Bio-Plex *Manager*™ *Software 4.1 User Guide*

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1. Bio-Plex Suspension Array System Overview

The Bio-Plex suspension array system is a flow-based dual-laser system for simultaneously identifying and quantitating up to 100 different analytes in a single biomolecular assay (xMAP technology) . The system detects and measures molecules bound to the surfaces of fluorescent microspheres, providing highly accurate, real-time digital analysis of serum or culture media samples as small as 50 µl. This allows for quantitative analysis of a wide variety of cell biology assays, including immunoassays, complex genetic assays, and enzymatic assays.

The Bio-Plex suspension array system is fully integrated, and consists of an array reader and microplate platform, validation and calibration reagents, a selection of cytokine and phosphoprotein assays, sample preparation reagents, and a computer running Bio-Plex Manager software.

Figure 1.   Bio-Plex array reader and microplate platform.

The assays contain sets of microscopic color-coded beads, each of which is conjugated with a different reactant.

Reactants can include:

- DNA
- Enzyme substrates
- Receptors
- Antigens
- Antibodies

These can be used to create, for example, a capture sandwich immunoassay.

To perform a multiplex reading, samples are mixed with conjugated microsphere and reactant mixtures and fluorescent reporter molecules are added. The assays are loaded into the wells of a 96-well microtiter plate and the plate is inserted into the microplate platform. The platform and array reader are controlled by a computer running Bio-Plex Manager software.

The detection system in the array reader uses two lasers to analyze the microspheres in a flow stream. The first laser identifies each microsphere and its associated analyte based on the fluorescent signature of the microsphere, and the second measures the amount of analyte using the reporter molecules attached to the analytes. When the reading is complete, Bio-Plex Manager displays the raw data and generates detailed summary reports.

Components

A certified Bio-Rad service engineer will install the complete Bio-Plex suspension array system at your site, including the array reader, platform, and computer. The system setup procedure is described in the Bio-Plex Suspension Array System Hardware Instruction Manual.

The Bio-Plex suspension array system includes the following components:

- Array reader
- Microplate platform

- Bio-Plex MCV Plate III (required for use with Bio-Plex Manager 4.0 and later)
- A Pentium®-class PC, preinstalled with operating system, Microsoft® Excel, Microsoft® Internet Explorer, Microsoft.net, and Bio-Plex Manager Workstation software
- Software package, containing Bio-Plex Manager CD-ROM, user guide, and a hardware protection key
- Instrument manual for array reader and microplate platform
- Sample tube holders (two sizes, large and small)
- Sheath fluid bottle
- Sample needles (two long)
- Protective shield
- Needle adjustment tool
- Waste bottle
- Communications cable to connect the reader to the computer
- Communications cable to connect the microplate platform to the computer
- Computer monitor
- Power cords
- Computer keyboard
- Computer mouse
- HTF (High Throughput Fluidics), if selected

The Bio-Plex suspension array system comes with these reagents:

- Bio-Plex Calibration Kit (CAL1 and CAL2)
- Bio-Plex Validation Kit 4.0 (optics, fluidics, reporter, and classify components)
- Sheath fluid

Advantages

With the Bio-Plex suspension array system, you can:

- Simultaneously quantitate up to 100 different analytes from culture media and serum samples as small as 50 μl.

- Automatically analyze all the samples in a 96-well microtiter plate—yielding up to 9,600 data points—in about 30 minutes.
- Instantly customize your experiments by mixing Bio-Plex assays or creating your own assays.
- Analyze results, prepare reports, and print and/or export data immediately after each reading.
- Provide a complete electronic audit trail of data generation and analysis in a secure digital environment with multilevel account access, compliant with the Code of Federal Regulations, title 21, Part 11, "Electronic Records; Electronic Signatures."

For More Information…

For more information on the principles and concepts of the Bio-Plex suspension array system, see **Basic Concepts** on page 187, or visit our Web site at http://www.bio-rad.com/bio-plex.

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2. Bio-Plex Manager Software Overview

Bio-Plex Manager software runs on a computer installed with the Windows® 2000 or XP operating system, and requires a hardware protection key, also known as a HASP key, installed on either the computer itself or the computer network system (see page 12). The software features a standard Windows® interface, with pulldown menus, toolbars, and keyboard shortcuts.

Bio-Plex Manager comes in two editions—Standard Edition and Security Edition—and with three available licenses: Workstation, Desktop, and Network. These editions and licenses are described below. The computer included with the Bio-Plex suspension array system comes preinstalled with a compatible operating system and Bio-Plex Manager Standard Edition, Workstation license.

Software Editions

Bio-Plex Manager software comes in two editions:

- **Standard Edition** gives all users equal access to all features of the software with no restrictions and no electronic audit trail.
- **Security Edition** provides different levels of users access to different features of the software and creates a complete electronic audit trail of all data generation and analysis. Security Edition can be run in Secure Mode, with all the security features enabled, or Standard Mode, which behaves like the Standard Edition of the software. Note that Bio-Plex Manager Security Edition must be installed on the Windows® 2000 or XP Professional operating system for full security functionality. For more details, see Chapter 9.

Software Licenses

Bio-Plex Manager software is available with three different licenses:

- **Workstation license** enables the software to control the array reader and microplate platform and collect, analyze, and output data.
- **Desktop license** enables the software to analyze data files but not control the array reader and platform. The instrument communication and control functions described in Chapters 4, 5, and 7 are not available with this license.
- **Network license** provides Desktop licenses to multiple users over a computer network. Like the Desktop license, it enables the software to analyze data files but not control the array reader and platform.

Note on this User Guide

The main body of this user guide assumes that you are using the Standard Edition of the software with a Workstation license, unless otherwise noted. The Security Edition of the software and its user levels and restrictions are described in detail in Chapter 9.

Compatibility with Luminex® IS 2.3 Software

If you install Bio-Plex Manager 4.0 or later on a computer with Luminex® IS 2.3 software or later, note the following:

➢ To avoid communication conflicts with the array reader and platform, do not run Bio-Plex Manager and Luminex IS 2.3 at the same time.

➢ If you switch between the two software applications, **you must re-calibrate the array reader** before acquiring data.

Consult your Luminex documentation for instructions on using Luminex IS 2.3 software.

General Workflow

To collect, analyze, and output data using Bio-Plex Manager, you follow the general steps outlined below:

1. Start up and calibrate the array reader.

2. Open or create a protocol file, and specify the analytes that you want to detect, the layout of the 96-well plate, and information about your standards.

