html

CHAPTER 2:

BLItz System Specifications

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- Dip and Read Assays 9
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BLITZ SYSTEM SPECIFICATIONS

Figure 2-1 illustrates the BLItz system components (front and back).

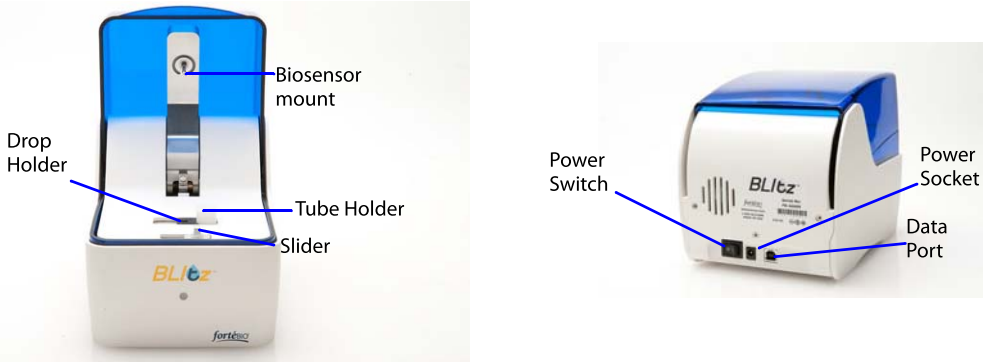


Figure 2-1: *BLItz System—Front and Back*

Table 2-1 lists and defines the BLItz system specifications.

Table 2-1: *BLItz System Specifications*

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

Table 2-1: *BLItz System Specifications (Continued)*

Specification	*Descriptions*
Sampling Volume	4 µL in drop holder; 250 µL in tube
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable fiber-optic biosensors
Optics and Mechanics	1-channel biosensor manifold, one (1) spectrometer
Shake Rate	Static or 1000–2600 rpm (default is **2200**)
Dimensions	6.8″ H x 6.0″ W x 8.7″ D (17.4 cm H x 15.3 cm W x 22.2 cm D)
Weight	7.2 lb (3.3 Kg)
Electrical Requirements	Power consumption: 8w (18W peak) Mains: AC 100–240 V, 0.2–0.1A, 50/60Hz, single phase
Connections	Power input, USB data output port
Software	BLItz Pro™ software for data acquisition and data analysis

BASIC FUNCTIONS OF THE BLITZ SYSTEM

Table 2-2 lists the basic functions of the BLItz system.

Table 2-2: *Basic Functions of the BLItz System*

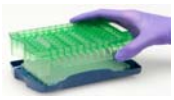
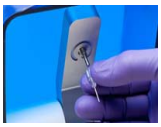
Function	
	**Hydrating Biosensors**—Perform hydration on the bench in the blue biosensor tray for, at least, ten minutes before use. Use the same buffer as that in your sample. For more information, refer to "Hydrating Biosensors" on page 42.
	Setting Up an Experiment—Select an **Experiment** type from menu (in the left pane) of the BLItz Pro software, input the experiment information and click **NEXT**. Then, follow the prompts to load sample and biosensor on the BLItz system.
	Placing a Drop of Sample in the Drop Holder Pipette a 4 µL drop of sample inside the dimple in the drop holder. Ensure that there are no air bubbles in the liquid.
	Placing and Removing Drop Holder—The drop holder is magnetic and easily snaps into place. Place the drop holder and press down gently to ensure a snug fit. Replace the drop holder when damaged or contaminated.
	Wiping sample with a swab (or other lint-free lab wipe)—At the end of a run, wipe the sample from the drop holder with a swab, or with the corner of a folded lab wipe. Rinse with buffer and blot with lab wipe thrice. For deep cleaning, use 0.5N HCl instead of buffer. Follow up with buffer rinse to remove all HCl.
	What is the use of the tube holder? The drop holder is the only sample location you should use in **Quick Yes/No**, **Create Standard Curve**, and **Quantitate Sample** experiments. In **Basic Kinetics** and **Advanced Kinetics** experiments, the tube holder is recommend for all baseline and dissociation steps, and for association steps longer than five (5) minutes. Use 250 µL of liquid in the tube for all experiments.
	Sliding Drop Holder and Tube to Read Position—Moving the slider to the right brings the drop holder in line with the black triangular mark and thus, to the read position. Moving the slider to the left brings the tube holder in line with the black triangular mark and thus, to the read position.

Table 2-2: *Basic Functions of the BLItz System (Continued)*

	Function
	**Mounting a Biosensor**—Apply a biosensor on to the biosensor mount by hand. Give a final quarter-turn-upward twist to ensure a snug fit. Do not let the tip of the biosensor come into contact with solid surfaces. Do not let the biosensor dry in air. This will result in loss of performance. Keep the biosensor hydrated until ready to use. After mounting a biosensor on the BLItz system, perform the experiment immediately.

BIOSENSORS

Dip and Read Assays

BLItz uses Pall ForteBio's Dip and Read™ label-free assays. These direct binding assays take place on a disposable biosensor made from a biocompatible matrix that is uniform, non-denaturing and minimizes non-specific binding (Figure 2-2). Only molecules that bind directly to the biosensor surface are detected, providing exceptional specificity for individual applications, even in crude media.

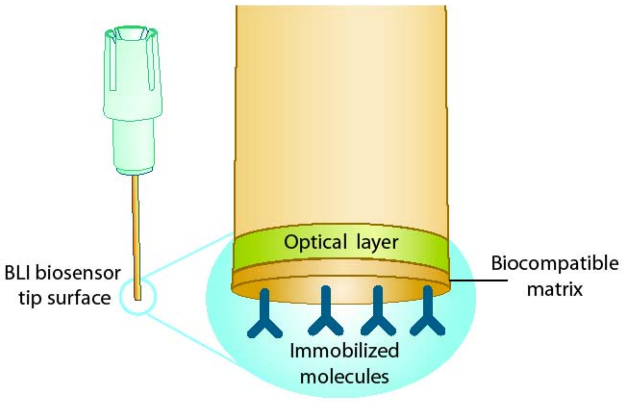


Figure 2-2: *Dip and Read Assays*

Biosensor Types

A variety of disposable, off-the-shelf biosensors are available for the BLItz system, making it easy to run a wide range of assays on the same system. The BLItz system uses the same Dip and Read biosensors that are used on BLItz's Octet platform, and over four million assays have been run to date. You can be confident that the assays you run on the BLItz system will provide the same high level of accuracy and precision. Our biosensors are manufactured in an ISO 9001:2008 certified facility. To find a current list of biosensors and to place an order, go to http://www.blitzmenow.com/biosensors.html.

***Table 2-3:** Biosensor Types*

Type	*Required Capture Molecule*	*Analyte Measured*	*Description*
Anti-hIgG-Fc	None	hIgG, human Fc-fused proteins	Binds specifically to the Fc portion of human IgGs and other proteins containing a human Fc region. Applications include quantitation of human IgG and proteins containing the human Fc region in cell line development, clone selection, process optimization, and production monitoring.
Anti-mIgG-Fv	None	mIgG, rat IgG, mouse-Fab, rat Fab	Binds specifically to the Fv(ab')2 portion of mouse and rat IgGs. Applications include quantitation of mouse and rat IgG in cell line development, clone selection, process optimization, and production monitoring.
Protein A	None	Many human and other IgG types	Binds with high affinity to the Fc region of human IgGs. Binds with moderate affinity to many subtypes of mouse and rabbit IgG. Applications include quantitation of IgG in cell line development, clone selection, process optimization, and production monitoring.
Protein G	None	Many murine and other IgG types	Binds with high affinity to the Fc region of murine, rat, goat, and bovine IgGs. Binds with moderate affinity to many subtypes of human IgG. Applications include quantitation of IgG in cell line development, clone selection, process optimization, and production monitoring.
Protein L	None	Most mouse, rat, human IgG and Fab	Binds with high affinity to most mouse, rat and human immunoglobulins containing a kappa light chain. Does not bind goat, bovine, rabbit or sheep IgG. Applications include quantitation of FAb fragments and of IgG in serum-based culture.
Anti-Penta-HIS (HIS1K)	None	His-tagged proteins, peptides	Uses the Qiagen Penta-HIS antibody to bind with high affinity HIS tagged recombinant proteins. Applications include quantitation and kinetics of HIS-tagged proteins.

Table 2-3: *Biosensor Types (Continued)*

Type	*Required Capture Molecule*	*Analyte Measured*	*Description*
Ni-NTA	None	HIS-tagged proteins, peptides	Quantitation and kinetic analysis of HIS-tagged proteins. Designed for use in buffer and diluted complex media.
Anti-GST (GST)	None	GST-tagged proteins, peptides	High affinity anti-GST antibody on biosensor binds GST-tagged proteins. Applications include quantitation of GST-tagged proteins and kinetics of proteins and peptides binding to GST-tagged proteins captured on biosensor.
High Precision Streptavidin (SAX) or Streptavidin (SA)	Biotin-tagged peptides, oligos, proteins	Proteins, peptides, oligos	Streptavidin-coated biosensor. Immobilizes biotinylated antibodies, proteins and nucleic acids to form a stable surface. Quantitation applications include antibody and protein quantitation in cell line development, clone selection, process optimization and production monitoring. Kinetic applications include protein and antibody kinetic and affinity analysis (k_a, k_d, K_D), and epitope binning.
Amine Reactive (AR2G)	Proteins, peptides, oligos	Proteins, antibody fragments	Second generation carboxylate functionalized surface allows covalent coupling of proteins via EDC/s-NHS mediated amide bond formation. Kinetic applications include protein and antibody kinetic and affinity characterization (k_a, k_d, K_D). Requires AR2G Assay Kit (P/N 18-5095).
Anti-hIgG-Fc Capture (AHC)	hIgG, human fc fusion protein	Proteins, peptides, antibody fragments	Immobilization of human IgG or other human Fc-containing proteins by binding to the human Fc region. Kinetic applications include protein and antibody kinetic and affinity characterization (k_a, k_d, K_D) and epitope binning.
Anti-mIgG-Fc Capture (AMC)	mIgG, mouse fc fusion protein	Proteins, peptides, antibody fragments	Binds the Fc portion of IgG1, IgG2a and IgG2b for capture-based immobilization. Applications include kinetic analysis of antibody-antigen interactions (k_a, k_d, K_D) and off-rate screening. IgG3 should be evaluated on a case-by-case basis.
Aminopropylsilane (APS)	Proteins, peptides	Proteins, peptides	Adsorption of proteins and membrane fractions through hydrophobic moieties. Kinetic applications include adsorption of proteins and membrane fractions through hydrophobic moieties for kinetic and affinity analysis (k_a, k_d, K_D).

BLI Technology

The BLI (bio-layer interferometry) technology (Figure 2-3) used by the BLItz system provides real-time data on protein interactions. The BLItz system emits white light down the biosensor, and then collects any light reflected back. Reflected wavelengths are affected by the thickness of the coating on the optical layer. Some wavelengths show constructive interference (blue), others show destructive interference (red).

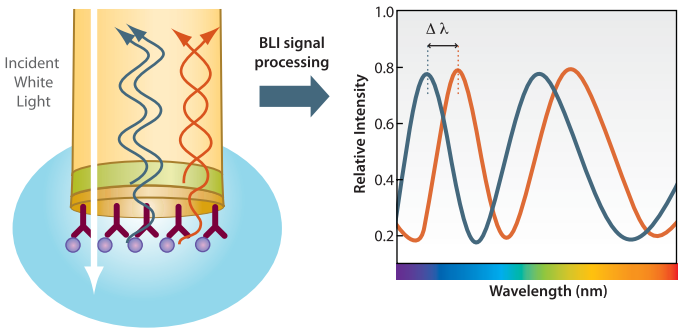


Figure 2-3: *BLI Technology—Displaying Constructive and Destructive Interference*

This interference is captured by a spectrometer as a unique spectral signature and is reported in relative intensity units (nm). Any change in the number of molecules bound to the biosensor causes a shift in the interference pattern that is measured in real time. This wavelength shift is a direct measure of the change in optical thickness (nm) of the biological layer.

Want to learn more about BLI technology? Call us at 1-888-628-3875 or email at fortebio_support@pall.com.

CHAPTER 3:

BLItz Installation and Usage

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INSTALLING THE BLITZ SYSTEM

IMPORTANT: *Users running the Octet system and software may install and run the BLItz system and BLItz Pro software on the same computer. For specific instructions, see Appendix A. For seamless operation of the Octet and BLItz systems, it is recommended that Octet users upgrade to at least versions 6.4.1.3 and 7.0.1.3 of the Octet software. lease contact the technical support team by calling 1-888-628-3875 or email fortebio_support@pall.com for more information.*

IMPORTANT: *Install the BLItz Pro software first before plugging in the BLItz system.*

IMPORTANT: *To install the BLItz system and BLItz Pro software, you should first install the software on your computer and go through the steps outlined below in the order specified.*

To install and configure the BLItz system and BLItz Pro™ software in your lab:

1. Remove the BLItz system, power cord, USB cable, and BLItz Pro software CD from the box. Place the BLItz system on a dry, level surface with minimal vibration, and away from direct sunlight.
2. Store the Sample Diluent, hydrochloric acid and IgG reagents at 2–8 °C right away.
3. Insert the BLItz Pro Software CD in a laptop or desktop computer and run the **BLItzInstallCD.exe** file. For minimum computer specifications, see Table 1-1 on page 3.
4. Click **Install BLItz Pro Software** (Figure 3-1) to install the BLItz Pro software.
5. Click **Install BLItz Driver** (Figure 3-1) to install the BLItz driver.

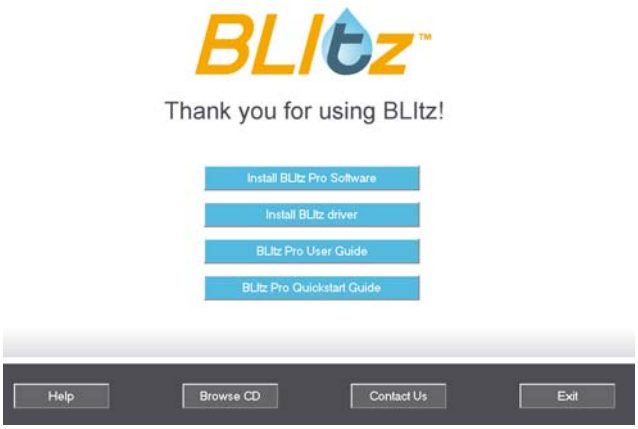


Figure 3-1: *Installing the BLItz Pro Software*

6. Connect the BLItz to a power source and computer using the cables provided. If using a desktop computer, ensure that the USB port is on the back of the computer. Do not connect to a USB port on the front side of the computer (Figure 3-2).

Figure 3-2: *BLItz System (Back)*

7. Place a drop holder in its slot on the BLItz system (Figure 3-3).

Figure 3-3: *Placing a Drop Holder on the BLItz System (press gently to ensure a snug fit)*

8. Power on the BLItz system and wait for the Windows plug-and-play manager to assign drivers.

IMPORTANT: *Keep the BLItz system powered on for, at least, 30 minutes, prior to running a sample.*

9. Run the **BLItz Pro** software (on the desktop, double-click the **BLItz Pro** icon).

BLITZ PRO SOFTWARE USER INTERFACE

This section describes the BLItz Pro software user interface (Figure 3-4) elements.

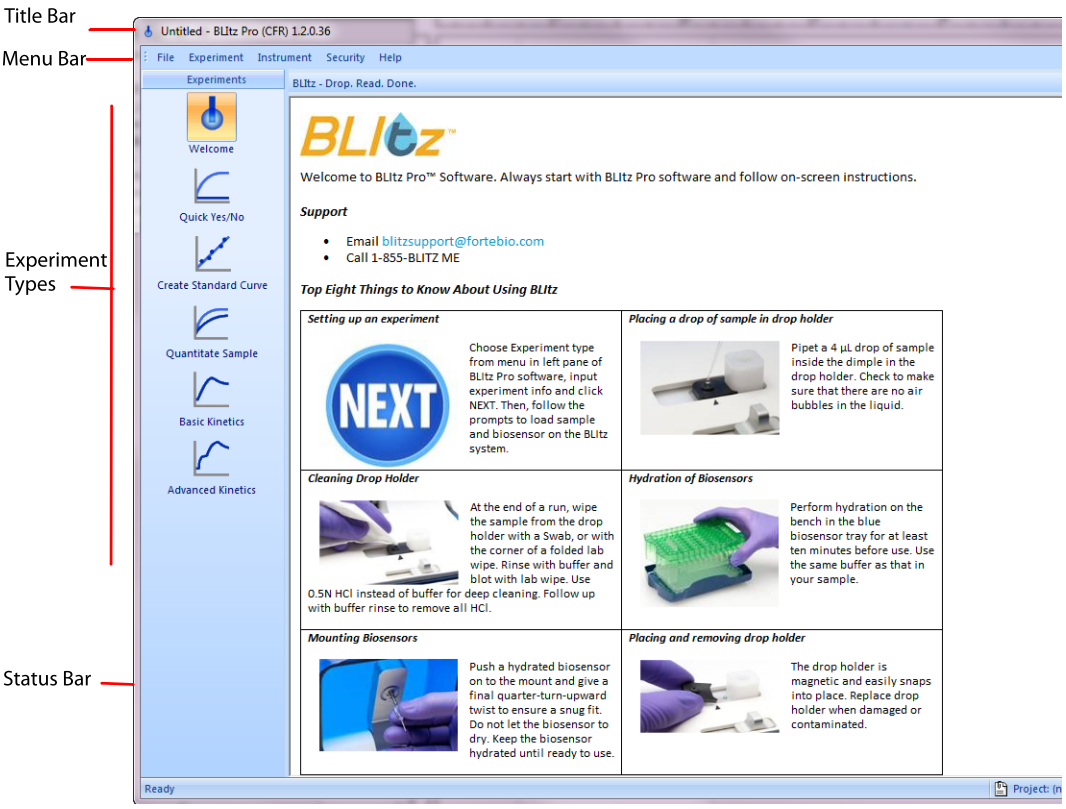
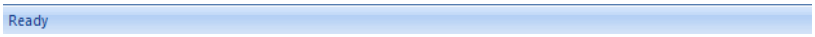


Figure 3-4: *BLItz Pro Software Welcome Page*

Toolbars

Table 3-1 describes the BLItz Pro software toolbars.

Table 3-1: *BLItz Pro Software Toolbars*

Experiment	*Description*
Title Bar	The **Title Bar** (Figure 3-6) is located at the top of the GUI window page and refers to the file name and BLItz Pro software version. Until the user saves the file, the file name is "Untitled". ***Figure 3-5:*** *BLItz Pro Title Bar*
Main Menu Bar	The **Main Menu Bar** (Figure 3-6) is located at the top of the GUI window, and displays the main menus available in the BLItz Pro software. See "Menu Commands" on page 18. for a definition of main menu bar commands. ***Figure 3-6:*** *BLItz Pro Main Menu Bar*
Status Bar	The **Status Bar** (Figure 3-7) is located at the bottom of the GUI window page and displays current system and experiment status. See Table 3-7 for a definition of the status bar icons.  ***Figure 3-7:*** *Status Bar*

Menu Commands

File Menu

The **File** menu (Figure 3-8) allows users to open and save method files, view experiments, print files, and set system and software options. Table 3-2 describes the menu commands and their respective functions.

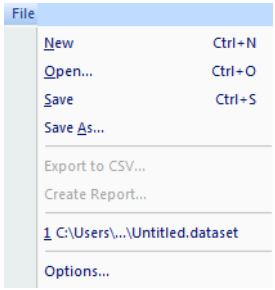


Figure 3-8: *File Menu*

Table 3-2: *File Menu Commands and Functions*

Menu Command	*Function*
New	Opens a new file after clearing out the existing experiment information that may exist on the software screen from a previous run. ***NOTE:*** *This menu option performs the same action as clicking the* **New Experiment** *icon.*
Open	Opens a previously saved experiment.
Save or **File > Save As**	Saves an experiment data file.
Export to CSV	Saves the results table to a .csv file that can be opened in a spreadsheet application.
Create Report	Creates a report of the experiment in PDF file format.

Table 3-2: File Menu Commands and Functions (Continued)

Menu Command	Function	
Options	Defines the system options available. To view the BLItz system options: 1. On the main menu, click **File** > **Options**. 2. On the Options dialog box (see Figure 3-9), confirm the default settings or enter new settings, and click **OK**.	
	Data Options—Significant digits	Specifies the number of decimal places (from two to nine) for the computed data. The default setting is **4**.
	Shaker—Shaker Speed (rpm)	Shaker speed (rpm)—Sample platform orbital shaking speed (rotations per minute). The default setting is **2200**. The shaker can be set to speeds in the range of 1000–2600 rpm. ***NOTE:*** *The default of* **2200** *rpm should be used in all kinetics experiments. The default speed also works well for the Quick Yes/No, Create Standard Curve and Quantitate Sample experiments. In these experiments, to work with high concentration samples, you may reduce the shake speed to lower levels.*
	Notification Sounds	When enabled (checked), plays sounds through computer speakers at the end of the hydration process, the end of a step, or at the end of a run. Click **Reset Sounds** to revert back to the default sound settings.

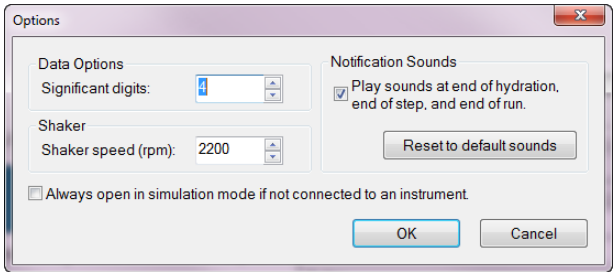


Figure 3-9: Options Dialog Box

Table 3-2: *File Menu Commands and Functions (Continued)*

Menu Command	*Function*
	Click the **Always open in simulation mode if not connected to an instrument** check box if you anticipate to work offline.
Exit	Closes the software after prompting you to save any changes.

Experiment Menu

The **Experiment** menu (Figure 3-10) displays the experiment modes available to run. Once you launch the BLItz Pro software, you may begin your experiment using one of the experiment modes available in the left pane (Figure 3-4: on page 16).

Table 3-3 describes the menu commands and their respective functions.

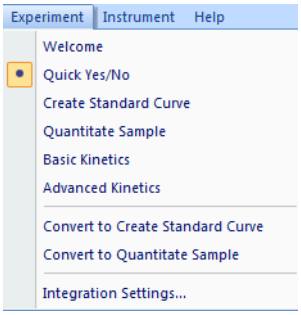


Figure 3-10: *Experiment Menu*

Table 3-3: *Experiment Menu Commands*

Menu Command	*Function*
Welcome	Displays the **Welcome** page upon launching the BLItz Pro software. Provides a high-level overview of the BLItz system and BLItz Pro software capabilities.
Quick Yes/No	Opens the **Quick Yes/No** mode for experimentation. Determines the presence of an analyte with a simple dip and read. Can include positive/negative control samples to compare the unknown results.
Create Standard Curve	Opens the **Create Standard Curve** mode for experimentation. Runs multiple standard concentrations to create a standard curve. For best results in quantitation, proteins used as standards should be identical to unknown and should be measured in the same sample milieu.

Table 3-3: Experiment Menu Commands (Continued)

Menu Command	Function
Quantitate Sample	Opens the **Quantitate Sample** mode for experimentation. Quantitates unknown samples against a standard curve.
Basic Kinetics	Opens the **Basic Kinetics** mode for experimentation. Generates binding curves for proteins of known. Use this mode to run kinetics experiments when the ligand is already bound to the biosensor, either as supplied by Pall ForteBio, or through an offline incubation of biosensor with ligand on the bench top.
Advanced Kinetics	Opens the **Advanced Kinetics** mode for experimentation. Enables ligand loading, followed by the generation of binding curves, and measures kinetic constants (k_a, k_d, K_D) for proteins of known concentrations.
Convert to Create Standard Curve	Switches the current mode to the Create Standard Curve mode. Use if the data generated in a Quantitate Sample experiment needs to be transferred to the Create Standard Curve mode for treatment as standard samples.
Convert to Quantitate Sample	Switches the current mode to the Quantitate Sample mode. Use if the data generated in a Create Standard Curve experiment must be transferred to the Quantitate Sample mode for quantitation.
Integration Settings	**Only available for Quantitate Experiments**. Optimizes the integration time at the start of each run. A short step is inserted at the start of each run where you are prompted to dip the biosensor in the tube to optimize the integration time to be used during the run.

Instrument Menu

The **Instrument** menu (Figure 3-11) resets or stops the BLItz Pro system during an experiment. Table 3-4 describes the menu commands and their respective functions.

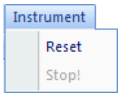


Figure 3-11: Instrument Menu

Table 3-4: Instrument Menu Commands

Menu Command	Function
Reset	Resets the system.
Stop!	Stops the current experiment run.

Security Menu

An additional **Security** menu (Figure 3-13) displays when running BLItz Pro CFR software.

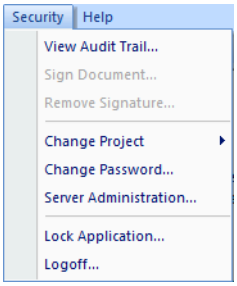


Figure 3-12: *Security Menu*

Table 3-5: *Security Menu Commands*

Menu Command	*Function*
View Audit Trail	Displays a historical log of user, system and software events recorded during user sessions.
Sign Document	Allows you to electronically sign documents. Once a document is signed, no further changes are allowed to the data file. Up to two signatures are allowed per data file.
Remove Signature	Allows you to remove the signatures assigned to the documents.
Change Project	During an active session, allows you to switch to another project.
Change Password	Allows you to change the user password.
Server Administration	When launching ForteBio GxP Server for the first time, you will be prompted to create an Administrator user account and to set the connection to clients. Please consult with your IT administration, if applicable, for configuring the BLItz Pro CFR for network use.
Lock Application	Locks the BLItz Pro CFR software during a user session to prevent another user from interrupting a session or experiment. When the application is locked, any experiments started will continue to run.
Logoff	Ends a user session.

Help Menu

The **Help** menu (Figure 3-13) displays the links to BLItz user guides, the BLItz Pro software version, and license information. Table 3-6 describes the menu command and its respective function.

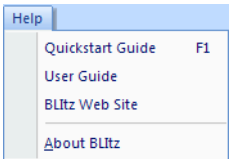


Figure 3-13: *Help Menu*

Table 3-6: *Help Menu Commands*

Menu Command	*Function*
Quickstart Guide	Opens the latest PDF of the *BLItz System Quickstart Guide*.
User Guide	Opens the latest PDF of the *BLItz System and BLItz Pro Software User Guide*.
BLItz Web Site	Opens your browser to the BLItz web site (http://www.blitzmenow.com).
About BLItz	Displays the BLItz properties and license information.

Icons

Table 3-7 list and defines the BLItz Pro software icons and status markers.

Table 3-7: *BLItz Pro Software Icons*

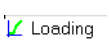
Icon	*Function*
	BLItz Pro—Represents the BLItz Pro software.
	Ready—Indicates that the BLItz Pro software is ready to use.
	Drop Holder—Indicates that the drop holder is in the read position.
	Tube—Indicates that the tube position is in the read position.
	Hydrate Biosensor—A timer to use for the biosensor hydration step. Set up the hydration time and click the **Hydrate Biosensor** icon to start. You may stop the hydration timer by clicking the **Stop** icon. Otherwise, after hydration, the timer runs down to zero (0) and the icon changes to **Hydration Done**. The window shakes until the **Hydration Done** icon is clicked.
	Stop—Stops the hydration or experiment.
	Hydration Done—Displays when the hydration process is complete. Is preceded by a ringtone to indicate completion. See the Table 3-2 on page 18 about notification sounds. The window shakes until the **Hydration Done** icon is clicked.
	New Experiment—Creates a new experiment, or clears your existing experiment for a new experiment. ***NOTE:*** *This menu option performs the same action as clicking the* ***File*** *>* ***New*** *command sequence.*
	Next—Guides you to the next step of the experiment run process.
	Initial Baseline—Mandatory step type used in Advanced Kinetics experiments.
	**Loading**—Step type used in Advanced Kinetics experiments to load ligand on biosensors.

Table 3-7: BLItz Pro Software Icons (Continued)

Icon	Function
Baseline	**Baseline**—Step type used in Basic and Advanced Kinetics experiments for the Baseline immediately preceding the Association step. ***NOTE:*** *Baseline and Dissociation steps should be performed in the tube.*
Association	**Association**—Step type used in Basic and Advanced Kinetics experiments to monitor the binding of an analyte to a ligand on the biosensor. Association should be monitored in tube for incubation times more than five (5) minutes. Up to five (5) minutes of association can be performed in the drop holder or tube.
Dissociation	**Dissociation**—Step type used in Basic and Advanced Kinetics experiments to monitor the dissociation of an analyte from a ligand. Dissociation should be monitored in the tube only.

UNINSTALLING THE BLITZ PRO SOFTWARE

To uninstall the BLItz Pro software:

1. From the Windows **Start** menu, click the icon (or the start button) and click **All Programs** > **ForteBio** > **Uninstall BLItz Pro**.

   You are prompted to confirm the request remove the BLItz Pro software and all of its components.

2. Click **Yes** to confirm the removal of the BLItz Pro software and all of its contents.
3. Click **OK** to exit.

CHAPTER 4:

21 CFR Part 11 Compliance

BLItz Pro CFR Software 28

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BLITZ PRO CFR SOFTWARE

The BLItz Pro CFR software provides additional support for GMP and GLP laboratory users. This version of the software includes features, such as user account management, audit trails, and electronic signatures. Use of BLItz Pro CFR software requires the additional installation of the ForteBio GxP Server software module, version 8.0 or higher.

This chapter describes how to use the ForteBio GxP Server module and the additional features enabled in the BLItz Pro CFR software.

NOTE: *BLItz Pro CFR files may be opened in the non-CFR version of the software in a read-only session. Non-CFR files may be opened and re-saved in BLItz Pro CFR software. The audit trail notes that the file was generated outside the CFR software, and subsequent actions will be captured.*

FORTEBIO GXP SERVER MODULE

Launching the ForteBio GxP Server for the First Time

When launching the ForteBio GxP server for the first time, you will be prompted to create an Administrator user account and to set the connection to clients. Please consult with your IT administration, if applicable, for configuring the BLItz Pro CFR for network use.

Should the BLItz Pro CFR and ForteBio GxP server reside only on the same computer, the "Localhost" option may be checked, and a port number chosen that is not currently used by another software application. If the ForteBio GxP server will be installed on a separate computer from the BLItz Pro CFR software, the computers must be connected over a network.

Assigning Additional Users

To assign additional users:

1. In the **Users** tab of the software, right-click and select a new user.
2. Enter the new user information and click **OK**.

   Figure 4-1 displays a blank New User dialog box for you to complete for the new user. Figure 4-2 displays a completed version of the users created.

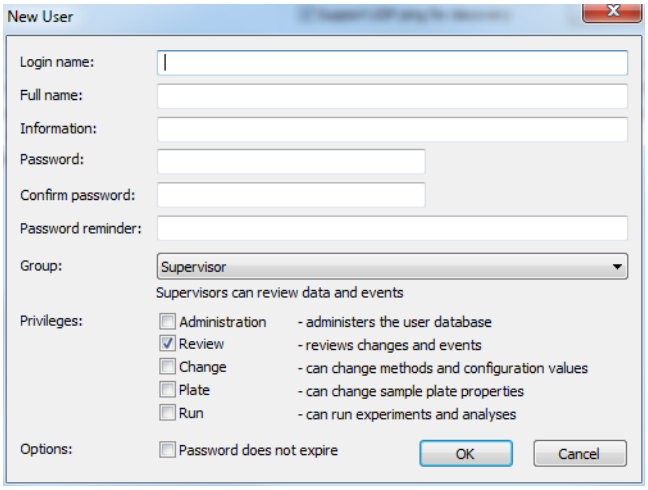

Figure 4-1: *New User Configuration*

NOTE: *Login names must be unique, and duplicates will not be allowed by the ForteBio GxP Server. Additionally, you can assign permissions to the user based either on a pre-set privileges based on user Group, or assign individual sets of privileges to enable particular workflows.*

NOTE*: The ForteBio GxP server is shared by the BLItz and Octet platforms. The "Plate" privilege has no designated function with the BLItz system because it does not accept samples in a microplate format.*

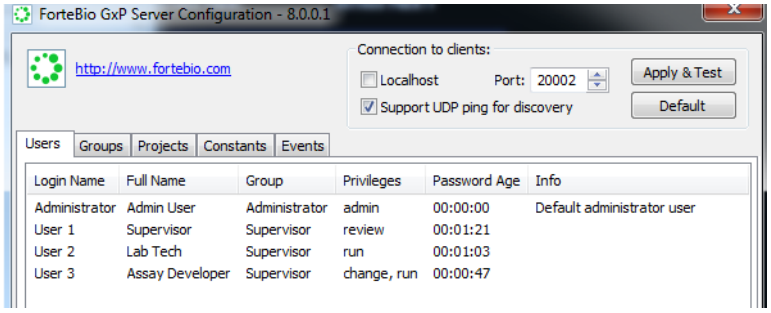

Figure 4-2: *Assigning Additional (New) Users*

Assigning Privileges by Group

Software permissions can be assigned to individual users based on the Group to that they belong. In the **Groups** tab of the ForteBio GxP server, there are five pre-assigned Groups. The name and specific privileges for each Group can be modified by double-clicking the Group of interest. To create a new group or assign privileges by group, right-click in the dialog box to expose and select this option.

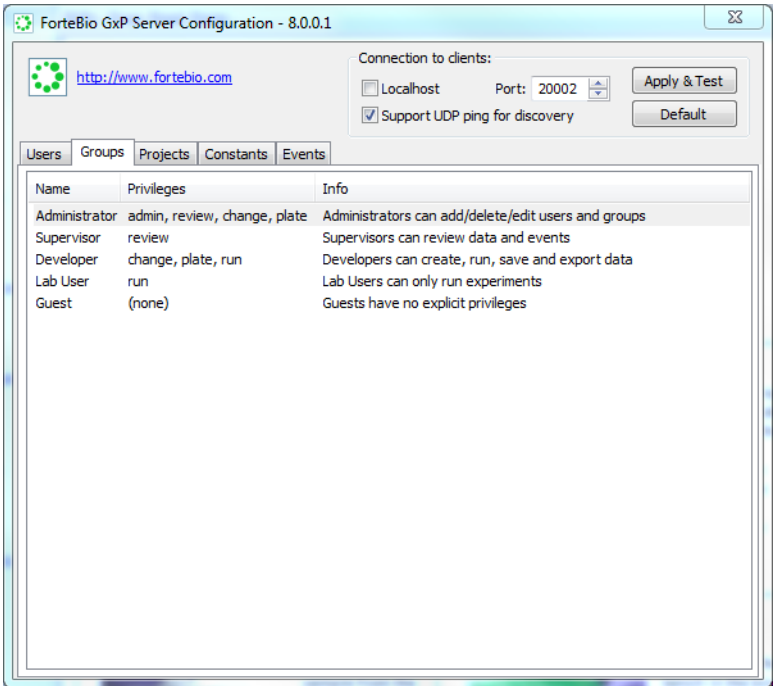

Figure 4-3: *ForteBio GxP Server Groups Tab*

Creating Projects for Sorting Events

For additional sorting of the audit trail of events, the ForteBio GxP server introduces a projects concept. Method files used in BLItz Pro can be associated with specific projects, and the Events log filtered by project.

To create a new project, in the **Projects** tab, right-click to configure settings and create new projects.

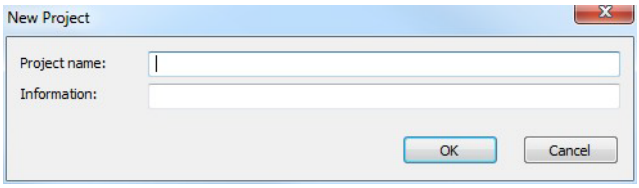

Figure 4-4: *Creating a New Project*

Setting Constants for Advanced Options

The **Constants** tab contains global security settings that affect all users and enhance security:

- **PasswordTTL** is the lifetime set for user passwords. The value entered is in days.
- **PasswordMinLength** sets the minimum number of characters required for a password. If set to 0, a password is not required to log in. This is not recommended.
- **PasswordSecure** affects the complexity of passwords. A value other than zero (0) requires a minimum length of three characters and the inclusion of at least one alphabetic, one numeric, and one punctuation character.
- **CredentialsTTL** allows for offline use of the BLItz Pro CFR software in a networked environment. The value entered sets the maximum amount of time BLItz Pro CFR can be used in the absence of reconnecting to the ForteBio GxP server. Users are authenticated locally until the connection to the ForteBio GxP server is restored, and changes to the audit trail are integrated.
- **UserIdleMin** logs a user out of the BLItz Pro CFR software after a specified amount of time. The value is in minutes.