3. Run the selected protocol.

4. Review the results.

5. Print and/or export the data.

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┌─────────────────────────────────────────────┐
│     Start up and calibrate array reader       │
└─────────────────────────────────────────────┘
                      ↓
┌─────────────────────────────────────────────┐
│   Select analytes, standards, and plate format │
└─────────────────────────────────────────────┘
                      ↓
┌─────────────────────────────────────────────┐
│               Run the Protocol                │
└─────────────────────────────────────────────┘
                      ↓
┌─────────────────────────────────────────────┐
│     Generate reports and review results       │
└─────────────────────────────────────────────┘
                      ↓
┌─────────────────────────────────────────────┐
│           Print and export the data           │
└─────────────────────────────────────────────┘
```
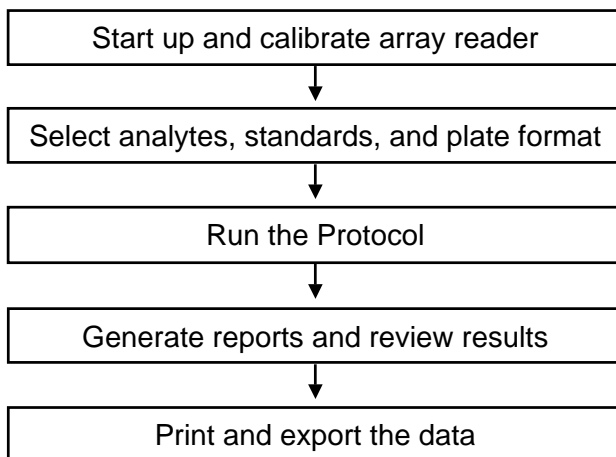
Figure 2.   Workflow for Bio-Plex Manager

The *Quick Guide*, which displays automatically at startup, is designed to guide you through the typical Bio-Plex Manager workflow, from start up and calibration through shut down. Commands such as **Start Up** and **Calibrate** open dialog boxes that contain step-by-step instructions to guide you through the procedures.

Types of Files

There are two main types of files created and used by Bio-Plex Manager:

- **Protocol files** (see page 49) contain the settings and controls for reading a microplate.
- **Results files** (see page 115) contain the data from each reading and tools and reports for analyzing that data.

The application database file (**bioplexdata.mdb**) is installed on your hard drive or file server when you install the software. This database contains calibration, validation, and instrument operations information. See page 11 for more details.

Key Software Features

The key features of Bio-Plex Manager software include:

- Graphical user interface
- Complete control of the array reader and microplate platform
- Automated maintenance, calibration and validation functions
- Automatic error detection and logging system
- Bead map and histogram
- Raw data reports
- Data reduction
- Logistic curve-fitting algorithms
- Calculation of unknown concentrations and ratios
- Export to Microsoft$^®$ Excel, text files, and XML format
- Microplate platform heater control
- Complete electronic audit trail of data generation and analysis (Security Edition)
- Different levels of user access (Security Edition)
- Secure documents—Protocol files, Results files, log files (Security Edition)

3. Software Installation

The Bio-Plex Manager CD can be used as described in the following section to install, reinstall, or upgrade Bio-Plex Manager software.

System Requirements

Component	Minimum	Recommended
Operating system	Windows® 2000 or XP	Windows® 2000 or XP Professional
Processor	Pentium III or equivalent, 933 MHz	Pentium III or higher, 1 GHz or higher
Hard disk space	4 GB	40 GB
System memory	256 MB	512 MB
Screen resolution	1024 × 768	1024 × 768
Screen colors	256 colors	24-bit True Color
Ports for connecting instrument (required for Workstation license only)	2 RS232 serial ports	1 RS232 serial port and 1 USB port
Port for connecting HPK	1 USB port	1 USB port
Other software	Internet Explorer 6.0 or later Microsoft Excel 2000 or later	Internet Explorer 6.0 or later Microsoft Excel 2000 or later

Required Screen Resolution

Your computer screen resolution must be set to at least 1024 × 768 pixels for correct display of the Bio-Plex Manager interface. The status bar and some dialog boxes will not display properly at lower resolution. If your display is currently set to a lower resolution:

1. Go to the Windows **Start** menu, select **Settings**, and select **Control Panel**.

2. Open the **Display** control panel.

3. In the **Display Properties** dialog, select the **Settings** tab.

4. Drag the **Screen Area** slider to the right (**More**) until you have selected **1024 by 768 pixels**. Click on **OK** to accept the settings.
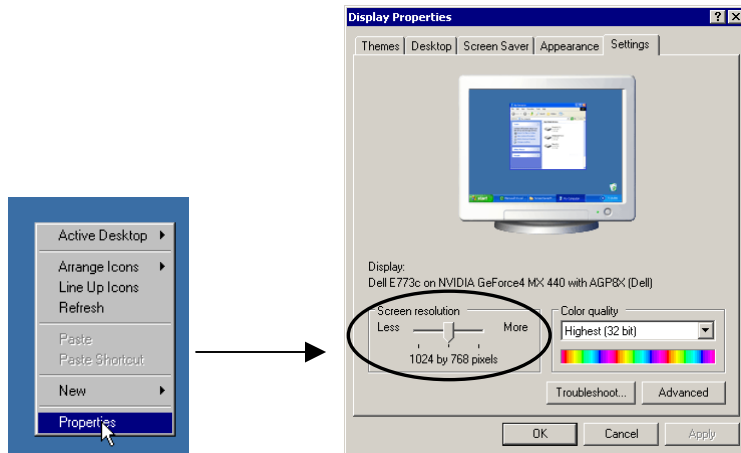
Figure 3.   Changing the screen settings.

Installing the Software

To install, reinstall, or upgrade the software, insert the Bio-Plex Manager CD into the CD-ROM drive on your computer.

➢ Before reinstalling Bio-Plex Manager, we recommend that you first *uninstall* any existing version of Bio-Plex Manager on your computer.

➢ We recommend that you turn off any anti-virus protection software before installation. Such software, if active, can greatly slow the progress of the installation. If you are unable to turn off your anti-virus protection software, allow 15 minutes for complete installation. Do not cancel the installation during this period.

The **Bio-Plex Manager Installation Program** will open, displaying a navigation screen for performing the installation.

Microsoft.net

Microsoft.net is automatically installed on your computer when you install Bio-Plex Manager. It is required to support the logistic curve fitting features of Bio-Plex Manager.

Bioplexdata.mdb File

During installation, you will be prompted to save the application database file (**bioplexdata.mdb**) to a location on your hard drive or a file server. This database file contains logs of calibration, validation, and instrument operations activity for your instrument.

- For more information about the Calibration Log, see page 31.
- For more information about the Validation Log, see page 40.
- For more information about the Instrument Operations Log, see page 47.

You can save the bioplexdata.mdb file to any folder on your computer; the default location is the Bio-Plex Manager application folder. If your computer is connected to multiple instruments, each instrument must have a separate bioplexdata.mdb file saved in a different folder.

For more information about the calibration, validation, and instrument operations logs in Bio-Plex Manager 4.1, Security Edition, see page 153.

➢ Note that the bioplexdata.mdb file is not compatible with versions of Bio-Plex Manager earlier than 4.0. If you have an earlier version of the software, installing version 4.0 or later will copy the data from your existing database file (bioplex.mdb) into the new database. A copy of your old database will remain in the application folder.

Luminex LXR Directory and Files

A directory called **Luminex** is automatically created in the **Program Files** folder on your computer during installation. Inside this folder is a folder called **LXR** that contains various applications for monitoring and communicating with the array reader and platform. A Windows® service called LXService is also installed and started. This service enables automatic communication with the instrument when you open Bio-Plex Manager.

Hardware Protection Key (HASP Key)

A hardware protection key, also known as a HASP key, is required to run Bio-Plex Manager. The HASP key determines the license (Workstation, Desktop, or Network) and the edition (Standard or Security) of Bio-Plex Manager.

Workstation or Desktop HASP keys must be attached to a USB port on the computer running the software. Network HASP keys must be attached to a USB port on the network file server computer.

The HASP key has a driver that is automatically installed when you install Bio-Plex Manager.

Uninstalling

To uninstall Bio-Plex Manager from your computer, use the Windows **Add/Remove Programs** function. Click on the Windows **Start** button, select **Settings**, select **Control Panel**, double-click on **Add/Remove Programs**, and follow the instructions for removing the program.

4. Getting Started

Before starting Bio-Plex Manager, make sure the Hardware Protection Key (see page 11) is attached to your computer, and switch on the array reader and the microplate platform. This turns on the optics inside the array reader and enables communication between the array reader and software.

➢ Avoid using other applications while Bio-Plex Manager is communicating with the array reader. Running other applications may interrupt communication between Bio-Plex Manager and the array reader.

Starting Bio-Plex Manager

To start Bio-Plex Manager, click on the application icon on your desktop or select **Bio-Plex Manager 4.1** from the **Programs** directory on your Windows *Start* menu.

Figure 4.   Bio-Plex Manager 4.1 desktop icon.

The software will open and connect to the array reader and platform.

Communication to the Array Reader and Microplate Platform

The computer running Bio-Plex Manager software is connected to the array reader by a serial or USB cable, and to the microplate platform by a serial cable. See the

Bio-Plex Suspension Array System Hardware Instruction Manual or the manual sent with the Luminex 100 IS 2.3 system for a description of the cable connections.

Connecting to the Array Reader and Platform

Communication between the computer and the array reader and microplate platform is automatically established when you open Bio-Plex Manager. If Bio-Plex Manager is unable to communicate with the instrument, a dialog box will prompt you to check the connections.
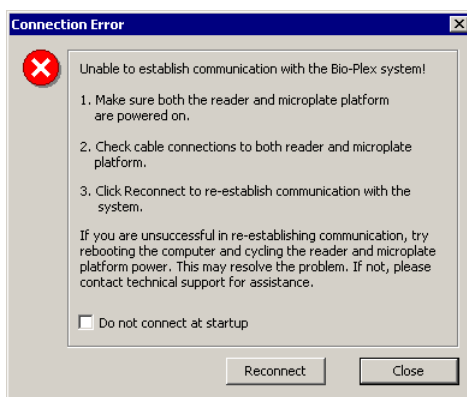
Figure 5.   *Connection Error* dialog.

Make sure the instrument is turned on and check the cable connections, then click on **Reconnect** in the dialog. If you are still unable to connect, try restarting the computer, array reader, and platform. If you experience further difficulties, contact Bio-Rad technical support (see page 4).

If you do not want to automatically connect to the array reader and platform when you open the software, select the **Do not connect at startup** checkbox. This is useful if you are not connected to an instrument or want to use the software without connecting to the instrument. To re-enable automatic connection at startup, select **Reconnect** from the *Instrument* menu (see next section).

Disconnecting and Reconnecting

Communication between the computer and the array reader and platform is closed when you exit Bio-Plex Manager. If you need to disconnect from the array reader and platform without shutting down Bio-Plex Manager software (e.g., to troubleshoot the instrument), go the *Instrument* menu and select **Disconnect.**

To reconnect to the array reader and platform while Bio-Plex Manager is running, select **Reconnect** from the *Instrument* menu. This will also enable automatic connection each time you start the software, if it has been disabled (see previous section).

See the Bio-Plex system hardware manual or the Luminex 100 IS 2.3 system manual for specific instructions.

Menu and Toolbars

Bio-Plex Manager includes a menu bar and main toolbar at the top of the application window.

The pulldown menus contain all the major functions of the software. Note that these menus change depending on whether you are displaying a Protocol window, a Results window, or neither.

The main toolbar includes the major instrument controls, including **Start Up, Shut Down, Wash, Unclog,** etc. The various Protocol and Results windows also contain their own toolbars, with commands specific to those windows. These are described in greater detail in the following chapters.

Status Bar

The instrument **status bar** shows the current state of the instrument (Calibration, Warm Up, Ready, Pressurizing, etc.). It includes a **Cancel** button for canceling the current operation (see page 46).

Figure 6.   Instrument status bar.

When the array reader is performing an operation, the time remaining for the operation will be shown in the instrument status bar.

The Bio-Plex Manager software status bar is below the instrument status bar, and provides information about the command under the cursor. This information is continuously updated as you move your cursor over the software window.

The software status bar also shows the current user and, if you are using the Security Edition of the software, whether you are in Secure Mode (shown as a "locked" symbol 🔒) or Standard Mode (shown as "unlocked" 🔓). See Chapter 9 for more information about Bio-Plex Manager Security Edition.

The software status bar also shows the Caps Lock, Scroll Lock, and Num Lock keyboard status.

Quick Guide

When Bio-Plex Manager first opens, a *Quick Guide* toolbar is displayed in the upper right corner of the screen. The *Quick Guide* is designed to guide you through the typical workflow when using the system, from start up and calibration through shut down.
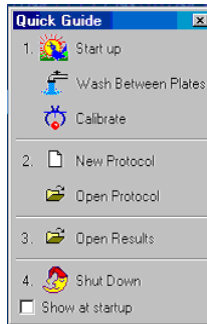
Figure 7.   Bio-Plex Quick Guide.

By default, the *Quick Guide* opens automatically when you start Bio-Plex Manager. To disable this feature, deselect the **Show at Startup** checkbox at the bottom of the guide.

To open the *Quick Guide* after it has been closed, select **Quick Guide** from the *View* menu.

Sample Needle Adjustment

See the Bio-Plex Hardware Manual or the Luminex manual for instructions on installing the sample needle.

The height of the array reader sample needle must be adjusted when (1) the style of microtiter plate has changed or (2) when the sample needle is replaced. The sample needle height is the same for flat bottom or filter plates. Note that, for round bottom plates, the needle must be raised so that it is slightly above the bottom of the round bottom well.

➢ If you adjust the sample needle for use with round bottom plates, you will not be able to use the MCV Plate III without readjusting the needle to filter-plate height.

With the array reader and platform switched on, go to the *Instrument* menu, go to the *Set Up* submenu, and select **Adjust Needle**. The *Adjust Needle* dialog box will open.

17
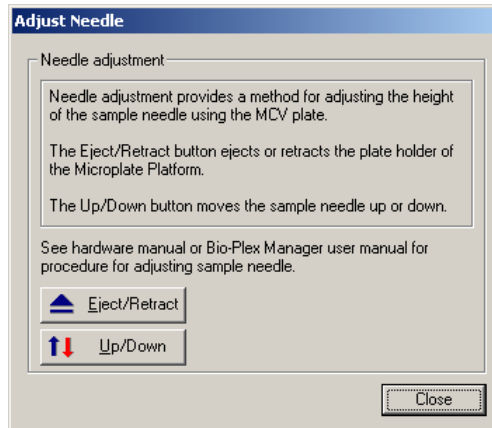
Figure 8.   *Adjust Needle* dialog.

Then follow the steps below:

1. Click on the **Eject/Retract** button to eject the plate holder, and place the MCV Plate III on the microplate platform with the arrow pointing toward the array reader.

2. While holding the platform door open, click on the **Eject/Retract** button again to retract the plate.

3. Tape the access door of the microplate platform open. You must be able to see inside the access door.

> **Warning:** DO NOT stick your hands and fingers inside the platform at any time.

4. Click on the **Up/Down** button in the *Adjust Needle* dialog to lower the needle into the needle adjustment well in the MCV Plate III.

5. Using the hex key needle adjustment tool (supplied with the array platform), loosen the screw at the top of the needle so that the needle housing can move up and down freely.

6. Move the knurled dial on the needle arm and manually move it downward so that it just touches the bottom of the needle adjustment well. Use a flashlight to inspect the needle through the platform door as you adjust it. Move the needle up and down gently a couple of times to verify that the needle is just touching the bottom of the well.

7. Tighten the needle set screw so that it is no longer possible to manually move the needle.

8. Click on the **Up/Down** button in the dialog a couple of times to make sure that the needle moves freely up and down. The needle should not push down on the MCV Plate III.

9. Finally, remove the tape so that the platform door can open and close normally, and click on the **Close** button to close the *Adjust Needle* dialog.

5. System Controls

This chapter covers the Bio-Plex Manager commands that control basic system functions, such as instrument start up, warm up, calibration, validation, and shut down.

➢ The Start Up, Warm Up, and Calibration functions must be performed prior to running an assay with Bio-Plex Manager, and all together take about 45 minutes.

⚠ When performing the following operations, the sheath fluid container and the waste fluid container should be closely monitored.

⚠ The sheath fluid bottle must be placed at the same level as the array reader, unless you are using the Bio-Plex High-Throughput Fluidics (HTF; see caution below) or Luminex Sheath Delivery System (SDS). The fluid level should be below the air inlet connection and above the sheath outlet connection. Always check the sheath fluid level before starting a run or procedure.

⚠ If you are using the Bio-Plex HTF or Luminex SDS, it should sit on the counter next to the array reader, while the sheath fluid cube should be placed ∼3–4 feet below the array reader (e.g., on the floor).

⚠ The waste fluid container receives waste from the system. **Do not allow the waste container to overflow!** Empty the waste bottle each time the sheath fluid bottle is filled. The waste container should be placed on the bench next to the instrument. Never place this container on top of the instrument. All waste containers should have vented caps.

Bio-Plex MCV Plate III

Bio-Plex Manager 4.0 and later require the use of the Bio-Plex MCV Plate III (MCV stands for maintenance, calibration and validation). This plate contains wells marked

for the different types of fluids used in validation, washing, calibration, and other functions. The Bio-Plex MCV Plate III has been modified from the previous MCV plate to work with Bio-Plex Validation Kit 4.0, with its enhanced Reporter and Classify bead sets. It also includes an open needle well for ease of needle adjustment.

➢ Bio-Plex Manager 4.0 and later require the use of the Bio-Plex MCV Plate III, the Bio-Plex Validation Kit 4.0, and the Bio-Plex Calibration Kit.
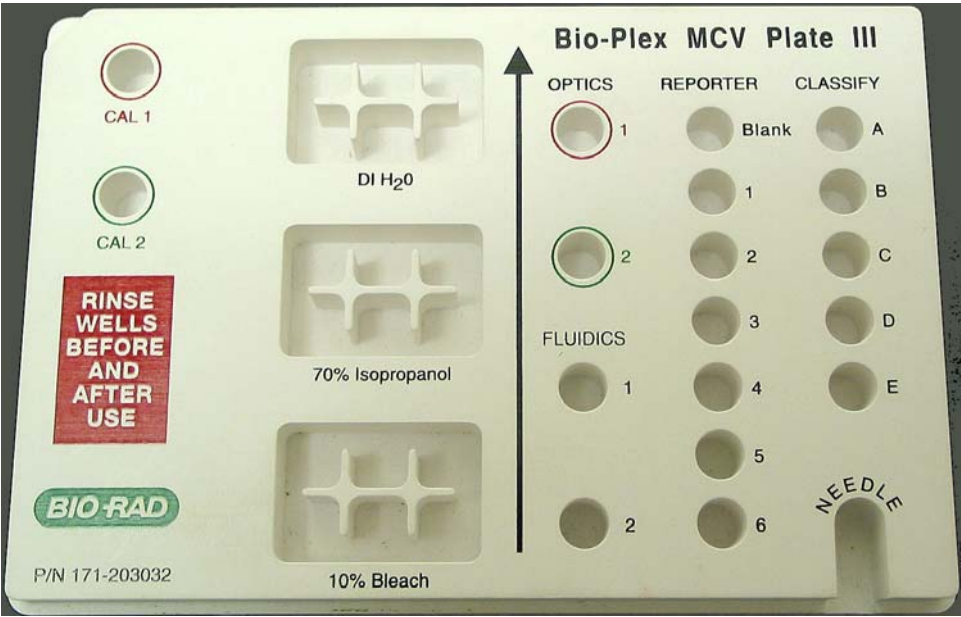
Figure 9.   Bio-Plex MCV Plate III.

When a particular procedure (e.g., Calibration) requires you to add solutions to the Bio-Plex MCV Plate III, the dialog box describing the procedure will include a diagram of the MCV Plate III with the wells to be loaded highlighted in blinking yellow.

Wells highlighted in yellow
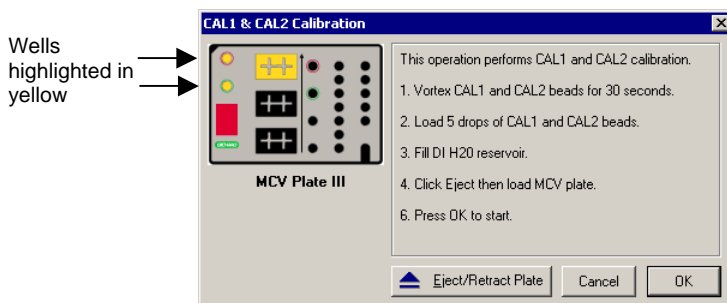
Figure 10.   Highlighted wells in diagram indicate wells to be filled.

Start Up

Start up is a series of fluidics functions that prepares the array reader to acquire data. This process requires the Bio-Plex MCV Plate III, distilled water, and 70 percent isopropanol. Start up takes approximately 10 minutes.

➢ You do not need to wait for the array reader optics to warm up before performing the start up procedure.

Click on the **Start Up** button   on the *Quick Guide* or main toolbar or select the command from the *Instrument* menu. Follow the step-by-step directions in the dialog box for preparing the MCV Plate III.
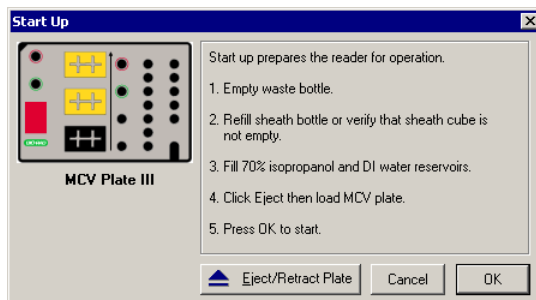
Figure 11.   *Start Up* dialog.

Insert the prepared plate into the microplate platform and click on **OK** to begin the start up process.

Optics Warm Up and Shut Down 🔥

To ensure accurate and reproducible results, the optics (i.e., the lasers) in the array reader must warm up for at least 30 minutes prior to calibration, validation, and reading assays. Optics warm up begins when you first turn on the array reader.

You can proceed with the array reader start up procedure described in the previous section while the optics are still warming up. However, if you try to perform calibration, validation, or an assay reading, you will receive a warning message.

You can cancel the warm up procedure using the **Cancel Operation** command on the *Instrument* menu or the **Cancel** button in the status bar; however, this is not recommended. Results for identical readings may vary if the optics have not reached optimal operating temperature.

➢   If you attempt to start a reading during warm up, you can eject the plate carrier using the **Eject/Retract Plate** command ⏏ on the main toolbar and remove the plate for storage until warm up is complete without canceling the warm up procedure.

If the array reader is idle for more than four hours, the optics will automatically power down (though the array reader itself will remain on). Depending on the length of the shut down, a full 30-minute warm up period may be required before more readings can be taken.

To begin warm up after automatic power down, click on the **Warm Up** button
on the main toolbar or select the command from the *Instrument* menu.

If you attempt to perform a reading after automatic power down, you will receive a
warning message and the optics will begin warming up.

Calibration

Calibration of the array reader is essential for optimal performance and day-to-day
reproducibility of results. Calibration is required:

- Each day after the start up procedure is complete and the optics have
  warmed up.

- If the array reader temperature changes by more than 2º C during the course
  of the day (if the temperature changes by more than 2º C, a message box
  will prompt you to recalibrate).

- Before data acquisition if you switch between Bio-Plex Manager and
  Luminex IS 2.3 software installed on the same computer (see page 6).

➢ Before calibrating, make sure that optics warm up is complete.
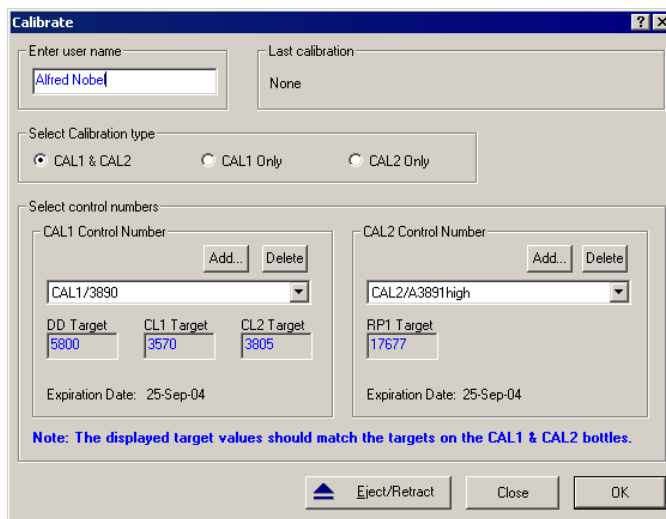
The Bio-Plex Calibration Kit contains calibration microspheres (CAL1 and CAL2
beads) with stable fluorescent intensities in the RP1, CL1, and CL2 wavelength
ranges. The calibration process uses these microspheres to adjust voltage settings for
optimal and consistent microsphere classification and reporter readings over time
and across different instruments. Current calibrated settings are automatically
applied to any new session.

Calibration using Bio-Plex Manager requires the Bio-Plex MCV Plate III, the CAL1
beads and CAL2 beads from the Bio-Plex Calibration Kit, and DI water. The CAL1
beads calibrate the array reader's doublet discriminator and classification channels,
while the CAL2 beads calibrate the array reader's reporter channel for reporter
fluorescence detection.

Opening the Calibration Dialog Box

To begin calibration, click on the **Calibrate** button 🐞 on the *Quick Guide* or main toolbar, or select **Calibrate** from the *Instrument* menu. The *Calibrate* dialog box will open.

Figure 12.   *Calibrate* dialog.

At the top of the dialog box, enter your name in the field. If you are using the Security Edition of the software in Secure Mode, your user name will be listed and grayed out.

The time and date of the last calibration using Bio-Plex Manager are listed, as is the temperature at the time of that calibration. If the temperature has not changed by more than 2º C in a single day, it is not necessary to recalibrate the instrument.

Next, select the calibration type by clicking on the **CAL1 & CAL2**, **CAL1 only**, or **CAL2 only** button. You should perform both CAL1 and CAL2 calibration daily.

Calibration Microsphere Control Numbers

Under *Select Control Numbers*, you can either select existing control numbers for your CAL1 and CAL2 microspheres or enter new control numbers.

Select existing control numbers from the pulldown list, and the target values for the control numbers will appear in the appropriate fields.

When you receive a new Bio-Plex Calibration Kit, you must add the new CAL1 and CAL2 control numbers and target values to the *Calibrate* dialog. These numbers are printed on the bottles containing the beads.

➢ It is critical that you enter the correct target values for your CAL1 and CAL2 calibrators. Entering incorrect values will result in an incorrectly calibrated array reader, which will adversely impact assay results.

To add a new CAL1 control number, click on the **Add** button under *CAL1 control number* in the *Calibrate* dialog. The *Add New CAL1 Control Number* dialog box will open.

Figure 13.   Adding a new CAL1 control number.

In the dialog, enter the control number from the CAL1 bottle in the **Enter Control Number** field. If the expiration date is printed on the bottle, select the option button next to the date field under **Expiration Date** and click on the pulldown button next to the field to open the calendar selection box.
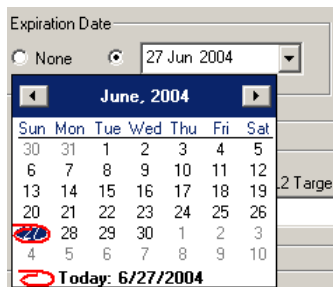
Figure 14.   Calendar selection box

Scroll through the calendar using the scroll buttons at the top. Select a particular date by clicking on it.

Next, enter the DD, CL1, and CL2 target values for the control number, as printed on the bottle, in the appropriate fields.

➢   If you are using a Luminex-type assay from another manufacturer, do not use the DD target value specified in the manufacturer's manual. Use only the DD target value on the Bio-Plex CAL1 bottle.

When you are done, click on **Add** to close the dialog box and save your changes.

To add a new CAL2 control number, click on the **Add** button under *CAL2 control number* in the *Calibrate* dialog box. The *Add New CAL2 Control Number* dialog box will open.

Figure 15.   Adding a new CAL2 control number.

In the dialog, enter the control number from the CAL2 bottle in the field. Select the expiration date, if printed on the bottle, as described above. Next, enter the High RP1 or Low RP1 target value for the control number, as printed on the bottle, in the **RP1** field.

➢   The CAL2 calibration bottle label lists two different RP1 target values: Low RP1 and High RP1. Bio-Plex phosphoprotein assays require calibration using the High RP1 target value, while Bio-Plex cytokine assays may be calibrated with either the Low or High RP1 target value. Other Luminex-type assays typically require calibration with the Low RP1 target value. Check with your assay supplier.

When you are done, click on **Add** to close the dialog box and save your changes. Your new control numbers will be added to the selection lists in the main *Calibrate* dialog.

To delete a particular control number, first select it in the *Calibrate* dialog box, then click on the **Delete** button.

Calibration Set Up

When you have specified your control numbers of calibration microspheres and specified the type of calibration you want to perform (CAL1 & CAL2, CAL1 only, or CAL2 only), click on **OK** in the *Calibrate* dialog.

Another dialog box will list step-by-step instructions for preparing the Bio-Plex MCV Plate III.
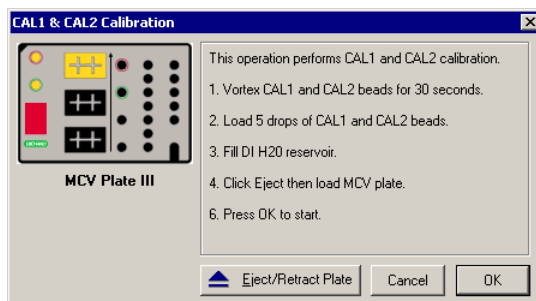
Figure 16.   Preparing the Bio-Plex MCV Plate III for CAL1 & CAL2 calibration.

When preparing the MCV Plate III, note the following:

- **Important:** Before vortexing, remove the calibration beads from 2–8°C (36–46°F) storage and allow to warm to room temperature. Vortex each bottle for 30 seconds. Proper resuspension of the microspheres is essential for efficient calibration.
- Never dilute the calibration beads, and be careful to limit their exposure to light. Store at 2–8° C immediately following calibration.
- Load 5 drops of beads (approximately 200 μl) per reservoir (CAL1 or CAL2).
- The DI H_2O well of the MCV Plate III holds about 3 ml of distilled water.

Performing the Calibration

Click on the **Eject/Retract Plate** button to eject the microplate platform plate carrier, place the MCV Plate III in the carrier with the arrow facing toward the platform, then retract the plate. Click on **OK** to start the calibration process.

As calibration proceeds, the status bar at the bottom of the Bio-Plex Manager window will monitor the progress of calibration. The number of beads per second should be 100 or higher; fewer beads per second may indicate a problem with the fluidics system. See the troubleshooting guide at the end of this manual.

Number of beads per second should be 100 or higher
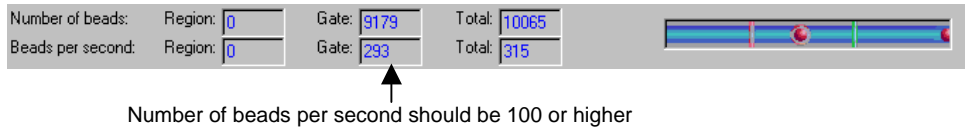
Figure 17.   Status bar during calibration.

Because calibration beads are highly concentrated, calibration is followed by three wash cycles using distilled water.

An alert box will notify you whether calibration has succeeded or failed. If the calibration process failed, check the MCV Plate III to verify that the correct beads were added to the wells. Then perform a fluidics **Wash** (page 45) and repeat the above steps. For more information, consult the **Troubleshooting** guide on page 165.
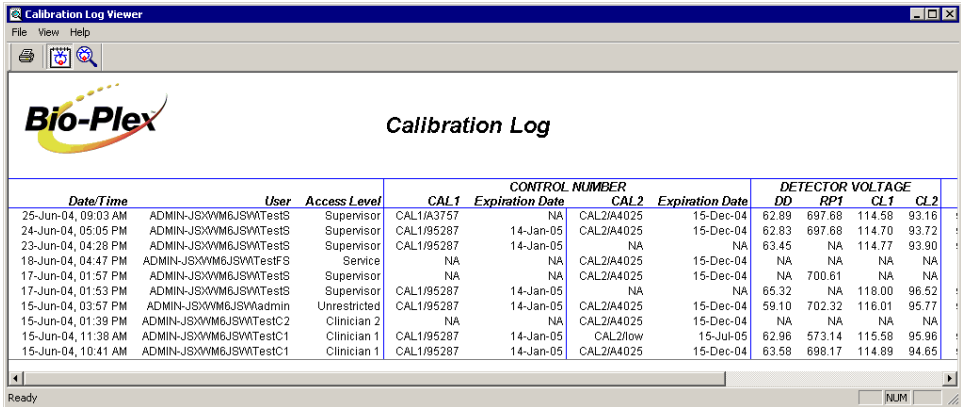
Calibration Log

The Calibration Log provides a list of past calibration dates and times, results, instrument settings, and other data obtained by Bio-Plex Manager. This data is stored in a log file called **bioplexdata.mdb**, which by default is saved in the main Bio-Plex Manager application folder on your computer.

➢ Note that this database is not compatible with versions of Bio-Plex Manager earlier than 4.0. If you have an earlier version of Bio-Plex Manager, installing version 4.0 or later will copy the data from your existing database (bioplex.mdb) into the new database. A copy of your old database will remain in the application folder.

To open the Calibration Log, go to the *View* menu and select **Calibration Log**. The *Calibration Log* viewer will open.

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Figure 18.   Calibration Log viewer.

Each calibration is listed by date and time. If you are using Bio-Plex Manager Security Edition in Secure Mode, the **User** and **Access Level** columns list information about the user who was logged into the application when each calibration was performed. The **Result** column notes the result of each calibration, including whether it passed or failed.

➢ If you see a drastic change in a detector's voltage from one calibration to the next, it could indicate a problem with the instrument. A steadily increasing detector voltage may indicate that the laser is decreasing in intensity.

On the toolbar, click on **Calibration Detail** 🔍 to display additional information for each calibration, including detector gain. Click on **Calibration Log** 📊 to return to the default view.

For CAL1-only calibrations, the RP1 column will display a entry of NA (not applicable). For CAL2-only calibrations, the DD, CL1, and CL2 columns will display entries of NA.

To print the calibration report, select **Print** from the *File* menu. **Print Preview** displays the report as it will appear in a printout. **Print Setup** allows you to select some standard print settings.

Wash Between Plates 🚰

You should wash the fluidics lines between each plate reading to prevent traces of sample or other debris from building up inside the system. Click on the **Wash Between Plates** button 🚰 on the *Quick Guide* or main toolbar, or select the command from the *Instrument* menu. A dialog box will guide you through the steps for preparing the MCV Plate III for a wash procedure.
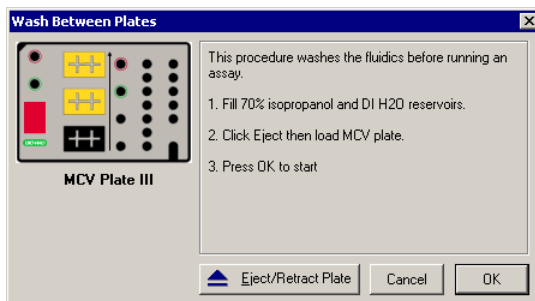
Figure 19.   Steps for washing between plate readings.

Add 70% isopropanol and distilled water to the appropriate reservoirs, insert the plate in the microplate platform, and click on **OK**. This procedure performs a series of different fluidics operations and will take several minutes. After initial system pressurization (5–20 seconds), the time remaining in the operation will be displayed in the Bio-Plex Manager status bar.

Remove Air Bubbles ❄

Microscopic air bubbles in the cuvette may cause a sudden shift in the bead regions during an assay reading. If the array reader detects such a shift, the reading will stop and you will be prompted to perform an alcohol wash to force air bubbles out of the system.

Click on the **Remove Bubbles** button ❄ on the main toolbar or select the command from the *Instrument* menu. A dialog box will guide you through the steps for preparing the MCV Plate III for the procedure.
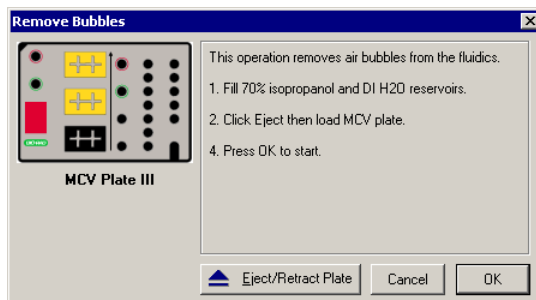
Figure 20.   Steps for removing air bubbles from the fluidics system.

Add 70% isopropanol and distilled water to the appropriate reservoirs, insert the plate in the microplate platform, and click on **OK**. This procedure performs a series of different fluidics operations and will take several minutes. After initial system pressurization (5–20 seconds), the time remaining in the operation will be displayed in the Bio-Plex Manager status bar.

Unclog 📭

If the array reader detects an unusually low bead count during a reading, you will be prompted to perform an **Unclog** operation to remove possible obstructions from the fluidics lines. Click on the **Unclog** button 📭 on the main toolbar or select the command from the *Instrument* menu. An instruction box will guide you through preparation of the MCV Plate III.
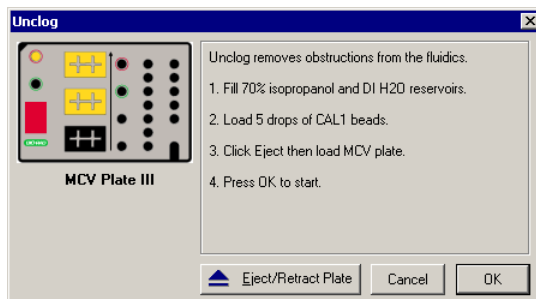
Figure 21.   Steps for unclogging the fluidics system.

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Add 10% bleach solution and distilled water to the appropriate reservoirs, add 5 drops of CAL1 beads to the CAL1 reservoir, insert the plate in the microplate platform, and click on **OK**. This procedure performs a series of fluidics operations and reads a sample of CAL1 beads to verify that the fluidics are operating properly. It will take several minutes. After initial system pressurization (5–20 seconds), the time remaining in the operation will be displayed in the Bio-Plex Manager status bar.

Validation

Validation of the array reader is a formal process for documenting that the instrument is fit for its intended use and that it is kept in a state of maintenance and calibration.

> ➢ Validation is performed after **Start Up** and **Calibration** have been performed. Validation is dependent on successful calibration for accuracy.

You should perform a validation reading:

- Once a month
- Each time you move the array reader, or
- To diagnose any problems with the array reader that cannot be solved by other procedures (calibrating, washing, unclogging, etc.).

Validation Kit

Bio-Plex Manager requires the use of Bio-Plex Validation Kit 4.0 to perform validation. It must be used in conjunction with the Bio-Plex MCV Plate III. Each Bio-Plex Validation Kit consists of reagents and procedures to evaluate:

- Optical alignment
- Reporter channel performance
- Efficiency of multiplexing
- Integrity of fluidics

The Bio-Plex Validation Kit validates the operation of all of the primary components of the array system, and can also be used to discriminate between assay and instrumentation problems.

Validation Kit Control Number

Each Bio-Plex Validation Kit has a control number linked to the specifications for the kit. You must use the mini-CD included in the kit to install the control number on your computer. After installation, you can select the control number in Bio-Plex Manager as described in the following section.

See the Bio-Plex Validation Kit manual for more information.

Setting Up a Validation Run

To set up a validation reading, click on the **Validation** button on the main toolbar or select the command from the *Instrument* menu. The *Validate* dialog box will open.
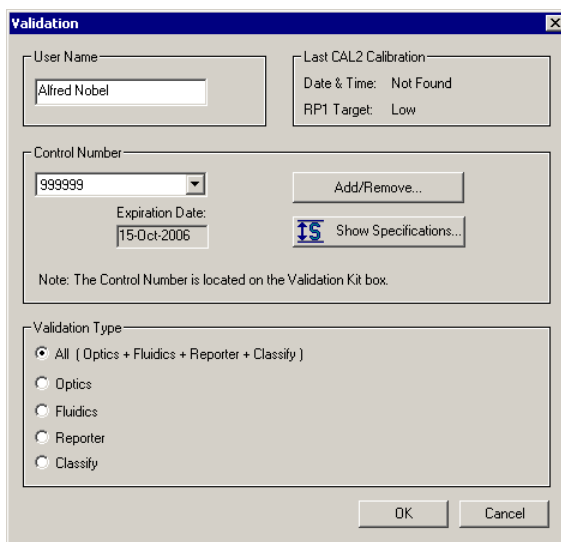
Figure 22.   *Validate* dialog.

Enter your user name in the top field. If you are using the Security Edition of the software in Secure Mode, your user name will be listed and grayed out.

Control Number Selection

Select the Control Number for your specific Bio-Plex Validation Kit from the
Control Number pulldown list. The expiration date for the control number will be
listed below the field.

If the control number is not available in the list, or the list is too long and you want
to limit it to the control numbers you are using, click on the **Add/Remove** button.
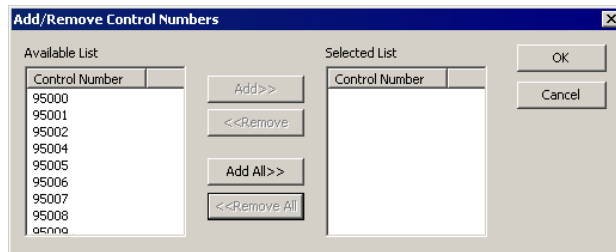
Figure 23. *Add/Remove Control Numbers* dialog.

The *Add/Remove Control Numbers* dialog lists all the available validation Control
Numbers. To make Control Numbers available in the *Validate* dialog pulldown list,
you must add them to the **Selected List**. To select all the numbers in the **Available
List**, click on the **Add All>>** button. To add control numbers individually, double-
click on them, or select multiple numbers using Shift + click and Ctrl + click key
combinations and click on the **Add>>** button. Use the **<<Remove** and **<<Remove
All** buttons to unselect them. At least one Control Number must remain in the
Selected List.

When you click on **OK**, the Control Numbers in the **Selected List** will be added to
the pulldown list in the *Validate* dialog.

To view the specifications for a Control Number, select the number from the
pulldown list and click on the **Show Specifications** button. The *Control Number
Specifications* dialog box will list the validation specifications.
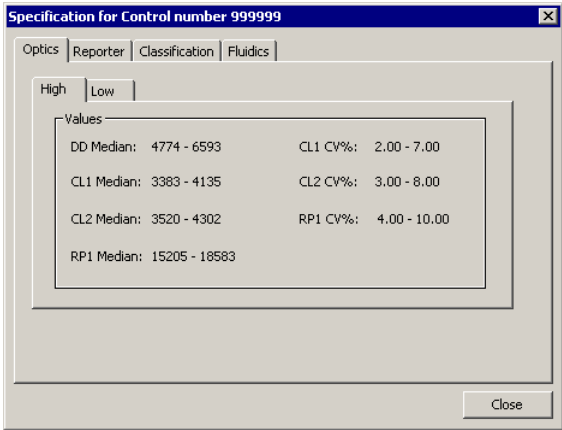
Figure 24.   *Control Number Specifications* dialog.

Validation Type Selection

In the *Validate* dialog, select the type of validation you want to perform using the **Validation Type** option buttons: All, Optics, Fluidics, Reporter, or Classify.

Performing a Validation Run

When you have made your selections in the *Validate* dialog, click on **OK**. The dialog box for the selected type of validation will open.
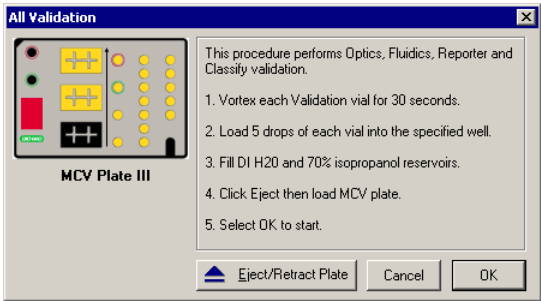
Figure 25.   Dialog box for performing all validation types.

See the Bio-Plex Validation Kit manual for information about the bottles in the Validation Kit, and instructions on loading the Bio-Plex MCV Plate III for validation. When you have prepared the plate according to the instructions, click on **Eject/Retract Plate** in the dialog box, place the plate on the plate carrier, and click on **OK**.

Validation Results

After a run, the *Validation Results* dialog box will automatically open. You can also view this dialog by going to the *View* menu and selecting **Validation Results**.
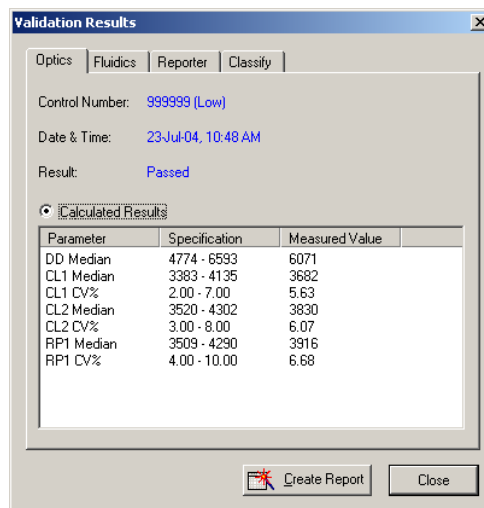
Figure 26.   *Validation Results* dialog.

The *Validation Results* dialog box includes tabs for each type of validation. If you selected and performed All Validations, all the tabs will be available. Otherwise, only the tab for the validation you performed (Optics, Fluidics, Reporter, or Classify) will be available and displayed.

Click on **Create Report** to generate and display a Microsoft® Excel spreadsheet showing the validation results. Note that Microsoft® Excel 2000 or higher must be installed on your computer for this function to work (Excel comes preinstalled on all computers supplied with the Bio-Plex suspension array system).

See the Bio-Plex Validation Kit 4.0 manual for interpreting the results of a validation run.

Validation Log

Each validation run is recorded in the Validation Log. This log is stored in a secure database file called **bioplexdata.mdb**, which by default is saved in the main Bio-Plex Manager application folder on your computer.

➢ Note that this database is not compatible with versions of Bio-Plex Manager earlier than 4.0. If you have an earlier version of Bio-Plex Manager, installing version 4.0 or later will copy the data from your existing database (bioplex.mdb) into the new database. A copy of your old database will remain in the application folder.

To view this log, go to the *View* menu and select **Validation Log**.

Figure 27.   *Validation Log* viewer.

The validation results are listed by date and time in the upper window, and the specifications associated with each Control Number are listed in the lower window. If you are using Bio-Plex Manager Security Edition in Secure Mode, the **User** and

Access Level columns list information about the user who was logged into the application when each validation was performed.

Use the buttons in the upper left corner of the window to view the different validation types (Optics ![icon], Fluidics ![icon], Reporter ![icon], and Classify ![icon]).

To print the Results window, click on the **Print Report Results** button ![icon]. To print the Specifications window, click on the **Print Specifications** button ![icon].

To show or hide the specifications data in the *Validation Log* viewer, click on the **Show/Hide Specifications Window** button ![icon].

To create a spreadsheet report for a particular validation run, click on the row for the run in the Results window. It will appear selected, as will the specifications associated with the Control Number for that run in the lower window. Then click on **Create Report** ![icon]. A Microsoft® Excel spreadsheet will be automatically generated and displayed showing all the validation data for that run. Note that Microsoft® Excel 2000 or higher must be installed on your computer for this function to work.
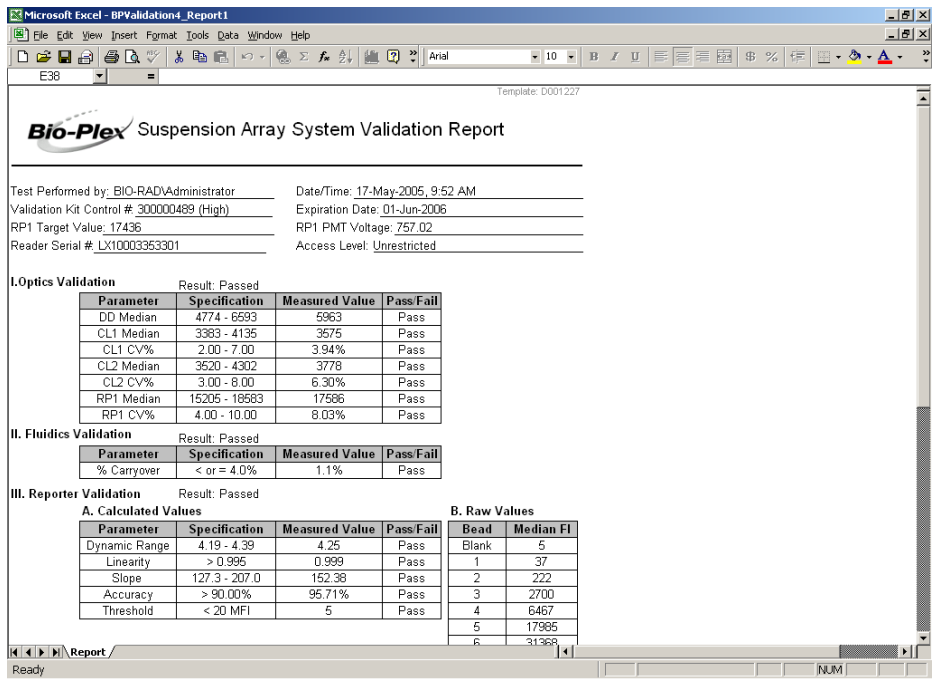
Figure 28. *Validation Results* spreadsheet (Microsoft® Excel).

Platform Heater 🌡️

The microplate platform has a heater for warming samples. The heater has an operating range of 35ºC to 60ºC (95ºF to 140ºF), and can be controlled through Bio-Plex Manager.

To access the heater controls, select **Platform Heater** from the *Instrument* menu, or click on the button on the main toolbar 🌡️.
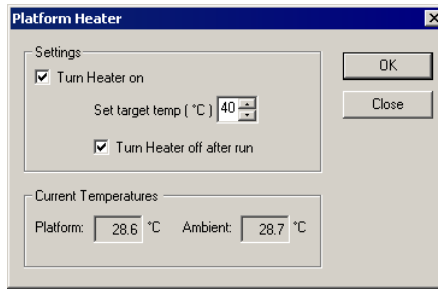
Figure 29. *Platform Heater* dialog.

In the *Platform Heater* dialog, click on the **Turn Heater on** checkbox to turn on the heater, and set the target temperature within the range 35–60ºC using the scroll buttons.

To automatically turn the heater off at the end of a run, select the **Turn Heater off after run** checkbox.

The dialog box and the Bio-Plex Manager status bar will indicate the current platform temperature. The status bar will also indicate whether the heater is turned on.
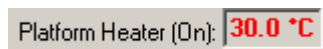
Figure 30. Status bar with heater on.

If the heater is in the process of warming up or cooling down, the temperature will be shown in red. If the heater is on and has reached the specified temperature, the temperature will be shown in green. If the heater is off and is at ambient temperature, the temperature will be displayed in black.

➢ If you initiate a run before the heater has warmed up or cooled down (i.e., the temperature is shown in red), you will have the option of waiting until the specified temperature is reached or proceeding with the run immediately.

Instrument Information 🄸

The array reader continuously monitors its internal temperature, voltage, pressure, and other systems for diagnostic purposes. You can access this information by clicking on the **Information** button 🄸 on the main toolbar or selecting **Information** from the *Instrument* menu.
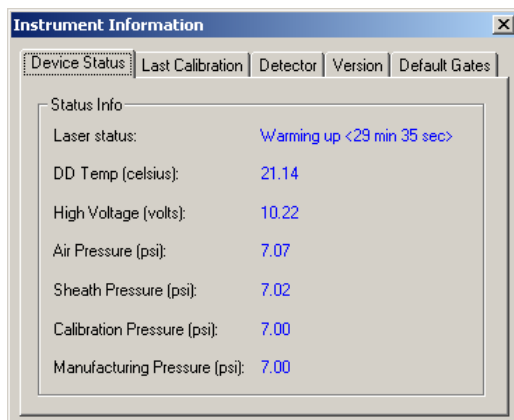
Figure 31.   *Instrument Information* dialog.

Click on the tabs in the dialog to access different information about the instrument. See the hardware manual for detailed instrument specifications.

Eject/Retract Plate ▲

The **Eject/Retract Plate** command ▲ on the main toolbar and *Instrument* menu ejects and retracts the plate carrier in the microplate platform. Note that every function that requires insertion of a plate into the platform (e.g., **Start Up, Wash, Calibrate,** etc.) includes an **Eject/Retract** button in its dialog box.

Additional Instrument Functions

The *Additional Functions* submenu under the *Instrument* menu contains secondary instrument fluidics functions. Selecting one of these commands will open a dialog box to guide you through the procedure. Many of these functions require the use of the Bio-Plex MCV Plate III.

➢ Major fluidics functions such as **Wash Between Plates**, **Remove Bubbles**, **Unclog**, etc. consist of combinations of the following functions.

Wash

The **Wash** command performs a single fluidics system wash using DI water. It can be used to remove traces of sample in the fluidics pathway. Note that this procedure is less comprehensive than **Wash Between Plates** (page 33)

Select **Wash** from the *Additional Functions* submenu of the *Instrument* menu and follow the steps in the dialog box.

Drain

This command completely drains the fluidics system of the array reader—for example, if you need to move the instrument. Make sure you have enough room in your waste fluid container to prevent overflow.

Prime

This command primes the fluidics system with sheath fluid.

Sanitize

This command performs a comprehensive cleansing of the fluidics system using 10 percent bleach and DI water.

Alcohol Wash

This command performs a single fluidics system wash using 70 percent isopropanol.

Alcohol Flush

This command performs a comprehensive cleaning of the fluidics system using 70 percent isopropanol.

Back flush

This command flushes sheath fluid through the fluidics system in the opposite direction of sample flow, to remove particle obstructions from the cuvette.

Cancel Operation

The **Cancel** button, located in the status bar at the bottom of the Bio-Plex Manager window, can be used to cancel many of the instrument functions, including calibration, validation, or any of the fluidics functions. Click on the **Cancel** button to cancel the current operation. An alert box will prompt you to confirm the cancellation.

Figure 32.   Cancel button in the status bar.

Shut Down

The shut down procedure consists of a series of functions designed to clean the fluidics lines and prevent a build up of debris within the system. To initiate this process, select **Shut Down** from the main toolbar or *Instrument* menu, and follow the instructions in the dialog box.
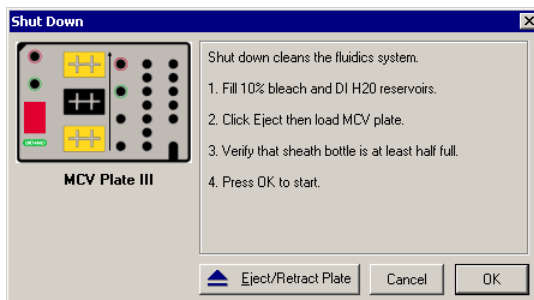
Figure 33.   *Shut Down* dialog.
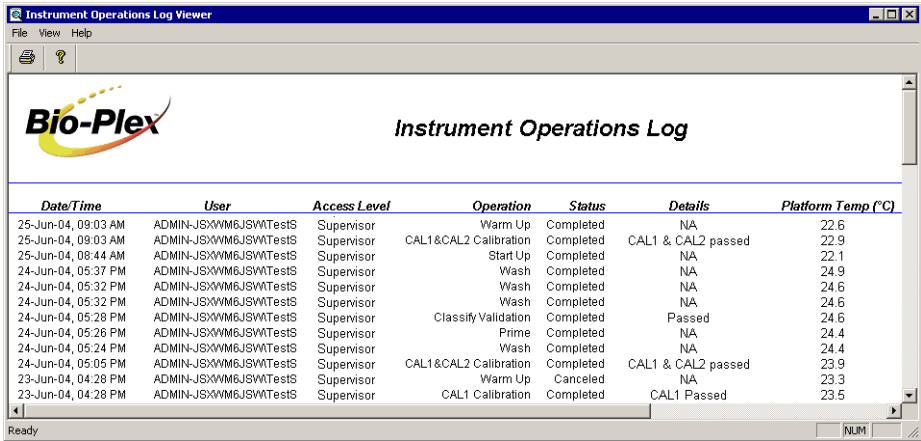
Instrument Operations Log

Each instrument operation you perform with Bio-Plex Manager is recorded in the Instrument Operations Log. This log is stored in a secure database file called **bioplexdata.mdb**, which by default is saved in the main Bio-Plex Manager application folder on your computer.

➢ Note that this database is not compatible with versions of Bio-Plex Manager earlier than 4.0. If you have an earlier version of Bio-Plex Manager, installing version 4.0 or later will copy the data from your existing database (bioplex.mdb) into the new database. A copy of your old database will remain in the application folder.

To view this log, go to the *View* menu and select **Instrument Operations Log**. The log viewer will open.

Figure 34.   Instrument Operations Log

Each instrument operation is listed by date and time. If you are using Bio-Plex Manager Security Edition in Secure Mode, the **User** and **Access Level** columns list information about the user who was logged into the application when each operation was performed.

To print the log, click on the **Print** button ⏚.

To copy the information in the log to the Windows® clipboard and paste it into another document, drag your cursor over the rows and columns in the table to select them, and use the Ctrl + C key command to copy the data.

6. Preparing a Protocol

Protocol Files

A **Protocol file** contains the parameters of a Bio-Plex Manager reading. It specifies the analytes used in the reading, the plate wells to be read, sample information, the values of standards and controls, and instrument settings.

Protocol files also contain the controls for performing the reading. When the reading is complete, the settings in the Protocol file are copied into the Results file, along with the data from the reading.

You can save Protocol files and reuse or modify them for other readings. Raw data from the most recent reading is stored in the Protocol file, and you can generate a new Results file from this data.

Creating/Opening Protocol Files

To create a new Protocol file, click on the **New** button on the main toolbar or *Quick Guide* or select **New** from the *File* menu. A new Protocol window will open.

To open an existing Protocol file, click on the **Open** button on the *Quick Guide* or main toolbar or select **Open** from the *File* menu. Locate and select the file using the standard Windows® *Open* dialog.

Standard Protocol files have the file extension ***.pbx**. Secure Protocol files, created using Bio-Plex Manager Security Edition, have the file extension ***.spbx**. For more information about Secure Protocol files, see page 153.

➢ Secure Protocol files can only be opened as **read-only** files using the standard (non-Security Edition) version of Bio-Plex Manager.

➢ Protocol files created or modified by Bio-Plex Manager 4.0 or later cannot be opened by earlier versions of the software.

You can generate a new Protocol file from the settings stored in a Results file (see the next chapter for information about Results files). With the Results file open, select **New Protocol from Results** from the *File* menu. A new Protocol window will open.

Saving Protocols

To save the settings in a Protocol, click on the **Save** button 🖫 on the main toolbar or select **Save** from the *File* menu.

After you have performed a reading (see **Running the Protocol** on page 87), the data from that reading is also saved with the Protocol file. This data is overwritten in the Protocol file whenever you rerun the Protocol. To preserve the data from multiple readings, you must save separate Results files (see page 115).

Use **Save As** on the *File* menu to save a Protocol under a different name. You can create a new Protocol by opening an existing Protocol, modifying the settings, selecting **Save As**, and specifying a new name for the modified Protocol.

Reducing the File Size of Protocols

Protocol files that include all the data from a reading can be very large (>5 megabytes). Much of this file size is due to the raw bead event data from the reading, which is used to generate the bead map and histogram displays. If you require a smaller file size (e.g., you want to send the Protocol file via email), you can save a copy of the file *without* this raw bead event data, but with the other numerical data from the reading intact.

➢ We strongly recommend keeping a copy of the original Protocol file with the raw bead event data intact. This data is necessary for changing the DD gate range in the Results file (see page 120), and can also be exported in XML file format from the Results file (see page 143) for further analysis.

In the *Save As* dialog box, select the **Compressed mode** checkbox to save the Protocol file without the bead map and histogram data. All other data will be preserved.

Select this checkbox to reduce the file size of a Protocol containing reading data
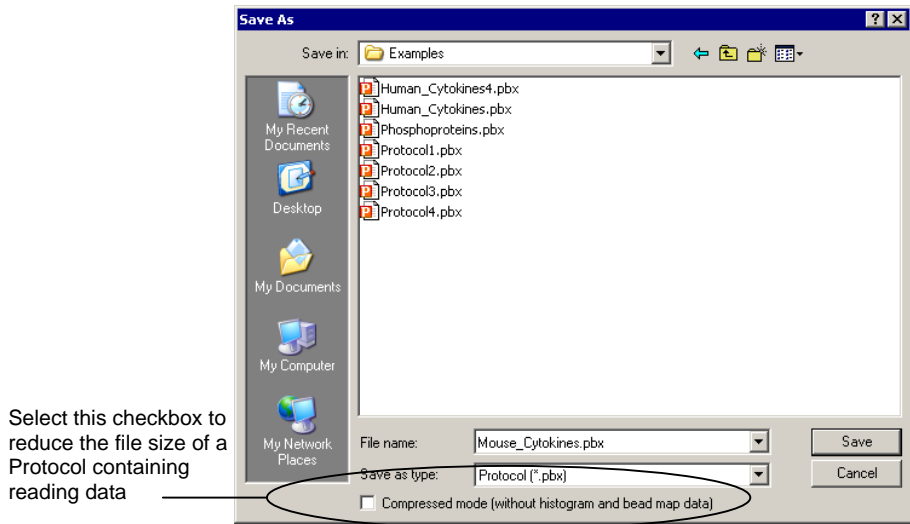
Figure 35.   *Save As* dialog for Protocols.

➢   Note that this compressed file option is not available for Secure Protocol files generated using the Security Edition of the software (see page 153).

Protocol Window

The Protocol window is divided into a series of subwindows, each containing different controls and settings. Navigate through the different subwindows using the buttons along the left side of the Protocol window.

Click on the buttons to select different settings.
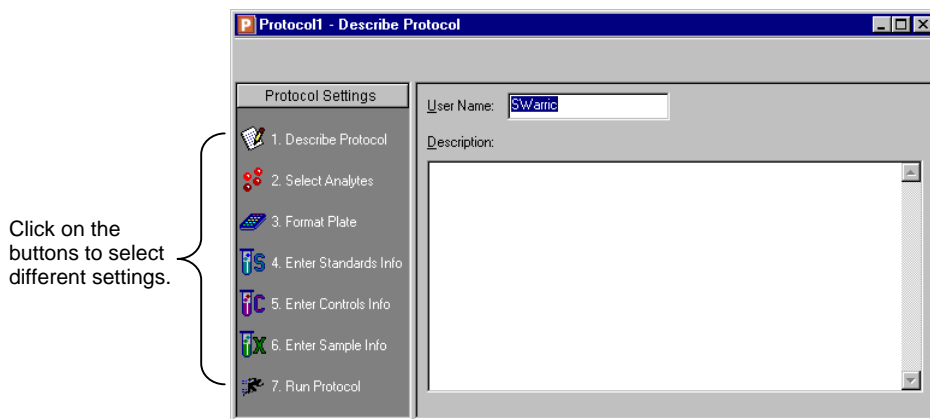
Figure 36.   Protocol window.

The buttons are numbered to guide you through the selection process:

1.   Describe Protocol (optional) (see page 53).

2.   Select Analytes (see page 53).

3.   Format Plate (see page 59).

4.   Enter Standards Info (see page 71).

5.   Enter Controls Info (see page 82).

6.   Enter Samples Info (see page 84).

7.   Run Protocol (see page 87).

Click on each setting button and enter the necessary information, then select the next setting. After all the settings have been specified, you are ready to run the Protocol.

Step 1. Describe Protocol

The Protocol description window is displayed when you first open a Protocol. (Otherwise, click on the **Describe Protocol** button to display this window.) Entering a description of your Protocol is optional.

If you are using the Standard Edition of the software, the **Author** field contains your computer login name. If you are using the Security Edition, the field contains your user name. Enter a new name in the field, if desired, and enter a description of the assay reading in the **Description** field.

Step 2. Select Analytes

In this step, you select the analytes that you want to detect in the reading.

> ➤ During a reading, the array reader will always detect *all* the analytes in the sample, including any that you have not selected. However, analytes that were detected but not selected will by default not appear in the final reports and tables. After a reading, you can go back and correct your selections in the Protocol window and/or the Results file, and the detected analytes will appear in the tables.

Click on the **Select Analytes** button to view the analyte selection window.
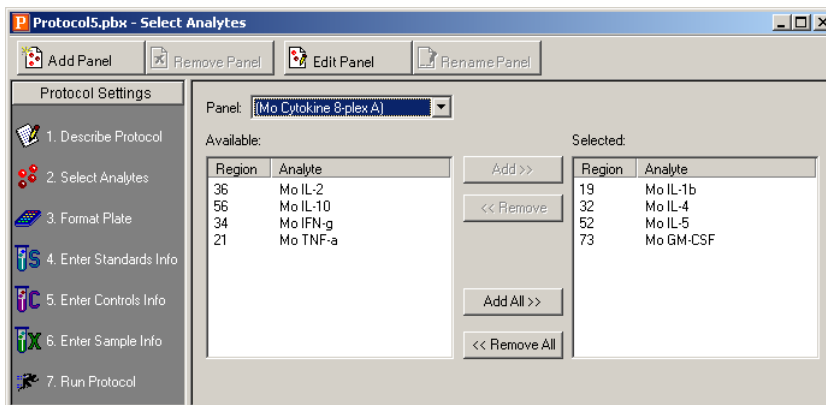
Figure 37.   *Select Analytes* window.

Bio-Plex Manager groups analytes by panels. Preconfigured panels of analytes are built into the software; these panels correspond to off-the-shelf Bio-Plex assays, and include human, mouse, and rat cytokines and phosphoproteins. You can add to these existing panels of analytes, or create new panels that contain only the analytes that you use in your experiments.

First you select the panel of analytes that you want to detect, and then you select the specific analytes.

Selecting Analytes

Go to the **Panel** pulldown menu and select from the list of preconfigured panels. The analytes in the selected panel will be displayed in the *Available* list.

Each analyte is listed by name and region number. The **region number** refers to the region of a fluorescent color map used to identify the analyte's bead set. Each bead set is embedded with specific quantities of two fluorescent dyes; the combination of these fluorochromes, as detected by the array reader, places the bead set within a unique region on the color map, thereby identifying the set and its associated analyte.

To move an individual analyte into the *Selected* list, double-click on it. Double-click on an analyte in the *Selected* list to move it back.

Hold down the Shift or Ctrl key and click on multiple analytes in the *Available* list to select them as a group. Then click on the **Add >>** button to move them over to the *Selected* list. Use the **<<Remove** button to return multiple selected analytes back to the *Available* list.

Use **Add All>>** to move all the available analytes into the *Selected* list, and **<<Remove All** to move them back.
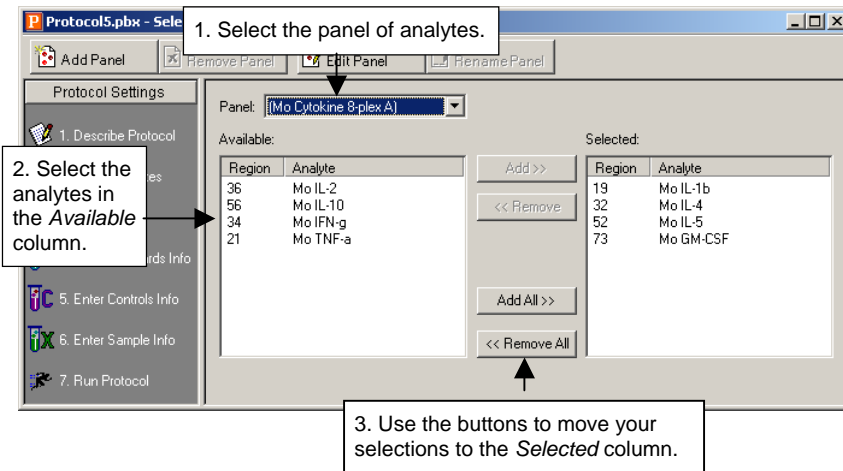
Figure 38.   Selecting the analytes to be detected.

➢ Some analytes share the same region. Only one analyte per region can be added to the *Selected* list.

Customizing Analytes and Panels

Bio-Plex Manager comes preconfigured with a selection of panels and analytes that Bio-Rad provides as Bio-Plex assays. You can edit these and/or create new ones.

➢ If you have assay kits for analytes that are not included in Bio-Plex Manager, you will need to add these analytes using the commands described in this section.

Creating a New Panel of Analytes

Click on the **Add Panel** button in the toolbar to create a new panel of analytes.
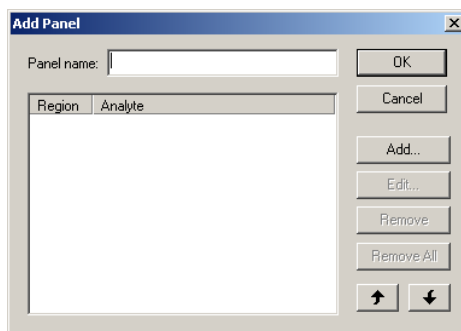
Figure 39. Creating a new panel of analytes.

Enter a name for the panel in the top field, then click on **Add** to add analytes to the panel. The *Add Analyte* dialog box will open.
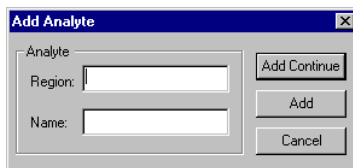
Figure 40. Adding a new analyte.

Enter the bead region number of the first analyte in the **Region** field, and the analyte name in the **Name** field.

➢ The bead region number must be correct for proper detection of analytes. Confirm that this number is correct before proceeding.

Click on **Add Continue** to add the analyte to the panel and continue adding more analytes. When you have entered your last analyte, click on the **Add** button to add it to the list and close the *Add Analyte* dialog.

After you have added several analytes to the panel you are creating, you can use the **Sort** buttons at the bottom of the *Add Panel* dialog to sort them. Click on an analyte in the list to select it, then click on the appropriate arrow to move it up or down in the list.
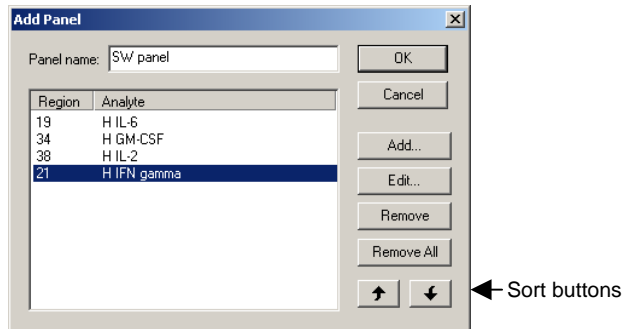
Figure 41.   Sorting analytes.

You can also remove an analyte from the list by selecting it then clicking on the **Remove** button, or remove all analytes by clicking on the **Remove All** button.

To edit an analyte in the list, select it, then click on the **Edit** button. A dialog box will open in which you can change the analyte region and name.

When you are finished creating the panel, click on **OK** to save your changes and return to the Protocol window.

Editing a Panel of Analytes

To edit an existing panel of analytes, first select it from the **Panel name** pulldown menu, then click on the **Edit Panel** button . The *Edit Panel* dialog box will open.
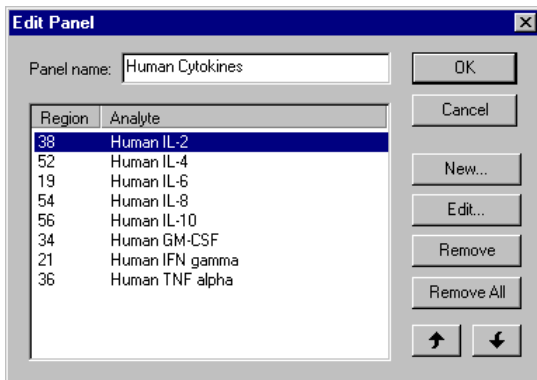
Figure 42.   Editing a panel.

Use the **New, Edit, Remove, Remove All**, and **Sort** buttons as described above to change the analytes in the panel.

If you also change the **Panel Name**, the changes will be saved under the new name, and the old panel will not be overwritten.

➢ The preconfigured Bio-Plex panels (*Human Cytokines, Mouse Cytokines,* etc.) can be edited but not overwritten; therefore, you must change the panel name to preserve your changes.

When you are satisfied with your changes, click on **OK**.

Deleting and Renaming Custom Panels

To delete a custom panel of analytes, first select it from the **Panel name** pulldown menu, then click on the **Remove Panel** button ⊠.

To rename a custom panel of analytes, first select it from the **Panel name** pulldown menu, then click on the **Rename Panel** button. Enter a new name in the pop-up box, and click on **OK**.

Combining Panels of Analytes

To combine entire panels of analytes (e.g., *Human Cytokines I* and *Human Cytokines II*) without going to the trouble of creating custom panels:

1. Select the panels individually from the pulldown list.

2. Use the **Add All>>** command to move the analytes from each panel into the *Selected* list.

3. Save the entire Protocol as you would normally.

Your analyte selections are saved when you save the Protocol.

Step 3. Format Plate

After you have selected the analytes, you are ready to specify the format of your 96-well microtiter plate using the plate template in the Protocol window. The formatting in the plate template tells the array reader which wells to read, and tells Bio-Plex Manager how to analyze the different sample types in each well.

➢ Only formatted wells will be read by the array reader. Be careful to format **all** the wells that you want to read before running a Protocol.

Click on the **Format Plate** button in the Protocol window to display the plate template and the formatting controls.
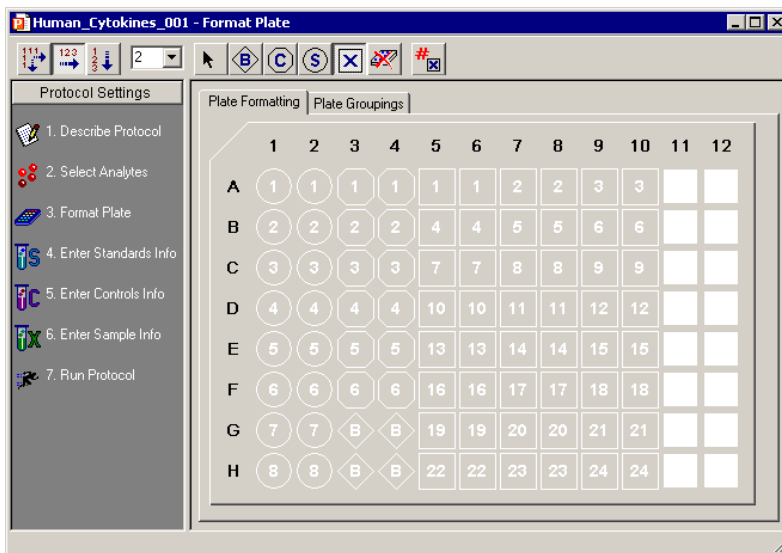
Figure 43.   The plate template and formatting toolbar
(*Plate Formatting* view).

The *Format Plate* window has two views: the *Plate Formatting* view and the *Plate Groupings* view. You can toggle between these views using the tabs above the plate diagram. As you toggle, the plate template will remain the same, but the available commands will toggle between formatting controls and grouping controls.

- **Plate formatting** tools are used to *define the types of wells* in a plate (sample, control, standard, blank, etc.).

- **Plate groupings** tools are used to *organize the well types into groups*, with one member of each group defined as the Reference (or Primary) member, as in a Western blot kinase assay. The ratio of each member's fluorescent intensity to the fluorescent intensity of the Reference can then be calculated.

➢ Plate formatting is required to perform a reading, because only formatted wells will be read by the array reader, whereas plate groupings are optional and can be defined later.

Plate Formatting

To format the well types on the plate, make sure that the *Plate Formatting* tab is selected. Then use the buttons on the toolbar above the plate template to define the wells on the microtiter plate. These formatting commands are also located on the *Format Options* menu.

Figure 44.   Plate formatting tools (*Plate Formatting* view).

The different well types are:

- Unknown sample
- Standard
- Control
- Blank
- Undefined*

*Remember that all undefined wells will not be read by the array reader.

Defining Unknown Sample Wells

To define wells containing unknown samples, first click on the **Unknown Sample** button ⊠, then click or drag on the wells in the template. Use the **Autofill** buttons as described on the following page for defining multiple unknown wells and/or replicate groups of unknowns.

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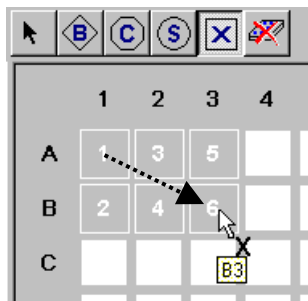
Figure 45. Defining unknown sample wells.

Note that the cursor changes to an *Unknown Sample cursor* as you move it over the template. As you click on each well, the wells are marked with a square and a number at the center. The square identifies the well as containing an unknown sample, and the number identifies the specific sample.

The **Set Number of Unknown Samples** command (only available in the Security Edition of the software) is described on page 157.

Well Numbering and Replicate Groups

For unknowns, standards and controls, wells with different numbers contain different samples. Wells with the same number contain the same sample, and are defined as a **replicate group**. For each replicate group, a mean value is calculated in the *Report* table, along with the standard deviation and coefficient of variation.

Autofilling Well Numbers

To define multiple single wells as different samples, you can click on them individually, or select **Autofill Across** 123→ or **Autofill Down** ↓ and drag the cursor over the wells to number them sequentially.