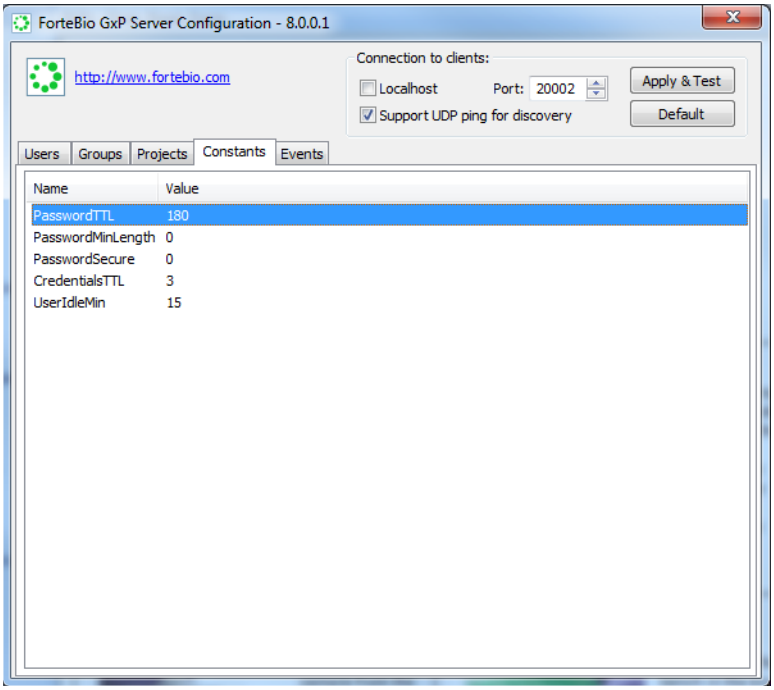

Figure 4-5: *Constants Tab—Advanced Options*

Viewing Events in the Audit Trail

The **Events** tab contains the global audit trail. The events can be sorted by user, project, and machine (i.e. computer used) in the drop-down menus. The list can additionally be ordered by clicking any of the column headers. For example, to sort events by user, click the **Login Name**. The default method of sorting is by date/time.

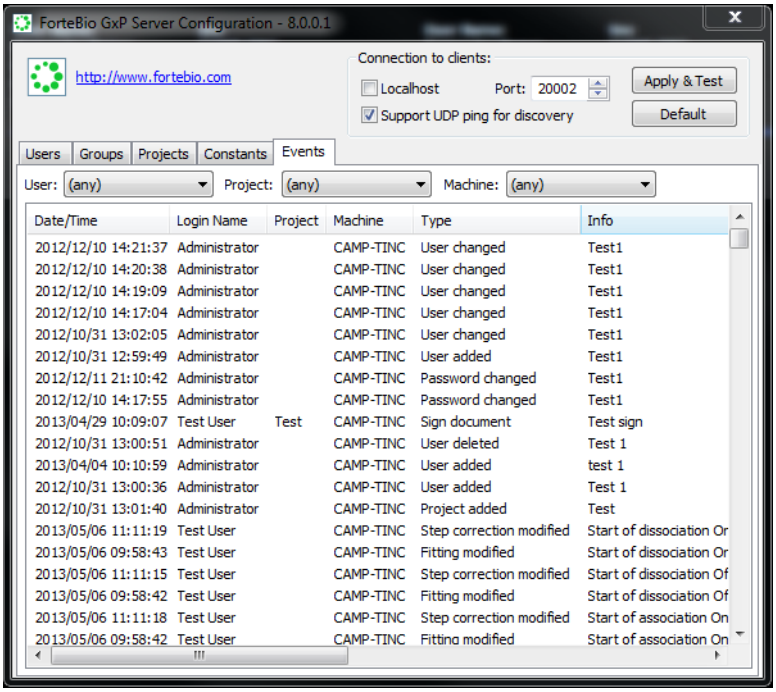

Figure 4-6: *Viewing Events in the Audit Trail*

LAUNCHING THE BLITZ PRO CFR SOFTWARE FOR THE FIRST TIME

When launching the BLItz Pro CFR software for the first time, perform the following steps:

1. On your desktop, navigate to and click the BLItz Pro software icon.
2. When the Login dialog box displays, click **...** (ellipsis) to select a **Server** location (Figure 4-7).

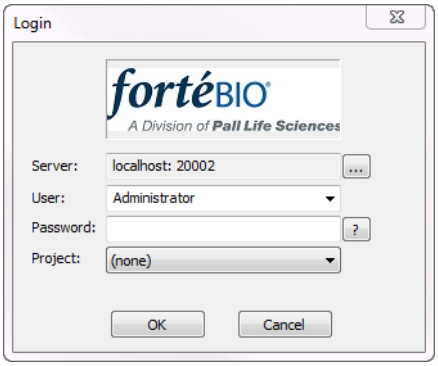

Figure 4-7: *Launching the BLItz Pro CFR Software for the First Time*

3. Confirm that the **Server** location is correct.
   a. Click **Default** to recall the default server settings of localhost and Port 2002.

      Local host—If the local computer is to be used as the GxP Server module host, click the **Localhost** check box. Change the **Port** number if necessary.

      Remote host on same subnet—If the GxP Server module is hosted on the same subnet, deselect the **Localhost** check box and click **Find**. A list of potential GxP Server module addresses will be listed. Choose the location and click **OK**.

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

   b. When the Server module host location has been selected or entered, click **OK** to save changes and exit the **Authentication Server** dialog box.
4. On the Login dialog box, from the **User** drop-down list, select a login name.

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

---

5. Enter your password in the **Password** text box. (Click **?** for a password reminder if needed.)
6. Select a project from the **Project** drop-down list, if required.
7. Click **OK**.

RESTRICTED PRIVILEGES

The main differences users of the software notice is the availability of fields in the BLItz Pro CFR software based on assigned privileges. The general workflows enabled by the pre-set privileges assigned to Groups are as follow:

- **Users** can modify descriptors in Run Settings for an experiment, but cannot alter the run time and shaker settings. Once data has been acquired, changing sample descriptors, such as Sample ID and Concentration, cannot be altered. Users can also load standard curves for quantitation, but cannot save their own.
- **Developers** have all of the permissions of Users, but can modify the run time and shaker settings for an experiment, as well as modify sample descriptors after the data has been acquired. Developers can also save standard curves.
- **Supervisors** can open files and make changes to settings that affect only the display.
- **Administrators** have all of the previously mentioned privileges.

COMPLIANCE FEATURES

Software features to support compliance can be accessed by clicking the **Security** menu.

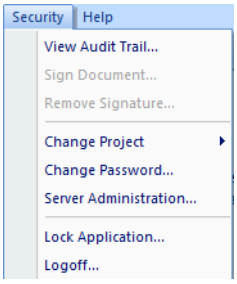

Figure 4-8: *Security Menu*

Viewing the Audit Trail

The Audit Trail displays a historical log of user, system and software events recorded during user sessions. To view the Audit Trail (see example in Figure 4-9), click **Security** > **View Audit Trail**.

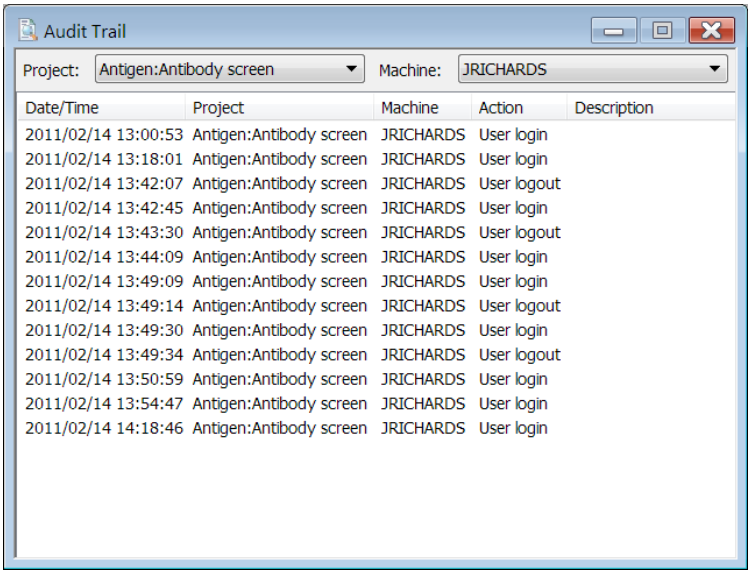

Figure 4-9: *Audit Trail*

Electronic Signatures

The BLItz Pro CFR software also adds support for electronic signatures. To sign a data file, on the **Security** menu, select **Sign Document** or click the lock icon. A password is required to authenticate the signing, and a prompt will follow to capture the meaning of the signature.

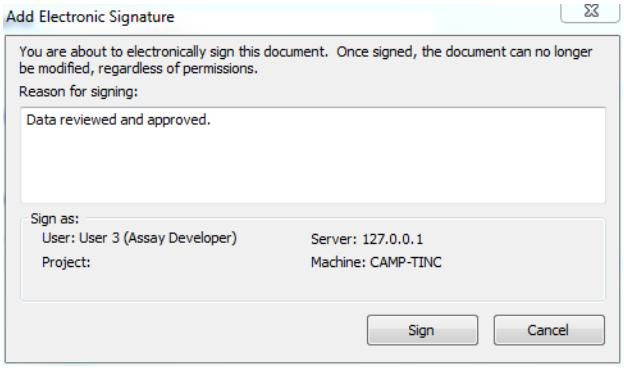

Figure 4-10: *Electronically Signing a Document*

Once a document is signed, no further changes are allowed to the data file. Up to two signatures are allowed per data file.

Changing Projects During a User Session

During an active session, users can switch to another project:

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

   A list of projects assigned to your user account displays with the active project highlighted.

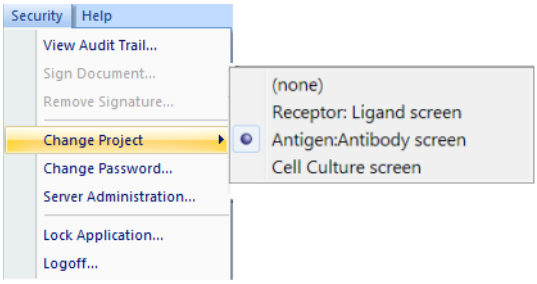

Figure 4-11: *Changing Projects*

2. Select the desired project from the list.

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

Changing the User Password

To change the user password:

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

   The **Change Password** dialog box displays.

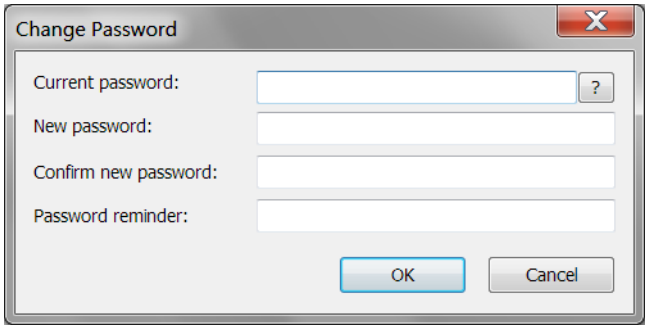

Figure 4-12: *Change Password Dialog Box*

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

Locking the Application

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

To lock the application:

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

   The software is placed in locked mode immediately and the **Application Locked** dialog box displays (Figure 4-13).

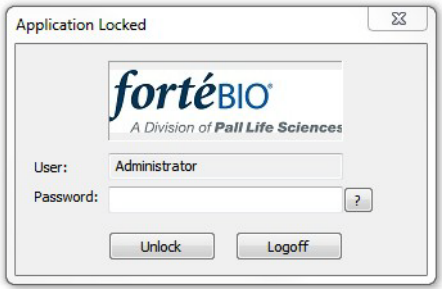

Figure 4-13: *Application Locked Dialog Box*

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

Ending a User Session

To end a user session:

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

CHAPTER 5:

Working with Experiments

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INTRODUCTION

The section provides a set of specific instructions based on the Starter kit included with your BLItz system. We highly recommend to new users that you first perform the tests illustrated in this guide to familiarize yourselves with BLItz system operations before embarking on your own experiments. The specific experiment instructions should help you in designing and running your own experiments.

ACCESSING BLITZ EXPERIMENT MODES

To access the BLItz experiment modes:

1. Launch the BLItz Pro™ software: on the desktop, double-click the **BLItz Pro** icon.

   The **BLItz Pro Welcome** page (Figure 5-1) displays with the **Experiments** listed in the left pane.

2. You can access any Experiment type by clicking the icons in the left pane, or by clicking the **Experiment** menu.

   For a description of the **Experiment** menu commands and their respective functions and experiment type definitions, see Table 3-3 on page 20.

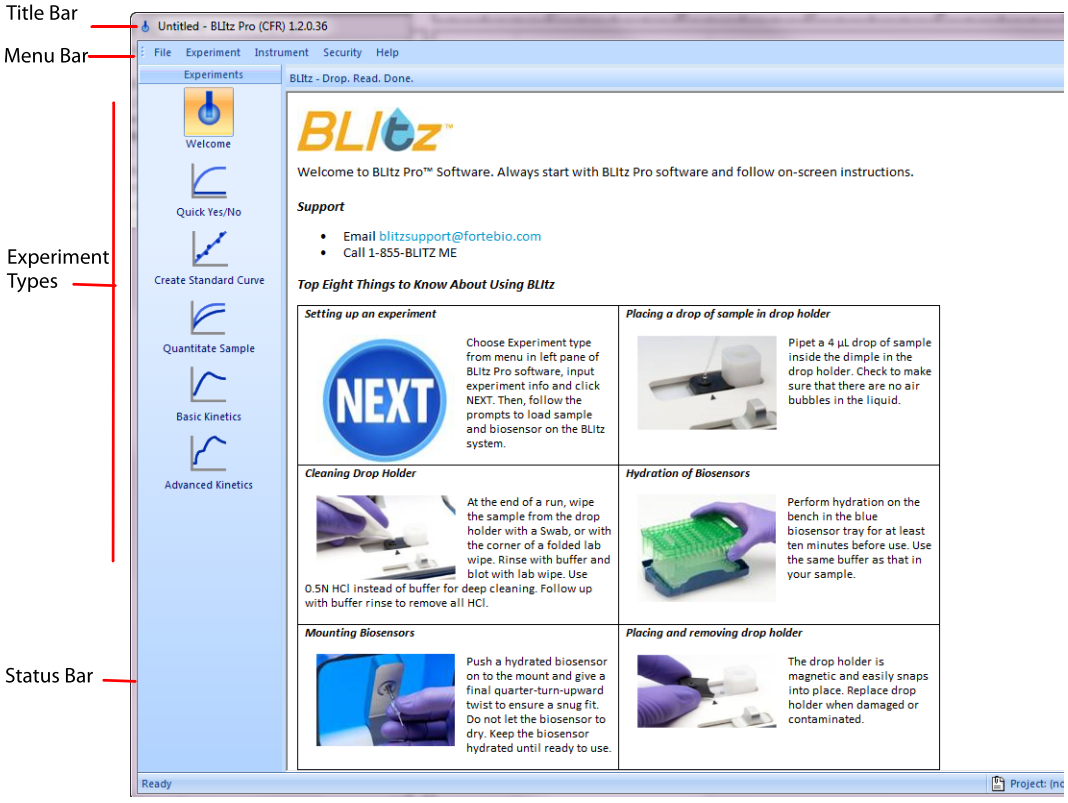
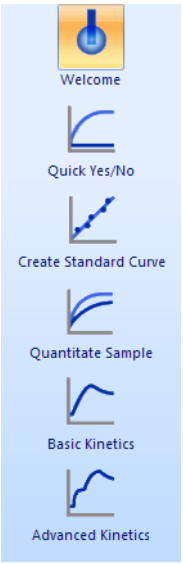


Figure 5-1: *BLItz Pro Software Welcome Page*

Defining Experiment Types

The BLItz Pro software's Experiments menu lists the experiment types:



- **Quick Yes/No.** Determines the presence of an analyte with a simple dip and read. Can include positive/negative control samples to compare to the unknown results.
- **Create Standard Curve.** Runs multiple standard concentrations to create a standard curve. For best results in quantitation, proteins used as standards should be identical to unknown and should be measured in the same sample matrix.
- **Quantitate Sample.** Quantitates unknown samples against a standard curve.
- **Basic Kinetics.** Generates binding curves for proteins of known concentrations and measures kinetic constants (k_a, k_d, K_D).
- **Advanced Kinetics.** Enables ligand loading, followed by the generation of binding curves for proteins of known concentration, and measures kinetic constants (k_a, k_d, K_D).

BEFORE YOU START YOUR EXPERIMENT

- Allow all required reagents to warm to room temperature before running your experiments on the BLItz system.
- Handle biosensors carefully. Do not touch the tip of the biosensor or bring into contact with surfaces.
- Hydrate only the biosensors needed for an experiment. Store unused biosensors dry on the benchtop in the biosensor tray.
- Do not leave a hydrated biosensor to dry. Keep it dipped in buffer until ready for use.
- Store hydrated biosensors for up to 48 hours in a refrigerator (2–8 °C) and warm to room temperature before re-use.
- Dispose used biosensors in a sharps container.
- Use 4 µL of sample in the drop holder. Use 250 µL of sample or buffer in the tube. (A tube is used in Basic Kinetics and Advanced Kinetics experiments.)
- Ensure that there are no bubbles in the sample. Bubbles may cause erratic signals.
- Between experiment steps, when lifting the BLItz system cover, lift all the way up to ensure proper electronic communication with the BLItz Pro software.

Navigating Through the BLItz System

The BLItz Pro software's **NEXT** button is an important tool in communicating with the BLItz system. After entering Experiment Settings and Run Settings, click the **NEXT** button to start an experiment. The software starts communicating with the BLItz system to guide you through the process of adding samples and mounting biosensors on the BLItz system to complete your experiment.

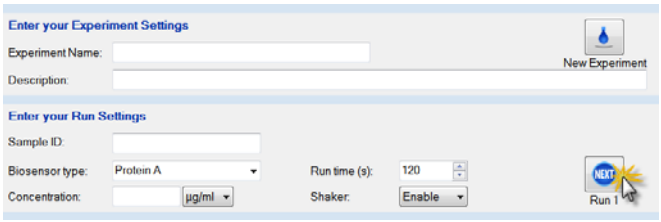


Figure 5-2: *Entering Experiment and Run Settings*

***NOTE**: Clicking **NEXT** displays **User Action Required** messages that guide you through the steps of your experiment. It is imperative to read the action messages and follow the instructions.*

Hydrating Biosensors

***IMPORTANT:** Biosensors must be hydrated before use in any experiment.*
** Hydrate only those sensors to be used in an experiment.*
** Hydrate just before running an experiment.*
** Hydrate for at least 10 minutes.*
** Do not leave the biosensors to dry on the biosensor mount.*

Pall ForteBio's biosensors should be dipped in buffer to hydrate the bio-layer at the tip and prepare it for use in a run. Hydration is performed on the bench top in the blue tray provided. A 96-well microplate is required for the hydration.

In the BLItz Pro software, each experiment mode reminds you to perform this hydration step. The timer included in the software may be used to time your hydration. Alternatively, you may use a lab stop watch to time your hydration. You do not need to hydrate exactly for 10 minutes; a minimum of 10 minutes is sufficient.

Use the following procedure to set up biosensor hydration:

1. Ensure that all reagents to be used on the BLItz system are warmed to room temperature.
2. Add 200 µL of sample diluent or buffer only in wells of a 96-well microplate corresponding to the biosensors that will be used in the experiment; for example, if using biosensors A1 to A4 from the biosensor tray, add buffer only to wells A1 to A4 in the 96-well microplate.
3. Remove the clear lid of the Protein A biosensor tray.
4. Lift the green biosensor rack carefully out of the blue biosensor tray holder (Figure 5-3)—avoiding touching of the biosensor tips on any solid surface.