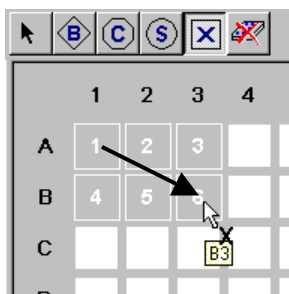
Figure 46.   Defining multiple sample wells, left to right.

Note that **Autofill Across** numbers the wells sequentially left to right, then top to bottom, while **Autofill Down** numbers them sequentially top to bottom, then left to right.

Defining a Replicate Group

To define multiple wells as a replicate group, select **Turn Off Autofill** from the formatting toolbar or *Format Options* menu, then drag your cursor over several wells that contain the same sample. The wells will be labeled with the same number.
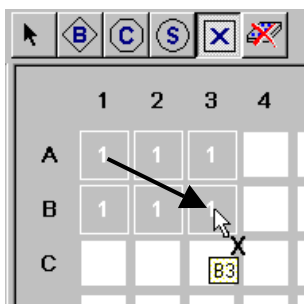
Figure 47.   Defining a replicate group of unknowns.

For each replicate group, a mean value is calculated, along with the standard deviation, standard error, and coefficient of variation.

Autofilling Replicate Groups

If you have multiple replicate groups of wells in rows or columns, you can autofill these by selecting **Autofill Across** or **Autofill Down** as before, and then entering the number of wells in the replicate group (up to 96) in the number field next to the autofill buttons. (You can also select a well number up to 10 from the pulldown menu.)

Figure 48.   Entering the number of replicate wells in an autofill sequence.

Now when you drag your cursor, the sequential sets of replicate wells will be defined.
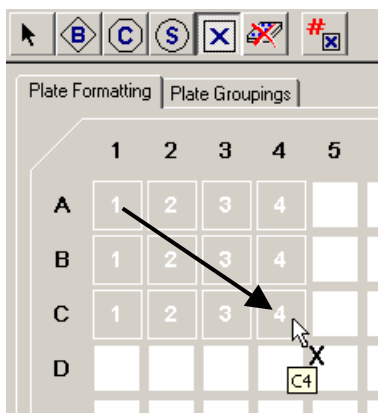
Figure 49.   Autofilling a sequence of replicate groups.

For each replicate group, a mean value is calculated, along with the standard deviation, standard error, and coefficient of variation.

Changing a Replicate Group

To change the replicate group of a well or group of wells:

1. Click on the **Select** tool ↖, and click or drag on the wells that you want to change. The wells will appear highlighted.

2. Right-click on the selection, and select **Set Replicate Group** from the context menu.

3. In the pop-up box, select the desired replicate group number from the pulldown list.

4. Click on **OK**. The selection will be changed to the specified group number.
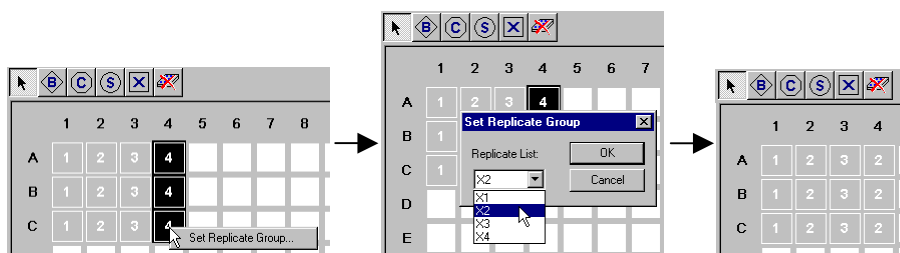
Figure 50. Selecting a different replicate group

Resequencing Well Numbers

If your well numbering is out of sequence and you want to correct it, select **Resequence Well Names** from the *Format Options* menu. All the wells in the plate will be renumbered sequentially, based on whether you have select **Autofill Across** or **Autofill Down**. This is a cosmetic change, and will not affect the analysis in any way.

Defining Standard Wells

Standard wells contain analytes of known concentration, which are used to generate a standard curve of fluorescent intensity versus analyte concentration. The regression equation for the curve is then used to calculate the concentrations of your unknowns.

To define the wells containing standards in your microtiter plate, click on the

Standard button ⑤, then click or drag on the wells in the template. Use the

Autofill buttons as described on page 62 for defining multiple standard wells and/or replicate groups of standards.
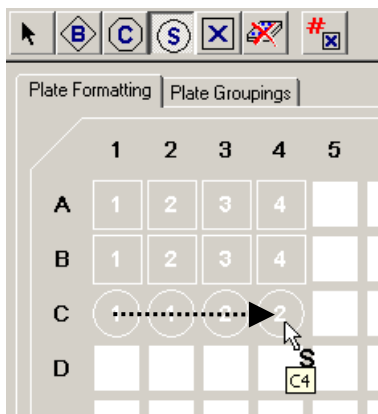
Figure 51.   Defining a row of standard wells.

Note that the cursor changes to a *Standard cursor* as you move it over the template. Standard wells are marked in the template by a circle with a number inside it.

➢ After you have defined the standard wells, you are ready to enter the concentrations of the standards as described on page 71.

Defining Control Wells

Control wells contain samples of known concentration; the expected versus observed concentrations of the controls can be calculated at the end of the reading.

To define the wells containing controls in your microtiter plate, click on the **Control** button ⓒ, then click or drag on the wells in the template. Use the **Autofill** buttons as described on page 62 for defining multiple control wells and/or replicate sets of controls.
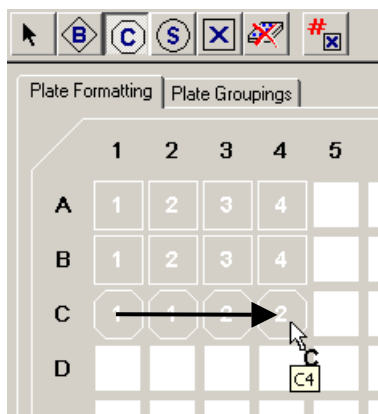
Figure 52. Defining a row of control wells.

Note that the cursor changes to a *Control cursor* as you move it over the template. Control wells are marked in the template by an octagon with a number inside it.

➢ After you have defined the control wells, you are ready to enter the concentrations of the controls as described on page 82.

Defining Blank Wells

In certain types of assays such as the Bio-Plex phosphoprotein assay, it may be useful to subtract the assay background from the readings of standards, controls, and unknown samples. To do this, you can prepare "blank" wells containing all the assay components except sample. Wells prepared as blanks are read along with the rest of the assay, and then Bio-Plex Manager subtracts the mean background reading of these wells from the fluorescent intensity values of the wells containing standards, controls, and unknowns.

To format blank wells in the plate template, click on the **Blank** button , then click or drag on the blank well(s) in the template. Note that the cursor changes to a *Blank cursor* as you move it over the template.
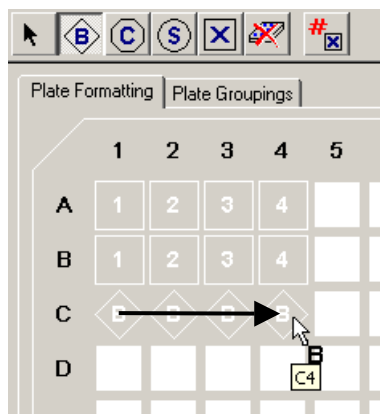
Figure 53.   Defining a row of blank wells.

Blank wells are marked in the template with a diamond shape containing a letter B.

Multiple blank wells in a template are treated as a single replicate group. A mean value, standard deviation, standard error, and coefficient of variation are calculated for the group, and the mean is used as the background value.

Deleting or Changing Well Formatting

To delete the formatting from a well or set of wells, select **Undefined** from the toolbar or *Format Options* menu, then click or drag on the well(s). To clear the formatting from all the wells in the template, select **Clear Plate** from the *Format Options* menu.

To change the formatting of a well or group of wells, simply select the appropriate tool and overwrite the old formatting, as if you were defining the wells for the first time.

Plate Groupings

After you have formatted the wells (see previous section), you can organize them into groups and identify one member of each group as the Reference, or Primary member. You can then calculate the ratio of each member's fluorescent intensity to the fluorescent intensity of its Reference. This type of analysis is similar to that performed in Western blotting.

Click on the *Plate Groupings* tab to display the plate groupings tools.
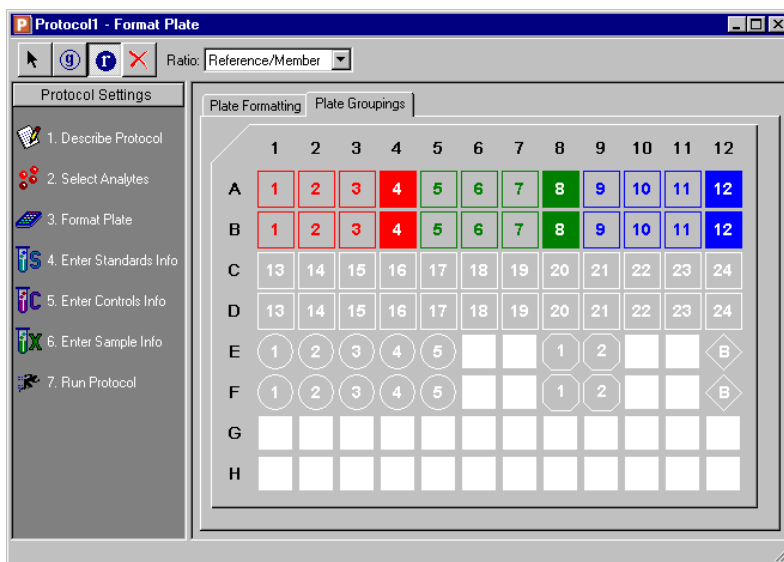
Figure 54.   *Plate Groupings* view.

The buttons on the toolbar are used to group the wells on the microtiter plate diagram and identify the Reference well(s). These formatting commands are also located on the *Format Options* menu.
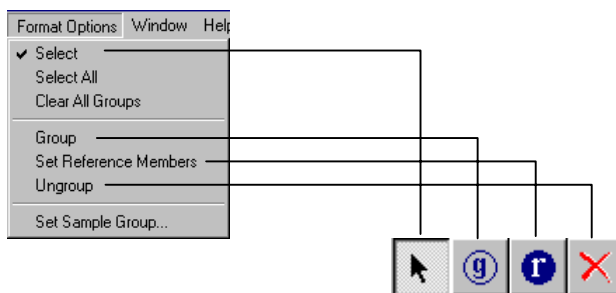
Figure 55.   Plate groupings tools (*Plate Groupings* view).

First, select the ratio you want to calculate for each group—**Reference/Member** or **Member/Reference**—using the **Ratio** field pulldown list.

Defining a Group

To define an assay group, click on the **Group** button ⑨ and drag the cursor across the defined wells in the assay. Note that:

- Wells must be defined as Samples, Standards, Controls, or Blanks before they can be grouped.

- You can group across well types (e.g., a single group can include Standards, Samples, Controls, and/or Blanks).

- Replicate wells will be automatically placed in the same group (even if you drag over only one well in the replicate set).
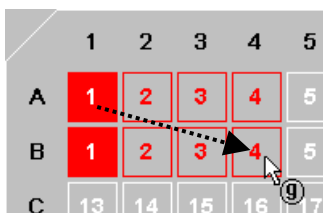
Figure 56.   Defining a group of wells.

The grouped wells will change color to indicate their grouping, and the first member of the group will be highlighted to indicate that it is defined as the Reference well.

Each group you create will be designated with a different color.

To remove individual members of a group or the entire group, click on the **Ungroup Samples** button ✕ and click or drag over the members/group.

Note that you can also use the **Select** tool ➤ to first select a group of wells, and then use the **Set Sample Group** command on the *Format Options* menu or context menu to select the group.

Defining the Reference

To change the Reference member of the group, click on the **Reference** button ⓡ and click on the desired Reference member in the group. For replicates, all replicate

wells of the same number within the group will be automatically designated as the same Reference.

You can also use the **Select** tool ➤ to first select the Reference well within a group, and then use the **Set Sample as Reference Member** command on the context menu to set the Reference.

Printing the Plate Format

To print the plate format for your records, select **Print** from the *File* menu, or click on the **Print** button 🖶 on the main toolbar. For a preview of the printed format, select **Print Preview** from the *File* menu.

Step 4. Enter Standards Info 🧪

Standards are analytes of known concentration, which can be used to generate a standard curve of values using one of the several regression methods built into Bio-Plex Manager. This curve can be used to calculate the concentrations of your unknowns.

The standards you select will depend on the performance characteristics of your assay as well as the type of curve-fitting model you want to use. Due to variations in assay preparation, we recommend using a minimum of **eight** standard concentrations to generate the standard curve. See the Bio-Plex cytokine assay manual for recommendations of specific standard concentrations.

The minimum number of non-zero standards required for each type of regression method available in the software is shown in the following table:

Method	Minimum number of standards
Logistic-5PL	6
Logistic-4PL	5
Cubic spine	4
Linear (linear and semi-log)	2
Point to point (linear and semi-log)	2

Before entering the concentration values of your standards, you must select the analytes that you will be analyzing in the reading as described on page 53. If your

current plate contains your standards, you must also identify the standard wells as described on page 65.

Click on the **Enter Standards Info** button in the Protocol window to enter information about your standards.

Figure 57.   Entering information about standards.

The *Enter Standards Info* window allows you to define standards on your current microtiter plate, and/or import external standards data from another plate. Use the tabs above the data fields to select between standards on the current plate and external standards.

➢   You can define standards on the current plate and import external standards in the same Protocol. This is useful if you want to run standards for some analytes on the current plate, but include external standards for other analytes in your concentration calculations. Note that, if external standards and current standards are defined *for the same analyte,* the external standards will always be used to calculate the concentrations of that analyte.

Selecting External Standards

This section describes how to select a Results file from which to import external standards data. If you are not using external standards, you can skip this section and proceed to **Entering Standards Information** on page 74.

Click on the *Select External Standards* tab. In the window, click on the **Fill Available List** button, navigate to the directory containing the Results file with your standards of interest, and select the file. (Note that the *Open* dialog will only display Results files.)
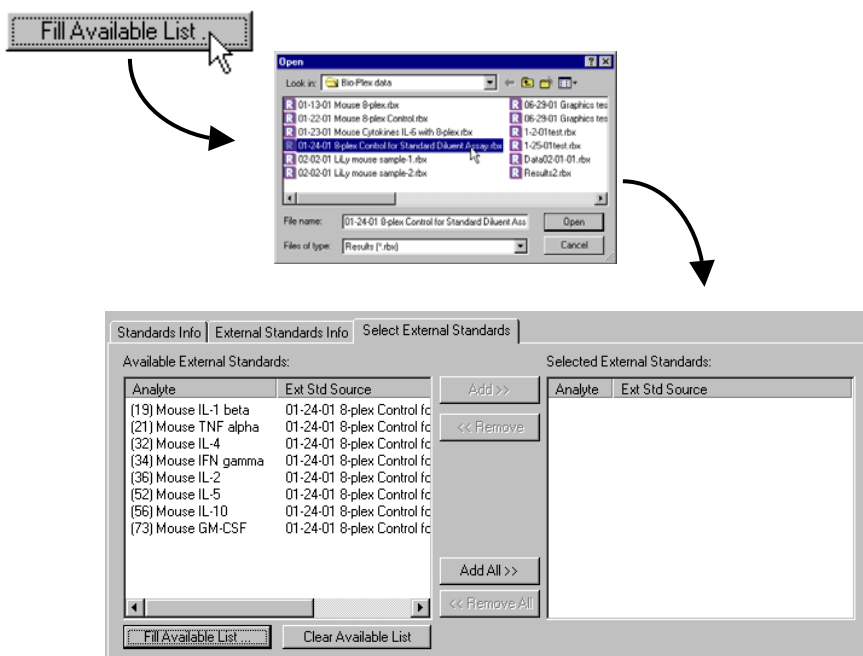
Figure 58.   Selecting the Results file containing your external standards.

The *Available External Standards* list will be populated with the standards from the selected file. To add additional standards to the list, repeat the procedure, selecting a different file.

To delete all standards from the *Available* list, click on the **Clear Available List** button.

To make the available external standards accessible in the current Protocol, you must add them to the *Selected External Standards* list (right-hand pane). To select all the standards in the *Available* column, click on the **Add All>>** button. To add available standards individually, double-click on them, or select multiple analytes using Shift + click and Ctrl + click key combinations and click on the **Add>>** button. (Use the **<<Remove** and **<<Remove All** buttons to unselect them.)

➢ Each external standard you select must match the region of a selected analyte in the Protocol. For example, if you selected analytes in regions 19, 32, and 34 in the *Select Analytes* window (page 53), you can only select external standards in those same regions. (Note that the analyte name does not have to match—only the region.) Also, each analyte must come from a different region.

When you are finished, the *Selected* list should list all the analytes that you want to use as external standards, and their source file(s).
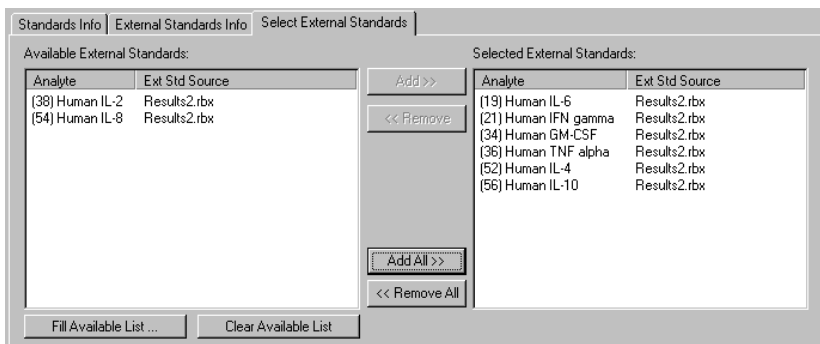
Figure 59.   Selected external standards.

When you are done, click on the *External Standards Info* tab to enter additional information about your external standards.

Entering Standards Information

This section describes how to enter information about your standards. To enter information for standard wells defined on the current plate, make sure that the *Standards Info* tab is selected. To enter information for external standards, first select

the external standards as described in the previous section, and then click on the *External Standards Info* tab.

➢ The fields under these tabs are the same, except that you cannot alter the concentrations under the *External Standards Info* tab (they will appear grayed out), as shown in Figure 60.

Figure 60.   *Standards Info* window (top) and *External Standards Info* window (bottom) (external standards concentrations cannot be changed).

The analytes that you selected in the *Select Analytes* window will be available under the **Analyte** pulldown list. Select the first analyte that you will be using as a standard.
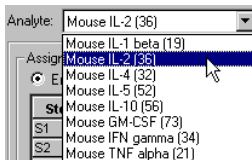
Figure 61.   Selecting a standard analyte.

➢ For the settings described on the following pages, you can select different settings for each analyte, or select the same settings for all analytes.

Entering Concentrations (for Current Standards Only)

After you have selected an analyte, you are ready to enter the concentrations of the standards on your current plate. If you are using external standards, the concentrations will be automatically entered, and you can skip to the next section on selecting concentration units (page 78). To enter concentrations, make sure the *Standards Info* tab is selected.

To enter the concentrations manually, select **Enter Manually**. To calculate them automatically from a dilution series, select **Enter Automatically**.

Entering Concentrations Manually

If you selected the **Enter Manually** option button, the table will list your standard wells (S1, S2, S3, etc.). These are the wells that you defined in the *Format Plate* window (page 59). For the selected analyte, enter the concentration for each standard in the concentration column.

Figure 62.   Entering standard concentrations manually.

If the **Same conc. values for all analytes** checkbox is selected, the concentration values you enter will be applied to all the analyte standards. Deselect the checkbox if you want to enter different concentrations for each analyte standard.

You can also enter additional information about your standards in the description column for each well.

➢ You can use the **Cut, Copy** and **Paste** commands on the *Edit* menu to copy your descriptions and concentrations between analytes. You can also copy and paste from and to other applications, such as Microsoft® Word or Excel. Click on a cell, row, or column in the table to copy and paste.

If you are entering different concentrations for each analyte standard, repeat the procedure by selecting each analyte from the pulldown menu as described above.

Calculating Concentrations Automatically

If a dilution series was created for the standards, the software can automatically calculate the concentrations of each standard. Select the analyte, and then select the **Enter Automatically** option button.

First, select the appropriate **Most Concentrated Standard** well number. (The most concentrated standard must be in either the first standard well or the last.)

Figure 63.   Entering standard concentrations automatically.

Then enter the concentration of the most concentrated standard in the **Concentration of S1** field and the dilution factor for the remaining standards in the **Dilution Factor** field.

➢ Calculations are made by dividing by the dilution factor, so do not enter fractional values for the dilution factor. If each standard is half the concentration of the previous standard, enter 2 for the dilution factor. If each standard concentration is one-fourth the previous, enter 4, and so on.

Click on the **Calculate** button when all the information is entered. The concentrations in the table will be automatically updated.

If the **Same conc. values for all analytes** checkbox below the concentrations table is selected, the concentration values will be applied to all the analyte standards. Deselect the checkbox if you want to enter different concentrations for each analyte standard, and repeat the procedure.

Concentration Units

You can enter the units of concentration in the **Concentration Units** field (note that units are not required and will not affect calculations). These units will appear in your reports. If all your analytes have the same concentration units, select the **Same units for all analytes** checkbox.

Regression Method

Using the **Std Regression Curve** pulldown list, choose from among seven different regression methods to generate the standard curve (page 136):

- Logistic-5 PL
- Logistic-4 PL
- Linear
- Cubic spline
- Point-to-point
- Linear (Semi-log)
- Point-to-point (Semi-log)
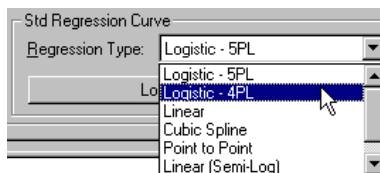
Figure 64.   Selecting a regression method.

You can change your selection later in the *Standard Curve* window of the Results file. See page 136 for a more detailed discussion of the different regression methods.

You can also select the regression curve axis scale from four different combinations of linear and logarithmic. Select the axis scale from the **Axis Transformation** pulldown list.
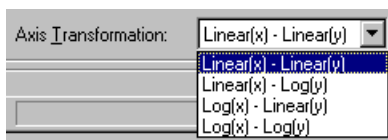
Figure 65.   Selecting a regression curve axis transformation.

Note that the axis transformation only changes how the curve is displayed, not the calculated values.

➢ The regression method and axis transformation you select in the Protocol window
will be used to calculate your initial results. However, these settings will not affect
the reading, and you can change these settings and recalculate your results after a
reading is complete.

Finally, if you want to use different regression methods for different analytes,
deselect the **Same regression type for all analytes** checkbox. Then, when you select
a different analyte from the pulldown list, you can select a new regression method as
well.

Logistic Weighting

If you select a logistic curve fitting method (Logistic 5PL or Logistic 4PL), the
software will perform automatic weighting of the points in the curve. Click on the
Logistic Weighting button to access the weighting algorithm controls.
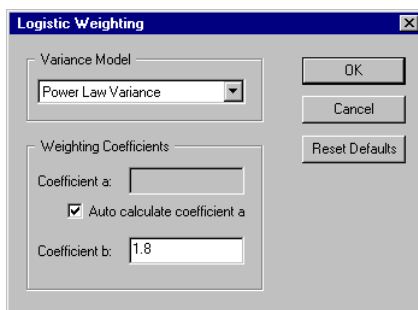
Figure 66. *Logistic Weighting* dialog.

There are eight models for calculating the variance in the logistic weighting
algorithm:

- Linear Variance
- Logarithmic Variance
- Exponential Variance
- Power Law Variance (default)
- Linear CV
- Logarithmic CV

- Exponential CV
- Power Law CV

Coefficient a

Weighting coefficient a will be automatically calculated using the following equation:

$$a = .002 * pow ((m\_YMax + m\_YMin) / 2 + blank,.2)$$

Alternatively, you can enter your own value by deselecting the **Auto calculate coefficient a** checkbox and entering a value in the **Coefficient a** field.

Coefficient b

Weighting coefficient b has a default value of 1.8. You can enter a different value in the **Coefficient b** field.

Recovery Percentage Range

Because the standard curve (page 136) is critical for calculating the concentrations of your unknowns, Bio-Plex Manager includes a mechanism for assessing the fit of the curve to the standards. This is the recovery percentage.

For each analyte standard, an observed concentration is back-calculated from the standard curve and the fluorescent intensity. This is divided by the expected concentration as entered in the *Enter Standards Info* window and multiplied by 100 to give the recovery percentage:

$$Recovery\ percentage = Observed\ conc/Expected\ conc * 100$$

Recovery percentages are also used with controls (page 82) to determine the overall accuracy of an assay.

In the *Enter Standards Info* window, use the **Acceptable Recovery Percentage Range** pulldown menu to select an acceptable recovery range.

Figure 67.   Selecting the recovery percentage range.

For example, if you select a range of 70–130%, the observed concentration should be within 70–130% of the expected concentration.

The actual recovery rate for each standard and control is shown in the **Obs/Exp*100** column of the *Report* table (see page 130). Concentrations that fall outside this range are flagged as ******* in the **Conc in Range** column of the *Report* table.

Step 5. Enter Controls Info 🔧

Controls are samples of known concentration whose values are measured during a reading in the same manner as the unknown samples. You can then compare the calculated concentrations of the controls after a reading with the expected concentrations to determine the overall accuracy of the assay. (This is sometimes called the "spiked" recovery method, because you are "spiking" your assay with known quantities of analytes.)

In this step, you enter the concentration values of your controls. First, you must select the analytes that you will be analyzing in the reading as described on page 53, and identify the plate well(s) containing your controls as described on page 66.

➢   Only formatted wells will be read by the array reader. Be sure to define all your control wells before running a Protocol.

Click on the **Enter Controls Info** button in the Protocol window to enter information about your controls.

Figure 68. *Enter Controls Info* dialog.

The analytes that you selected in the *Select Analytes* window will be available under the **Analyte** pulldown menu. Select the first analyte that you will be using as a control.

The table will list your control wells (C1, C2, C3, etc.). These are the wells that you defined in the *Format Plate* window (page 59). Enter the concentration of the selected analyte in the **Conc** column for each well.
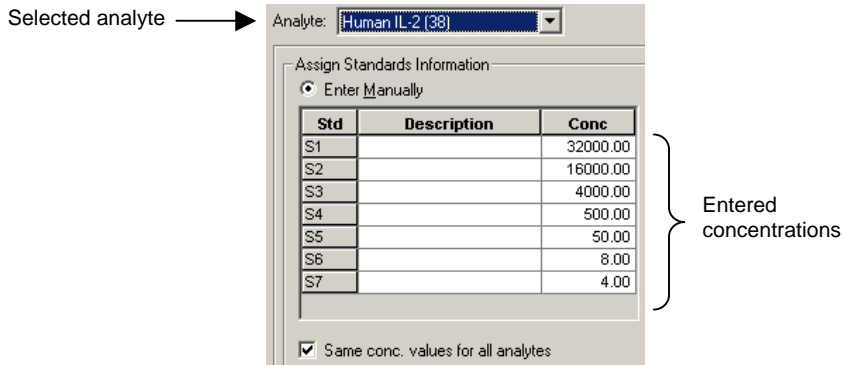

Figure 69. Entering concentrations of the controls.

If the **Same conc. values for all analytes** checkbox is selected, the concentration values you enter will be applied to all the available control analytes. Deselect the checkbox if you want to enter different concentrations for each control analyte.

You can enter additional information about your controls in the description column for each well.

If you are performing a dilution of your controls, you can enter a dilution factor for each control in the **Dilution** column. Note that a control diluted 1 in 2 would have a dilution factor of 2. This feature allows for the performance parallelism studies, whereby a control diluted by different dilution factors is analyzed to verify that the final concentration is the same for all of the samples.

Analyte:	Mo IL-2 (36)		

Assign Control Information

Ctrl	Description	Conc	Dilution
C1		1000.00	1.00
C2		1000.00	2.00
C3		1000.00	3.00
C4		1000.00	4.00

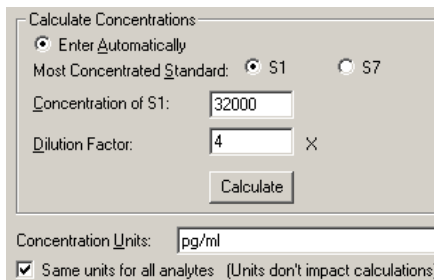Figure 70.   Controls with different dilution factors.

If you are entering different concentrations for each analyte standard, repeat the procedure by selecting each analyte from the pulldown menu as described above.

To enter the same dilution factor for all controls, enter the value in the **Dilution Factor** field, and click on the **Set All Dilution Factors** button. The dilution factor you enter for one analyte is automatically applied to all analytes.

➢ You can use the **Cut, Copy** and **Paste** commands on the *Edit* menu to copy your descriptions, concentrations, and/or dilutions between analytes. You can also copy and paste from and to other applications, such as Microsoft® Word or Excel. Click on a cell, row, or column in the table to copy and paste.
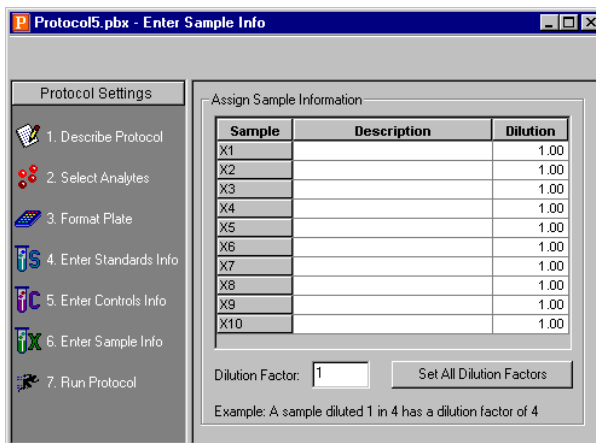
Step 6. Enter Sample Info (optional) 📗x

Before entering information about your unknown samples, select the analytes that you will be analyzing in the reading as described on page 53, and then identify the plate well(s) containing your unknown(s) as described on page 61.

➢ Only formatted wells will be read by the array reader. Be sure to define all your unknown sample wells before running a Protocol.

Click on the **Enter Sample Info** button in the Protocol window to enter information about your unknown samples.

Figure 71.   *Enter Sample Info* dialog.

The information table will list all the unknown sample wells as defined in the plate template for the reading (page 61).

You can enter a description and a dilution factor for each unknown in the appropriate columns. Note that a sample diluted 1 in 2 would have a dilution factor of 2. To enter the same dilution factor for all unknowns, enter the factor in the **Dilution Factor** field and click on the **Set All Dilution Factors** button.

➢ You can use the **Cut, Copy** and **Paste** commands on the *Edit* menu to copy your descriptions and/or dilutions from and to other applications, such as Microsoft® Word or Excel. Click on a cell, row, or column in the table to copy and paste.

7. Running the Protocol 🏃

After you have prepared the Protocol as described in the previous chapter, you are ready to run the Protocol. Click on the **Run Protocol** button 🏃 in the Protocol window to select the final settings and run the Protocol.

⚠️ When performing the following operations, the sheath fluid container and the waste fluid container should be closely monitored.

⚠️ The sheath fluid bottle must be placed at the same level as the array reader, unless you are using the HTF (see caution below). The fluid level should be below the air inlet connection and above the sheath outlet connection. Always check the sheath fluid level before starting a run or procedure.

⚠️ If you are using the HTF, it should sit on the counter next to the array reader, while the sheath fluid cube should be placed ~3–4 feet below the reader (e.g., on the floor).

⚠️ The waste fluid container receives waste from the system. **Do not allow the waste container to overflow!** Empty the waste bottle each time the sheath fluid bottle is filled. Do not place it on the instrument. All waste containers should have vented caps.

Run Protocol Window

The *Run Protocol* window has controls for performing the run, and two views for displaying the status of the run: the *Raw Data* table view and the histogram/bead map view. Click on the **Show/Hide Histogram/Bead Map** button 📊 to display only the *Raw Data* table, or the histogram/bead map display with the *Raw Data* table below it (maximize the window to see both the table and the display).
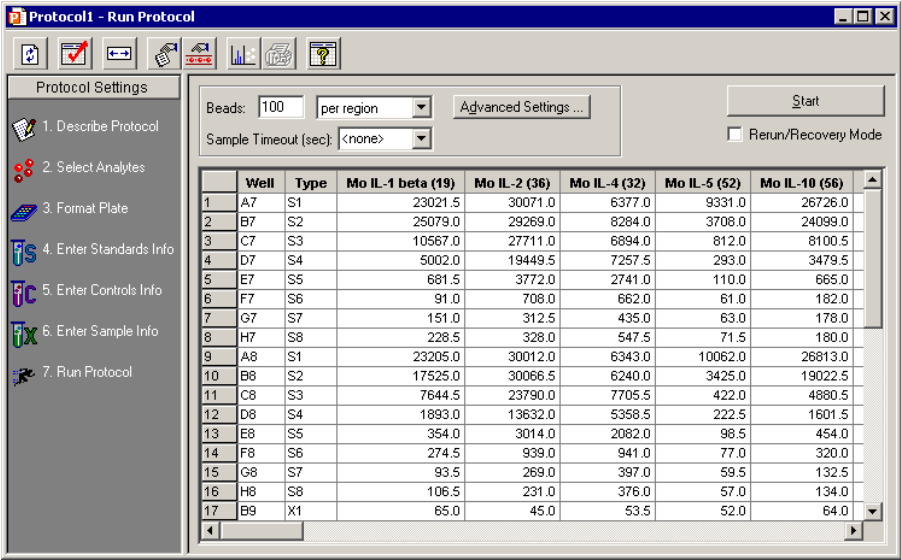
Figure 72.   *Run Protocol* window—*Raw Data* table view.
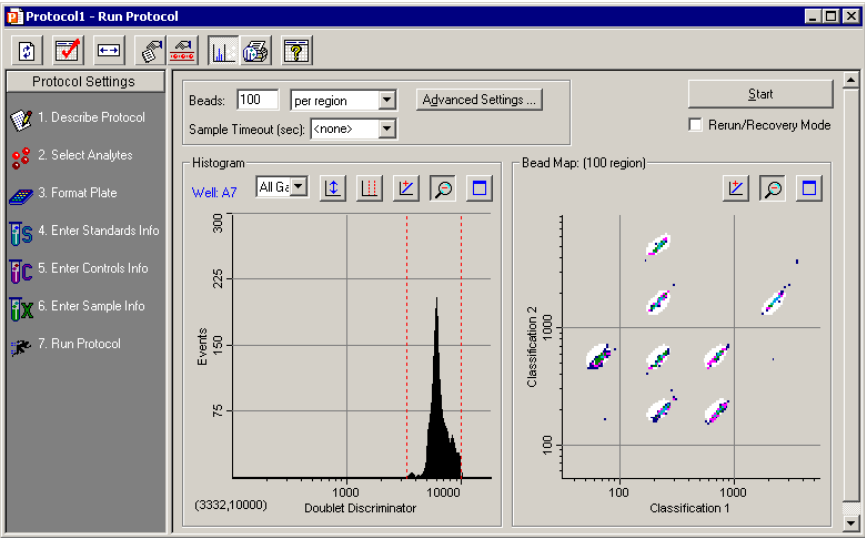
Figure 73.   *Run Protocol* window—Histogram/bead map view.

The bead map and histogram (see page 98) provide graphical data about a run, and are continually updated throughout the run. The *Raw Data* table (see page 108) displays the raw data of the reading after it is complete.

The controls for performing the run are described in the following sections.

Bead Count

The **Beads** field lists the number of beads to be detected before a reading is complete. Note that this number can be beads detected **per region** or **total** beads detected, depending on the pull-down menu selection.

If you select **per region** as the counting method, you can enter a number between 1 and 10,000 in the **Beads** field. For most assays, including the Bio-Plex cytokine assay, we recommend a value of 100 beads per region. This ensures that at least 100 beads in each region will be acquired before sample acquisition is complete.

If you select **total** as the counting method, you can enter a number between 1 and 100,000 in the **Beads** field. Note that if you choose to measure total beads, the instrument will stop acquisition as soon as it has acquired the total number of beads in any combination of bead sets. If you are detecting multiple analytes, this means that some bead sets may be underrepresented in the final tally.

➢ For the Bio-Plex phosphoprotein assay, we recommend specifying 25 beads per region to avoid triggering a low bead count warning.

Sample Timeout

The **Sample Timeout** field works in conjunction with the **Beads** field to ensure that a well reading does not continue for an extended length of time. If the specified bead count in the **Beads** field is not reached for a particular well (i.e., because the well does not contain all of the selected analytes)*,* the array reader will continue the well reading until the time limit specified in the **Sample Timeout** field.

You can enter a time limit in seconds from 0 to 200, or make a selection from the pulldown list. Select <none> or enter 0 if you want no time limit. The Sample Timeout value for each well will be noted in the *Raw Data* table.

Advanced Settings

Click on the **Advanced Settings** button to access additional reader settings and controls.
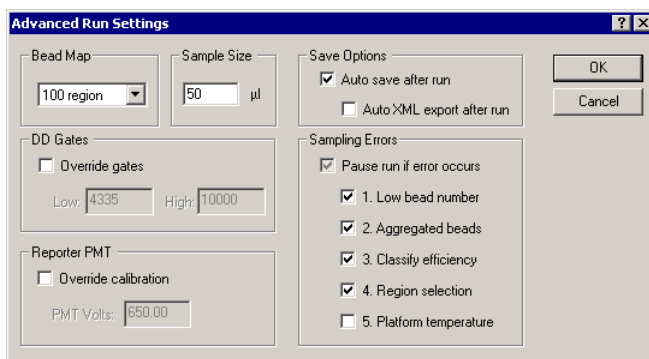
Figure 74. *Advanced Run Settings* dialog.

Bead Map Selection

Bio-Plex Manager identifies each bead in an assay by its classification region—that is, the region in the bead map where the bead falls based on its fluorescence as measured by the classification channels in the array reader (see page 104). The default bead map is the 100-region map, which is appropriate for assays that use xMAP microspheres (*e.g.*, Bio-Plex assays and Bio-Plex COOH beads). A 50-region map and 25-region map are also available. The 50-region map includes alternating regions from the default 100-region map. The 25-region map also includes select regions from the default 100-region map. The result is that the regions are larger and more spread out.