Figure 5-3: *Biosensor Tray*

NOTE*: Use the black hydration microplate provided in the demo kit, in place of the clear microplate shown in Figure 5-3.*

5. Place the hydration microplate inside the blue biosensor tray holder.
6. Carefully lower the green biosensor rack on top of the hydration microplate in the biosensor tray holder to begin hydrating the biosensors.
7. Select a hydration time of **10 minutes** (the default) or more, and click the **Hydrate Biosensor** icon (Figure 5-4).

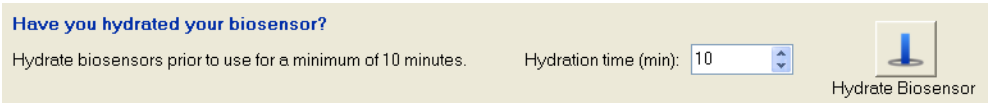


Figure 5-4: *Hydrate Biosensors*

IMPORTANT: *Prior to any experiment performed on the BLItz system, the biosensors must be hydrated for a minimum of 10 minutes to ensure active protein on the biosensor tip. Biosensors should be hydrated in a solution buffer that is as similar to the sample matrix as possible.*

NOTE*: The hydration timer in the BLItz Pro software may be used to time the biosensor hydration process. Users may also use other lab stop watch methods to monitor the hydration time.*

The **Hydrate Biosensors** icon changes, indicating that the hydration process begins. The **Hydration time (min)** field timer counts down the 10 minutes. You can click the **Stop Hydration** icon at any time to stop the hydration process (Figure 5-5).

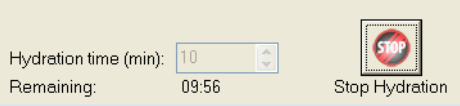


Figure 5-5: *Stop Hydration Process*

After hydration, the timer runs down to zero (0) and the icon changes to **Hydration Done** (Figure 5-6). The window shakes until the **Hydration Done** icon is clicked.

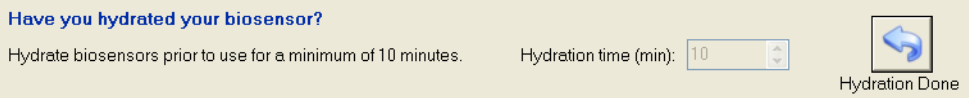


Figure 5-6: *Hydration Done*

NOTE*: After hydrating, leaving a biosensor on the biosensor mount or in air to dry will affect performance of the biosensor. If you do not plan to perform an experiment immediately, leave the biosensor tip to hydrate.*

8. Access any Experiment type by clicking the icons in the left pane, or by clicking the **Experiment** menu (Figure 3-10: on page 20).

RUNNING A QUICK YES/NO EXPERIMENT

When using the Starter kit to perform a **Quick Yes/No** experiment, perform the following steps for human IgG samples binding Protein A biosensors.

To develop a **Quick Yes/No** experiment for your samples on the BLItz system, consider these guidelines:

- Test a negative control and positive control sample along with your unknown.
- Use an appropriate biosensor type from Pall ForteBio's list of off-the-shelf biosensors. If one is not readily available, you may prepare a custom biosensor by loading your ligand on any one of the biosensors available.
- Use the drop holder for all **Quick Yes/No** measurements. Use 4 µL of sample diluent.
- Start with the default Run time value of **120** seconds to test your positive and negative controls. You may then optimize the Run time as needed. Typically, longer run times allow more binding of analyte to the biosensor, thus providing greater signal.
- Keep the Shaker in **Enable** mode to allow good mixing of sample. You can tweak the shaker rate via **File** >**Options** menu to a value between 1000 and 2600 rpm to suit your needs. Typically, increasing shaker rate leads to faster binding rates.
- Always input experiment info first and click **Next**. Follow the on-screen prompts to load sample and biosensor on the BLItz system.

The following experiment is an example of a **Quick Yes/No** experiment, where hIgG is the analyte you will use to prepare three (3) samples: positive control (1000 µg/mL), "unknown" (100 µg/mL), and negative control (sample diluent). The 100 µg/mL hIgG sample will be considered a mock "unknown" sample to show how BLItz can evaluate the presence of your protein in a sample. The negative control (sample diluent) will also be used as the sample blank (reference).

Everything you need is in the demo kit:

- Protein A biosensors
- hIgG stock (10 mg/mL)
- sample diluent
- 96-well, black flat-bottom microplate

To run a **Quick Yes/No** experiment:

1. Click the **Quick Yes/No** icon

to display the **Quick Yes/No** experiment mode (Figure 5-7).

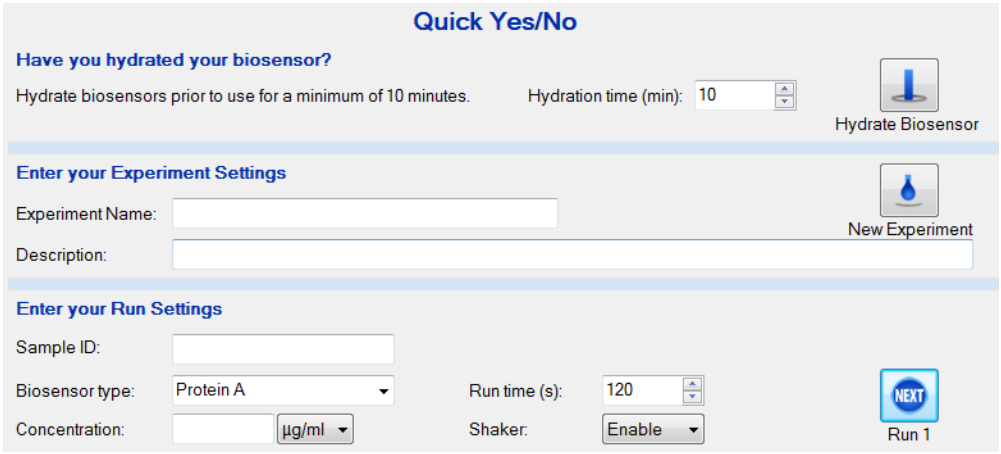


Figure 5-7: *Quick Yes/No Experiment*

Preparation

2. Ensure that all reagents to be used on the BLItz system are warmed to room temperature.
3. Hydrate three (3) biosensors, as described in “Hydrating Biosensors” on page 42.
4. Prepare the hIgG test samples as follows:
   - 1000 µg/mL hIgG (positive control): 20 µL of stock hIgG + 180 µL of sample diluent
   - 100 µg/mL hIgG (unknown sample): 10 µL of 1000 µg/ml solution + 90 µL of sample diluent

---

NOTE: *hIgG samples should be run in this order:*
Run 1: sample blank (negative control)
Run 2: 100 µg/mL (unknown)
Run 3: 1000 µg/mL (positive control)

Experiment

5. Input **Run Settings** for **Negative Control**, as shown in Figure 5-8.

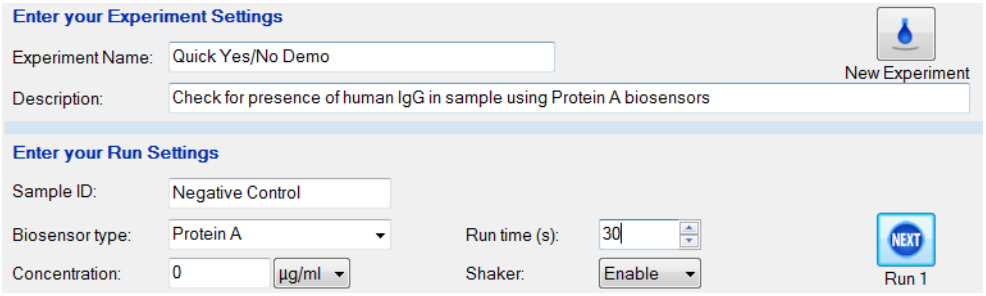


Figure 5-8: *Entering Run Settings for Negative Control*

   a. Enter a Run time of **30 seconds**.
   b. Click NEXT.

6. Follow the on-screen instructions (Figure 5-9).

Figure 5-9: *User Action Required—Experiment Operations*

7. Add 4 µL of the sample diluent for the first run into the drop holder (Figure 5-10).

Figure 5-10: *Adding 4 µL of Sample into Drop Holder*

8. Load the Protein A biosensor on to the biosensor mount; give it a final quarter-turn upward twist to ensure a snug fit (Figure 5-11).

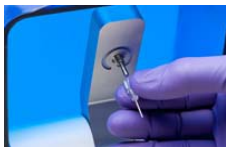


Figure 5-11: *Mounting a Biosensor*

9. Move the slider to the right to move the drop holder to the read position (Figure 5-12).

Figure 5-12: *Moving Drop Holder to Read Position*

10. Close the BLItz system cover to start data acquisition each time the BLItz Pro software prompts you (Figure 5-13).

Figure 5-13: *Closing the BLItz System Cover*

Data is collected in real-time on screen. The sensorgram (Figure 5-14) displays the results for Negative Control.

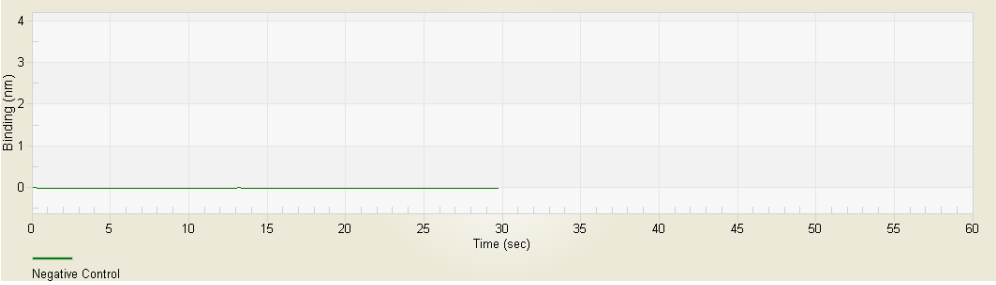


Figure 5-14: *Data Collected in Real-Time—Binding Signal Measured in nanometers (nm) as a Function of Time (seconds)*

After the run, a **Save As** dialog box displays and prompts you to save the experiment file (Figure 5-15).

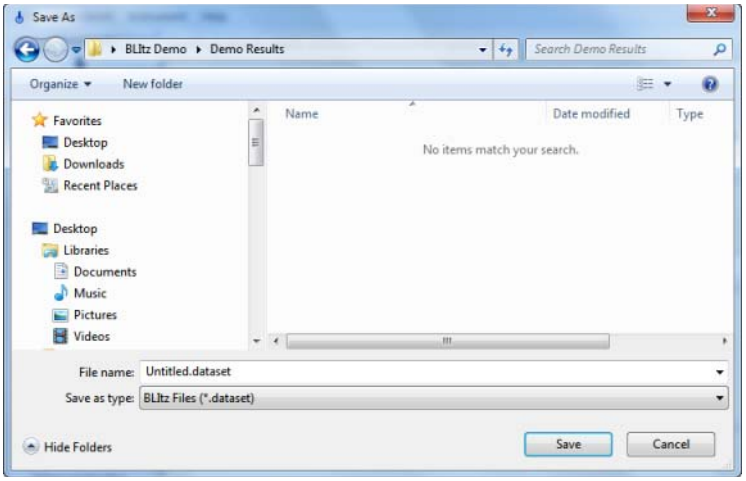


Figure 5-15: *Saving Dataset File*

11. Enter a file name and **Save** the dataset.
12. Lift the cover, discard the used biosensor, wipe the drop holder with a swab or Kimwipe (Figure 5-16), and rinse 3X with sample diluent—wiping each time with a swab or Kimwipe.

The best way to rinse 3X with sample diluent is to take, at least, 20 µL of buffer in a pipet and add small aliquots into the drop holder followed by blotting with a Kimwipe.

Figure 5-16: *Wiping the Drop Holder with a Kimwipe*

13. Perform subsequent sample runs by repeating steps 4 to 11 for each sample:
    - Run 2: 100 µg/mL (unknown)
    - Run 3: 1000 µg/mL (positive control)

Data

14. Inspect the **Run** data.

    The sensorgram (Figure 5-17) displays the results after all runs. The unknown sample displays binding signal, indicating the presence of human IgG.

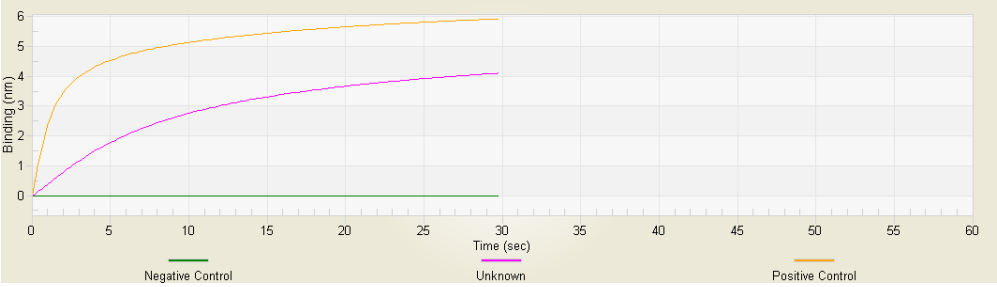
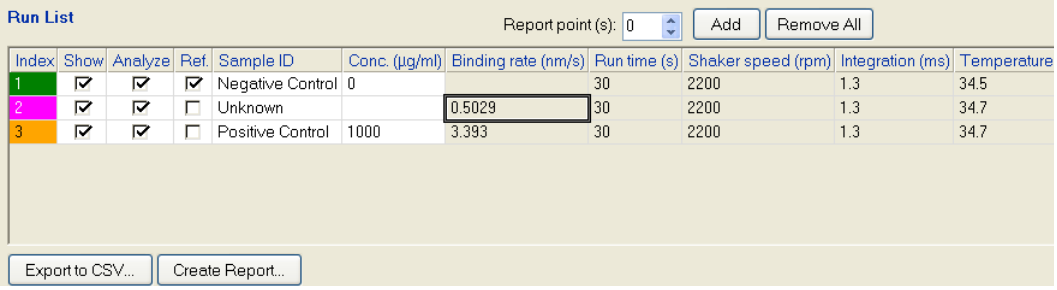


Figure 5-17: *Inspecting the Run Data*

15. Check the Run List table for the Binding Rate value for the unknown sample.

    A higher value than that of the Negative Control should mean that protein is present in the unknown sample (Figure 5-18).



Index	Show	Analyze	Ref.	Sample ID	Conc. (µg/ml)	Binding rate (nm/s)	Run time (s)	Shaker speed (rpm)	Integration (ms)	Temperature
1	☑	☑	☑	Negative Control	0		30	2200	1.3	34.5
2	☑	☑	☐	Unknown		0.5029	30	2200	1.3	34.7
3	☑	☑	☐	Positive Control	1000	3.393	30	2200	1.3	34.7

Figure 5-18: *Binding Rate Values in the Run List Table*

Table 5-1 lists and defines the binding rate values in the Run List table.

Table 5-1: *Binding Rate Values in the Run List Table*

Parameter	*Description*
Report point (s)	Specifies a time point at which to report signal for all samples in the graph. • **Add**—Adds a report point to the table. • **Remove All**—Removes all the report points from the table.
Index	Numbered order of the curves processed. The index is useful to sort back to the original order.
Show	Controls whether the curve is displayed in the Run Data window.
Analyze	Allows you to analyze the results. If not checked, this box excludes the sample from further analysis.

Table 5-1: Binding Rate Values in the Run List Table (Continued)

Parameter	*Description*
Ref.	Designates a sample for reference subtraction. If a single sample is chosen, that will be subtracted from all other sample curves. If multiple samples are chosen as reference, the average will be subtracted from all other sample curves.
Sample ID	The sample ID entered during experiment setup.
Conc. (µg/ml)	The molar concentration of the sample used in the association step. The molar concentration is entered by the user or computed by the molarity calculator during experiment setup.
Binding rate (nm/s)	The rate of sample binding to the biosensor computed by the BLItz Pro software using the binding rate equation specified.
Run time (s)	The duration of data analysis seconds.
Shaker speed (rpm)	The sample platform orbital shaking speed (rotations per minute). This value is set in **File** > **Options**.
Integration	Reports the time intervals the spectrometer was exposed to light in acquiring the data.
Biosensor Type	The biosensor chemistry utilized in the experiment. By default, Protein A is used.
Information	Information about the biosensor that was entered in the BLItz system.
Status	Indicates if a run is interrupted. For example, if a user stops a run, the status is shown as "Terminated by User".
Export to CSV	Exports the data in the graph to .csv files, one for each sample.
Create Report	Creates a report of the experiment in a PDF file format.

Result

The "unknown" sample, which we know is a 100 µg/mL hIgG solution, displayed a binding signal that indicates the presence of protein. In just a few minutes, you have successfully tested a sample for presence of protein. Bravo!

CREATING A STANDARD CURVE

When using the starter kit to perform a Create Standard Curve experiment, follow the instructions provided below for human IgG samples binding Protein A biosensors.

To create a standard curve for your samples on the BLItz system, consider these guidelines:

- Use standards that are representative of the analyte/unknown in your Quantitate Sample experiment.
- Use a sample milieu that is representative of that in which the analyte/unknown will be present in your Quantitate Sample experiment.
- Start with a broad concentration range of standard samples that will cover the analyte/unknown samples that you wish to quantitate.
- When optimizing experiment conditions, use fewer standard samples distributed over the concentration range. After optimizing experiment conditions, populate more standard concentrations to maximize reliability of the standard curve.
- Run standards from low to high concentrations to minimize interferences from carryover between samples. Cleaning the drop holder according to recommendations should typically prevent such carryover.
- Use an appropriate biosensor type from Pall ForteBio's list of off-the-shelf biosensors. If one is not readily available, you may prepare a custom biosensor by loading your ligand on any one of the biosensors available.
- Use the drop holder for all Create Standard Curve runs. Use 4 µL of standard samples.
- Start with the default Run time value of 120 seconds. You may then optimize the Run time as needed. Typically, longer run times allow more binding of analyte to the biosensor, thus providing greater signal.
- Keep the Shaker in **Enable** mode to allow good mixing of sample. You may tweak shaker rate in **File** > **Options** to a value between 1000 and 2600 rpm to suit your needs. Typically, increasing shaker rate leads to faster binding rates.
- Always input experiment info first and click **Next**. Follow the on-screen prompts to load sample and biosensor on the BLItz system.
- To fit a curve to the data, choose from the available options. When in doubt about which curve fit is best, try all three options.

The purpose of this experiment is for using samples of known concentration to determine the signal the system shows for each concentration.

This experiment should display a signal vs. concentration graph, which is the standard graph. This is essential for using with the Quantitate Sample experiment. When you perform a Quantitate Sample experiment, you should have already performed the standard curve experiment. If you do not do the Creating a Standard Curve experiment first, the system will generate some data, but you will not know the details of the data, which is necessary to use for the Quantitate Sample experiment. In this way, you can make assumptions from the concentration provided.

In this example **Standard Curve** experiment, hIgG is the analyte you will use to prepare four (4) standards ranging from 15.6–1000 µg/mL. With sample diluent as the reference, there will be a total of five (5) samples measured on the BLItz system.