Consult your bead assay manual for instructions on selecting the appropriate map for your beads.

➢ If you select the 50- or 25-region maps, the software will automatically verify whether the analytes selected in the protocol can be accommodated within the selected regions. If not, you will receive a warning message, and you must change either your analyte selections or **Bead Map** field selection before you can run the protocol.

Sample Size

The **Sample Size** is the amount of sample that will be aspirated from the microplate well by the reader. The default sample size of **50 µl** is recommended. You can specify a sample size as small as 10 µl or as large as 200 µl.

➢ To avoid air uptake, make sure that the sample needle is properly aligned (see page 17) and each well to be read contains at least 125 µl of sample. The maximum sample volume per well is approximately 140 µl. (The array reader rinses an additional 160 µl into each well after a reading is complete; the maximum well capacity is 300 µl.)

Save Options

You can automatically save the Results file generated by the reading. With the **Auto Save After Run** checkbox selected, when you click on **Start** to begin a reading, you will be prompted to enter a name for the Results file.

➢ In Bio-Plex Manager Security Edition, Secure Mode, this checkbox is selected and cannot be deselected (see page 159).

If the **Auto Save After Run** checkbox is selected, you can also select the **Auto XML Export After Run** checkbox. With this checkbox selected, Bio-Plex Manager will automatically generate an XML file and save it to a folder or open it in an application after the reading (these options are specified in the *XML Export Properties* dialog). The XML file will have the same name as the Results file. For more information on XML files, see page 143.

Sampling Errors

During a reading, the system will continuously monitor the flow of beads, the bead count, the bead regions, and the platform temperature. If the system detects a problem in any of these areas, it can stop the reading and trigger a warning. This feature is enabled if any or all of the checkboxes under *Sampling Errors* are selected.

Select **Pause Run If Error Occurs** to select all the possible error conditions, or select/deselect the individual error condition checkboxes. By default, these are not selected (i.e., the run will not pause for any error condition).
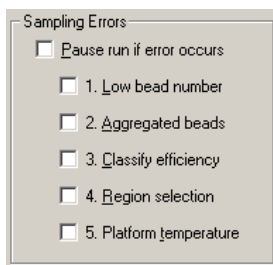
Figure 75.   Error condition checkboxes

➢ All errors will be logged in the *Raw Data* and *Report* tables, whether or not the checkboxes are selected (see page 110).

The reading errors and their possible causes are listed in the following table:

Error	Possible Cause*
1. Low bead number	Too few beads in the assay; buffer volume in well is too low; plate was not shaken properly before analysis; microbubbles in the cuvette.
2. Aggregated beads	Bead clumping in the assay; sheath fluid is empty; waste reservoir is overfull.
3. Classify efficiency	Microbubbles in the cuvette; beads have become photobleached; percentage of beads outside the selected bead region is too high.
4. Region selection	Incorrect beads were selected in the Protocol or assay; too few beads are present in the assay.
5. Platform temperature	Platform temperature has fluctuated +/- 2ºC during the reading.

* See **Troubleshooting Guide** on page 165 for a detailed list of reading errors, causes, and suggested solutions.

With the checkboxes selected, the reading will stop and an alert box will describe the problem and suggest steps for correcting it. After you have performed the suggested steps, you can rerun the reading.

➢ If you are rerunning wells in Rerun/Recovery Mode, you may want to disable the **Low bead number** checkbox, since wells that have already been read once contain fewer beads.

DD Gates

During a reading, a doublet discriminator (DD) channel measures the amount of light scatter from particles that flow past the red laser. The light scatter is directly proportional to particle size, and an internal DD "gate" is designed to identify particles that are smaller or larger than a single microsphere, including microspheres that are clumped or aggregated.

In the **Advanced Run Settings** dialog, the default **DD Gates** range for 100-region and 50-region bead maps (**Low** = 4335, **High** = 10000) is designed to accommodate

the standard 5.5-micron beads distributed by Bio-Rad. If you are using different-sized beads, you can change the size of the gate by selecting the **Override gates** checkbox and entering new values in the **Low** and **High** fields.

You can also change the range by dragging the red lines on the *Run Protocol* window histogram (see page 101).

➢ The values in the **Low** and **High** fields are channel numbers corresponding to the amount of light scatter from a particle. If you want to manually change the range, you will need to know the approximate channel numbers corresponding to the size of the beads you are using.

➢ You can change the DD gate range in the Protocol before a reading or in the Results file after a reading (see page 120). If you change the DD gate range in the Results file, a record of the range during the reading will be preserved.

➢ You can only set the gates on the DD channel, not the RP1, CL1, or CL2 channel.

➢ For Bio-Plex assays or Luminex-type assays from other manufacturers, the default DD gate settings should work well. However, if you are developing your own assay, the position of the DD peak may shift and thus the DD gates may need to be shifted accordingly. Note that you can adjust the DD gate range after a reading in the Results file and re-analyze the data.

PMT Voltage

Users who want to manually set the PMT voltage on the reporter detector can select the **Override calibration** checkbox in the *Advanced Run Settings* dialog and enter a new voltage in the **PMT Volts** field.

➢ Note that the PMT voltage is set during calibration, and should only be adjusted by users who know the precise PMT value that they want to specify. If you change the PMT, you will no longer have day-to-day and unit-to-unit reproducibility of results. The PMT voltage will be reset to the calibrated values after the plate is read and the results generated.

Plate Loading Guidelines

Before starting the run, note the following plate handling guidelines and warnings:

Protect your assay microspheres from light. Once photobleached, the beads are no longer usable. Proper care of microspheres must be observed to maintain the benefits of your product warranty.

When using a filter plate, avoid touching the bottom of the wells when wrapping the plate. Sample may wick or leak from the bottom of the well, causing problems when reading the plate.

Make sure that you have added at least 125 μl of sample to all the wells specified in your plate template before starting a reading. If the array reader attempts to draw sample from an empty well, air will be sucked into the sample loop and injected into the flow chamber. As a result, bubbles will form in the cuvette and interfere with the analysis. If this happens, perform a **Remove Bubbles** procedure (page 33) and rerun the Protocol.

Shake the microplate on a plate shaker for 30 seconds prior to performing a reading.

Running the Protocol

When you have selected and reviewed all your Protocol options and prepared your microplate, click on the **Start** button. The *Run Protocol* dialog will open.
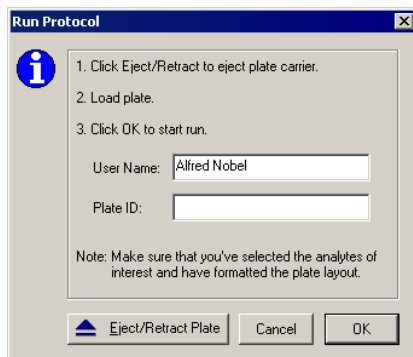
Figure 76.   *Run Protocol* dialog.

Enter your user name in the field. If you are using the Security Edition of the software in Secure Mode, your user name will be entered and cannot be changed.

If your microplate has a barcode number or other type of ID number, enter it in the **Plate ID** field.

Click on **Eject/Retract Plate** to insert the plate into the reader, and click on **OK** to begin the reading.

If the **Auto Save After Run** checkbox is selected, you will be prompted to enter a name for the Results file to be generated.

The following conditions may delay the start of a reading:

- If the array reader optics have not warmed up, the reading will be delayed until warm up is complete.
- If the pressure is too low in the fluidics system, you will receive a warning message prompting you to check the sheath fluid level, hose connections, etc.
- If the platform heater is not at the specified temperature, a warning message will appear and you will be prompted to wait for the temperature to change.

When the reading begins, the sample needle will lower, draw sample from a plate well, and retract to its original position. The sample will be drawn from the needle into the sample loop and the reading will begin.

After initial system pressurization (5–20 seconds), a typical well reading may take up to 2 minutes; in most cases, individual wells are read in a matter of seconds.

Status Bar

As the sample is analyzed, the status of the reading will be displayed in the status bar at the bottom of the Bio-Plex Manager window.
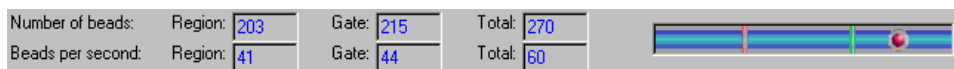
Figure 77.   Status bar during a reading.

The status bar provides a running count of both the **Number of beads** and the **Beads per second**. Within these categories, the array reader keeps track of the following information:

- The **Region** fields monitor only those beads that fall within one of the regions you selected in the Protocol (page 54).
- The **Gate** fields monitor all beads that pass an internal discriminator "gate" designed to eliminate particles that are smaller or larger than a microsphere (including microspheres that are clumped or aggregated).
- The **Total** fields report all particles measured by the array reader, including those that do not pass the internal discriminator gate.

Manually Stopping a Reading

While the sample is being read, the **Start** button will change to a **Stop** button in the Protocol window. You can click on **Stop** to cancel the reading. An alert box will notify you that you can generate a report with the incomplete results or rerun the Protocol as described on page 113.

Generating Results from a Protocol

The reading stops when you click on **Stop**, when an error stops the reading, or when all the samples have been analyzed. At the end of the reading, the sample needle

lowers and the syringe pump purges the sample loop and needle with sheath fluid, sending a fraction of sheath fluid (approximately 160 μl) back into each well.

At the end of a complete reading, the Results will be automatically calculated and displayed.

Note that the Results file will be automatically saved if you selected **Auto save after run** (page 91) in the Protocol. Otherwise, you must save your Results as a separate step (see the following chapter).

To generate a new Results file from a Protocol, select **New Results from Protocol** from the *Run Protocol* toolbar or the *File* menu.

Histogram and Bead Map

The histogram and bead map provide a graphical display of the raw data from a reading. These graphs are continuously updated during the reading in the *Run Protocol* window of a Protocol file. They also can be displayed after a reading in the *Raw Data* window of a Results file.

The histogram and bead map share window space with the *Raw Data* table (see page 108). If the histogram and bead map are not visible, click on the **Show/Hide Histogram/Bead Map** button to display them.

Well number
selected
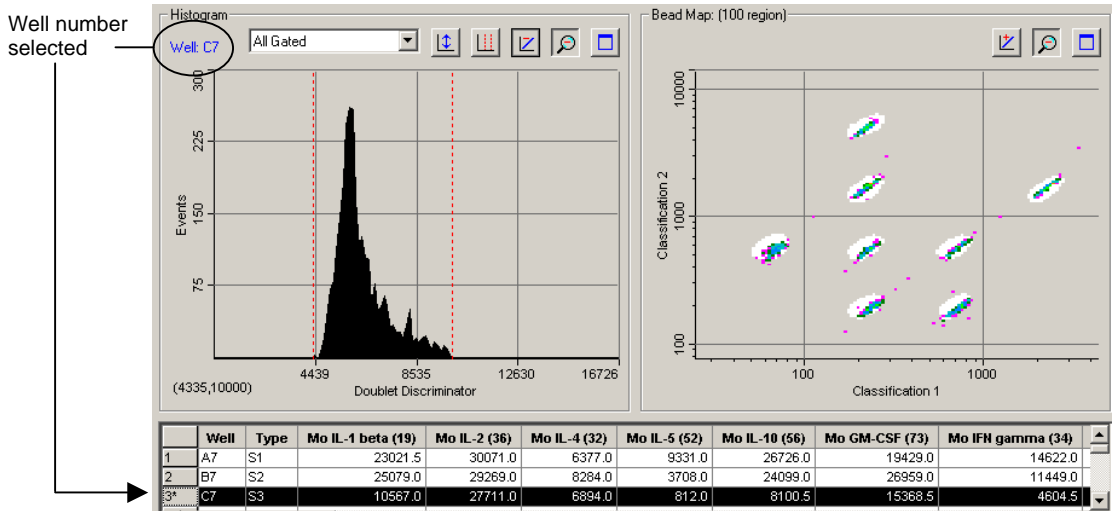
Figure 78.   Histogram and bead map.

You can see some of the rows of the *Raw Data* table below the histogram and bead map display if you maximize the window.

During a run, the well number being read is displayed in blue in the upper left corner of the histogram. After the run, select the different wells to display by selecting rows in the table below the graphs.

When viewing the histogram and bead map, you can choose to display all analytes, each analyte separately, or all beads that pass through the discriminator gate (see page 93). Make your selection using the pulldown menu above the histogram.
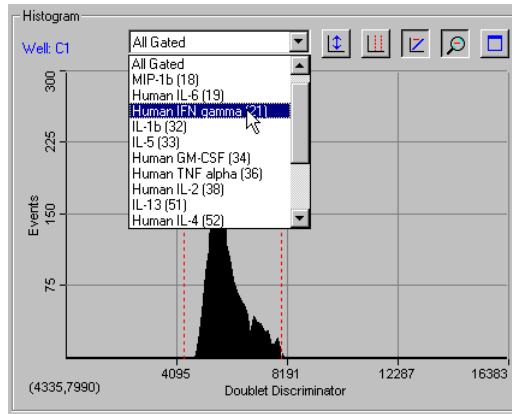
Figure 79.   Selecting the analyte(s) to display.

To print the histogram and/or bead map, click on the **Print Histogram/Bead Map** button 🖨️. This opens a small dialog in which you can select the histogram, bead map, or both to print.

Histogram

The histogram plots the number of events per channel number for the selected well, analyte(s), and channel type. An event is generated when a particle such as a bead or aggregated beads passes through the path of the lasers. Each event generates signals in different channels:

- The fluorochromes embedded in each bead generates signals in the Classification 1 and Classification 2 channels.
- The fluorescent molecules bound to the analytes generate a signal in the Reporter 1 channel.
- The light scatter of each bead generates a signal in the Doublet Discriminator channel. The amount of light scatter is directly proportional to bead size.

The signals are reported as channel numbers. In the histogram, the Y-axis is the events, and the X-axis is the channel numbers from 1 to 32766 for the selected channel type.

DD Gate Range

The **DD Gates** range specified under the *Run Protocol* **Advanced Settings** (see page 93) is shown on the histogram as two red lines perpendicular to the X-axis. In the Protocol window, you can change the DD gate range by positioning your cursor over the red lines and dragging them to the right or left. Note that this will also change the range as listed in the **Advanced Settings** dialog.
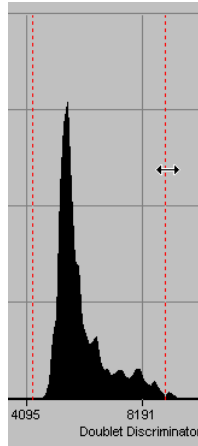
Figure 80.   Changing the DD gate range.

You can show or hide the gate lines by right-clicking on the histogram and selecting **Show** or **Hide** from the **Gate** context menu.

To reset the gates to their default values, click on the **Restore Default Gates** button 📊 above the histogram.

Selecting the Channel Type

You can change the histogram to display different channel types. Click on the histogram with the right mouse button and select **X-Axis** from the context menu.
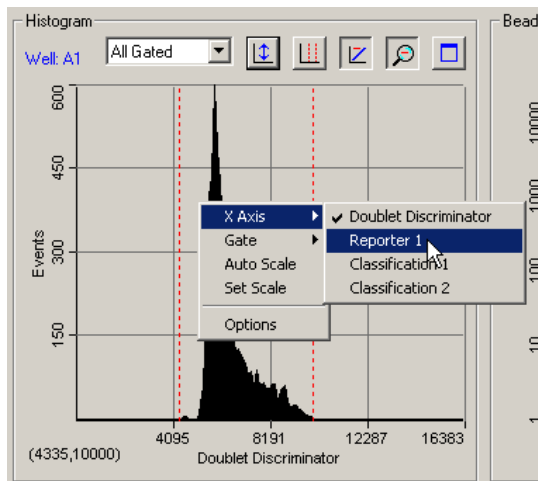
Figure 81.   Histogram right-click context menu.

From the **X-Axis** submenu, select from the following channel types:

- The **Doublet Discriminator** channel measures the forward light scatter from particles as they pass through the red laser. Light scatter correlates to particle size; this channel allows you to discriminate single beads ("singlets") from aggregated beads in the histogram. This is the default selection.

- The **Reporter 1** channel measures fluorescence from the reporter molecules bound to the analytes on each bead as they pass through the green laser. The amount of fluorescence per bead type is directly proportional to the amount of analyte present in the assay. This can be useful for viewing the distribution of the fluorescence signal of a particular analyte.

- The **Classification 1** channel measures fluorescence from the first classification dye embedded in each bead as it passes through the red laser.

- The **Classification 2** channel measures fluorescence from the second classification dye embedded in each bead as it passes through the red laser.

Note that changing the channel type will change the channel numbers and the histogram display.

Scaling the Histogram Data

If the histogram is too big or too small to fit comfortably within the display range, click on the **Auto Scale** button ⬍ on the histogram toolbar. The height of the Y-axis will change to better fit the data. You can also select this command from the right-click context menu.

Select **Set Scale** from the right-click histogram context menu to manually set the range of the Y-axis. In the pop-up box, enter the maximum number of events to display on the Y-axis.

The default view of the data is log scale. From the toolbar, click on the **Log/Linear** button ✕ to change the display from log to linear. Click again to return to log.

Magnifying and Resizing the Histogram

Click on the **Zoom** button 🔍 on the histogram toolbar to magnify the display. With **Zoom** selected, the cursor will change to a "hand" symbol 🖐 when you position it over the histogram. Drag the hand cursor to view different magnified regions of the histogram.

Click on the **Maximize** button ☐ to maximize the histogram portion of the window within the larger Protocol window. Click on the **Restore** button ⧉ to return the histogram to its default state.
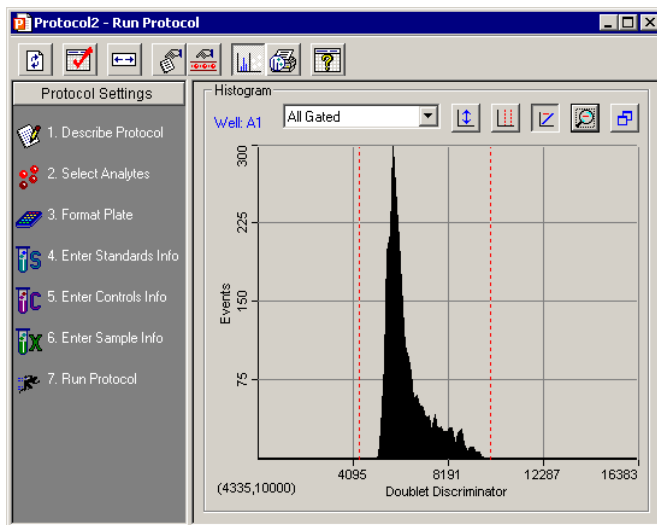
Figure 82.   Histogram maximized in the Protocol window.

Bead Map

The bead color map is a density dot plot of the events in a reading for a selected well. Different colored dots in the map represent different numbers of events at those data points. The number of available regions in the bead map (100, 50, or 25) is listed above the map, and is based on the selection in the *Advanced Settings* dialog (see page 90).

In the default bead map, the X-axis is the **Classification 1** channel and the Y-axis is the **Classification 2** channel. These channels measure the embedded fluorochromes in each bead, which are used to identify the bead set and corresponding analyte. The resulting data point clusters in the map represent the different bead sets in the assay and their associated analytes

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➢   Each bead set generates a cluster of data points, rather than a single point, because of minute variations in fluorochrome levels and intensity among the beads in a set.

The white areas on the map indicate the expected regions of the selected analytes. If you move your cursor over these areas, the name of the analyte will appear in a pop-up. The data point clusters should fall within these regions.

Each cluster of dots that falls within a white region represents a unique bead set (analyte) within an assay
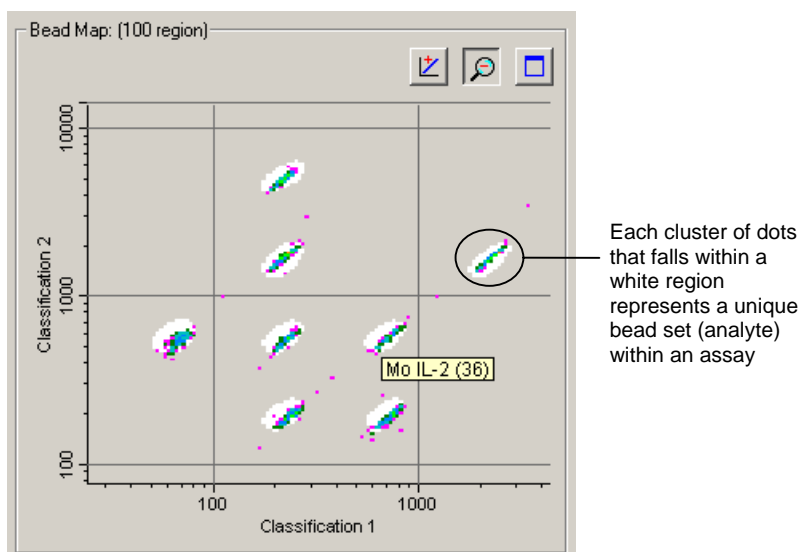
Figure 83. Bead map, with pop-up indicating the analyte region under the cursor.

If one or more clusters do not fall within the white areas, it may indicate a problem with the beads such as photobleaching, or the analyte selections in the Protocol may be incorrect. If all the data clusters have shifted out of the white regions, there may be microbubbles in the cuvette. See 0 on page 165 for guidance with troubleshooting.

In this way, the map can be used to confirm that the bead sets are correctly selected, measured and identified.

Changing the X- and Y-Axes of the Map

You can change the channel types of the axes. Right-click on the bead map and select one channel from the **X-Axis** submenu, and the other channel from the **Y-Axis** submenu. The available channels are:

- **Doublet Discriminator**—This channel measures light scatter from the beads as they pass through the red laser. Light scatter correlates to particle size.

- **Reporter 1**—This channel measures the reporter molecules bound to the analytes on each bead.

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- **Classification 1**—This channel measures the first classification dye embedded in each bead.
- **Classification 2—**This channel measures the second classification dye embedded in each bead.

If you are developing your own assay, you may want to plot the Reporter 1 channel against the Doublet Discriminator channel to evaluate the distribution of the reporter signal. This yields information about the specificity of the antibodies in the assay.
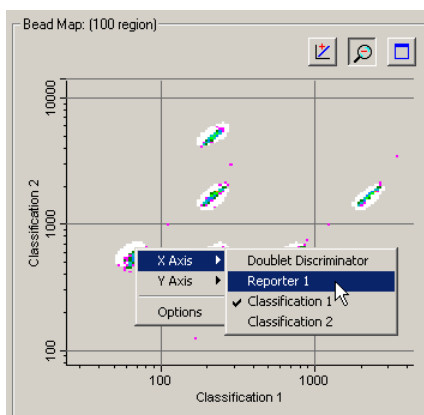
Figure 84.   Changing the X-axis.

Scaling the Bead Map Data

The default view of the data is log scale. From the toolbar, click on the **Log/Linear** button ⬚ to change the display from log to linear. Click again to return to log.

Magnifying and Resizing the Bead Map

Click on the **Zoom** button ⬚ in the bead map toolbar to magnify the display. With **Zoom** selected, the cursor will change to a "hand" symbol ⬚ when you position it over the map. Drag the hand cursor to view different magnified regions of the bead map.

Click on the **Maximize** button ☐ to maximize the bead map portion of the window within the larger Protocol window. Click on the **Restore** button 🗗 to return the bead map to its default state.

Bead Map Display Options

To access additional Bead Map display settings, right-click on the bead map and select **Options**.

Density Filtering

Each colored dot in the bead map represents a certain number of events measured by the reader at that data point. Different colors are used to distinguish between different numbers of events. You can adjust the display so that data points with low numbers of events are not shown, or a certain number of events is required before the data point color changes.
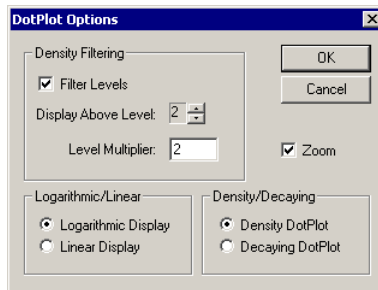
Figure 85.   Bead map display options.

➢ These settings only change the bead map display, and do not affect the underlying data in any way.

First, enter a number in the **Level Multiplier** field. This number is used exponentially to determine the number of events required before a data point changes color. For the default value of 2, the color level will change at 2 events, 4 events, 8 events, 16 events, etc.

If you want to filter lower numbers of events from the display, select the **Filter Levels** checkbox and use the arrow keys next to **Display above level** to select the

exponent level below which no data points will be displayed. For example, if the **Level Multiplier** is set to 2, and **Display above level** is set to 3, data points are not displayed until 8 or more events are registered; the first color level occurs at 8 events, the second color level occurs at 16 events, the third color level occurs at 32 events, etc.

The color levels in the display are, in order, dark blue, purple, dark green, light blue, blue, light green, orange, black, and brown.

Logarithmic/Linear Display

Select whether you want to display the bead map data on a logarithmic or linear scale using these option buttons.

Raw Data Table

The *Raw Data* table displays the raw numerical data of a reading, as well as all instrument settings and information related to the system hardware and software used in the reading (version numbers, voltage, temperature, gain, etc.).

➢ Note that the instrument hardware and software information previously included in the *Run Info* window is now provided in the *Raw Data* table.

This table is available in both the *Run Protocol* window and in the *Raw Data* window of the Results file. (Note that you can only print and export the *Raw Data* table from a Results file, not a Protocol window. See page 121.)

The first few lines of this table are visible below the bead map and histogram. Alternatively, hide the bead map/histogram display by clicking on the **Show/Hide Histogram/Bead Map** button to maximize the table.

	Well	Type	Human IL-2 (38)	Human IL-4 (52)	Human IL-6 (19)	Human IL-8 (54)	Human IL-10 (56)	Hu
1	A1	S1	30624.5 (114)	27006.5 (134)	29012.0 (116)	28236.0 (171)	30960.0 (100)	
2	B1	S2	31651.0 (121)	28427.0 (121)	28489.0 (117)	29672.0 (125)	31771.0 (115)	
3	C1	S3	30068.0 (113)	25496.0 (120)	18464.0 (107)	29496.0 (155)	29109.5 (112)	
4	D1	S4	12722.5 (112)	2619.5 (114)	2679.0 (103)	16132.0 (131)	8667.5 (119)	
5	E1	S5	721.5 (114)	165.0 (139)	215.0 (109)	1092.5 (163)	1356.0 (133)	
6	F1	S6	178.5 (128)	103.5 (154)	74.5 (122)	179.0 (147)	655.0 (157)	
7	G1	S7	148.0 (126)	104.5 (122)	55.5 (101)	132.0 (113)	621.0 (104)	
8	H1	B	113.0 (117)	104.0 (101)	42.0 (103)	85.5 (130)	618.5 (119)	
9	A2	S1	31739.0 (107)	28795.0 (127)	30648.0 (100)	30168.0 (130)	31875.5 (100)	
10	B2	S2	32018.0 (102)	29070.5 (112)	29775.0 (116)	30915.5 (132)	32022.0 (100)	
11	C2	S3	30433.0 (101)	24834.0 (132)	17395.0 (130)	29556.0 (119)	29054.0 (123)	
12	D2	S4	11596.0 (136)	2526.5 (150)	2273.0 (110)	15478.5 (110)	8633.0 (105)	
13	E2	S5	733.0 (115)	169.0 (134)	230.0 (112)	1065.0 (125)	1414.0 (110)	
14	F2	S6	202.0 (108)	104.5 (110)	74.0 (114)	197.0 (111)	716.0 (126)	
15	G2	S7	122.0 (120)	94.0 (127)	49.5 (112)	126.0 (154)	512.5 (116)	
16	H2	B	115.5 (140)	96.0 (141)	45.0 (121)	81.5 (140)	534.5 (100)	
17	A3	X1	28695.0 (115)	24950.5 (126)	25251.0 (130)	26184.5 (159)	28006.0 (101)	
18	B3	X2	28028.5 (102)	25142.5 (127)	24400.0 (135)	26554.0 (139)	27278.0 (107)	
19	C3	X3	30522.0 (117)	14236.5 (126)	9452.0 (102)	30237.0 (140)	25728.0 (111)	
20	D3	X4	6089.0 (143)	942.5 (126)	941.0 (127)	11316.0 (129)	4297.0 (125)	
21	E3	X5	376.5 (100)	116.0 (129)	91.0 (131)	764.0 (149)	451.0 (129)	

Figure 86.   Raw data from a reading.

During a reading, the table is filled in line by line as each well is read. After the reading is complete, all the data is displayed.

In the table, the fluorescent intensities (FI) of the analytes you selected are listed for each well number.

➢ During a reading, the array reader will always detect *all* the analytes in the sample, including any that you have not selected. However, analytes that were not selected in the Protocol will not be included in the table, even if they were detected. After a reading, you can go back and change your selections under *Select Analytes*, and the new analytes, if detected, will appear in the table.

For on-line Help information about the *Raw Data* table, click on the **Display Help for Table Legend** button .

Bead Statistics

To display statistical data for the individual beads as well as the entire well, click on the **Show/Hide Bead Statistics Columns** button on the table toolbar. The number of beads collected for each analyte will be displayed in parentheses after the analyte fluorescent intensity.

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Human IL-6 (19)	Human IFN gamma (21)
10744.00 (65)	11739.00 (75)

Fluorescent intensity   Bead count

Figure 87.   Analyte fluorescent intensity and bead count.

With **Show/Hide Bead Statistics Columns** selected, the following columns are also displayed:

- **Total**—Total bead count in the well.
- **Region**—Number of beads that fell within defined regions.
- **Gate**—Number of beads that passed the DD gate (see page 93).
- **% Agg Beads**—Percentage of aggregated beads in the well (100 − (Gate/Total*100)).

Region	Gate	Total	% Agg Beads
2058	2128	2385	11

Figure 88.   Well data.

➢ The number in the **Region** column will be flagged by an asterisk (*) if that well did not acquire the number of beads per region specified for the reading.

Sampling Error Codes

During a reading, the flow of beads, the bead count, the bead regions, and the platform temperature are continuously monitored. The *Sampling Errors* column reports errors in any of these areas for each analyte. These errors will be reported in the table even if you chose not to pause the run due to an error condition (see page 92).

The sampling error codes in the column are listed in the following table.

Error Code:	Indicates:
1	Low bead number detected in the well
2	Aggregated beads detected in the well
3	Bead classification efficiency problem detected in the well
4	Region selection problem detected in the well
5	Platform temperature problem detected

Raw Data Display Options

To select the columns to display in the *Raw Data* table, as well as other display

settings, click on the **Raw Data Display Options** button or select the command from the *Table Options* menu.
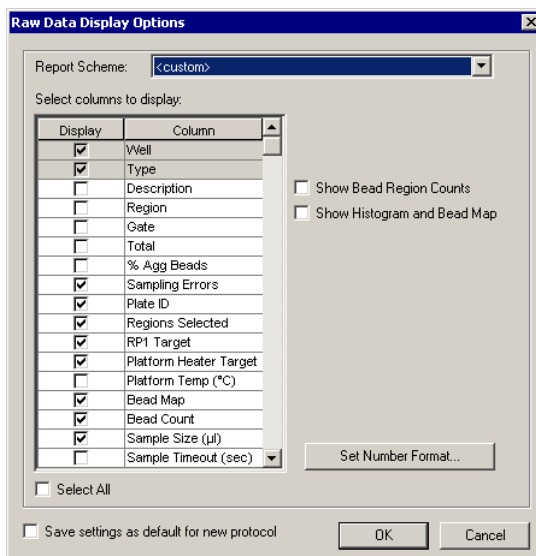
Figure 89.   *Raw Data Display Options* dialog.

In the *Raw Data Display Options* dialog, select the columns to display using the checkboxes. You can also choose between predefined report settings by selecting

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Standard or **Diagnostic** in the **Report Scheme** field; these selections will display different sets of columns.

Select the **Show Bead Region Counts** and **Show Histogram and Bead Map** checkboxes to set these display options as the default for the *Raw Data* table.

Select the **Save Settings as Default for New Protocol** checkbox to use the selections in this dialog as the default for new Protocol files.

Set Number Format

In the *Raw Data Display Options* dialog, click on the **Set Number Format** button to select the number format for each column in the *Raw Data* table.
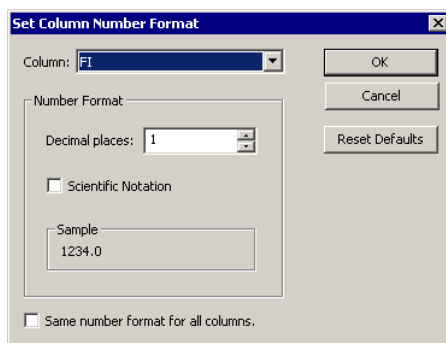
Figure 90.   *Set Column Number Format* dialog.

In the dialog, select the column from the pulldown list, then specify the number of decimal places for the value in that column in the **Decimal Places** field. Select the **Scientific Notation** checkbox to display the number in scientific notation. Select the **Same Number Format for All Columns** checkbox to use the selected number format for all the columns.

➢   Note that changing the number format only changes how the numbers are displayed and printed, and does not affect calculations in any way.

Click on **OK** to make the changes, or **Reset Defaults** to return all columns to their default number formats.

Other Table Formatting

To display the description of each sample in the table, click on the **Show/Hide Description Column** button 🖋 above the table. The *Description* column will be displayed.

To automatically size the columns to fit the width of the text, click on the **Autofit** button ↔ on the toolbar.

Copying the Raw Data

You can copy the contents of the table to the Windows clipboard. Select rows, columns, cells, or the entire table, then select **Copy** from the *Edit* menu. The values you copy can be pasted into other Windows® applications.

Rerun/Recovery Mode

After a reading has stopped—because of an error or user intervention, or because the reading is complete—the **Rerun/Recovery Mode** checkbox in the *Run Protocol* window will be selected. This allows you to rerun some or all of the samples.

With **Rerun/Recovery Mode** selected, checkboxes will appear next to each well number in the *Run Protocol* table. Select the checkboxes next to the wells that you want to read again.

Figure 91.   Selecting wells to rerun.

➢ To rerun wells in a filter plate, you must first vacuum the excess fluid from the wells and resuspend the remaining beads in 125 µl. (The beads will remain on top of each filter plug after vacuuming.)

Vacuum the wells of the filter plate using the vacuum filter apparatus and resuspend the beads in each well with 125 µl of resuspension buffer. See the Bio-Plex Cytokine assay manual for detailed instructions.

Each well may be read twice without adversely affecting the results.