NOTE: *A standard curve should be created with protein or antibody identical to, and in the simple matrix, as the unknown samples that will be quantitated.*

Everything you need is in the demo kit:

- Protein A biosensors
- hIgG stock (10 mg/mL)
- sample diluent
- 96-well, black flat-bottom microplate

To run a **Create a Standard Curve** experiment:

1. Click the **Create Standard Curve** icon

to display the **Create Standard Curve** mode (Figure 5-19).

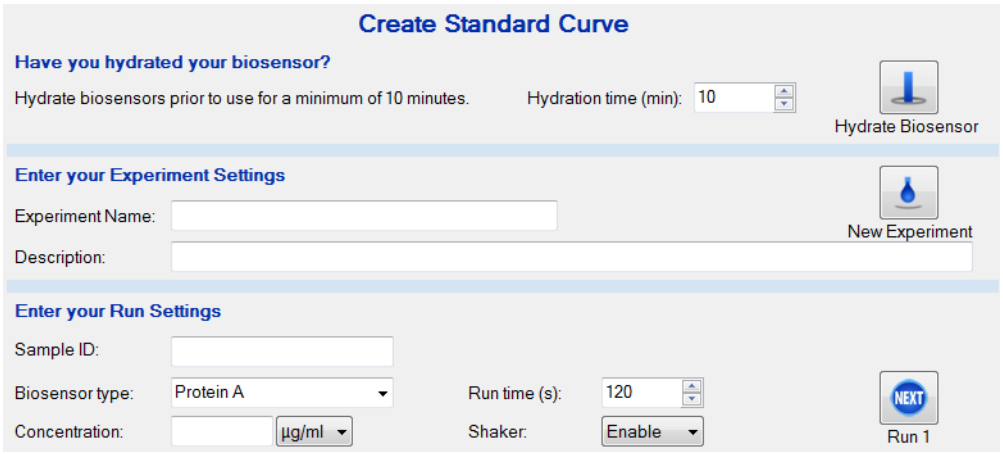


Figure 5-19: *Create Standard Curve*

Preparation

2. Ensure that all reagents to be used on the BLItz system are warmed to room temperature.
3. Hydrate five (5) biosensors, as described in "Defining Experiment Types" on page 41.
4. Prepare the hIgG test samples as follows:
   - 1000 µg/mL hIgG: 20 µL of stock hIgG + 180 µL of sample diluent. Label this as "**Stock A**".
   - Using **Stock A**, perform the following dilutions:

- Step 1— 4-fold dilution: 10 µL Stock A + 30 µL sample diluent for a [hIgG] = 40 µL of 250 µg/mL
- Step 2—4-fold dilution: 10 µL 250 µg/mL + 30 µL sample diluent for a [hIgG] = 40 µL of 62.5 µg/mL
- Step 3—4-fold dilution: 10 µL 62.5 µg/mL + 30 µL sample diluent for a [hIgG] = 40 µL of 15.6 µg/mL

NOTE: *hIgG samples should be run in this order:*
Run 1: 0 µg/mL (Reference)
Run 2: 15.6 µg/mL
Run 3: 62.5 µg/mL
Run 4: 250 µg/mL
Run 5: 1000 µg/mL (Stock A)

Experiment

5. Input **Run Settings** per Figure 5-20 and click NEXT.

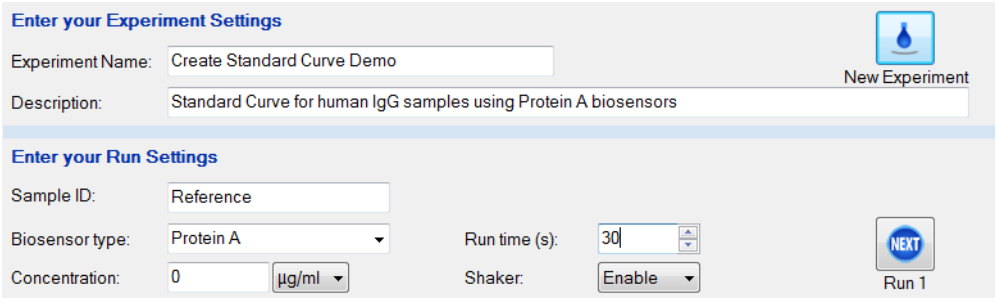


Figure 5-20: *Entering Run Settings*

6. Follow the on-screen instructions (Figure 5-21).

Figure 5-21: *User Action Required—Experiment Operations*

7. Add 4 µL of the sample diluent for the first run into the drop holder (Figure 5-22).

Figure 5-22: *Adding 4 µL of Sample Diluent into Drop Holder*

8. Load the Protein A biosensor on to the biosensor mount; give it a final quarter-turn upward twist to ensure a snug fit (Figure 5-23).

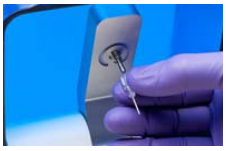


Figure 5-23: *Mounting a Biosensor*

9. Move the slider to the right to move the drop holder to the read position (Figure 5-24).

Figure 5-24: *Moving Drop Holder to the Read Position*

10. Close the BLItz system cover to start data acquisition each time the BLItz Pro software prompts you (Figure 5-25).

Figure 5-25: *Closing the BLItz System Cover*

Data is collected in real-time on screen (Figure 5-26). The sensorgram shows the results after the first run.

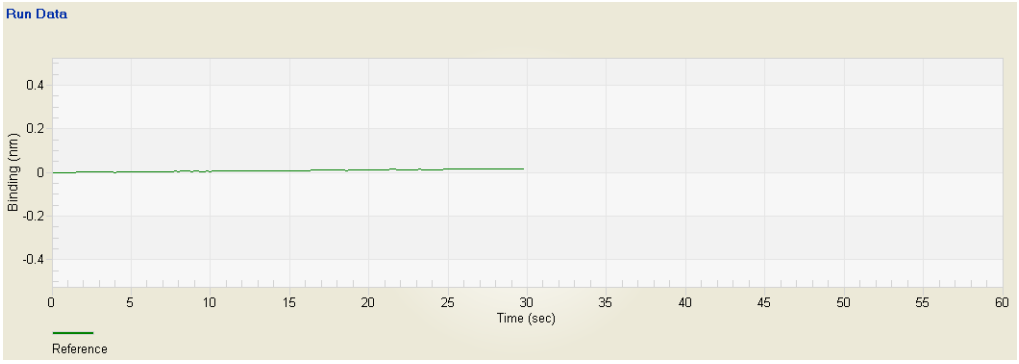


Figure 5-26: *Data for Run 1 Collected in Real-Time*

After the run, a **Save As** dialog box displays and prompts you to save the experiment file (Figure 5-27).

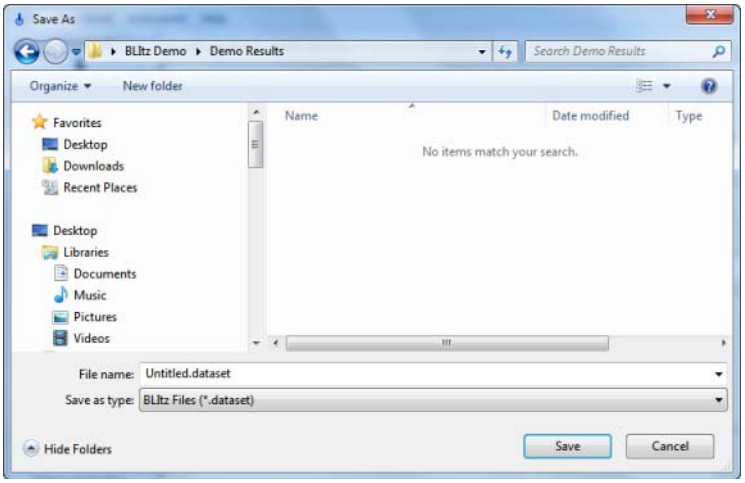


Figure 5-27: *Saving Dataset File*

11. Enter a file name and **Save** the dataset.
12. Lift the cover, discard the used biosensor, wipe the drop holder with a swab or Kimwipe (Figure 5-28), and rinse 3X with sample diluent—wiping each time with a swab or Kimwipe.

Figure 5-28: *Wiping the Drop Holder with a Kimwipe*

13. Perform subsequent sample runs as follows, repeating steps 4 to 12 for each sample:
    - Run 2: 15.6 µg/mL
    - Run 3: 62.5 µg/mL
    - Run 4: 250 µg/mL
    - Run 5: 1000 µg/mL

Data

14. Inspect the Run data. The sensorgram (Figure 5-29) displays the results after five (5) runs.

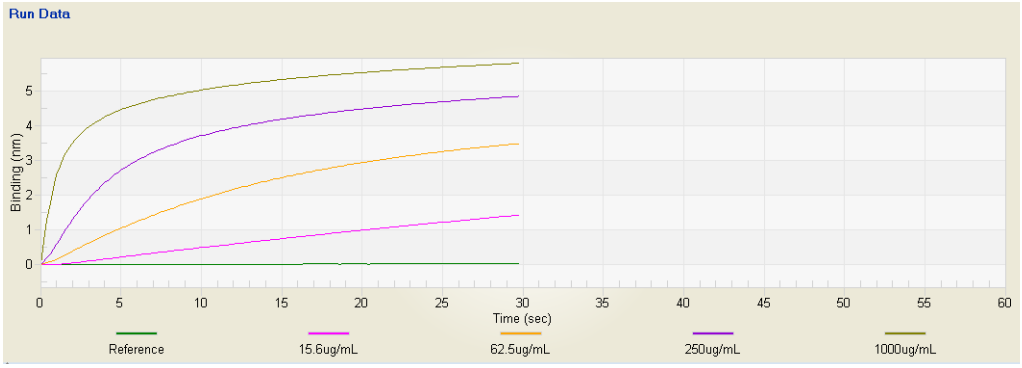


Figure 5-29: *Inspecting the Run Data*

15. Click **Save** to save your experiment.

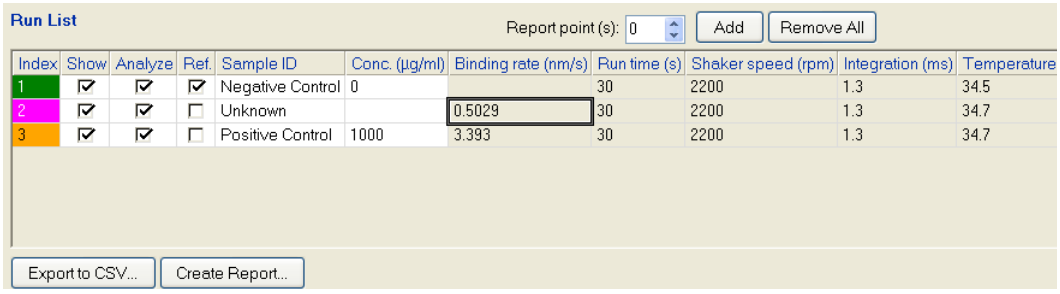


Figure 5-30: *Binding Rate Data in Run List Table*

Table 5-2 lists and defines the binding rate values in the Run List table.

Table 5-2: *Binding Rate Values in the Run List Table*

Parameter	*Description*
Report point (s)	Specifies a time point at which to report signal for all samples in the graph. • **Add**—Adds a report point to the table. • **Remove All**—Removes all the report points from the table.
Index	Numbered order of the curves processed. The index is useful to sort back to the original order.
Show	Controls whether the curve is displayed in the Run Data window.
Analyze	Allows you to analyze the results. If not checked, this box excludes the sample from further analysis.
Ref.	Designates a sample for reference subtraction. If a single sample is chosen, that will be subtracted from all other sample curves. If multiple samples are chosen as reference, the average will be subtracted from all other sample curves.
Sample ID	The sample ID entered during experiment setup.
Conc. (µg/ml)	The molar concentration of the sample used in the association step. The molar concentration is entered by the user or computed by the molarity calculator during experiment setup.
Binding rate (nm/s)	The rate of sample binding to the biosensor computed by the BLItz Pro software using the binding rate equation specified.
Run time (s)	The duration of data analysis seconds.
Shaker speed (rpm)	The sample platform orbital shaking speed (rotations per minute). This value is set in **File** > **Options**.
Integration	Reports the time intervals the spectrometer was exposed to light in acquiring the data.
Biosensor Type	The biosensor chemistry utilized in the experiment. By default, Protein A is used.
Information	Information about the biosensor that was entered in the system.
Status	Indicates if a run is interrupted. For example, if a user stops a run, the status is shown as “Terminated by User”.
Export to CSV	Exports the data in the graph to .csv files, one for each sample.
Create Report	Creates a report of the experiment in a PDF file format.

16. Click **Save Standard Curve** (Figure 5-31) to save the standard curve, rather than the experiment file itself

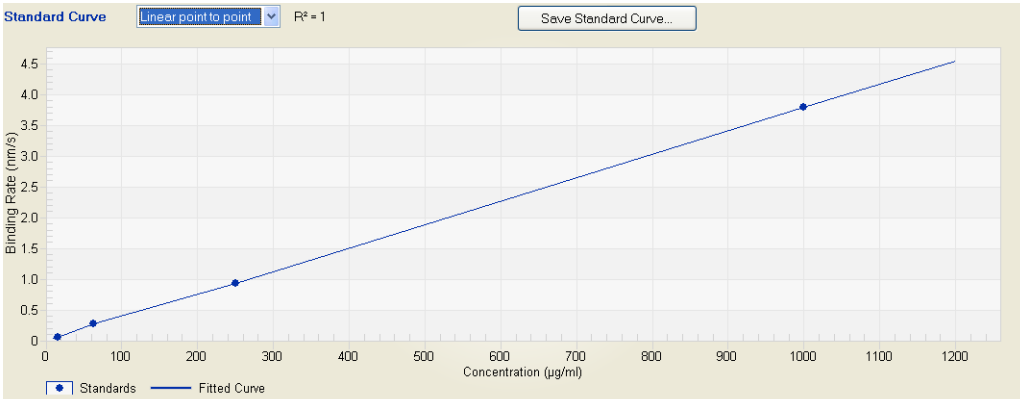


Figure 5-31: *Save Standard Curve*

The drop-down menu provides curve fit options of:

- (a) Linear point-to-point
- (b) Linear
- (c) 5-PL unweighted

Other general notes include:

- **R2 and Chi^2 values** report goodness of fit.
- Standard curve equation and parameters (slope, y-intercept) are shown depending on the curve fit selected.
- **Save Standard Curve** saves the standard curve as a .fsc file.

Result

Your standard curve has been generated. You are now ready to perform quantitation experiments. The 4-point standard curve that you ran just now is meant to serve as a demonstration of the standard curve experiment. If you create a standard curve for your samples, it is important to have standards that cover the entire dynamic concentration range of interest and populate enough data points in frequent intervals. Typically, a 2X or 3X dilution series is recommended.

RUNNING A QUANTITATE SAMPLE EXPERIMENT

When using the starter kit to perform a Quantitate Sample experiment, follow the instructions provided below for human IgG samples binding Protein A biosensors.

To measure concentration of proteins in your samples on the BLItz system, consider these guidelines:

- Create a standard curve that is representative of your unknown samples first before testing your unknowns. You may choose to perform the standard curve experiments after the Quantitate Sample experiments, but you will not be able to obtain concentrations of your unknowns until the standard curve is available.
- Ensure that the sample milieu in the unknowns and the standards are the same.
- Use the same experiment parameters such as Run time and Shaker speed for the Quantitate Sample experiment as that used in the creating the standard curve.
- Use an appropriate biosensor type from Pall ForteBio's list of off-the-shelf biosensors. If one is not readily available, you may prepare a custom biosensor by loading your ligand on any one of the biosensors available.
- Use the drop holder for all Quantitate Sample measurements. Use 4 µL of sample.
- Always input experiment info first and click **Next**. Follow the on-screen prompts to load sample and biosensor on the BLItz system.

This mode allows you to quantitate unknown samples against a standard curve. This experiment assumes that you have already created a standard curve (see "Creating a Standard Curve" on page 52).

In this sample mode, you will prepare two (2) samples of hIgG of known concentrations, and use the BLItz system to calculate their concentrations and confirm that the system reliably measures protein concentrations.

Everything you need is in the demo kit:

- Protein A biosensors
- hIgG stock (10 mg/mL)
- sample diluent
- 96-well, black flat-bottom microplate

To run a Quantitate Sample experiment:

1. Click the **Quantitate Sample** icon

to display the **Quantitate Sample** mode (Figure 5-32).

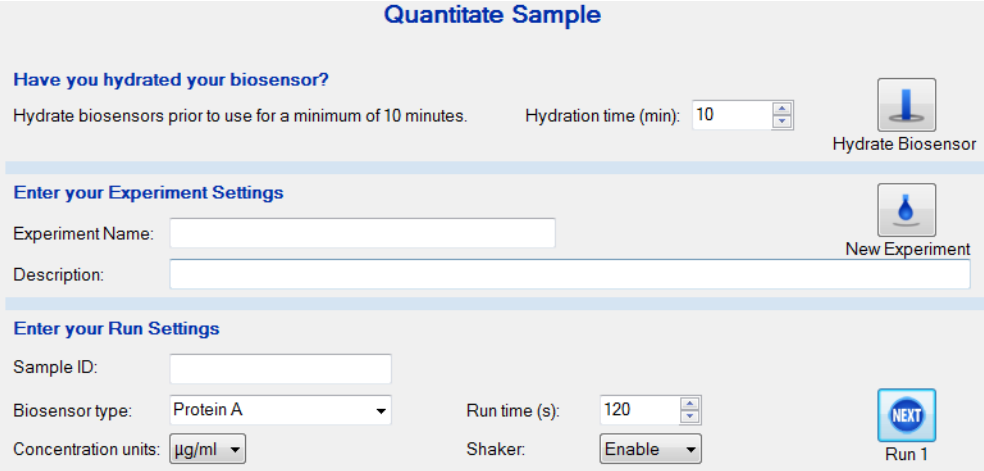


Figure 5-32: *Quantitate Sample*

Preparation

2. Ensure that all reagents to be used on the BLItz system are warmed to room temperature.
3. Hydrate two (2) biosensors, as described in "Defining Experiment Types" on page 41.
4. Prepare hIgG "unknowns" as follows:

   Using the Stock A (1000 µg/mL) that you created to perform the standard curve experiment, perform the following dilutions:

   - 5-fold dilution: 8 µL Stock A + 32 µL sample diluent for a [hIgG] = 200 µg/mL
   - 4-fold dilution: 10 µL 200 µg/mL + 30 µL sample diluent for a [hIgG] = 50 µg/mL

NOTE: *hIgG samples should be run in this order:*
Run 1: 50 µg/mL
Run 2: 200 µg/mL

Quantitate Samples

5. Input **Run Settings** per Figure 5-33 and click

.