Because there are fewer beads in a well that has already been run once, be sure to select 100 beads per region or fewer in the *Run Protocol* settings (see page 89). Also, click on the **Advanced Settings** button and deselect the **Low bead number** error condition checkbox (page 90). Otherwise, a low bead error may stop the reading.

When you are ready, click on **Start** to begin the reading.

8. Analyzing the Results

Results Files

After a reading is complete, a **Results file** is generated and automatically opens. The Results file contains the data from a reading, the Protocol parameters used to collect that data, and analysis tools for interpreting the data. It includes all the information necessary to analyze raw data, including a description of the assays, well types, concentrations, dilutions, and the regression method used for calculating the concentrations of unknowns from standards. It also includes information about the instrument and the run conditions at each well at the time of data acquisition.

In the Results file, you can change many of the parameters copied from the original Protocol file—including the plate format, analyte selection, standards info, etc.—and recalculate the results. However, you can only change the parameters of wells that have already been read, and you can only calculate results based on the analytes that were present in the sample.

Note that the Results file is automatically saved after a reading if you selected **Auto save after run** in the Protocol window. Otherwise, you must save your Results file as a separate step.

To generate a Results file from an incomplete reading or a previous reading, open the Protocol file that contains the raw data for that reading and select **New Results from Protocol** from the *Run Protocol* toolbar or the *File* menu.

Opening a Results File

To open an existing Results file, click on the **Open** button on the *Quick Guide* or main toolbar or select **Open** from the *File* menu. Locate and select the file using the standard Windows *Open* dialog.

Standard Results files have the file extension ***.rbx**. Secure Results files, created using Bio-Plex Manager Security Edition, have the extension. ***.srbx**. See page 153 for more information about Secure files.

➢ Secure Results files can only be opened as **read-only** files using the Standard Edition of Bio-Plex Manager.

➢ Results files created or modified by Bio-Plex Manager 4.0 or later cannot be opened by earlier versions of the software.

Saving a Results File

To save a Results file, with the file open click on the **Save** button 🖫 on the main toolbar or select **Save** or **Save As** from the *File* menu.

Reducing the Size of a Results File

Results files that include all the data from a reading can be very large (>5 megabytes). Much of this file size is due to the raw bead event data from the reading, which is used to generate the bead map and histogram displays. If you require a smaller file size (e.g., to send via email), you can save a copy of the file *without* this raw bead event data, but with the other numerical data from the reading intact.

➢ We strongly recommend keeping a copy of the original Results file with the raw bead event data intact. This data is necessary for changing the DD gate range in the Results file (see page 120), and can also be exported in XML file format (see page 143) for further analysis.

In the *Save As* dialog box, select the **Compressed mode** checkbox to save the Results file without the bead map and histogram data. All other data will be preserved.

Select this checkbox
to reduce the size of
the Results file
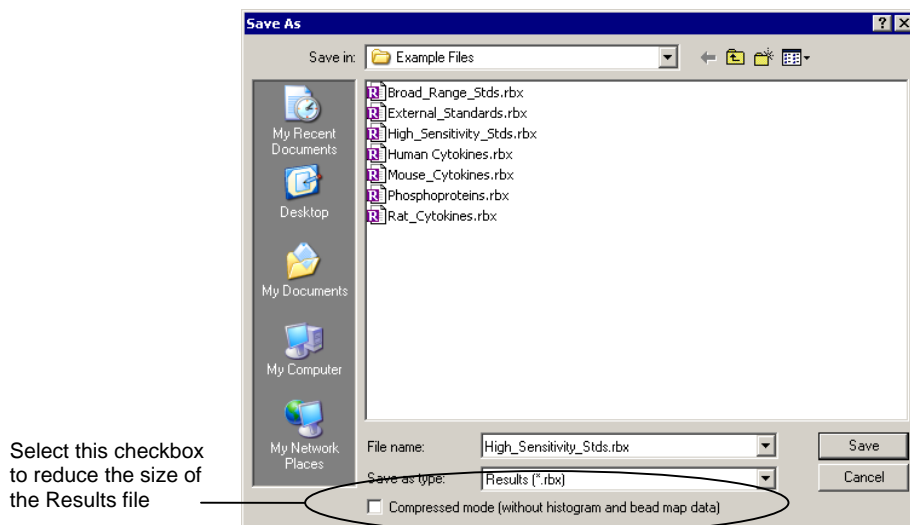
Figure 92.   *Save As* dialog for Results files.

➢ Note that this compressed file option is not available for Secure Protocol files
generated using the Security Edition of the software (see page 153).

Results Window

The Results window is divided into subwindows, each containing different options
for viewing, formatting, and calculating your data.

Click on these buttons to review/change the original Protocol settings.

Click on these buttons to view and calculate results.
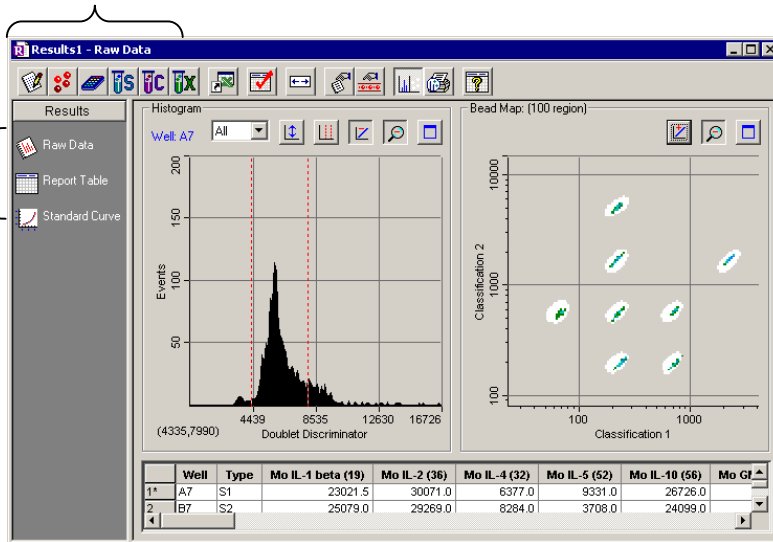
Figure 93.   Results window.

Navigate among the different subwindows using the buttons along the left side of the Results window:

- Raw Data [icon] (see page 121).
- Report Table [icon] (see page 123).
- Standard Curve [icon] (see page 136).

Viewing/Changing the Protocol Settings

In the Results window, you can review and in some cases change the plate formatting, analyte selections, sample concentrations, etc., from the original Protocol file to display different data or recalculate data in the Results file tables. These controls are located in the toolbar across the top of the Results window.

> Any changes that you make in the Results file will not change the original Protocol file or the data from the reading. They will only change how that data is displayed in the Results file tables.

Click on the **Describe Protocol** button ✒️ in the Results toolbar to review or change the description of the Protocol, as described on page 53.

Click on the **Select Analytes** button ⚫️ in the Results toolbar to review the analyte selection or select different analytes, as described on page 53.

> During a reading, the array reader will always detect *all* the analytes in the sample, including any that you have not selected. However, analytes that were detected but not selected will not appear in the final reports and tables. After a reading, you can go back and correct your analyte selections, and the detected analytes will appear in the tables.

Click on the **Format Plate** button 🟦 in the Results toolbar to review or change the formatting of the plate. Plate formatting is described on page 59.

> Only formatted wells are read by the array reader. You must define all the wells that you want to read before running a Protocol. However, you can change the formatting of a defined well (e.g., from unknown to standard) after a reading, and the analysis of that well will change.

Click on the **Enter Standards Info** button 🧪S in the Results toolbar to review or change the concentrations of the standards, as described on page 71.

Click on the **Enter Controls Info** button 🧪C in the Results toolbar to review or change the concentrations of the controls, as described on page 82.

Click on the **Enter Sample Info** button 🧪X in the Results toolbar to review or change the sample information, as described on page 85.

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Changing the DD Gate Range

You can change the DD gate range (see page 93) in the Results file and recalculate the data based on the new range. Note the original DD gate range set in the Protocol file and used during the reading will be preserved and can be displayed in the *Raw Data* table.

To reset the DD gate range, go to the *Edit* menu, open the *Reanalyze Results* submenu, and select **Change DD Gates**. The *Change DD Gates* dialog will open, displaying the histogram and bead map with controls for changing the DD gate range. (See page 98 for a complete description of other histogram and bead map controls.)
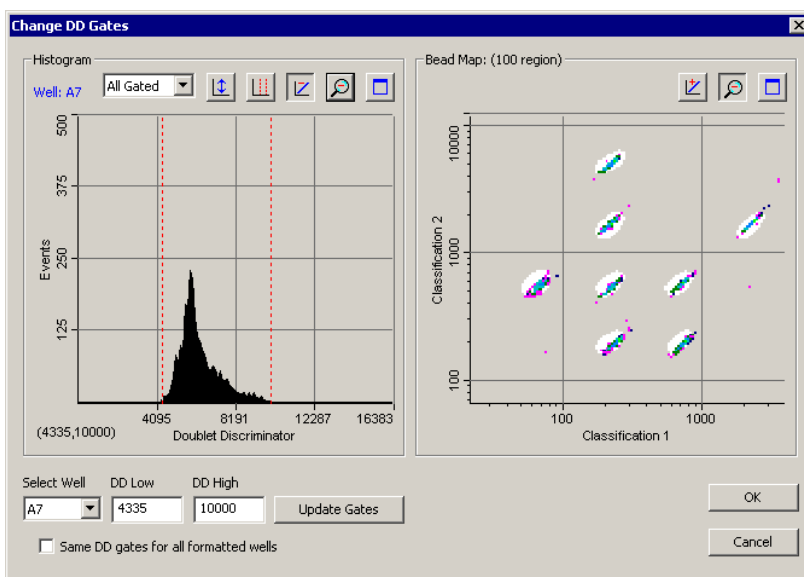
Figure 94. *Change DD Gates* dialog.

In the *Change DD Gates* dialog, first select the well that you want to change or select the **Same DD Gates for All Formatted Wells** checkbox. Also, you can select a specific analyte, all gated analytes, or all analytes from the pulldown menu above the histogram. Then either drag the red dashed lines in the histogram display to adjust the DD gates, or enter new values in the **DD Low** and/or **DD High** fields and click on the **Update Gates** button.

When you have made your changes, click on **OK**. The data in the *Raw Data* table (see next section) will be recalculated based on the new settings.

Raw Data

The *Raw Data* window in the Results file includes the same histogram and bead map and numerical *Raw Data* table as in the *Run Protocol* window. These features are described in the previous chapter on the following pages:

- Histogram and bead map—page 98.
- *Raw Data* table—page 108.
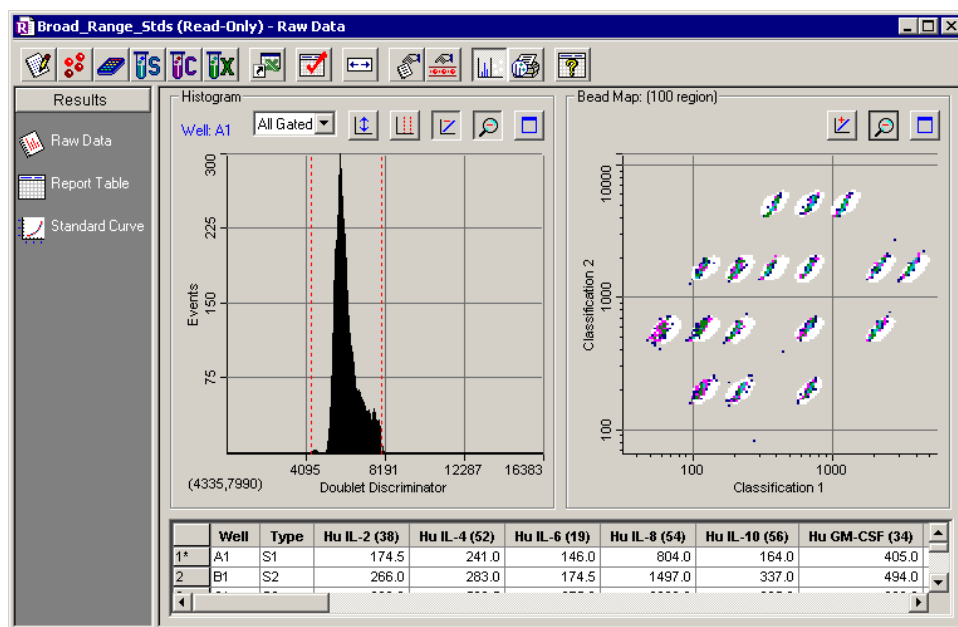
Figure 95.   *Raw Data* window in Results file.

The *Raw Data* window in the Results file includes additional exporting and printing functions not available in the Protocol window.

Setting the Plate ID

If you have an identification number for the microplate used in a reading, you can specify the ID number in the Results file. Right-click anywhere in the *Raw Data* table and select **Set Plate ID** from the context menu.
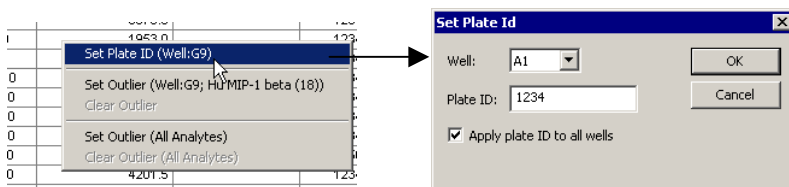
Figure 96.   *Set Plate ID* dialog.

In the dialog, enter the plate ID number in the **Plate ID** field. If you want to associate the same ID number with all the wells in the reading, select **Apply Plate ID to All Wells** checkbox. If you want to associate different ID numbers with different wells, select the well for this number from the **Well** pulldown list. Click on **OK** to save the changes.

The plate ID number is displayed for each well in the *Raw Data* table.

Exporting the Raw Data

To automatically export your raw data to Microsoft® Excel, click on the **Export to Excel** button in the toolbar or select the command from the *File* menu. This command will automatically launch Excel and display a spreadsheet containing your data.

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Figure 97.   Example of an Excel spreadsheet with exported data.

You can also export the raw data to a text file. With your raw data displayed, go to the *File* menu and select **Export Table to File**. This command will open a *Save As* dialog box in which you can specify a file name and location for the text file.

➢ You can copy any or all values in the table to the Windows clipboard. Select the entire table, or the desired rows, columns or entries, and select **Copy** from the *Edit* menu. The values you copy can be pasted into other Windows applications.

Printing the Raw Data

You can print the *Raw Data* table using the **Print** command on the main toolbar or *File* menu. **Print Preview** on the *File* menu displays the table as it will appear in the printout.

Report Table

The *Report* table provides detailed information about each of the analytes in your samples. Click on the **Report Table** button to open the *Report* table window.

Select analyte from the pulldown list
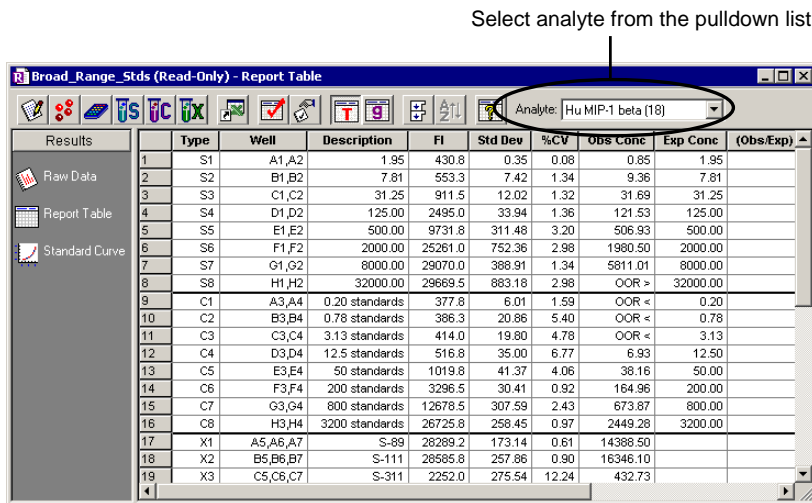
Figure 98.   *Report* table.

First, select the analyte that you want to review from the **Analyte** pulldown list above the table. All the data for that analyte in each defined well of the plate will be displayed in the table.

For on-line Help information about the *Report* table, click on the **Display Help for Table Legends** button ![button].

Report Table Columns

The columns in the table show the data for the selected analyte for each well or replicate group of wells.

The available *Report* table columns are listed below:

- **Type**—Sample type (unknown, standard, blank, etc.) and number, based on plate formatting.
- **Well**—Well number; all wells are listed for a replicate group (see next section).
- **Outlier**—Contains a checkbox for flagging the well or group of wells as an outlier. See page 129.

- **Description**—The description you entered for each standard, control, or sample in the Protocol. Click on the **Show/Hide Description Columns** button ⬚ to view or hide this column.

- **FI**—The median fluorescent intensity of the selected analyte.

- **FI – Bkgd**—The median fluorescent intensity minus background (measured using the defined blank wells) of the selected analyte.

- **Std Dev**—The standard deviation of a replicate group of wells.

- **Std Err**— The standard deviation of a replicate group of wells divided by the square root of the number of replicates.

- **%CV**— The coefficient of variation of a replicate group of wells. If this value is high, you may want to review the individual FI values and select the appropriate member of the replicate group as an outlier (see next section).

- **Obs Conc**—The observed concentration of the selected analyte, calculated from its fluorescent intensity and the standard curve. Note that the observed concentrations of standards are back-calculated from their fluorescent intensities and the standard curve.

- **Exp Conc**—The expected concentration of the selected analyte standard or control, as specified in the *Enter Standards Info* window or *Enter Controls Info* window, respectively.

- **(Obs/Exp)*100**— The recovery rate for the observed versus expected concentrations of the standard or control. See page 130.

- **Conc in Range**—An observed concentration (see above) that falls within the range of standard concentrations given a specified recovery range (see page 80). Values outside the range are flagged as ***.

- **Group**—The defined group of the selected well (see page 68).

- **Ratio**—The ratio of the FI values of a member/reference or reference/member in a group (see page 68).

- **Dilution**—The specified dilution factor of the selected analyte.

- **Bead Count**—The number of beads counted in region for the selected analyte.

- **Bead Mean**—The mean fluorescent intensity of the beads counted in region for the selected analyte.

- **Bead Std Dev**—The standard deviation of the bead mean.

- **Bead Std Err**—The standard error of the bead mean.

- **Bead %CV**—The coefficient of variation of the bead mean.

- **Trimmed Mean**—The bead mean excluding the top 5% and bottom 5% of fluorescent intensities.
- **Trimmed Std Dev**—The standard deviation of the trimmed mean.
- **Trimmed Std Err**—The standard error of the trimmed mean.
- **Trimmed %CV**—The coefficient of variation of the trimmed mean.
- **Sampling Errors**—Error codes for a reading. See page 110.

Report Table Options

To select the columns to display in the *Report* table, as well as other display settings, click on the **Report Table Options** button or select the command from the *Table Options* menu.
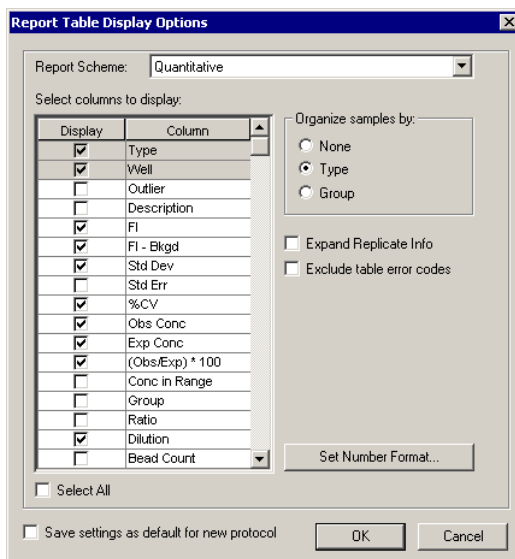
Figure 99.   *Report Table Display Options* dialog.

In the *Report Table Display Options* dialog, select the columns to display using the checkboxes. You can also choose between predefined report settings by selecting **Quantitative** or **Qualitative** in the **Report Scheme** field; these selections will display different sets of columns.

> ➢ The predefined set of Quantitative columns are designed to be used with cytokine assays, while the Qualitative columns are designed to be used with phosphoprotein assays.

Under *Organize samples by*, you can group the rows in the *Report* table by **Type** (standards, controls, unknowns, etc.), **Group** (if defined), or **None**. Note that **Type** and **Group** selections will override the **Sort** function in the *Report* table (see page 131). For example, if you select **Type** organization, any sorting you do will be within each type.

To review the data for each well in a replicate group (see page 126), select the **Expand Replicate Info** option.

To hide error codes in the table such as OOR, ***, and ----, as well as * before concentration values, select the **Exclude table error codes** checkbox.

Select the **Save Settings as Default for New Protocol** checkbox to use the selections in this dialog as the default in Results files generated from new Protocols.

Set Number Format

In the *Report Table Display Options* dialog, click on the **Set Number Format** button to select the number format for each column in the *Report* Table.
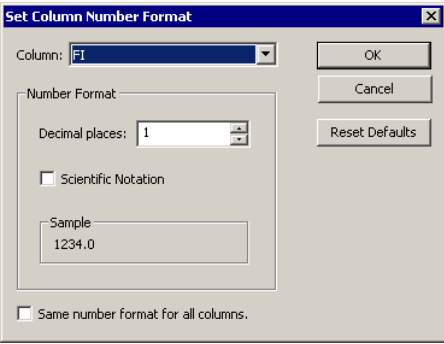
Figure 100. *Set Column Number Format* dialog.

In the dialog, select the column from the pulldown list, then specify the number of decimal places for the value in that column in the **Decimal Places** field. Select the **Scientific Notation** checkbox to display the number in scientific notation. Select the **Same Number Format for All Columns** checkbox to use the selected number format for all the columns.

➢ Note that changing the number format only changes how the numbers are displayed and printed, and does not affect calculations in any way.

Click on **OK** to make the changes, or **Reset Defaults** to return all columns to their default number formats.

Replicate Groups

If you have defined multiple wells as a replicate group in the *Format Plate* window, the wells will be listed together in the **Well** column, and mean data values for those wells will be reported in the relevant columns for the selected analyte (unless the wells have been flagged as outliers; see page 129).

Figure 101. Example of a standard with two replicate wells (A11 and A12).

To review the data for each well in a replicate group, click on the **Show Replicates** button.

	Type	Well	FI	FI - Bkgd
1	B	A1,A2	70.25	70.25
2		A1	69.00	69.00
3		A2	71.50	71.50
4	S1	A11,A12	23692.00	23621.75
5		A11	23706.00	23635.75
6		A12	23678.00	23607.75
7	S2	B11,B12	21500.00	21429.75
8		B11	21155.00	21084.75
9		B12	21845.00	21774.75
10	S3	C11,C12	3538.50	3468.25
11		C11	3594.00	3523.75
12		C12	3483.00	3412.75

Figure 102. Expanded data for replicate wells.

The mean values as well as the values for the individual wells will be reported for each replicate group.

Selecting Outliers

In some cases, you may want to exclude a well or replicate group of wells from statistical calculations (e.g., when the CV% of a set of replicates is high, or the observed concentration of an analyte is out of range). The **Outlier** column in the *Report* table window allows you to flag a well or replicate group of wells as an outlier.

Click on the **Show/Hide Outliers Column** button in the toolbar to show or hide the column.

Each row in the column contains a checkbox. When you select the checkbox, the data for the selected analyte in the well is excluded and the table is automatically recalculated.

Note that flagging a well as an outlier for the selected analyte does not flag it as an outlier for all analytes. To do this, right click on the outlier checkbox for the well, and select **Set Outlier (All Analytes)** from the context menu.

C7	G3,G4	☐	12070.3	307.33	2.4
C8	H3,H4	☐	26725.8	258.45	0.9
X1	A5,A6,A7	☑			
X2	B5,B6,B7	☐			
X3	C5,C6,C7	☐			
X4	D5,D6,D7	☐			
X5	E5,E6,E7	☐			
X6	F5,F6,F7	☐			
X7	G5,G6,G7	☐			
X8	H5,H6,H7	☐	1036.0	80.36	8.8

Context menu overlaying the table:
- Set Plate ID
- Set Outlier (grayed)
- Clear Outlier
- Set Outlier (All Analytes)
- Clear Outlier (All Analytes)
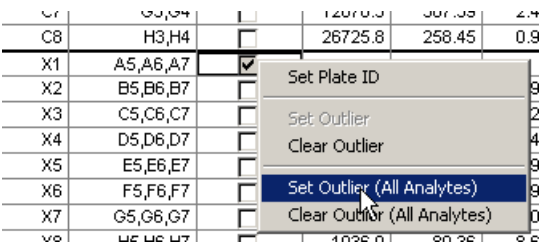
Figure 103. Selecting a well as an outlier for all analytes.

Now the well data for all analytes will be excluded from calculations. To remove the outlier flag for all analytes, right-click on the well select **Clear Outlier (All Analytes)** from the context menu.

If you flag a replicate group of wells as an outlier, data for the entire group is excluded. If you click on the **Expand Replicates Info** button ⊞, you can flag each well in the group as an individual outlier. If you do this, the outlier checkbox for the entire group will appear checked and grayed out, indicating that part of the group has been flagged.

Type	Well	Outlier
B	H1,H2	☑ (gray)
	H1	☐
	H2	☑

Group checkbox appears gray and selected
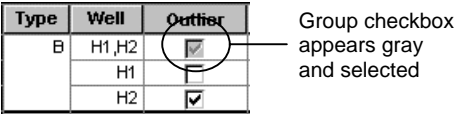
Figure 104. Flagging a single well in a replicate group as an outlier.

Obs/Exp*100 Column

Because the standard curve (page 136) is critical for calculating the concentrations of unknowns, the **Obs/Exp*100** column in the *Report* table allows you to assess how well the curve fits the standards. This column is also used with controls (page 82) to determine the overall accuracy of an assay.

To display this column, click on **Report Table Options** 📋 and select the **(Obs/Exp)*100** checkbox.

The **(Obs/Exp)*100** column shows the recovery rate for the observed versus expected concentration of each standard. This number should fall within the recovery

percentage range selected in the *Enter Standards Info* window (see page 81). For example, if you selected a recovery range of 80–120%, the observed (back-calculated) concentration of a standard should be within 80–120% of the expected concentration. Values outside this range are flagged in the **Conc in Range** column.

Conc in Range Column

The concentration in range column (**Conc in Range**) provides a method for determining which unknown concentrations are reliable based on the recovery range of the standards (see previous section). This column shows only observed concentration values that fall within the range of valid standards; values that do not fall in the range are displayed as ***. (The concentration in range values of the back-calculated standards are also shown, for comparison purposes.)

Obs Conc	Exp Conc	(Obs/Exp) * 100	Conc in Range
	32000.00		
7937.63	16000.00	50	***
3999.99	4000.00	100	3999.99
500.00	500.00	100	500.00
49.97	50.00	100	49.97
8.84	8.00	111	8.84
2.73	4.00	68	***
1556.37			1556.37
1662.21			1662.21
7232.12			***
335.06			335.06
38.24			38.24
7.20			***
*2.00			***

Back-calculated standard concentrations within a specified recovery percentage range of 80–120%

Concentrations of unknowns within the range of valid back-calculated standard concentrations

Concentrations of unknowns outside the range of valid back-calculated standard concentrations

Figure 105. *Conc in Range* column.

For example, if only standard concentrations from 1.9 to 8,100 ρg/ml fall within the specified recovery range, then only unknown concentrations that fall within this range will be displayed in the **Conc in Range** column.

Table Formatting

To sort the data in the table alphanumerically, click on the heading of the column that you want to sort by, then click on the **Sort** button 🔽. Clicking twice will invert the order.

To highlight the divisions between the unknowns, standards, controls, etc. in the report, click on the **Organize by Type** button ▦. With this button selected, any sorting you do will be within each type.

To highlight the divisions between defined groups (see page 68) in the report, click on the **Organize by Group** button ▦. With this button selected, any sorting you do will be within each group.

To automatically size the columns to fit the width of the text, click on the **Autofit** button ↔ on the toolbar.

Copying the Report Table

You can copy the contents of the table to the Windows clipboard. Select rows, columns, cells, or the entire table, then select **Copy** from the *Edit* menu. The copied values can be pasted into other Windows applications.

Exporting the Report Table

To export the data in the *Report* table to an Excel spreadsheet or workbook, or tab-defined text file, click on the **Export Report Table** button ▦ in the toolbar.
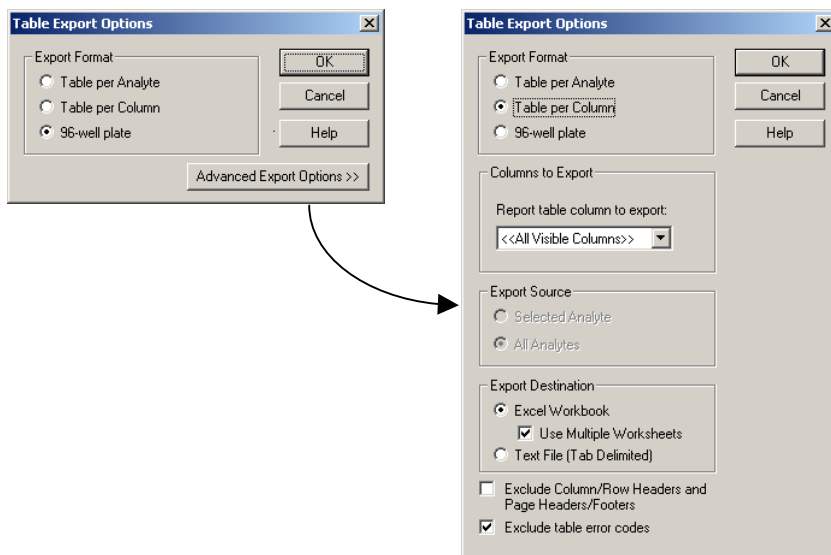
Figure 106. *Table Export Options* dialog.

The *Table Export Options* dialog will open.

Export Format

Select **Table per Analyte** to display all the data for each analyte on an individual spreadsheet.

Select **Table per Column** to display one column of data (e.g., FI) for all the analytes on an individual spreadsheet.

Select **96-Well Plate** to display one type of data for each analyte in a spreadsheet, laid out like the wells in a 96-well plate (rows A–H and columns 1–12). Note that this selection will export only one type of data at a time.

For additional export options, click on the **Advanced Export Options** button.

Advanced Export Options

If you selected **96-Well Plate** or **Table per Column** above, select the data to export under the **Report Table Column to Export** pulldown list.

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If you selected **96-Well Plate** or **Table per Analyte** above, under **Export Source**, select **Selected Analyte** to export only the data for the currently selected analyte in the *Report* table or **All Analytes** to export the data for all analytes in the table.

Under **Export Destination**, select **Excel Workbook** to export the data to a Microsoft® Excel file, or **Text File (Tab Delimited)** to export it to a tab-delimited text file (see Figure 107 for an example).

If you selected **Excel Workbook** and will be generating multiple tables of data, select the **Use Multiple Worksheets** checkbox to export each table to a different worksheet within the Excel workbook. Otherwise, all tables will appear on the same worksheet.

C:\Program Files\Bio-Rad\Bio-Plex Manager\Examples\Human Cytokine 16 plex.rbx												
FI – Bkgd												
Hu TNF alpha (36)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	31.00	31880.00	364.00	27041.00	32043.00	32214.50	32045.50	32735.00	32666.00	32735.00	32735.00	32735.00
B	33.00	29213.50	380.00	26205.00	31977.50	31616.00	31204.00	32735.00	30125.00	29382.00	27725.00	30507.50
C	29.00	29646.00	46.00	20294.00	20248.00	15367.00	23669.00	30275.00	4824.50	3129.00	3249.00	3843.00
D	---	28781.00	45.00	1370.50	1441.50	2077.00	1830.00	3462.50	268.50	206.50	205.50	257.00
E	30630.00	6489.00	47.00	113.00	87.00	119.00	140.00	267.50	38.50	31.00	31.00	37.00
F	31636.50	6372.00	20.00	11.00	13.00	24.00	14.00	38.00	18.00	18.00	15.00	10.00
G	31561.50	6606.00	20.00	4.00	1.00	9.00	5.50	19.00	-4.00	-4.00	0.50	-1.00
H	31243.00	372.00	20.00	-7.00	-7.00	-8.00	-5.50	0.00	32735.00	32735.00	32735.00	32735.00
Hu IL-4 (52)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	31.00	31880.00	364.00	27041.00	32043.00	32214.50	32045.50	32735.00	32666.00	32735.00	32735.00	32735.00
B	33.00	29213.50	380.00	26205.00	31977.50	31616.00	31204.00	32735.00	30125.00	29382.00	27725.00	30507.50
C	29.00	29646.00	46.00	20294.00	20248.00	15367.00	23669.00	30275.00	4824.50	3129.00	3249.00	3843.00
D	---	28781.00	45.00	1370.50	1441.50	2077.00	1830.00	3462.50	268.50	206.50	205.50	257.00
E	30630.00	6489.00	47.00	113.00	87.00	119.00	140.00	267.50	38.50	31.00	31.00	37.00
F	31636.50	6372.00	20.00	11.00	13.00	24.00	14.00	38.00	18.00	18.00	15.00	10.00
G	31561.50	6606.00	20.00	4.00	1.00	9.00	5.50	19.00	-4.00	-4.00	0.50	-1.00
H	31243.00	372.00	20.00	-7.00	-7.00	-8.00	-5.50	0.00	32735.00	32735.00	32735.00	32735.00

Figure 107. FI - Bkgd data for multiple analytes exported to a tab-delimited text file, 96-well plate format.

Select the **Exclude Column Headers/Footers and Page Headers/Footers** to exclude this information from the exported file.

The error codes symbols in the *Report* table (OOR< >, ***, ----, * before concentration values) can interfere with macros in some types of spreadsheets, including Excel spreadsheets. Select the **Exclude Table Error Codes** checkbox to exclude these symbols from the exported file. If this option is selected, table cells with error codes will be exported as empty cells.

If you are exporting to Excel, Microsoft® Excel will automatically launch when you click on **OK** and a spreadsheet will be created.

Figure 108. Example of an Excel spreadsheet, Table per Analyte format.

Figure 109. Example of an Excel spreadsheet, Table per Column format.

If you are exporting to a text file, a *Save As* dialog will open when you click on **OK**. Select the desired directory and name and click on **Save** to complete the operation.

Printing the Report Table

You can print the *Report* table for the selected analyte using the **Print** command 🖨 on the main toolbar or *File* menu. You can print the *Report* table for all analytes using the **Print All Analytes** command 🖨 on the main toolbar or *File* menu.

Print Preview displays the table as it will appear in the printout.

Standard Curve 📈

The *Standard Curve* window displays the standard curve for each standard analyte in the Results file, and allows you to change the regression model as well as various curve display controls for each analyte. (Curve settings are initially selected in the *Enter Standards Info* window in a Protocol; see page 71.)

Click on the **Standard Curve** button 📈 to open the *Standard Curve* window.
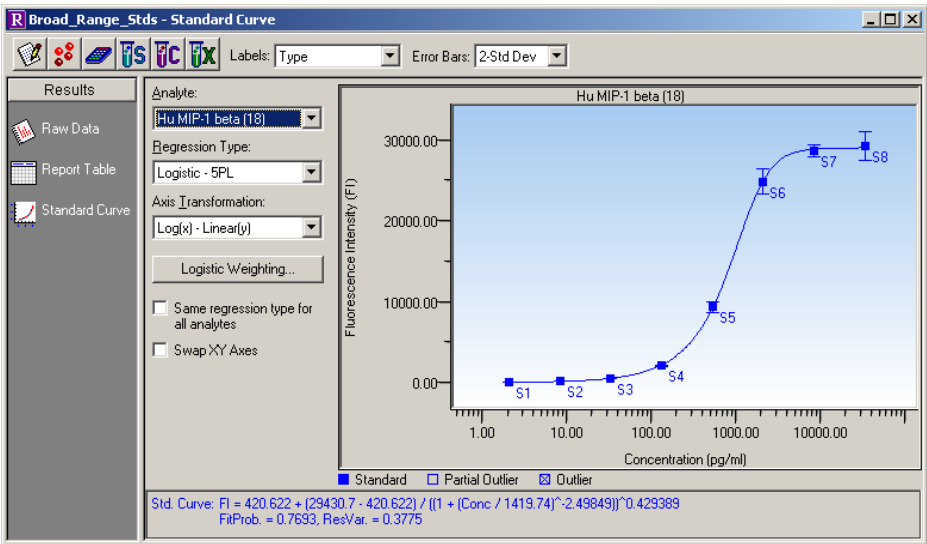
Figure 110. Standard curve.

In the graph, standards defined on the current plate will be displayed as blue squares, while external standards will be displayed as red circles. If both types of standards are used in the *Results* file, both will be displayed on the curve. Outliers and partial outliers will be displayed on the curve as indicated in the legend below the curve.

The equation for the selected regression method as well as some curve fit statistics (R^2, fit probability, residual variance) will be displayed below the standard curve, if possible. Equations for standards defined on the current plate are shown in blue, while equations for external standards are displayed in dark red. Any curve fitting errors will also be displayed below the curve in red.

Use the **Analyte** pulldown list to display the curve for a selected analyte.

Above the curve, use the **Labels** pulldown list to select from a variety of different labels for the points in the curve. If both external and current standards are displayed in the graph, labels will only be displayed for the external standards.

Use the **Error Bars** pulldown list to select from several different error bars (e.g., 1-Std Dev, 2-Std Err) to plot for each point in the curve. If there is only one replicate for a point, the bar will have a value of zero. If both external and current standards are displayed in the graph, error bars will only be displayed for the external standards.

Use the **Regression Type** pulldown list to choose from seven different regression models to generate the standard curve (see next section).

➢ Changing the regression type will automatically recalculate all the data in the *Report* table. If you are unsure whether your data has been recalculated, select **Refresh Calculations** from the *View* menu.

Use the **Axis Transformation** pulldown list to select the standard curve axis scale. Four different combinations of linear and logarithmic are available.

➢ Changing the axis transformation will only change the display of the curve—not the calculated data.

You can use a different regression model for each analyte. Deselect the **Same regression type for all analytes** checkbox, select a different analyte from the pulldown list, and select the regression type to use for that analyte.

Select the **Swap XY Axes** checkbox to change the X and Y axes of the curve.

To display all the data for each standard sample point in the curve, position your cursor over the point. A pop-up box will list the data.
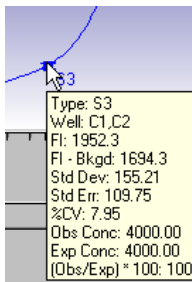
Figure 111. Pop-up data for a standard sample point in the curve.

Regression Methods