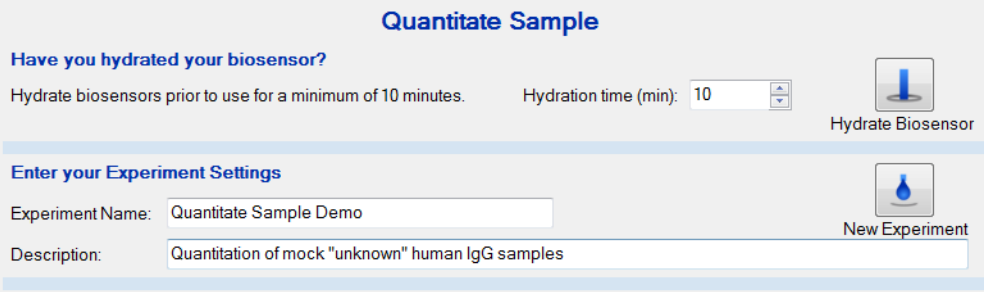


Figure 5-33: *Entering Run Settings*

6. Follow the on-screen instructions (Figure 5-34).

Figure 5-34: *User Action Required—Experiment Operation*

7. Add 4 µL of the sample diluent for the first run into the drop holder (Figure 5-35).

Figure 5-35: *Adding 4 µL of Sample Diluent into Drop Holder*

8. Load the Protein A biosensor on to the biosensor mount; give it a final quarter-turn upward twist to ensure a snug fit (Figure 5-36).

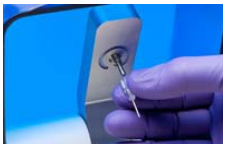


Figure 5-36: *Mounting a Biosensor*

9. Move the slider to the right to move the drop holder to the read position (Figure 5-37).

Figure 5-37: *Moving Slider to Bring Drop Holder or Tube to the Read Position*

10. Close the BLItz system cover to start data acquisition each time the BLItz Pro software prompts you (Figure 5-38).

Figure 5-38: *Closing the BLItz System Cover*

Data is collected in real-time on screen. The sensorgram (Figure 5-39) displays the results after the first run.

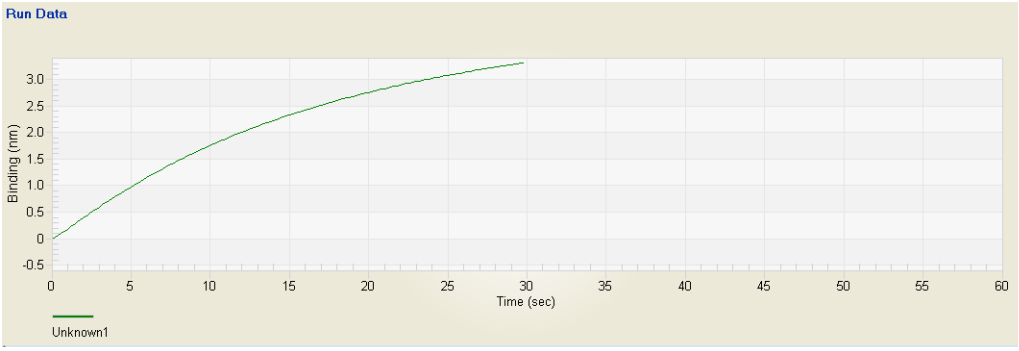


Figure 5-39: *Data Collected in Real-Time for Run 1*

After the run, a **Save As** dialog box displays and prompts you to save the experiment file (Figure 5-40).

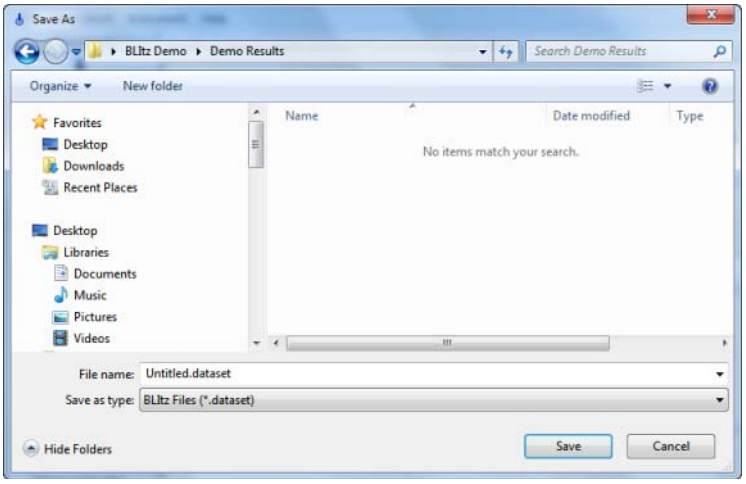


Figure 5-40: *Saving Dataset File*

11. Enter a filename and **Save** the dataset.
12. Lift the cover, discard the used biosensor, wipe the drop holder with a swab or Kimwipe (Figure 5-41), and rinse 3X with sample diluent—wiping each time with a swab or Kimwipe.

The best way to rinse 3X with sample diluent is to take, at least, 20 µL of buffer in a pipet and add small aliquots into the drop holder followed by blotting with a Kimwipe.

Figure 5-41: *Wiping the Drop Holder with a Kimwipe*

13. Perform subsequent "unknown" runs:

    Run 2: 200 µg/mL

Select Standard Curve

14. Click the **Load Standards** button on the Standard Curve graph (Figure 5-42) and select your standard curve (.fsc file) to load.

NOTE: *You must load the standard curve generated using the protocol described in this guide.*

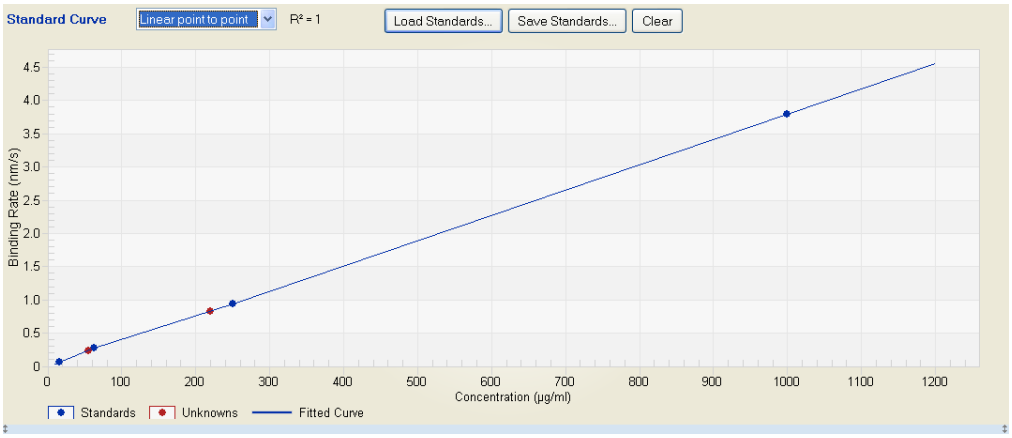


Figure 5-42: *Loading Standard Curve*

- The drop-down menu provides curve fit options of:
  - (a) Linear point-to-point:
  - (b) Linear
  - (c) 5-PL unweighted

Other general notes:

- **R^2 and Chi^2 values** report goodness of fit.
- Standard curve equation and parameters (slope, y-intercept) are shown depending on the curve fit selected.
- **Load Standards** loads an existing standard curve as a .fsc file.
- **Save Standards** saves the standard curve as a .fsc file.
- **Clear** clears the loaded standard curve from the graph.

Data

15. Click **Save** to save your experiment.
16. Inspect the interpolated concentrations. Unknown1 should be about 50 µg/mL and Unknown2 should be about 200 µg/mL.

    Figure 5-43 displays the interpolated unknown concentrations.

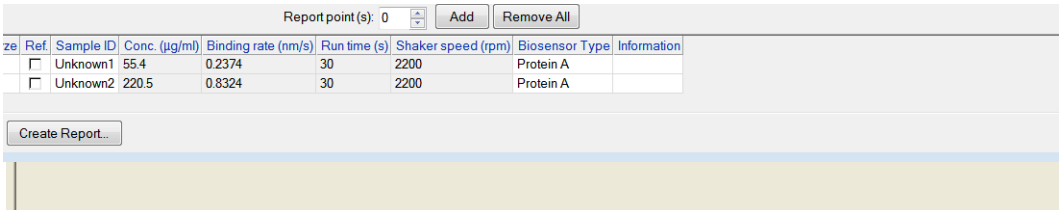


Figure 5-43: *Interpolated Unknown Concentrations in Table*

Table 5-3 lists and defines the binding rate values in the Run List table.

Table 5-3: *Binding Rate Values in the Run List Table*

Parameter	*Description*
Report point (s)	Specifies a time point at which to report signal for all samples in the graph. • **Add**—Adds a report point to the table. • **Remove All**—Removes all the report points from the table.
Index	Numbered order of the curves processed. The index is useful to sort back to the original order.
Show	Controls whether the curve is displayed in the Run Data window.
Analyze	Allows you to analyze the results. If not checked, this box excludes the sample from further analysis.
Ref.	Designates a sample for reference subtraction. If a single sample is chosen, that will be subtracted from all other sample curves. If multiple samples are chosen as reference, the average will be subtracted from all other sample curves.
Sample ID	The sample ID entered during experiment setup.
Conc. (µg/ml)	The molar concentration of the sample used in the association step. The molar concentration is entered by the user or computed by the molarity calculator during experiment setup.
Binding rate (nm/s)	The rate of sample binding to the biosensor computed by the BLItz Pro software using the binding rate equation specified.
Run time (s)	The duration of data analysis seconds.
Shaker speed (rpm)	The sample platform orbital shaking speed (rotations per minute). This value is set in **File** > **Options**.
Integration	Reports the time intervals the spectrometer was exposed to light in acquiring the data.

Table 5-3: Binding Rate Values in the Run List Table (Continued)

Parameter	Description
Biosensor Type	The biosensor chemistry utilized in the experiment. By default, Protein A is used.
Information	Information about the biosensor that was entered in the BLItz system.
Status	Indicates if a run is interrupted. For example, if a user stops a run, the status is shown as "Terminated by User".
Export to CSV	Exports the data in the graph to .csv files, one for each sample.
Create Report	Creates a report of the experiment in a PDF file format.

17. Click **Save** to save your experiment.

Result

In just a few minutes, you have measured the specific concentration of protein in your unknown samples.

RUNNING A BASIC KINETICS EXPERIMENT

When using the starter kit to perform a Basic Kinetics experiment, follow the instructions provided below for mouse IgG samples binding Protein A biosensors.

To measure kinetic parameters for your ligand-analyte interactions on the BLItz system consider these guidelines:

- Ligand indicates the binding partner loaded on the biosensor. Analyte refers to the binding partner present in solution.
- The Basic Kinetics mode is appropriate for use when the ligand has previously been loaded on the biosensor, either as provided by Pall ForteBio, or loaded offline on the bench top, or loaded on BLItz system in a previous experiment.
- Perform baseline and dissociation steps in an microcentrifuge tube. Perform association steps in either drop holder or tube for Duration of 5 minutes or less. When association step duration is more than five minutes, perform step in microcentrifuge tube.
- Use 250 µL of reagent in microcentrifuge tube and 4 µL of reagent in drop holder.
- When optimizing experiment conditions for measuring kinetic constants for a binding interaction, use fewer analyte concentrations distributed over the range of 100xK_D to 0.01xK_D. After optimizing experiment conditions, populate more concentrations to maximize reliability of the measured kinetic constants.

- Input concentration and molecular weight values, or, the molar concentration values for the analyte samples used. The BLItz Pro software needs these values to calculate kinetic constants.
- Keep the shaker in **Enable** mode for all kinetics experiments. In **File** > **Options**, leave the shaker speed at the default setting of 2200 rpm for all kinetics experiments.
- Use an appropriate biosensor type from Pall ForteBio's list of off-the-shelf biosensors. If one is not readily available, you can prepare a custom biosensor by loading your ligand on any one of the biosensors available.
- For the baseline step, duration of 30 to 60 seconds is typically recommended. You may modify this time as desired to achieve a stable Baseline before moving to the Association step.
- The duration for Association and Dissociation steps should be optimized based on the concentration of your analyte samples and the expected affinity of the interaction. Typically, high affinity interactions can require longer dissociation times.
- Always include a reference sample in your experiment to correct for background signal.
- Always input experiment info first and click **Next**. Follow the on-screen prompts to add reagents to the appropriate sample locations and to mount biosensor on the BLItz system.
- To fit a curve to the data, choose from the available options. To learn more about the differences between local and global fitting, consult available literature or contact BLItz technical support.

In this sample Basic Kinetics experiment, you will develop a series of real-time data graphs for multiple concentrations of mouse IgG analyte binding to pre-loaded Protein A ligand on the Protein A biosensors. You will analyze the collected data to measure kinetic constants (k_a, k_d, K_D). You will use 4 µL of sample on the drop holder for the association step and 250 µL of sample diluent in the tube for baseline and dissociation.

Everything you need is in the demo kit:

- Protein A biosensors
- mIgG stock (10 mg/mL)
- Sample diluent
- 96-well, black flat-bottom microplate
- Black 0.5 mL microcentrifuge tubes

To run a Basic Kinetics experiment:

1. Click the **Basic Kinetics** icon

to display the default **Basic Kinetics** mode (Figure 5-44).

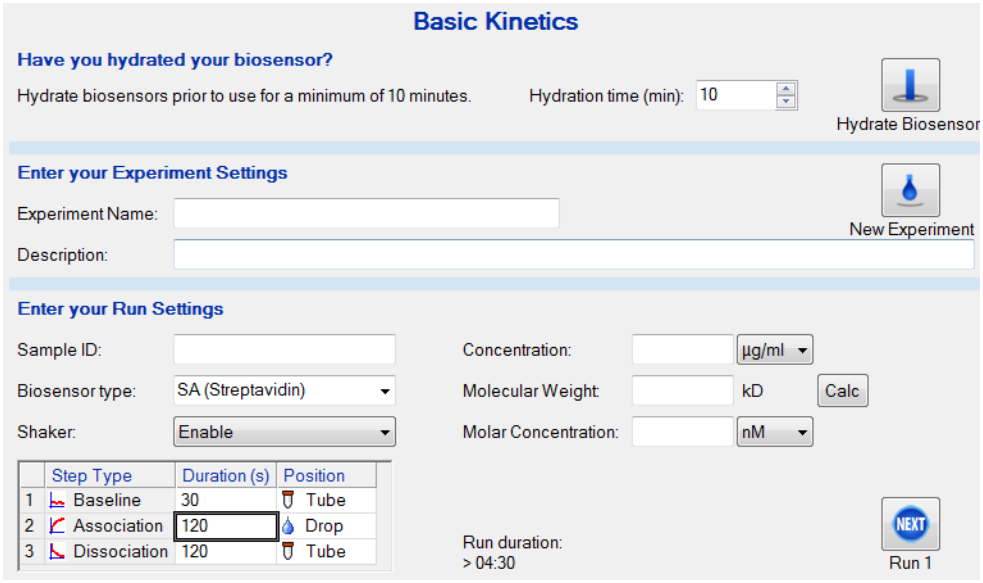


Figure 5-44: *Basic Kinetics Mode*

Preparation

2. Ensure that all reagents to be used on the BLItz system are warmed to room temperature.
3. Hydrate five (5) biosensors, as described in "Defining Experiment Types" on page 41.
4. Prepare mIgG sample dilutions:
   Using the mIgG stock (10 mg/mL), perform the following dilutions:
   - Step 1—20-fold dilution: 4 µL mIgG stock + 76 µL sample diluent for a [mIgG] = 0.5 mg/mL. Label this as "**Stock A**".
   - Step 2—50-fold dilution: 4 µL Stock A + 196 µL sample diluent for a [mIgG] = 10 µg/mL
   - Step 3— 2-fold dilution: 50 µL of 10 µg/mL + 50 µL sample diluent for a [mIgG] = 5 µg/mL
   - Step 4—2-fold dilution: 50 µL of 5 µg/mL + 50 µL sample diluent for a [mIgG] = 2.5 µg/mL
   - Step 5—2-fold dilution: 50 µL of 2.5 µg/mL + 50 µL sample diluent for a [mIgG] = 1.25 µg/mL

NOTE: *mIgG samples will be run in this order:*
Run 1: sample blank
Run 2: 1.25 µg/mL
Run 3: 2.5 µg/mL
Run 4: 5 µg/mL
Run 5: 10 µg/mL

Run Kinetics Experiment

5. Input **Run Settings** per Figure 5-45 and click NEXT.

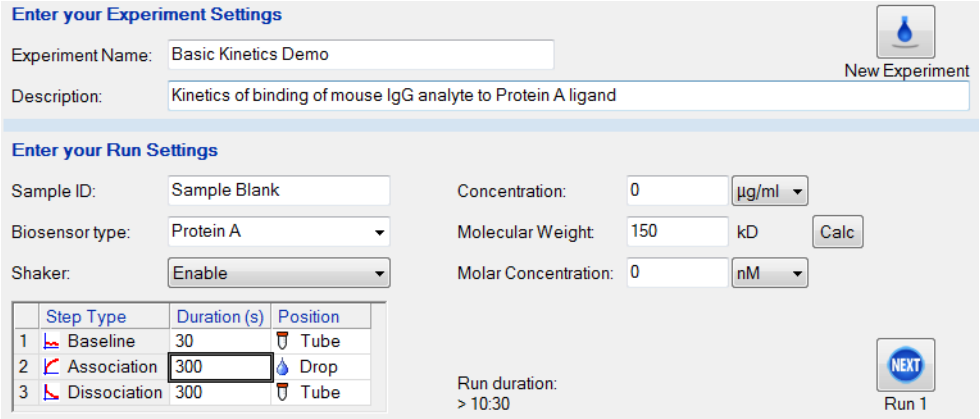


Figure 5-45: *Entering Run Settings*

Table 5-4 lists and defines the step types for Basic Kinetics experiments.

Table 5-4: *Step Types—Basic Kinetics Experiments*

Option	*Description*
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm. ***IMPORTANT:*** *An experiment MUST include a baseline step followed by a set of association/dissociation steps to be analyzed. The data analysis software recognizes the baseline/association/dissociation set of steps.*

Table 5-4: Step Types—Basic Kinetics Experiments (Continued)

Option	Description
Association	Calculates the k_{obs}. Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the k_d. Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.

***NOTE**: You can change the position of the Position defaults (Figure 5-46): click the **Position** field to change the position. All concentrations should use the same position for each step type.*

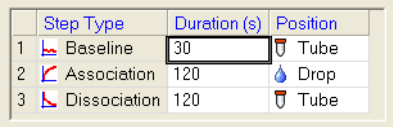


***Figure 5-46:** Step Type Default Settings*

6. Follow the on-screen instructions (Figure 5-47).

***Figure 5-47:** User Action Required—Experiment Operation*

7. Ensure that the sample diluent (4 µL in the drop holder) or (250 µL in the tube) are available for reading (Figure 5-48).

***Figure 5-48:** Perform Baseline and Dissociation Measurements in Tube and Association Measurement in Drop Holder*

8. Load the Protein A biosensor on to the biosensor mount; give it a final quarter-turn upward twist to ensure a snug fit (Figure 5-49).

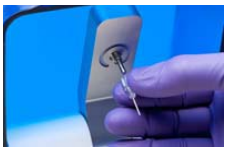


Figure 5-49: *Mounting a Biosensor*

9. Move the slider to the appropriate position to bring either the tube or drop holder to the read position, as appropriate for each step (Figure 5-50):
   a. left position for **Baseline**
   b. right position for **Association**
   c. left position for **Dissociation**

Figure 5-50: *Moving Slider to Bring the Drop Holder or Tube to the Read Position*

10. Close the BLItz system cover to start data acquisition each time the BLItz Pro software prompts you (Figure 5-51).

Figure 5-51: *Closing the BLItz System Cover*

Data is collected in real-time on screen. The sensorgram (Figure 5-52) displays the results after the first run.

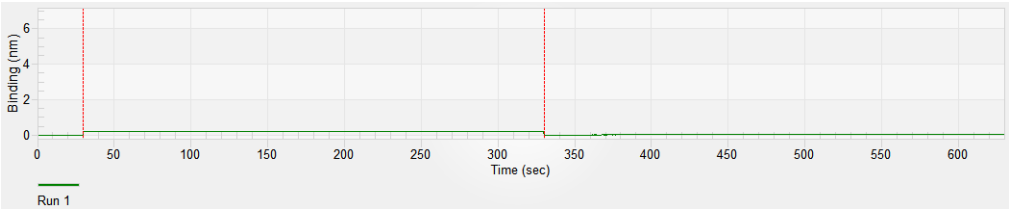


Figure 5-52: *Data for Run 1 Collected in Real-Time*

After the run, a **Save As** dialog box displays and prompts you to save the experiment file (Figure 5-53).

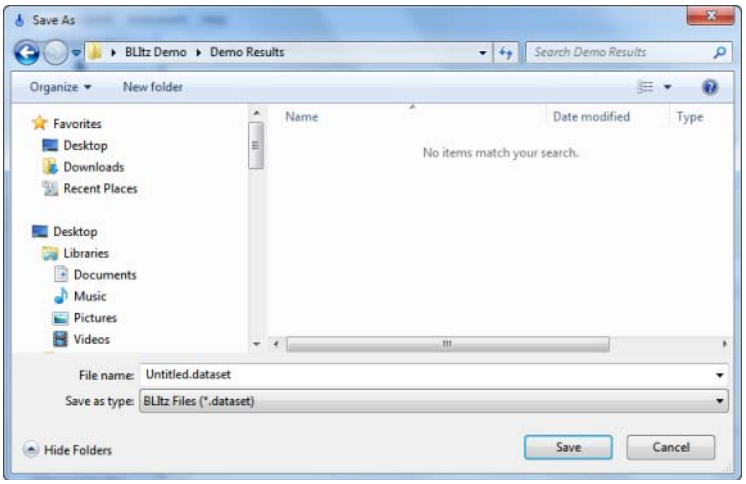


Figure 5-53: *Saving Dataset File*

11. Enter a file name and **Save** the dataset.
12. Lift the cover, discard the used biosensor, wipe the drop holder with a swab or Kimwipe (Figure 5-54), and rinse 3X with sample diluent—wiping each time with a swab or Kimwipe.

The best way to rinse 3X with sample diluent is to take, at least, 20 µL of buffer in a pipet and add small aliquots into the drop holder followed by blotting with a Kimwipe.

Figure 5-54: *Wiping the Drop Holder with a Kimwipe*

13. Perform subsequent sample runs, repeating steps 4 to 12 for each concentration:

    Run 2: 1.25 µg/mL

    Run 3: 2.5 µg/mL

    Run 4: 5 µg/mL

    Run 5: 10 µg/mL

Data

14. Observe the Run data and save.
    The sensorgram displays the results after five (5) runs (Figure 5-55).

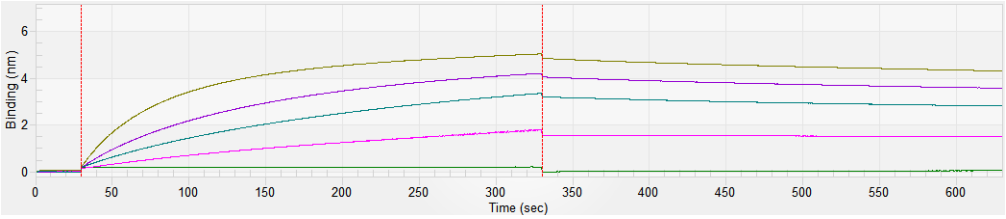


Figure 5-55: *Observing Run Data*

15. Select the settings in Figure 5-56 and click **Analyze** to display the results in Figure 5-57.

Figure 5-56: *Analysis Settings—Step Correction and Fitting (1:1)*

The **Step Correction** feature corrects misalignment between two steps due to system artifacts. The association step can be aligned to the dissociation step or to the baseline.

- **Start of association**—Moves the association step on the Y axis to align the beginning of the association step with the end of the adjacent baseline step.
- **Start of dissociation**—Moves the dissociation step on the Y axis to align the end of the adjacent association step with the beginning of the dissociation step.

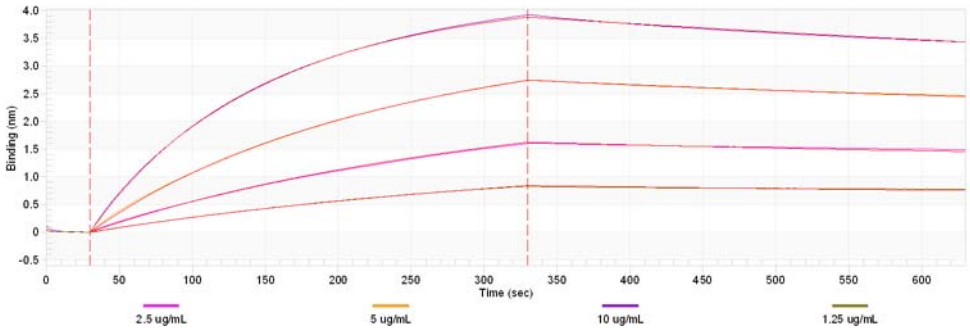
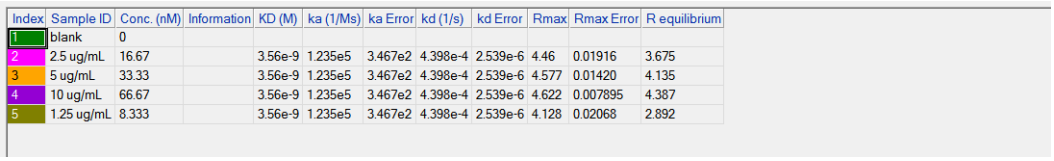


Figure 5-57: *Analysis Results—Red Line Overlying Data Are Curve Fits*

A model represents the mathematical model that is used to generate the fitted view. The Fitting 1:1 model fits one analyte in solution binding to one binding site on the surface. The Fitting 1:1 model can be one of the following options:

- **Fitting-Local—**If this option is selected, the BLItz computes kinetic constants for each curve. The constants that are calculated depend on the steps that are analyzed (Association only, Dissociation only, or Association and Dissociation).
- **Fitting-Global (Full)—**If this option is selected, an analysis includes all of the binding curve data in the group and the BLItz generates kinetic constants for the entire group. The kinetic constants that are calculated depend on the model selected.

You have just generated binding curves for mIgG against Protein A and a table of kinetic constants (Figure 5-58). Table 5-5 lists and defines the binding rate values.



Index	Sample ID	Conc. (nM)	Information	KD (M)	ka (1/Ms)	ka Error	kd (1/s)	kd Error	Rmax	Rmax Error	R equilibrium
1	blank	0									
2	2.5 ug/mL	16.67		3.56e-9	1.235e5	3.467e2	4.398e-4	2.539e-6	4.46	0.01916	3.675
3	5 ug/mL	33.33		3.56e-9	1.235e5	3.467e2	4.398e-4	2.539e-6	4.577	0.01420	4.135
4	10 ug/mL	66.67		3.56e-9	1.235e5	3.467e2	4.398e-4	2.539e-6	4.622	0.007895	4.387
5	1.25 ug/mL	8.333		3.56e-9	1.235e5	3.467e2	4.398e-4	2.539e-6	4.128	0.02068	2.892

Figure 5-58: *Binding Curve Results for mIgG against Protein A and Kinetic Constants*

Table 5-5: *Binding Rate Values in the Run List Table*

Parameter	*Description*
Index	Numbered order of the curves processed. The index is useful to sort back to the original order.
Sample ID	The sample ID entered during experiment setup.
Conc. (nM)	Concentration of analyte.
Information	Information about the biosensor that was entered.
K_D (M)	Measured affinity of interaction; affinity constant in Molar.
k_a (1/Ms)	Association rate constant.
k_a Error	Calculated standard error in the measured association rate constant.
k_d (1/s)	Dissociation rate constant.
k_d (Error)	Calculated error in the measured dissociation rate constant.
X^2	Measure of the goodness of curve fitting (not directly related to a parameter estimate). It is the sum of squared deviations, where the deviation is the difference between the actual data point and the fitted curve. Values close to zero indicate a good curve fit.
R^2	The coefficient of determination (COD). It is an estimate of the goodness of the curve fit and is not directly related to the estimate of a specific parameter. Values close to 1.0 indicate a good curve fit.

Table 5-5: *Binding Rate Values in the Run List Table (Continued)*

Parameter	*Description*
Rmax	Maximum response determined from the fit of the binding data.
Rmax Error	Calculated standard error in Rmax.
Requilibrium	Calculated response at equilibrium that is determined from a fit of the binding data.

Result

You have now generated rate constants and affinity constant for the mouse IgG2-Protein A binding interaction. Congratulations!

With this, your experiment is complete. We hope that BLItz's powerful experiment features combined with its simplicity is what you are looking for in your work.

RUNNING AN ADVANCED KINETICS EXPERIMENT

The Advanced Kinetics mode allows users to monitor the kinetics of a binding interaction by first loading one of the binding partners on a biosensor followed by probing a sample that contains the second binding partner. A typical experiment data graph is shown in Figure 5-59.

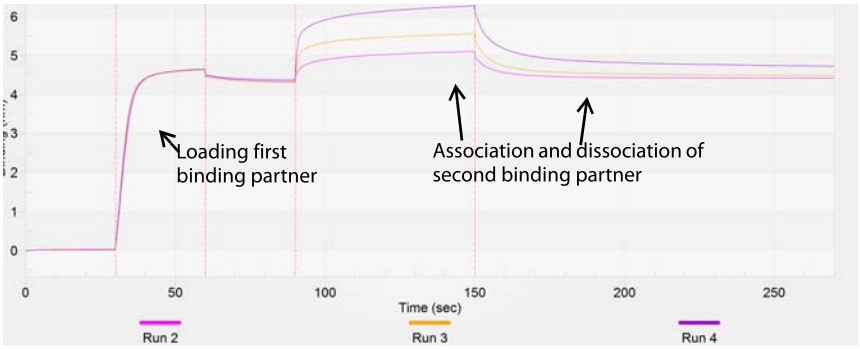


Figure 5-59: *Advanced Kinetics Data Graph*

The first protein can be bound to a biosensor in different ways. Human and Mouse antibodies or fc-fusion proteins can be bound to the Anti-human capture (AHC) and Anti-murine capture (AMC) biosensor, respectively. GST-fusion proteins can be bound to the Anti-GST biosensor. Other proteins that are functionalized with biotin can be bound to the Streptavidin biosensor. Unmodified proteins can be bound covalently to the 2nd generation amine reactive (AR2G) biosensor.

The Advanced Kinetics mode can be used to perform kinetic analysis of ligand-analyte interactions where the ligand is first loaded on biosensors on the BLItz system followed by monitoring the association and dissociation of analyte to the ligand. In addition, the

Advanced Kinetics mode can be used to set up custom binding assays involving multiple binding steps, such as the formation of a sandwich complex (capture antibody-antigen-secondary antibody).

The **Initial Baseline** (Figure 5-60) can typically be of 30 or 60 seconds duration, or until stable baseline is reached in the medium you choose for your experiment. The initial baseline is performed in the tube. The loading step allows you to load your ligand on the biosensor. The loading step can be performed on different biosensors listed in the Biosensor type field.

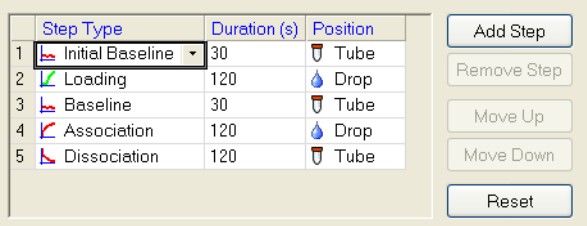


Figure 5-60: *Advanced Kinetics—Step Type List*

Table 5-6 lists the type of ligand required for loading to the biosensor.

Table 5-6: *Required Ligands*

Biosensor Type	*Required Ligand*
Streptavidin	Biotin-tagged proteins, oligos, peptides
Super Streptavidin	Biotin-tagged proteins, oligos, peptides
Anti-GST	GST-fused proteins
Anti-hIgG Fc Capture (AHC)	Human IgG, human Fc fusion proteins
Anti-mIgG Fc Capture (AMC)	Mouse IgG, mouse Fc fusion proteins
Amine reactive 2nd generation (AR2G)	Proteins, oligos, peptides
Amine reactive (AR)	Proteins, oligos, peptides
Aminopropylsilane (APS)	Proteins, peptides

To prepare biotin-tagged proteins, follow the minimum biotinylation ratio method described in *Technical Note 28*, *Biotinylation of Protein for Immobilization onto Streptavidin Biosensors*, available at www.blitzmenow.com/literature.html.

To load ligand on AR and AR2G biosensors, the Amine Coupling Second Generation Reagent Kit is required.

A Baseline step follows the loading step to establish a new baseline before association and dissociation steps with the analyte. In the Association step, the analyte in solution binds to the ligand on the biosensor. In the Dissociation step, the analyte bound to the biosensor dissociates into solution.

Table 5-7 lists the applicable rules when performing an Advanced Kinetics experiment.

Table 5-7: *General Step Type Rules for an Advanced Kinetics Experiment*

Step Type	*Position*
Initial Baseline	Tube
Loading	Drop holder or Tube if Duration is less than 300 seconds; only Tube if more than 300 seconds.
Baseline	Tube
Association	Drop holder or Tube if Duration is less than 300 seconds; only Tube if more than 300 seconds
Dissociation	Tube
Custom	Drop holder or Tube if Duration is less than 300 seconds; only Tube if more than 300 seconds.

NOTE: *In Advanced Kinetics, to create a successful experiment, there must (at a minimum) be* **Initial Baseline**, **Association**, *and* **Dissociation** *step, included in that order.*

To run an Advanced Kinetics experiment, you will need:

- Biosensors (choice of biosensor made by user)
- Ligand for loading on biosensor
- Analyte or sample (one or more concentrations, depending on type of experiment)
- Sample diluent
- 96-well, black, polypropylene flat-bottom microplate
- microcentrifuge tubes

To run an Advanced Kinetics experiment:

1. With the software launched, on the left pane, click the **Advanced Kinetics** icon

to display the default **Advanced Kinetics** mode (Figure 5-61).

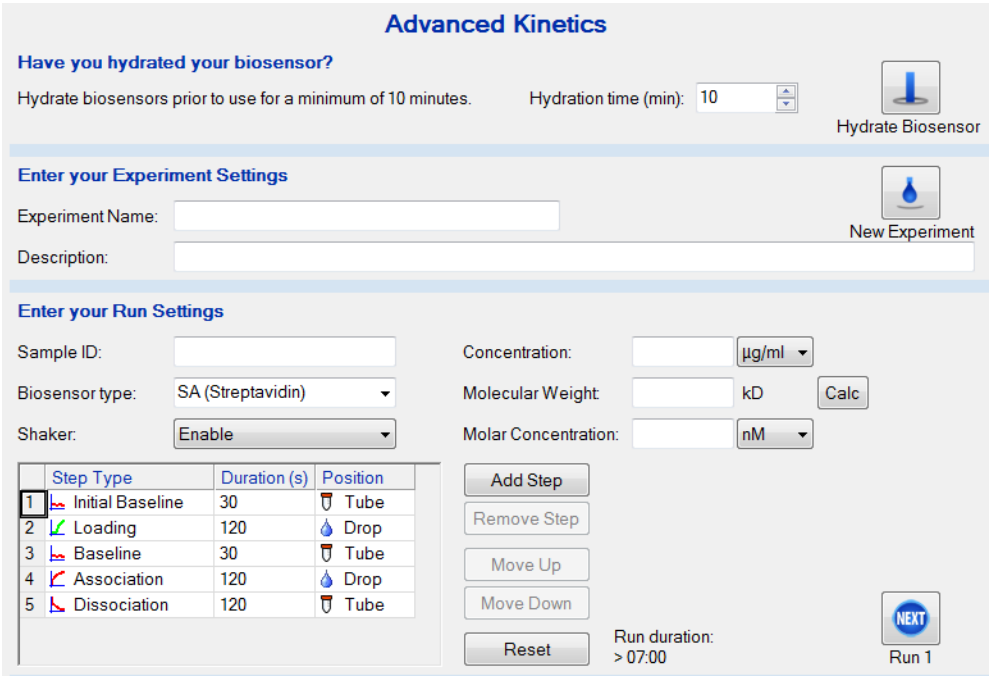


Figure 5-61: *Advanced Kinetics*

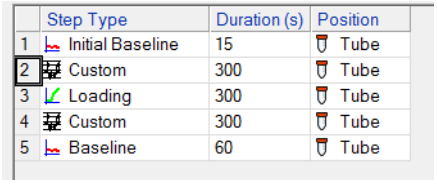
Preparation

2. Hydrate biosensors, as described in the "Defining Experiment Types" on page 41.
3. Prepare ligand solution for loading step. Perform dilution as required to bring the ligand solution to a concentration of 10–50 µg/mL.

NOTE: *When performing kinetics experiments on AR and AR2G biosensors, two separate Advanced Kinetics experiments are needed. The first Advanced Kinetics experiment should be performed according to the following protocol to load protein on the biosensors:*

---

a. Set up an experiment with the steps in Figure 5-62. In Step 2, activation of the hydrated biosensor with EDC/Sulfo NHS mixture is performed in a tube. In Step 3, the ligand is loaded on the activated biosensor either in the drop holder or the tube. In Step 5, the biosensor is quenched with quenching solution.

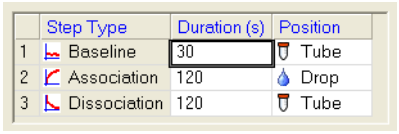


	Step Type	Duration (s)	Position
1	Initial Baseline	15	Tube
2	Custom	300	Tube
3	Loading	300	Tube
4	Custom	300	Tube
5	Baseline	60	Tube

Figure 5-62: *Step Types for This Experiment*

Table 5-8 lists and defines the step types for Advanced Kinetics experiments.

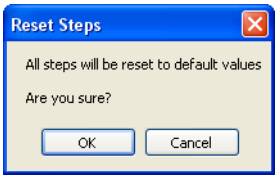
Table 5-8: *Step Types—Advanced Kinetics Experiments*

Option	*Description*
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm. ***IMPORTANT:*** *An experiment MUST include a baseline step followed by a set of association/dissociation steps to be analyzed. The data analysis software recognizes the baseline/association/dissociation set of steps.*
Association	Calculates the k_{obs}. Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the k_d. Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm. ***NOTE****: You can change the position of the Position defaults (Figure 5-63): click the* **Position** *field to change the position. All concentrations should use the same position for each step type.*  ***Figure 5-63:*** *Step Type Default Settings*

	Step Type	Duration (s)	Position
1	Baseline	30	Tube
2	Association	120	Drop
3	Dissociation	120	Tube

Table 5-9 lists and describes the available step type functions.

***Table 5-9:** Step Type Functions—Advanced Kinetics*

Task	*Description*
Add Step	To add a step type from the list, click the corresponding row in the Step Type list and click **Add Step**.
Remove Step	To delete a step type from the list, click the corresponding row in the Step Type list and click **Remove Step**.
Move Up	To move up a step type in the list, click the corresponding row in the Step Type list and click **Move Up**.
Move Down	To move down a step type in the list, click the corresponding row in the Step Type list and click **Move Down**.
Reset	To reset a step type from the list, click the corresponding row in the Step Type list and click **Reset**. A prompt displays (Figure 5-64) that all steps will be reset to default values. If this is the preference, click **OK**.  ***Figure 5-64:** Reset Steps*

***NOTE:** All biosensors needed for the experiment should be loaded one after another using this procedure and kept hydrated in solution until ready for the binding experiment with analyte.*

b. The analyte binding kinetics experiment can now be performed using the Basic Kinetics mode.

4. Prepare analyte solutions.

Run Experiment

5. Input **Run Settings** per Figure 5-65 and click NEXT.

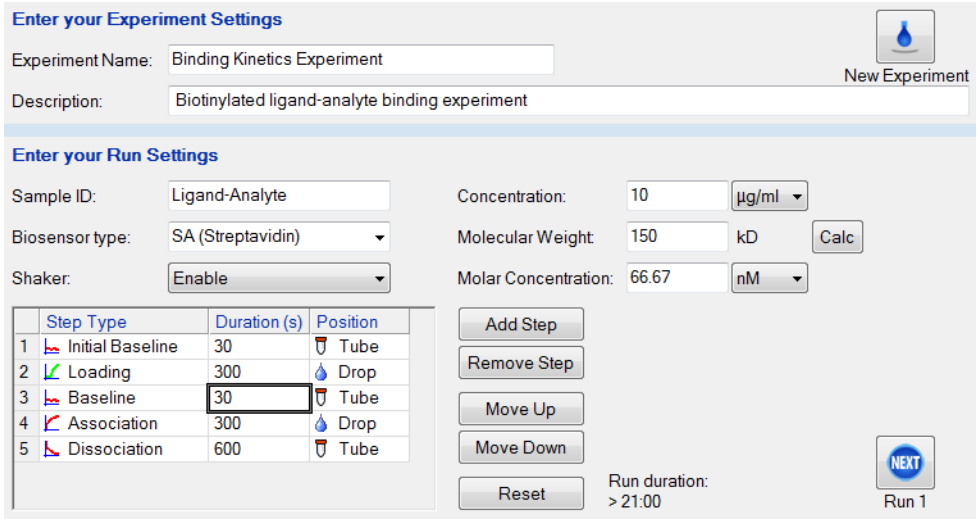


Figure 5-65: *Entering Run Settings*

6. Follow the on-screen instructions. For instructions on performing the functions prompted by the software, refer to "Basic Functions of the BLItz System" on page 8.
7. To clean the drop holder during the experiment, perform the following actions: Lift the BLItz system cover, discard the used biosensor, wipe the drop holder with a swab or Kimwipe, and rinse 3X with sample diluent—wiping each time with a swab or Kimwipe.
8. Repeat the experiment for all samples.
9. Save the experiment.

Data Analysis

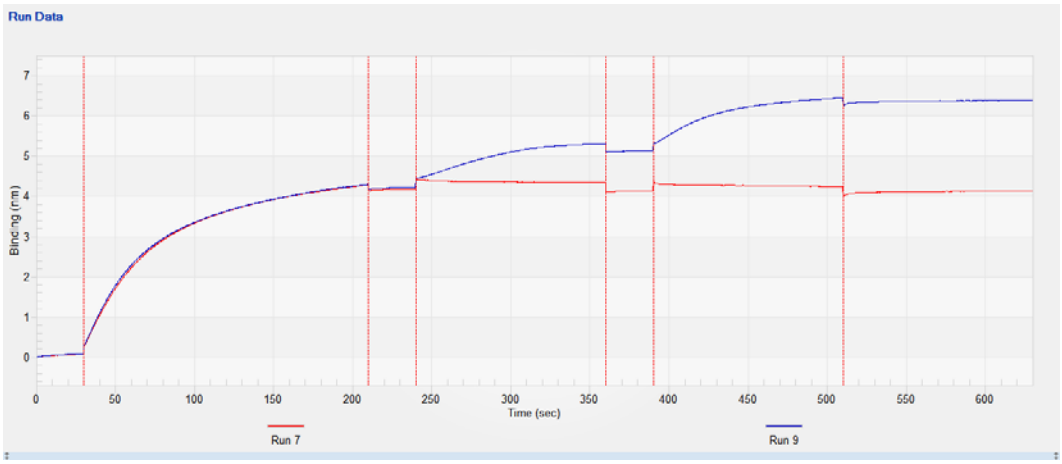


Figure 5-66: *Observing Run Data from an Advanced Kinetics Experiment*

10. Select **Step Correction and Fitting (1:1)** options and click **Analyze** to display curve fits and results.

MANAGING REPORTS

The BLItz software allows you to manage reports generated from the experiments you produce.

Generating a Report

To generate a report:

1. On the menu bar, click **File > Create Report**, or in the Run List section, click **Create Report**.
2. Confirm the location to which the file will be saved or specify a different location.
3. Click **Save**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the Run Data window by clicking the **Reference** (**Ref.**) check box (Figure 5-67) in the binding chart window. To view raw data, un-check the box.

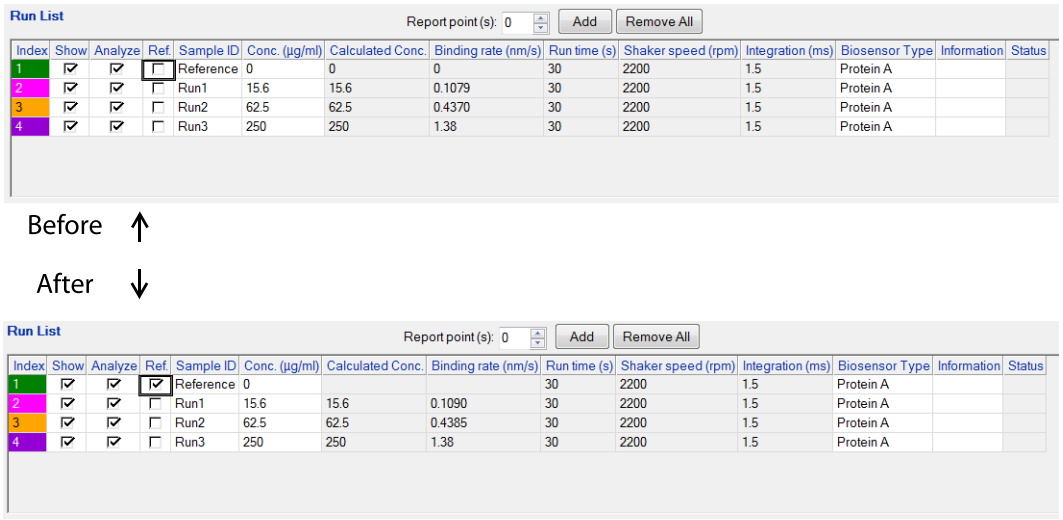


Figure 5-67: *Reference Check Box*

Viewing Inverted Data

The data displayed in the **Run Data window** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, right-click the chart and select **Flip Data** (see Figure 5-68); disable the check box to return to the default data display.

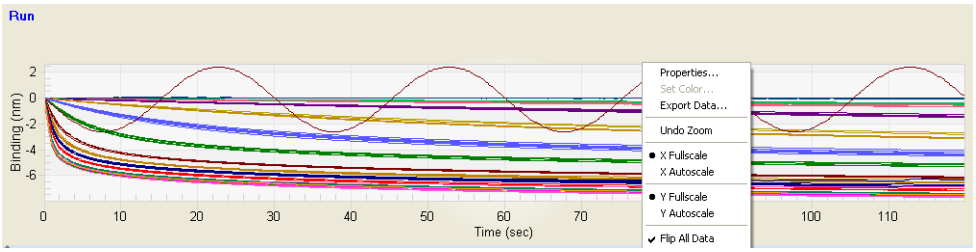


Figure 5-68: *Data Inverted Using Flip Data Function*

Table 5-10 lists and defines the right-click menu commands for the Run Data window.

Table 5-10: *Run Data Right-Click Menu Commands*

Menu Command	*Description*
Properties	Allows for adding a title and subtitle to the graph. Allows selection of the following for the graph legend: • **Run Index** (selected by default)—Numbered order of the curves processed. The index is useful to sort back to the original order. • **Sample ID**—The sample ID entered during experiment setup. • **Concentration**—The molar concentration of the sample used in the association step. The molar concentration is entered by the user or computed by the molarity calculator during experiment setup. • **Biosensor Type**—The type of biosensor chemistry. **NOTE:** *Text for **Sample ID**, **Biosensor Type**, or **Concentration** is taken from the initial experiment setup, and must be entered before the experiment is started.*
Set Color	Sets the color for the data results.

Table 5-10: Run Data Right-Click Menu Commands (Continued)

Menu Command	Description
Export Data	Allows for export of the graph image or the data. • For image export, the file format, image size and resolution, and destination can be specified (Figure 5-69).

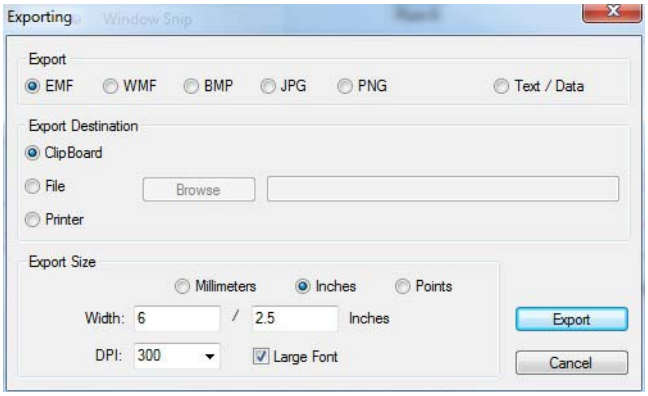


Figure 5-69: Image Export Settings

• For data export as text, additional options for selecting all data or selected data, and format for importing to other software can be selected (Figure 5-70). This is often done by selecting **All Data**, **Table** under Export style and **Subsets/Points** under Row vs Column in the Export dialog and then clicking the **Export** button (Figure 5-70). This data can then be pasted into Microsoft® Excel® or other software packages for additional analysis.

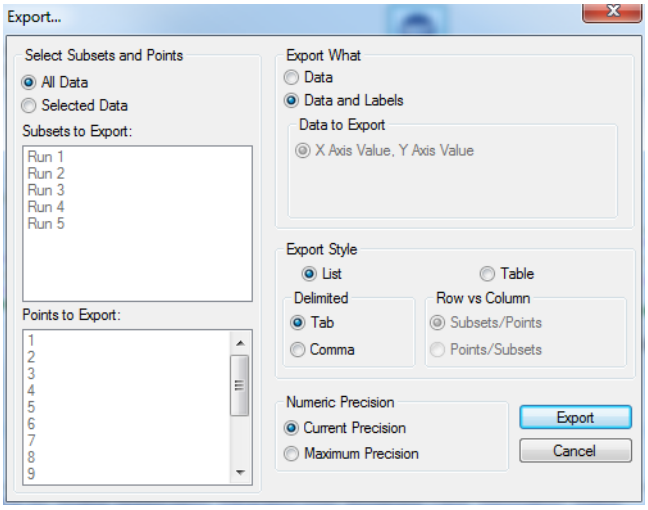


Figure 5-70: Image Export Settings

Table 5-10: *Run Data Right-Click Menu Commands (Continued)*

Menu Command	*Description*
X Fullscale	Scales the x axis to in all graphs to the range needed to accommodate all of the data.
X Autoscale	Scales the x axis to the data in each graph.
Y Fullscale	Scales the y axis to in all graphs to the range needed to accommodate all of the data.
Y Autoscale	Scales the y axis to the data in each graph.
Flip All Data	Inverts signals from positive to negative or from negative to positive. This is used most often when the observed nm shift is negative due to the presence of large analytes, such as phage, cells, and lipoparticles on the biosensor surface.

APPENDIX A:

Running the BLItz Pro Software on Computers Running Octet Systems

Running Only the BLItz System 88

Running Experiments on Octet and BLItz Systems 88

For Octet™ customers who also purchase a BLItz™ system, this section addresses the procedures with having the two systems share a computer. Those Octet customers include:

- Those that have the Octet v4.x software
- Those that have a relatively new v5.x. and v6.x software
- Those that have v7.0, v8.x and the latest v9.0 software

NOTE: *To have BLItz and Octet systems share a computer seamlessly, update your Octet software to v7.0.1.3 or higher. If you do not wish to update your Octet software, earlier versions of Octet software can still run on the same computer alongside BLItz Pro software. The following sections describe how to perform the procedure.*

RUNNING ONLY THE BLITZ SYSTEM

To run ONLY the BLItz system on the computer:

1. Ensure that the Octet software is closed. If the Octet software is running, close it.
2. Connect the BLItz system power and plug its USB cable into the computer.
3. Turn on the BLItz system.
4. Double-click the **BLItz Pro** desktop icon .

   The BLItz Pro software is launched, the BLItz system will initialize, and an "Initializing" prompt will display. The BLItz system's front light will blink; after the initialization process completes successfully, the "Initializing" prompt will close automatically, and the light will stop blinking.

   If no error message appears, you can start an experiment on the BLItz system; otherwise, refer to "Troubleshooting" on page 91.

RUNNING EXPERIMENTS ON OCTET AND BLITZ SYSTEMS

To run experiments on both an Octet system and a BLItz system on the same computer:

1. Ensure that the Octet software is closed, that the BLItz system is powered off, and that the BLItz Pro software is closed.
2. Double-click the Octet software desktop icon to launch the Octet software.

   The Octet software is launched, after the Octet system initializes properly. A "Ready. Date, Time" statement displays on the Instrument Status window (Figure A-1).

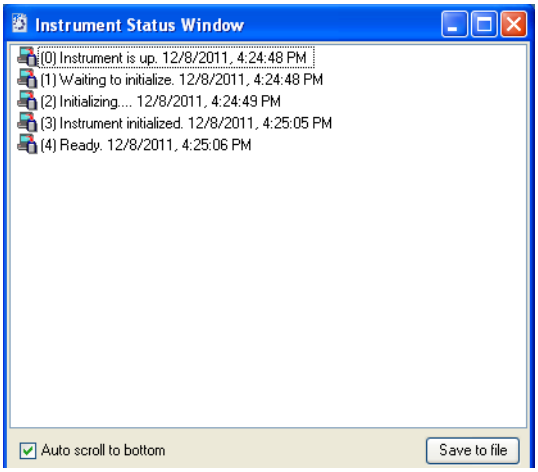


Figure A-1: *Instrument Status Window—Octet System*

3. Turn on the BLItz system.
4. Double-click the **BLItz Pro** desktop icon .

   The BLItz Pro software is launched, the BLItz system will initialize, and an "Initializing" prompt will display. The BLItz system's front light will blink; after the initialization process completes successfully, the "Initializing" prompt will close automatically, and the light will stop blinking.

   If no error message appears, you can start an experiment on the BLItz system; otherwise, refer to "Troubleshooting" on page 91.

CAUTION: *While running an experiment on an Octet system, do not click the Stop icon to abort the experiment. If this occurs: 1) close the Octet software and the BLItz Pro software, 2) power off the BLItz system, and 3) then re-launch the Octet software.*

CAUTION: *On the Octet software, do not click* **Instrument** > **Reset** *menu command to reset the Octet system. If an experiment was aborted on the Octet software: 1) close the Octet software and the BLItz Pro software, 2) power off the BLItz system, and 3) then re-launch the Octet software.*

APPENDIX B:

Troubleshooting

Troubleshooting BLItz System Errors with an Octet System 92
Scenario 1—The BLItz System Cannot Initialize Successfully 92
Scenario 2—The BLItz System Is Not Recognized 92

This appendix describes how to troubleshoot some scenarios of BLItz system errors.

TROUBLESHOOTING BLITZ SYSTEM ERRORS WITH AN OCTET SYSTEM

Scenario 1—The BLItz System Cannot Initialize Successfully

When you launch the BLItz Pro™ software, the BLItz™ system cannot initialize successfully (the BLItz system light keeps blinking), and either of the following error messages display (Figure B-1).

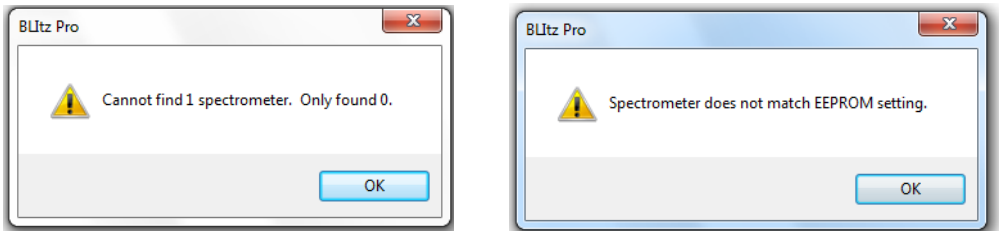


Figure B-1: *Error Message Possibilities*

How to Recover

1. Close the BLItz Pro software.
2. Switch off the BLItz system.
3. Ensure that the USB cable is connected properly between the BLItz system and the computer.
4. Switch on the BLItz system. Wait for five (5) minutes.
5. Turn on the BLItz Pro software.

NOTE: If the symptom still exists, restart steps 1 to 5 above.

Scenario 2—The BLItz System Is Not Recognized

When you launch BLItz Pro software, the following prompt (Figure B-2) displays.

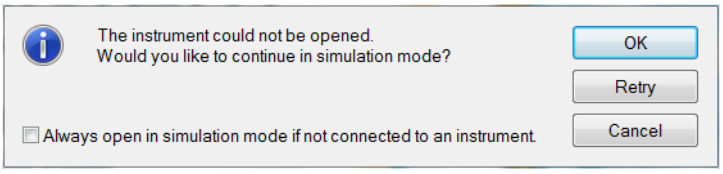


Figure B-2: *Simulation Mode Prompt*

How to Recover

1. Close the BLItz Pro software.
2. Ensure that the BLItz system is powered on (the light is on) and that the power and USB cable are properly connected to the BLItz system and computer.
3. Re-launch the BLItz Pro software until the system initialization completes successfully (no error message displays).

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