There are seven regression methods for calculating the standard curve:

- Logistic-5 PL
- Logistic-4 PL
- Linear
- Cubic spline
- Point-to-point
- Linear (semi-log)
- Point-to-point (semi-log)

The minimum number of non-zero standards required for using each type of regression method is shown in the following table:

Method	Minimum number of standards
Logistic-5PL	6
Logistic-4PL	5
Cubic spine	4
Linear (linear and semi-log)	2
Point to point (linear and semi-log)	2
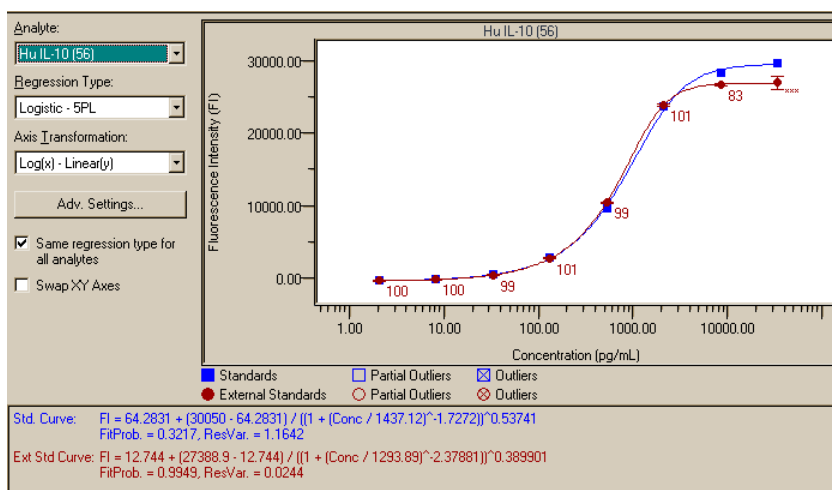
Figure 112. Example of Logistic-5PL curves for standards on the current plate and external standards.

If you do not have enough points for the selected method, an error message will be displayed in red below the standard curve.

Logistic-5PL

This version of the five parameter logistic equation has been developed by Brendan Scientific, creator of StatLIA immunoassay software. The Logistic-5PL regression type yields the best results for most assays; therefore, this is the default in Bio-Plex Manager. However, there may be cases where the Logistic-4PL equation gives slightly better results.

The Logistic-5PL equation is:

$$y = d + \frac{a - d}{\left[1 + \left(x/c\right)^b\right]^g}$$

where:

x is the concentration,

y is the response,

a is the estimated response at infinite concentration,

b is the slope of the tangent at midpoint,

c is the midrange concentration or midpoint,

d is the estimated response at zero concentration, and

g is an asymmetry factor.

Logistic-4PL

In certain cases, where the curve is a perfectly shaped "S," the Logistic-4PL regression may give better results. However, the Logistic-5PL regression has been shown to be the most robust routine for most Bio-Plex assays.

The Logistic-4PL equation is:

$$y = d + \frac{a - d}{1 + \left(x/c\right)^b}$$

where:

x is the concentration,

y is the response,

a is the estimated response at infinite concentration.

b is the slope of the tangent at midpoint,

c is the midrange concentration or midpoint, and

d is the estimated response at zero concentration,

Linear

The Linear equation is:

$$y = a + bx$$

Where *a* is the intercept and *b* is the slope of the line.

The simplest method for determining concentrations from a standard curve is to construct a plot using the linear portion of the response curve. The R-squared value may be used to determine the overall goodness of the linear fit. A linear regression with an R-squared value of greater then 0.99 is considered a very good fit.

The primary advantage of this method is that it is extremely simple. The primary disadvantage is that the linear range of concentrations is relatively small compared to that which may be obtained using nonlinear regression.

The Linear curve is available in linear and semi-log versions.

Cubic Spline

Cubic spline curves are smooth curves that go through every data point. The model is a cubic polynomial on each interval between data points. In some cases, a spline curve can work well as a standard curve for interpolation. However, because the curve is calculated individually for every pair of points, it does not correspond to any single equation.

The cubic spline curve is applicable to a wide range of assays; however, there are no built-in biochemical restrictions on the curve shape, and values cannot be extrapolated.[1,2]

Point-to-Point

A single equation is not available for the point-to-point method. The slope of each segment of the curve between data points is calculated independently.

The Point-to-Point curve is available in linear and semi-log versions.

Linear versus Logistic Regression Methods

In terms of performance, the primary difference between linear and logistic regression models is that the linear range in which concentrations can be accurately predicted is much smaller. In most cases, the overall response of the assay is best

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[1] The Immunoassay Handbook. David Wild, Editor. Nature Publishing Group, New York, NY. (2nd Edition, 2001).
[2] The GraphPad Guide to Nonlinear Regression. Harvey Moltusky, Ph.D. GraphPad Software, San Diego, CA.

modeled using a logistic fit and the Logistic-5PL model will yield the best results. For more information, see Bio-Rad bulletin 2861, available on our Web site (www.bio-rad.com) or from Technical Support (see page 4).

Nevertheless, in certain cases you may want to use linear regression to analyze the data. For most cytokines, the linear portion of the curve is well within the biological range of concentrations expected for patient samples. Thus, for serum the linear regression may be useful. The main advantage of a linear regression is that fewer points (as few as 2) can be used.

Copying the Standard Curve

To copy a bitmap image of the curve to the Windows clipboard, click in the *Standard Curve* window and select **Copy** from the *Edit* menu. You can then paste this image into your spreadsheets or other documents using the **Paste** command in your other applications.

You can also copy the regression method equation from below the standard curve by dragging your cursor over the equation to highlight it, and then selecting **Copy** from the *Edit* menu or right-click context menu.

Printing the Standard Curve

You can print the standard curve for the selected analyte using the **Print** command on the main toolbar or *File* menu. You can print the curve for all analytes using the **Print All Analytes** command on the main toolbar or *File* menu.

When you select a print command, a dialog box will open allowing you to specify the page layout of the printed curve.
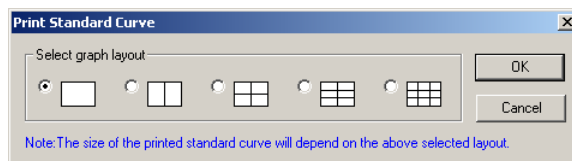
Figure 113. *Print Standard Curve* dialog.

The layout diagrams show the number of curves that can be printed on a page. If you are printing the curve for a single analyte, that curve will be sized based on the layout you select (e.g., the ⊞ layout will size the curve at one-quarter the full size of the page). The regression type will be printed below each curve.

Select a layout and click on **OK** to display the standard Windows® *Print* dialog.

XML Export

You can export all the information in a Results file as an XML-formatted text file for data sharing, import into Laboratory Information System databases, and data analysis using other applications.

> ➢ XML is a self-describing, universal file format compatible with a wide range of applications.

The XML document generated by Bio-Plex Manager includes all the data stored in the Results file, including but not limited to:

- Run parameters, including DD gate settings, instrument temperature settings, data limits, bead regions, etc.

- Run conditions, including time stamps per well, error conditions during the run, actual temperature during acquisition by well, etc.

- Complete data values and calculations (mean, median, standard deviation, coefficient of variation, etc.) per well

- Complete Protocol information, including analytes selected, plate formatting, standard values, run settings, etc.

- Raw bead event data (when selected)

- Standard curve information, including mathematical models used, how well the data fit the curve, etc.

- Instrument and software information, including version numbers

To export the Results data in XML format, go to the *File* menu and select **XML Document Export**.
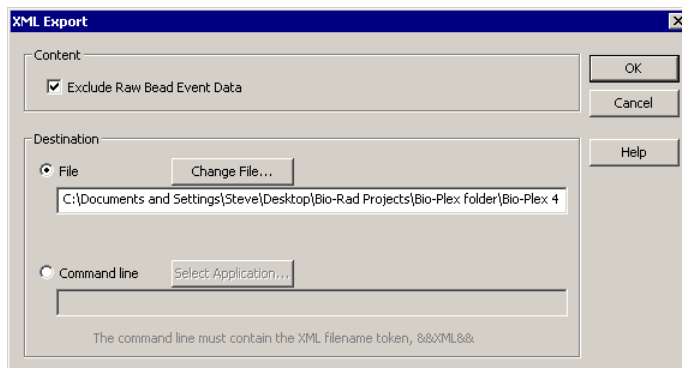
Figure 114. *XML Export* dialog.

In the *XML Export* dialog, select the **Exclude Raw Bead Event Data** checkbox to exclude this information from the export file.

Under **Destination**, specify whether you want to send the data to a file or display the data using another software application.

If you select **File**, you can use the default file name and location, or click on **Change File** to specify a new file name and location.

If you select **Command Line**, click on **Select Application** to locate the software application that you want to automatically launch when the file is generated. This application must accept a command line filename argument. After you locate and select the application, the application pathname will appear in the **Command Line** field enclosed in quotation marks, and the string "&&XML&&" will be automatically appended to it, as shown in Figure 115.

Note the following:

- If you manually enter the application pathname, it must be enclosed in quotation marks, as shown in Figure 115.
- The &&XML&& token must also be enclosed in quotation marks.
- If the application can be found by using the system executable search PATH, only the application name needs to be entered.
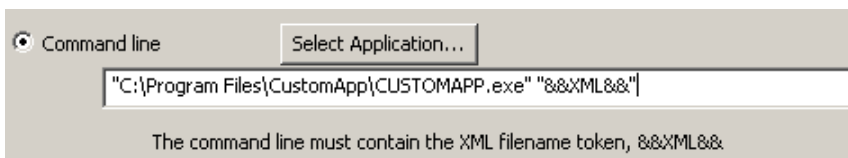
Figure 115. Example of a command line.

When you have made your selections, click on **OK**.
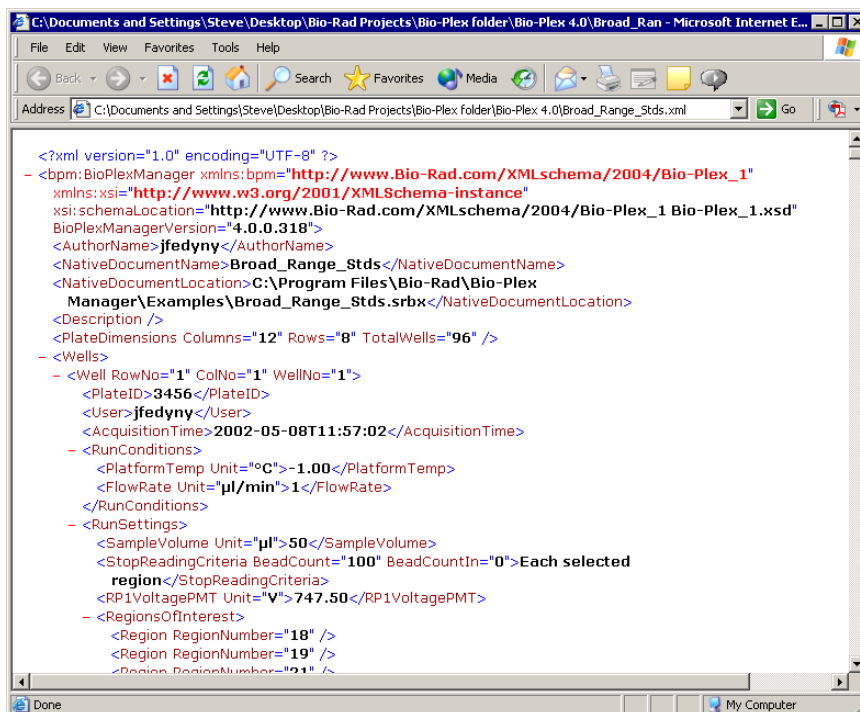
Figure 116. Example of an XML document.

You can set the default XML export settings using the *XML Export Properties* dialog. Go to the *File* menu and select **XML Export Properties** to open this dialog.
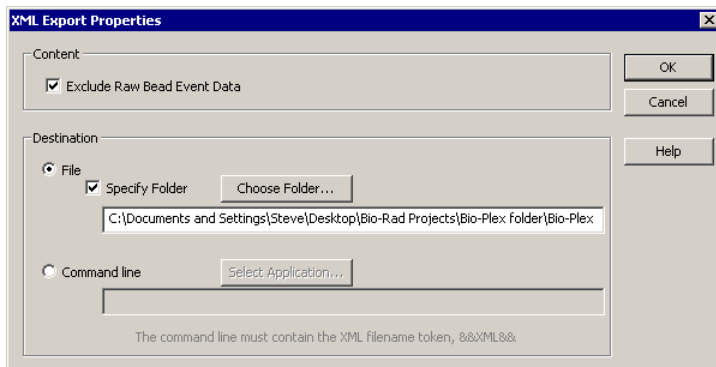
Figure 117. *XML Export Properties* dialog.

This dialog is similar to the *XML Export* dialog, except that it sets the default XML export settings and default folder where XML files will be saved. These settings are used if you select the **Auto XML Export After Run** checkbox in the *Advanced Run Settings* dialog (see page 91).

XML Schema File

An XML schema file named **Bio-Plex_2.xsd** is included in the main Bio-Plex Manager application directory. This schema file can be used to automatically validate the XML file.

9. Bio-Plex Manager Security Edition

Overview

Bio-Plex Manager Security Edition works in conjunction with the built-in security features of the Windows® 2000 and Windows® XP Professional operating systems to provide a secure environment for the maintenance, verification, and tracking of all electronic records generated by the application. These records include Protocol and Results files, and the Calibration, Validation, and Instrument Operations Logs. The tools provided in the software include:

- Access controls and authority checks via the use of user identification codes and passwords
- Electronic record security via the use of protected directories
- Time- and date-stamped audit trails
- Electronic signature of electronic records (user authentication)

When properly configured and administered, these tools ensure compliance with the rules for secure handling of electronic records as outlined in Title 21, Part 11 of the Code of Federal Regulations (CFR).

Background on 21 CFR Part 11

Effective August 20, 1997, the United States Food and Drug Administration (FDA) released Part 11 "Electronic Records; Electronic Signatures" of title 21 of CFR. This rule states the conditions under which the FDA considers electronic signatures and electronic records to be trustworthy, reliable, and equivalent to traditional handwritten signatures.

Bio-Plex Manager Security Edition is designed to enable organizations and institutions using the Bio-Plex suspension array system (or Luminex 100 IS 2.3 system) to comply with the rules laid out under 21 CFR Part 11. It enables system administrators to ensure that Bio-Plex Manager operates in compliance with 21 CFR

Part 11 within a "closed system." A closed system is defined as "*an environment in which system access is controlled by the persons who are responsible for the content of electronic records that are on the system*" (Section 11.3 (b) (4)).

Note that the security controls built into Bio-Plex Manager Security Edition must be properly configured and administered by the system administrator(s) in your organization. Otherwise, the system will not be secure and will not be in compliance with 21 CFR Part 11.

➢ Bio-Rad makes no claim that Bio-Plex Manager Security Edition is CFR compliant in and of itself, nor does it guarantee compliance for the user. Your organization must establish policies and standard operating procedures that work in conjunction with the tools provided by Bio-Rad to ensure compliance with 21 CFR Part 11.

Standard Mode Versus Secure Mode

Bio-Plex Manager Security Edition can run in Standard Mode, in which all the security and audit trail features are disabled and the software functions like the standard version of Bio-Plex Manager, or it can run in Secure Mode, with the security functions enabled. This chapter assumes that you are running the software in Secure Mode, unless otherwise noted.

➢ When you are in Secure Mode, the status bar displays a "locked" symbol 🔒. When you are in Standard Mode, the status bar displays an "unlocked" symbol 🔓.

System Requirements

Bio-Plex Manager Security Edition has the same system requirements as the Standard Edition, except that the security functions are not supported under the Windows® XP Home Edition operating system. The software must be installed on a computer running the Windows® 2000 or XP Professional operating system for full Secure Mode functionality.

Installing and Starting Bio-Plex Manager Security Edition

Security Edition is installed and starts up just like Standard Edition. After installation, the software will run in Standard Mode until an Administrator-level user (see next section) enables Secure Mode.

When you start the Bio-Plex Manager in Secure Mode, you will be prompted to log in with your user name and password.

➢ User names and passwords must be set up on your computer or computer network as described in the *Bio-Plex Manager Security Edition Configuration Setup Guide* before you can use the application in Secure Mode. This guide is provided with your Security Edition license.

Users, Passwords, and User Levels

In Secure Mode, Bio-Plex Manager Security Edition requires that users log in with a user name and password to run the software. User names and passwords are set up by your Windows® system administrator as described in the *Bio-Plex Manager Security Edition Configuration Setup Guide*.

➢ Contact your Windows® system administrator about issues regarding your user name and password.

Each user is assigned to a particular user level. There are six user levels in Bio-Plex Manager Security Edition, and each level gives the user access to specific features and functions of the software.

A table listing the specific features and functions accessible at each user level is provided in 0. The following is a general summary of each user level.

- **Administrator** — Users at this level can enable or disable Secure Mode. Administrator-level users also can view log files. Access to all other features and functions of the software is restricted.

- **Supervisor** — Users at this level have full access to all features and functions of the software, except that they cannot enable or disable Secure Mode.

- **Service** — Users at this level have full access to all features and functions of the software, except that they cannot enable or disable Secure Mode.

- **Clinician 2** — Users at this level can perform instrument operations, run existing protocols, and view Protocol files, Results files, and log files. They can also change the number of unknown samples in the plate format (using

the **Set Number of Unknown Samples** command) and enter sample descriptions. All other access is restricted.

- **Clinician 1** — Users at this level can perform instrument operations, run protocols, and view Protocol files, Results files, and log files, but cannot change any settings. All other access is restricted.

- **Reviewer** — Users at this level can view and sign Protocol and Results files, and can view log files. All other access is restricted.

User Level Restrictions

If you attempt to select a command or perform an action not allowed by your user level, you will receive a pop-up warning. In addition, many fields and settings that are restricted by your user level will appear grayed out in the application.

User Information

To display information about the current user, including user name, domain, full name, and access level, go to the *Tools* menu and select **User Info**. You can also click on the user name displayed in the application status bar.
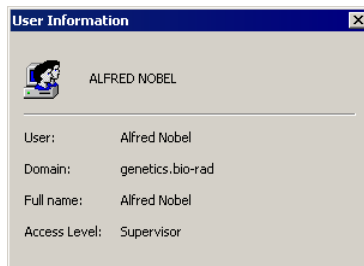
Figure 118. *User Information* dialog.

Enabling and Disabling Secure Mode

Only users with Administrator-level access can enable or disable Secure Mode. Enabling or disabling Secure Mode requires that you close all documents and restart the application.

When Secure Mode is enabled, the status bar will display the locked symbol.

Figure 119. Status bar showing disabled (left) and enabled (right) Secure Mode status

To enable Secure Mode, go to the *Tools* menu and select **Preferences**. In the *Preferences* dialog, select the **Enable Secure Mode** checkbox to enable Secure Mode, or deselect the checkbox to disable it.

If your user name and password are set up on a Windows® network server, enter the network domain where they reside in the **Domain to Be Used for Authentication** field. Contact your Windows® system administrator if you are unsure of your domain. Note that only users set up on the domain you specify will be able to use the software.

When you click on **OK**, you will be prompted to sign in as an Administrator-level user to authenticate the change. You will then need to restart the software.

User Authentication

Bio-Plex Manager Security Edition requires user authentication (entering a user name and password) at key points during operation. User authentication creates an electronic audit trail of user activity that cannot be altered or deleted.
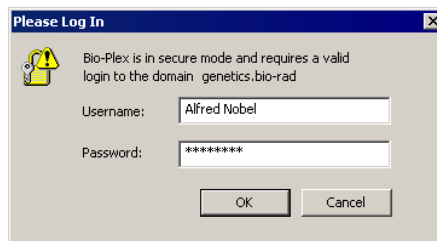
Figure 120. *Log In* dialog

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Users are required to enter their user name and password when they:

- Start the application
- Start a reading
- Unlock the application
- Sign a document, such as a Protocol or Results file

Different users may authenticate different operations. For example, a Clinician 1-level user may be logged into Bio-Plex Manager, but a Supervisor-level user may enter his user name and password to authenticate a particular reading. Note that in this case, the Clinician 1-level user will remain logged into the application.

Your organization must establish its own guidelines for user authentication of specific operations.

Electronic Records

Bio-Plex Manager Security Edition supports the creation and control of secure "electronic records," as defined by 21 CFR Part 11. In Bio-Plex Manager, the following are electronic records:

- Protocol files
- Results files
- Calibration Log (included in the bioplexdata.mdb file)
- Validation Log (included in the bioplexdata.mdb file)
- Instrument Operations Log (included in the bioplexdata.mdb file)

These records are only accessible through Bio-Plex Manager and its associated utilities.

File Security and Validation

The files used by Bio-Plex Manager Security Edition cannot be opened or edited using other programs. Bio-Plex Manager will check the integrity and validity of a file each time the file opens. If a file becomes damaged or shows signs of tampering with an outside application, it will not open in Bio-Plex Manager.

Calibration, Validation, and Instrument Operations Logs

These logs provide a permanent chronological record of all calibration, validation, and instrument operations events. You can view, copy, and print the data from these logs. They are available from the *View* menu in Bio-Plex Manager.

If the application is in Secure Mode, each event in a log will include the name and access level of the user who performed/authorized the action. If the application is in Standard Mode, the event will be flagged as having occurred outside Secure Mode and the access level will be labeled as "unrestricted."

These logs are stored in a database file called **bioplexdata.mdb**, which by default is saved in the main Bio-Plex Manager application folder on your computer (you can specify a different location during installation).

➢ Note that the bioplexdata.mdb database is not compatible with versions of Bio-Plex Manager earlier than 4.0. If you have an earlier version of the software, installing version 4.0 or later will copy the data from your existing database (bioplex.mdb) into the new database. A copy of your old database will remain in the application folder.

Secure Protocol and Results Files

Bio-Plex Manager Security Edition supports the creation of Secure Protocol and Results files. Secure files are documents that have been electronically "signed" by a user. Once signed, these files are controlled documents that cannot by overwritten by Bio-Plex Manager and that preserve a built-in audit trail of all saved changes.

Unsigned Protocol and Results Files

When you create a new Protocol file in Bio-Plex Manager Security Edition, it is initially unsigned and is not a Secure file. Protocol or Results files created with

previous versions of the software or created in Standard Mode are also unsigned, uncontrolled documents, and you will be notified that these files are not secure when you open them in Secure Mode. You can save and overwrite these files without restrictions and no audit trail will be generated for any changes.

If you make changes to a signed Protocol or Results file and save them as a new file, the new file will not be signed; however, the audit trail (page 159) will be preserved in the new file and the file will remain a Secure file.

➢ You cannot generate a new unsigned, uncontrolled Results file in Secure Mode; all Results files generated in Secure Mode must be signed.

Signed Protocol and Results Files

Protocol and Results files become Secure files when they are electronically signed using a user name and password. Once signed, these files become read-only; you can make changes to the file but they must be saved under a different file name. An audit trail of changes is built into each Secure Protocol and Results file.

Secure Protocol files have the file extension ***.spbx**. Secure Results files have the file extension ***.srbx**.

To sign a Protocol or Results file, go to the *File* menu and select **Sign Document**. The *Electronic Signature* dialog will open. (Note that this dialog will open automatically when a Results file is generated from a Protocol file.)
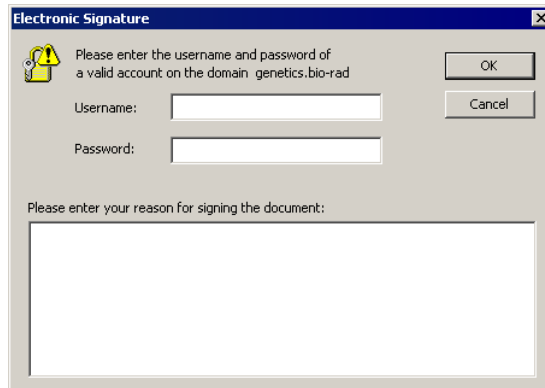
Figure 121. *Electronic Signature* dialog.

In the dialog, the signing party enters their user name and password in the appropriate fields. (Note that this may be someone other than the current user.) The reason for signing the document must be entered in the text field. Typical reasons may include review, approval, responsibility, or authorship.

When you click on **OK**, a *Save As* dialog will open.
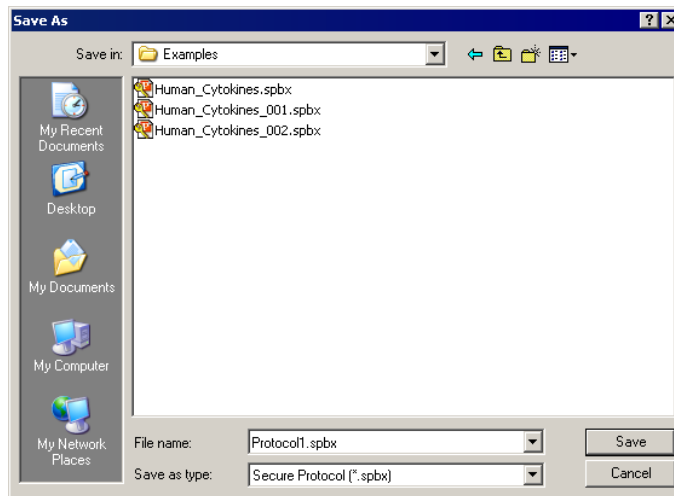
Figure 122. *Save As* dialog for Secure Protocol file.

Enter a name for the file or use the default name and click on **Save**.

After a file has been signed, it is flagged as **read-only** and cannot be modified. Any subsequent changes must be saved as a new file. Also, any changes made to a file after it has been signed will generate an audit trail documenting each change, as described starting on page 159.

A new file generated from a signed file can be signed again to verify and lock in any changes made to it. As before, the new signed file will be flagged as read-only and any subsequent changes must be saved under a new file name.

Document ID Number and Signature

Each signed Protocol and Results file has a unique document identifier (Document ID) number. To view this number, go to the *File* menu and select **Document Properties**.
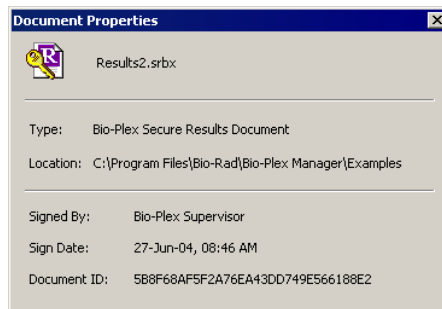
Figure 123. *Document Properties* dialog.

The *Document Properties* dialog also provides information about the signature.

The full name of the user who has signed the document is displayed in the title bar of the file window.
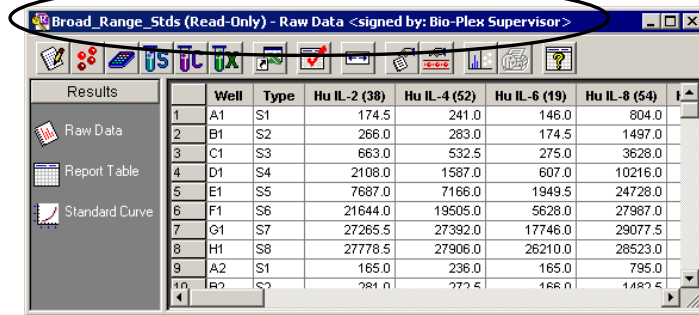
Figure 124. Signed and read-only status of file shown in title bar

All printouts and exports from a signed document also include the signature and Document ID number.
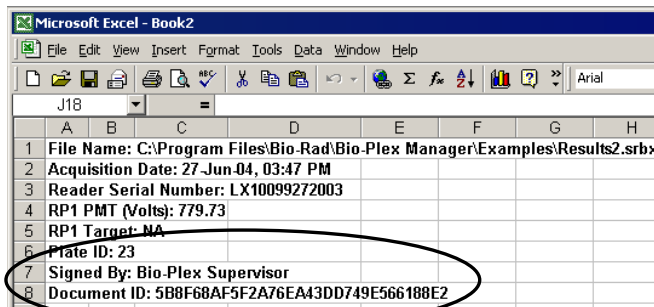
Figure 125. Example of a Document ID and signature in an Excel export file

Clinician 2 Users: Adjusting the Number of Unknown Samples in a Protocol

A Supervisor-level user can create a "master" Protocol file with a defined plate format, including a set number of unknown sample wells, and then a Clinician 2-level user can open the Protocol and adjust the number of unknown samples to be read in a particular reading. This enables Clinician 2-level users to use the same Protocol file in multiple readings with different numbers of samples.

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➢ Clinician 2-level users do not have access to the other plate formatting tools in Protocols. See pages 61–65 for information on defining unknown sample wells and replicates in Protocols.

With the Protocol file open and the *Format Plate* window displayed, click on the **Set Number of Unknown Samples** button #☒ in the formatting toolbar or select the command from the *Format Options* menu.

In the *Set Number of Samples* dialog, the maximum number of unknown samples defined in the plate template will be noted next to the field.
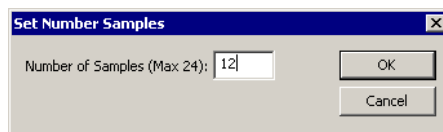
Figure 126. *Set Number of Samples* dialog.

➢ The maximum number of unknown samples is determined by the number of formatted unknown sample wells and the number of wells per replicate group defined in the template. For example, a template with 48 wells defined as unknowns and four wells per replicate will have a maximum number of 12 samples.

Enter the number of samples to use in a particular reading in the field and click on **OK**. The plate format will change to indicate the new number and layout of the sample wells. Only those wells will be read during a reading.

Note that you can only specify the number of unknown samples using this command—not standards, controls, or blanks.

If you save the Protocol file after changing the number of unknown samples, the number of samples you specified will become the new maximum number of samples. To preserve the original number of unknown samples, save the changed Protocol file under a different name.

Generating a Results File from a Protocol File

The Results file must be signed before a reading can begin. In Secure Mode, the **Auto Save After Run** checkbox in the *Run Protocol* window (see page 91) is automatically selected and cannot be deselected. You will be prompted to sign and save the Results file at the start of each reading.

➢ In Secure Mode, each reading, including reruns of plate wells, will generate a new Results file that cannot be overwritten.
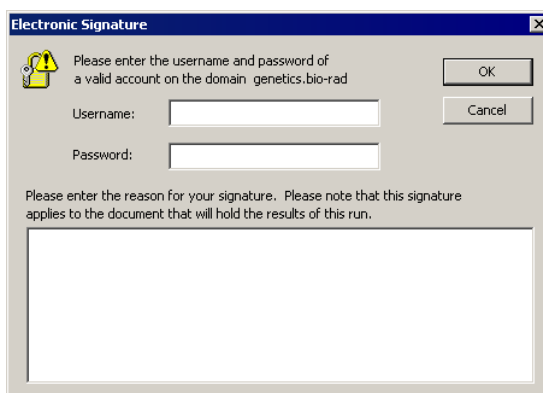
Figure 127. Electronic Signature required for a Results file before a reading

You will also be prompted to sign and save the Results file if you select the **New Results from Protocol** command from the *File* menu (see page 115).

Audit Trail

Any changes made or actions performed after a Protocol or Results file has been signed will automatically generate an audit trail documenting each change/action. If you make changes to a signed, read-only file and save it as a new file, the audit trail will be preserved in the new file (with the signature of the previous file noted as part of the audit trail). Also, the audit trail for a Protocol file will be preserved in the Results file generated from that Protocol.

All major actions and changes are audited. Examples of auditable actions include:

- Signing a file
- Changing Protocol settings (plate format, analyte selection, etc.)
- Changing the standard curve regression type, variance model, etc.
- Flagging outliers
- Performing readings (audited in the Results file)

Minor changes that affect the display only, such as selecting different columns in the Report Table or changing the axis transformation in the Standard Curve, are not audited.

Each change you make to a signed Protocol or Results file or a new file saved from a signed file must be documented in the *Reason for Change* dialog. The information in this dialog is used to generate the audit trail. The dialog opens automatically whenever you make a change and either save the document or switch between subwindows in the Protocol or Results file.

Figure 128. *Reason for Change* dialog, used to generate the audit trail
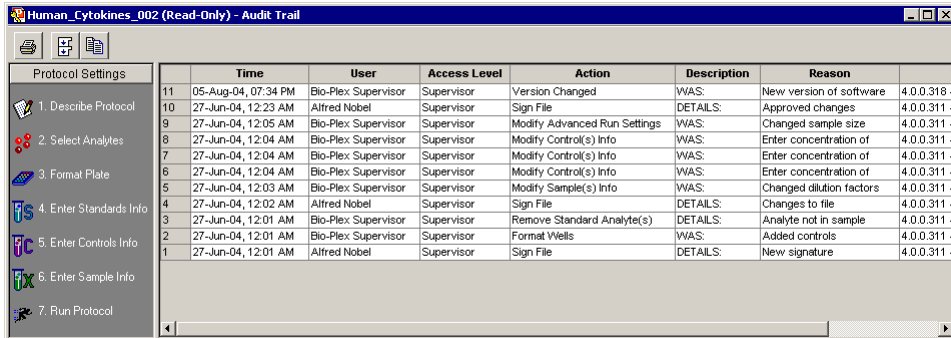
The dialog notes the date and time of each auditable action and describes the change/edit made. You must enter a reason for each change in the bottom field.

If you have made more than one change, use the **Next** and **Back** buttons to scroll through the changes. When you have entered a reason for each change, click on the **Finished** button.
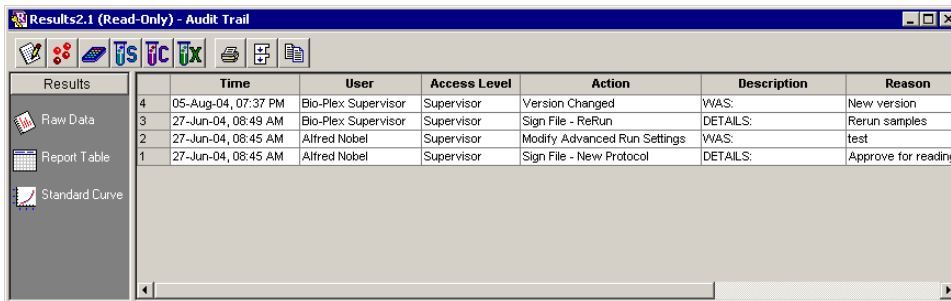
Viewing the Audit Trail

To view the audit trail for a Protocol or Results file, go to the *View* menu and select **Audit Trail**. The audit trail is displayed in a subwindow of the Protocol or Results file.

Figure 129. Audit trail for a Protocol file

Figure 130. Audit trail for a Results file

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The audit trail notes the date and time of each action, the user, the access level, the action, a description of the action (automatically generated by the software), the reason for the action (entered by the user), and the version of the software.

The audit trail also notes each time the file has been signed. In the example in Figure 131, note that each time the file was signed and made read-only, the audit trail was continued in the new file.

User	Access Level	Action
Alfred Nobel	Supervisor	Sign File
Bio-Plex Supervisor	Supervisor	Modify Advanced Run Settings
Bio-Plex Supervisor	Supervisor	Modify Control(s) Info
Bio-Plex Supervisor	Supervisor	Modify Control(s) Info
Bio-Plex Supervisor	Supervisor	Modify Control(s) Info
Bio-Plex Supervisor	Supervisor	Modify Sample(s) Info
Alfred Nobel	Supervisor	Sign File
Bio-Plex Supervisor	Supervisor	Remove Standard Analyte(s)
Bio-Plex Supervisor	Supervisor	Format Wells
Alfred Nobel	Supervisor	Sign File

New file:
Human_Cytokines_002.spbx

New file:
Human_Cytokines_001.spbx

New file:
Human_Cytokines.spbx

Figure 131. Example of a Protocol audit trail with signatures

To display the complete contents of the Description and Reason cells in the table, you can hold your cursor over a table cell and the information for that cell will be displayed in a context pop-up box. Or you can click on the **Expand Audit Entries** button in the audit trail window toolbar to expand all the cells.

Modify Control(s) Info	WAS:
Modify Sample(s) Info	WAS:
Sign File	DETAI
Remove Standard Analyte(s)	DETAI
Format Wells	WAS:
Sign File	DETAI

WAS:
Concentration:
All analytes:C1(0.0)
IS:
Concentration:
All analytes:C1(10.0)

Figure 132. Example of a pop-up information box in the audit trail

To copy the contents of the audit trail to the Windows® clipboard, click on the **Copy to Clipboard** button in the window toolbar. To print the audit trail, click on the **Print** button.

Protected Directories

Your Windows® system administrator may set up protected directories on your computer or computer network for the secure storage of Protocol and Results files. These protected directories must be set up using Windows® tools, and are keyed to your Windows® user name and password (which may be different than your Bio-Plex Manager user name and password). Consult your system administrator for information about protected directories.

Locking Bio-Plex Manager Security Edition

If you are stepping away from the computer and want to prevent anyone else from using Bio-Plex Manager Security Edition, you can lock the application. Once locked, the application can only be unlocked by the user who locked it or a Supervisor-level user.

➢ Locking Bio-Plex Manager will not shut down the application or end any instrument operations that are in progress.

To lock the application, go to the *Tools* menu and select **Lock Application**. You will be notified that the application is locked, and the *Log In* dialog will be displayed.

To unlock the application, enter your name and password and click on **OK**.

➢ If a Supervisor-level user unlocks the application, that Supervisor-level user will then be logged in as the current user.

Logging Off

When you close Bio-Plex Manager Security Edition, you will be automatically logged off. You can also log off without closing the application.

Note that you cannot log off while the array reader is in operation. Logging off will close all open documents. You will be prompted to save any changes before logging off.

To log off, go to the *Tools* menu and select **Log Off Application**. You will be notified that you have successfully logged off and the **Log In** dialog will be displayed.

Appendix

Troubleshooting Guide

Message/Problem	Cause	Solution
"Bio-Plex Manager has detected a problem with low bead number"	**Most Likely:**	
	Too few beads in the assay (5,000 per region recommended).	Check bead number calculations in assay.
	Plate not shaken for 30 seconds before analysis.	Remove plate from array reader and shake for 30 seconds.
	Buffer volume in wells is too low (must be at least 125μl).	Re-suspend wells in 125μl. Perform REMOVE BUBBLES.
	Microbubble in cuvette.	Perform REMOVE BUBBLES. Perform UNCLOG to verify fluidics integrity.
	Low/no sheath fluid.	Refill sheath fluid, check sheath connections. Perform START UP.
	Possible clog.	Perform UNCLOG and rerun. If unsuccessful, repeat. If still unsuccessful, contact technical support.
	Less Likely:	
	Incorrect needle height.	Adjust needle height.
	Incompatible plate type used.	Replace with flat bottom or filter plate and adjust needle height.
	Vacuum system not calibrated.	Calibrate vacuum system (see hardware manual).
	Red laser failure.	Contact technical support.
	Filter plate not flat.	Check filter plate flatness (see hardware manual).
	Incompatible suspension buffer used.	Check buffer compatibility (see hardware manual).

Message/Problem	Cause	Solution
"Bio-Plex Manager has detected a problem with selection of regions"	Incorrect bead regions were selected in the Protocol.	Compare bead regions in the assay with those selected in the Protocol.
	Incorrect regions were selected when preparing the assay.	Verify regions chosen during assay preparation.
	Too few beads in the assay in one or more regions.	Verify that the correct number of beads was used by referring to the specific assay preparation procedure.
" Bio-Plex Manager has detected a possible problem due to microbubbles in the cuvette or photobleached beads "	**Most likely:**	
	Calibration was performed before the array reader was warmed up.	Perform 30-minute WARM UP and recalibrate.
	Calibrated with incorrect CL1 or CL2 target values.	Check that the target values of the CAL beads match values entered in the software. If different, recalibrate.
	Array reader was calibrated with a dirty MCV Plate III.	Clean MCV Plate III and recalibrate.
	Misalignment of optics.	Perform OPTICS VALIDATION. Contact technical support if values are not within range.
	Less likely:	
	Microbubbles present in cuvette.	Perform REMOVE BUBBLES.
	Calibration beads are photobleached (do not expose to light for more than 1 hour).	Recalibrate with new CAL1 beads.

Message/Problem	Cause	Solution
"Bio-Plex Manager has detected a problem with bead aggregation"	Incorrect DD gate values used.	View histogram in Run Protocol window and make sure DD peak falls within the gate range. If necessary, adjust gates (see page 101).
	Incorrect DD target value used for calibration.	View histogram in Run Protocol window and make sure that the DD peak falls within the gate range. If running a Luminex-type assay from another manufacturer, use only Bio-Plex DD settings (targets and gates).
	Clumped beads present.	Vortex plate at 900 rpm for 1 minute.
	Sheath reservoir is empty.	Refill sheath reservoir, then perform START UP.
	Waste reservoir is overfilled.	Empty waste and reconnect.
	Incompatible suspension buffer used.	Check hardware manual for buffer compatibility.
"Check Link" in status bar of software	Array reader or microplate platform is not turned on.	Turn on array reader and microplate platform.
	Software is not communicating with array reader.	Close and restart Bio-Plex Manager.
	Cables between computer and array reader or microplate platform are loose/not connected.	Check cables for proper connections.
"Pressurizing" in status bar of software	Leak in sheath bottle or cap.	Tighten sheath cap.
Needle stuck in down position	Protective assay plate covering was not removed.	See hardware manual for procedure for raising needle stuck in down position. Then remove cover from assay plate.
	Needle guide is not screwed all the way in.	Tighten needle guide by turning tube clockwise until tight.
	Sample needle is bent.	Replace bent needle with a new needle (see hardware manual for procedure).

Message/Problem	Cause	Solution
No Assay Signal Detected	**Most likely:**	
	Error in assay preparation.	Make sure that streptavidin and cytokine standards have been added. Consult assay preparation procedure.
	Incorrect target values used in calibration.	Check that target values in the *Calibrate* dialog match the values on CAL1 and CAL2 bottles.
	Waste container overfilled.	Empty waste. Reconnect waste. Perform UNCLOG.
	Sheath reservoir low or empty.	Refill sheath reservoir. Perform START UP. Proceed with run.
	Less likely:	
	Waste line not connected properly.	Check waste line connection. Make sure that it clicks into place.
	Laser not functioning.	Perform calibration. If no signal detected, call technical support.
"Bio-Plex Manager has detected a change in sheath pressure"	**Most likely:**	
	Sheath reservoir cap not on securely.	Tighten sheath cap. Message should disappear within two minutes.
	Sheath bottle lines are not connected properly.	Make sure that all hoses are connected to the appropriate ports, and that they have clicked into place.
	Less likely:	
	Sheath fluid level above the AIR port on the sheath container.	Adjust sheath fluid level so that sheath fluid is below the AIR port of sheath bottle.
	Sheath bottle has a leak.	Try new sheath bottle. Call technical support for further assistance.
	High-throughput fluidics (HTF) system not turned on.	Turn on HTF and check to ensure that message disappears.
	Air Compressor not working.	Listen for air pump to turn on when WARM UP is selected. Contact technical support for further assistance.

Message/Problem	Cause	Solution
Bio-Plex Manager has detected a change in the temperature of the array reader. Please calibrate before running an assay to ensure accurate results.	Room temperature has changed.	Calibrate array reader.
The calibration was unsuccessful. Please repeat calibration. If calibration fails a second time, consult Troubleshooting Guide.	Calibration procedure failed.	Make sure CAL1 beads and CAL2 beads are placed in the appropriate wells (CAL1 in red well and CAL2 in green well). Repeat calibration. If problem persists, contact technical support.
The calibration was unsuccessful. Bio-Plex Manager has detected a problem with low bead number. Please repeat calibration.	Calibration procedure failed due to low bead number.	Run UNCLOG procedure, then repeat calibration. If problem persists, contact technical support.
Optics Validation Procedure shows value(s) outside of acceptable range(s).	Problem with optical component of array reader.	Repeat validation procedure. If values are still out of range, contact technical support.
Fluidics Validation Procedure shows value(s) outside of acceptable range(s).	Problem with fluidics component of array reader.	Repeat validation procedure. If values are still out of range, contact technical support.
Reporter Validation Procedure shows value(s) outside of acceptable range(s).	Problem with optical component of array reader.	Repeat validation procedure. If values are still out of range, contact technical support.
Classify Validation Procedure shows value(s) outside of acceptable range(s)	Problem with calibration or optical component of array reader.	Repeat validation procedure. If values are still out of range, contact technical support.

Message/Problem	Cause	Solution
Optics, Fluidics, Reporter, or Classify Validation Procedure fails due to low bead number	Beads not resuspended.	Resuspend beads and repeat validation procedure. If procedure still fails, contact technical support.
	Clog in instrument.	Perform UNCLOG operation and repeat validation procedure. If procedure still fails, contact technical support.
Platform heater not at target temperature	Insufficient warm-up time allowed.	Allow at least 15 minutes for platform heater to reach target temperature. If heater does not reach target in 30 minutes, contact technical support.
	Platform heater not functioning properly.	Contact technical support.
Platform heater not at ambient temperature	Insufficient cool-down time allowed.	Open platform door to facilitate cooling. An ice pack can also be placed on the platform plate holder to facilitate cooling.

Glossary

Accuracy	The closeness of a measured value to the actual value or the difference between expected and measured values. Using the Reporter Validation Kit, accuracy is defined as the percent difference between the measured regression curve and the expected or actual data points.
Aggregated microspheres	Microspheres that have associated. Two microspheres that have associated are called a doublet.
Analyte	The substance being measured, identified, quantitated or otherwise analyzed in an experiment. In the Bio-Plex system, analytes are molecules that are being quantitated.
Antibody	Immunological protein capable of binding a unique antigen via a specific binding site or epitope. Used in immunological assays to quantitate the amount of a specific analyte present.
Assay	The bead and reagent mixture that, when combined with sample, is loaded into microplate wells and is read by the array reader.
Background (noise)	Fluorescent intensity measured by the reporter channel that is due to non-specific binding of a fluorochrome and the electronic noise of the array reader. If you include blank wells in your microplates, you can calculate the average background intensity and subtract it from the intensity readings of your standards and unknowns.
Bead	A microsphere.
Calibration	A process that uses microspheres (calibrators) with a stable fluorescent intensity to adjust the gain settings in the array reader's detectors for optimal and consistent microsphere classifications and reporter readings, over time and across different array readers.
Calibrators	Microspheres with stable fluorescent intensities used to standardize the signal output of the array reader.
Capture antibody	An antibody used in sandwich immunoassays that binds or "captures" a specific analyte for quantitation.

Carboxylated microspheres	Microspheres with chemically modified surfaces such that they are covered with carboxyl functional groups (COOH) that allow covalent biomolecular attachment.
Classification channels	Fluorescent detection channels of the array reader that detect the fluorescent intensities of two dyes embedded in each microsphere. If the signals from the two dyes place the bead within a specified classification region, the bead is identified as a unique analyte in a sample.
Classification regions	Defined regions in a dot plot bead map that are used to identify beads in an assay based on the fluorescent signals from their embedded dyes.
Classify validation	A validation procedure for measuring the ability of the array reader to efficiently classify assay beads into specified regions.
Clumping	The aggregation of two or more microspheres.
Conjugated microspheres	A microsphere set that has been chemically modified so that its surfaces are covered with molecules that bind with the target analytes in an assay system.
Coupled microsphere	See conjugated microsphere.
Cytokine	An immunological protein that can signal a response in the immune system.
Detection antibody	An antibody used in capture sandwich immunoassays that binds a captured analyte to a fluorochrome, allowing for quantitation of the protein. Detection antibodies may be biotinylated, which allows for addition of a streptavidin-bound fluorochrome, or the detection antibody may be directly labeled with a fluorochrome.
Doublet	Two microspheres that have associated. The resulting signal of the associated microspheres is twice that of a single (singlet) microsphere.

Doublet discriminator	A channel that measures the amount of light scatter from particles that flow past the red laser. Light scatter is directly proportional to particle size, and the channel is designed to identify particles that are smaller or larger than a single microsphere, including microspheres that are clumped or aggregated.
Dynamic range	The calculated number of decades covered by the reporter channel scale of the array reader. Range is calculated using the Reporter Validation Kit.
Emission spectrum	The wavelength range of light emitted by an excited fluorochrome when its electrons fall from a higher to a lower energy state. Expressed in nanometers (nm).
Ex/Em	A common way of reporting a molecule's excitation and emission wavelengths (for example, Ex/Em = 488/520 nm).
Excitation spectrum	The wavelength range that excites a molecule's electrons to a higher energy state. Expressed in nanometers (nm).
External standard	A standard that has been imported from a different file.
Fit probability	A statistical measure of goodness of fit that incorporates the residual SSE. A fit probability of 1 indicates a perfect fit, while a fit probability of 0 indicates no fit.
Fluidics validation	The process of verifying the well-to-well carryover of beads in the array reader.
Fluorescence	The emission of light that occurs when the electrons of a fluorochrome drop to a lower energy state.
Fluorochrome	A fluorescent molecule.
Gate	An electronic method of discriminating or eliminating aggregated microspheres in the analysis of an assay. A gate separates singlet beads from aggregated beads.
Group	A group of wells defined on a plate. You can calculate the ratio of the fluorescent intensities of the group's member wells to the fluorescent intensity of a Reference well in the group.
Immunofluorescence	A technique that uses a covalently linked fluorochrome-antibody complex to detect or quantify a particular antigen.

Instrument Threshold	The instrument threshold is a measure of system noise, and is the reporter channel signal from a blank bead that contains no reporter dye.
Kinase	An enzyme that functions to phosphorylate specific proteins. Kinases are responsible for the activation of a variety of proteins through the process of phosphorylation.
Laser	A highly purified source of light used to excite fluorochrome electrons. An acronym for Light Amplification by Stimulated Emission of Radiation.
Linearity	A measurement of the coefficient of determination or R^2 value of a set of standard beads. An R^2 value of 0.995 or greater is an acceptable linearity value using the Bio-Plex validation kit.
Logistic regression	A regression model for binary (dichotomous) outcomes. The data are assumed to follow binomial distributions with probabilities that depend on the independent variables.
Median fluorescent intensity	A relative measurement of fluorescent intensity based on the median, a robust statistical measure.
Microparticle	A solid substance with a diameter in the micrometer range. Often used as a synonym for a microsphere.
Microsphere set	A set of multianalyte microspheres containing a unique mixture of two distinct fluorochromes to distinguish them from other multianalyte microspheres.
Microspheres	Latex spheres with a diameter in the micrometer range. Also called beads.
Multianalyte or multiplex	An analysis of several assays or tests performed simultaneously in the same reaction container.
Operational qualification	The process of determining that an instrument is fit for its intended use. The Bio-Plex Validation Kit is a tool for operational qualification.
Optics validation	The process for verifying the alignment of the optics assembly of the array reader.

Outlier	A well or replicate group of wells whose measured value is outside the normal range. You can exclude outliers from statistical calculations.
Panel	A group of related bead sets. For example, the Human Cytokine panel includes bead sets for Human IL-2, Human IL-4, Human IFN gamma, and other human cytokines. You can select multiple bead sets from the same panel or sets from different panels in the same multiplex assay.
PE	See phycoerythrin.
Phosphoprotein	An enzyme that functions to phosphorylate specific proteins. Phosphoproteins are responsible for the activation of a variety of proteins through the process of phosphorylation.
Photobleaching	A chemical reaction caused by exposure to light, in which a fluorochrome is converted into a differently fluorescent or non-fluorescent compound.
Photomultiplier tube	A light detector typically used in fluorescence detection systems, designed to convert a fluorescent signal into an electronic signal that can be quantitated.
Phycoerythrin (PE)	The fluorochrome used as the reporter molecule in Bio-Plex assays. Excitation = 546 nm, emission = 575 nm.
PMT	See photomultiplier tube
Precision	A measure of the reproducibility of replicate readings, usually represented by the coefficient of variation (CV%).
Protocol file (*.pbx or *.spbx)	A file containing the settings of a reading, including the microplate wells to read, the analytes to detect, sample size, etc. To perform a reading, you open a Protocol file, select the settings, and then run the Protocol. You can then save the Protocol settings and reuse or modify them. After a reading, a Results file is created containing the settings from the Protocol as well as the data results. The raw data from the most recent reading is also stored in the Protocol file. In Bio-Plex Manager Security Edition, Protocol files may be Secure files, in which case they have the file extension *.spbx.
Quality control	Procedures and guidelines that determine conformity to requirements.

Recovery percentage	A mechanism for assessing the fit of the standard curve to the actual standards. For each analyte standard, an observed concentration is back-calculated from the standard curve and the fluorescent intensity. This is divided by the expected concentration and multiplied by 100 to give the recovery percentage.
Recovery Range	The range of acceptable recovery percentages (see above). For example, a recovery range of 80–120% means that the observed concentration of a standard should be within 80–120% of the expected concentration. Concentrations of standards and unknowns that fall outside this range are flagged as unreliable.
Reference	A well within a group of wells defined on a plate that is used as a reference. You can calculate the ratio of the fluorescent intensity of the Reference well to the fluorescent intensities of the other wells in the group.
Region	The region of a fluorescent color map used to identify a particular bead set. Each bead set is embedded with specific quantities of two fluorescent dyes; the combination of these fluorochromes, as detected by the array reader, places the bead set within a unique region on the color map, thereby identifying the set and its associated analyte.
Reporter	A fluorescent molecule that is incorporated into an assay in such a way that the fluorescence intensity is directly proportional to the amount of analyte in an assay.
Reporter channel	The channel of the array reader that detects the fluorescent signal of the reporter molecule (phycoerythrin).
Reporter system	Any combination of molecules in solution that serves to quantitate the analytes in a particular assay.
Reporter validation	A standard method for verifying the performance of the reporter channel of the array reader. The five primary parameters that verify accurate performance of the reporter channel include linearity, dynamic range, sensitivity, accuracy and slope of the response.

Residual variance	A statistical measure of goodness of fit. This is the weighted sum of the squared errors between the observed response and the response predicted by the fitted curve (residual SSE), divided by the number of degrees of freedom. A small residual variance indicates a good curve fit.
Results file (*.rbx or *.srbx)	A file containing the results of a reading, including the raw data, the settings information from the Protocol, and tools for analyzing and exporting the data (tables, a standard curve, export functions, etc.). Each reading generates a new Results file. In Bio-Plex Manager Security Edition, Results files may be Secure files, in which case they have the file extension *.srbx.
Sample	A solution of analyte standards or unknowns that, when added to an assay, will bind to the microspheres in that assay and be identified and quantitated by the array reader.
Sampling error	A measure of the degree to which a sample differs from the entire population.
Sandwich immunoassay	Immunoassay for the quantitation of a specific analyte through the use of monoclonal antibodies (Ab). A sandwich is formed when an analyte of interest binds to a capture Ab, and then a second antibody (detection Ab) binds to a different epitope of the protein.
Secure file	A Protocol or Results file that has been electronically "signed" by a user. Once signed, this file is a controlled document that cannot by overwritten by Bio-Plex Manager and that preserves a built-in audit trail of all saved changes. If you make changes to a Secure file and save it as a new file, the new file will not be signed; however, the audit trail will be preserved in the new file and the file will remain a Secure file.
Sensitivity	The lowest detectable signal above instrument noise. Noise may be attributed to the lasers, the detectors and the amplification electronics of the array reader.
Signal	The detectable measurement unit of a reporter molecule.
Signal-to-noise ratio	The ratio of a specific assay signal to the underlying noise in the assay. Signal to noise is typically used to measure the sensitivity of an assay.

Singlet(s)	A single microsphere or a population of single microspheres. Compare to doublets which are two microspheres associated together.
Slope	Defined as the rise over the run when plotting a series of points to form a line. Using the Reporter Validation Kit, the slope of the response is related to the dynamic range of the array reader and yields information about the response of the photomultiplier tube (PMT).
Spectral address	The unique fluorescent emission spectra of a microsphere.
Standard deviation	The spread in individual data points (e.g., in a replicate group) to reflect the uncertainty of a single measurement.
Standard error	The standard deviation of a set of replicates divided by the square root of the number of replicates.
Unconjugated microspheres	A microsphere set that has been chemically modified so that its surfaces are covered with some functional group, but that has not been chemically prepared for use as a component in an assay system. These base microspheres do not carry biomolecular reactant.
Uniformity	The reproducibility of a signal over a series of replicate measurements.
Validation	A formal process for documenting that an instrument is fit for its intended use and is kept in an appropriate state of maintenance and calibration.
Validation kit	A set of reagents and procedures designed to verify the performance of the array reader apart from an assay. Bio-Plex Validation Kit 4.0 assesses the alignment of the optics assembly, measures the performance of the reporter channel, evaluates the fluidics system, and verifies the classify efficiency of the array reader.
Variance	The mean square deviation of a variable around the average value.

Table Error Codes

Certain data that cannot be measured or calculated will be flagged in the *Raw Data* and *Report* tables. Columns and their possible error codes are listed below:

Column	Error Code	Cause
FI	***	No data present
	---	Marked as an outlier
FI – Bkgd	***	No data present (negative values, if any, will be displayed)
	---	Marked as an outlier
SD	***	No data present
CV%	***	No data present
Obs Conc (4PL/5PL)	***	No data present
	OOR>	Out of Range - Above asymptote of equation
	OOR<	Out of Range - Below asymptote of equation
	OOR	Out of Range - Math Error
	*Value	Above or below standards FI
Obs Conc (Linear))	***	No data present
	OOR	Negative extrapolated data
	*Value	Positive extrapolated data or Data above/below the standards FI
Exp Conc	***	No data entry - this value is input by user
Obs/Exp*100	***	OOR displayed in Obs Conc column
Conc In Range	***	Obs Conc is not within the specified recovery range of standards conc (the recovery range is specified by the user; see page 81)
Sampling Error	1	Low bead number detected in the well
	2	Aggregated beads detected in the well
	3	Classify efficiency problem detected in the well
	4	Region selection problem detected in the well
	5	Platform temperature problem detected in the well

Security Edition: User Access by Function

The following table lists the functions and user levels in Bio-Plex Manager Security Edition. An X indicates that the user level has access to the function.

Function	Admin	Supervisor	Service	Clinician 2	Clinician 1	Reviewer
Functions that Apply to Whole Application						
Opening Files		X	X	X	X	X
Viewing All Screens (Not Modify)		X	X	X	X	X
View Audit Trail		X	X	X	X	X
Printing		X	X	X	X	X
Save		X	X	X	X	
Save As		X	X	X	X	
Exporting		X	X	X	X	X
Electronically Sign Document		X	X	X	X	X
Functions that Apply to Instrument Control						
Start Up		X	X	X	X	
Shut Down		X	X	X	X	
Warm Up		X	X	X	X	
Remove Bubbles		X	X	X	X	
Wash Between Plates		X	X	X	X	
Run Calibration		X	X	X	X	
Enter Calibration Lot/Exp Date		X	X	X	X	
Run Validation		X	X	X	X	
Enter Validation Lot/Exp Date		X	X	X	X	
Instrument - Set Up		X	X	X	X	
Instrument – Reconnect		X	X	X	X	
Instrument Info		X	X	X	X	
View Operation Log	X	X	X	X	X	X

Function	Admin	Supervisor	Service	Clinician 2	Clinician 1	Reviewer
View Calibration Log	X	X	X	X	X	X
View Validation Log	X	X	X	X	X	X
Functions that Apply to Protocol File — General						
New Protocol File		X	X			
New Results from Protocol		X	X	X	X	
Functions that Apply to Protocol File, Describe Protocol View						
Author Field		X	X			
Description Field		X	X			
Functions that Apply to Protocol File, Select Analytes View						
Select /Remove Analytes		X	X			
Add/Delete/Rename/Edit Panel		X	X			
Functions that Apply to Protocol File, Format Plate View						
Formatting Wells		X	X			
Change Number of Unknown Samples		X	X	X		
Functions that Apply to Protocol File, Enter Standards Info View						
Enter Standards Info (Description & Concentration)		X	X			
Enter Std Curve Regression Method		X	X			
Enter Std Curve Adv Settings		X	X			
Enter Std Concentration Units		X	X			
Enter Recovery Range		X	X			
Select External Standards		X	X			
Functions that Apply to Protocol File, Enter Sample Info View						
Enter Sample Info (Sample Description)		X	X	X		
Enter Sample Info (Sample Dilution)		X	X	X		
Functions that Apply to Protocol File, Enter Controls Info View						
Enter Controls Info		X	X			
Functions that Apply to Protocol File, Run Protocol View						
Start a Run		X	X	X	X	
Rerun/Recovery Mode		X	X	X	X	

Function	Admin	Supervisor	Service	Clinician 2	Clinician 1	Reviewer
Select Beads to Count		X	X			
Select Bead Count Method (Per Well/Per Analyte)		X	X			
Set Sample Timeout		X	X			
Advanced Settings		X	X			
Change Raw Data Display Options		X	X	X	X	X
Print Histogram & Bead Map		X	X	X	X	X
Histogram - Restore Default Gates		X	X			
Histogram – Adjust DD Gates		X	X			
Histogram – Autoscale		X	X	X	X	X
Histogram - Log/Linear View		X	X	X	X	X
Histogram – Zoom		X	X	X	X	X
Histogram - Max/Restore		X	X	X	X	X
Bead Map - Log/Lin		X	X	X	X	X
Bead Map – Zoom		X	X	X	X	X
Bead Map - Max/Restore		X	X	X	X	X
Functions that Apply to Results File — General						
New Protocol from Results		X	X			
Change DD Gates		X	X			
Refresh Calculations		X	X	X	X	X
Modify Plate ID		X	X			
Functions that Apply to Results File, Describe Protocol View						
Author Field		X	X			
Description Field		X	X			
Functions that Apply to Results File, Select Analytes View						
Select /Remove Analytes		X	X			
Add/Delete/Rename/Edit Panel		X	X			
Functions that Apply to Results File, Format Plate View						
Formatting Wells		X	X			
Functions that Apply to Results File, Enter Standards Info View						
Enter Standards Info (Description & Concentration)		X	X			

Function	Admin	Supervisor	Service	Clinician 2	Clinician 1	Reviewer
Enter Std Curve Regression Method		X	X			
Enter Std Curve Adv Settings		X	X			
Enter Std Concentration Units		X	X			
Enter Recovery Range		X	X			
Select External Standards		X	X			
Functions that Apply to Results File, Enter Sample Info View						
Enter Sample Info (Sample Description)		X	X			
Enter Sample Info (Sample Dilution)		X	X			
Functions that Apply to Results File, Enter Controls Info View						
Enter Controls Info (Desc, Conc, & Dilution)		X	X			
Functions that Apply to Results File, Raw Data View						
Change Raw Data Display Options		X	X	X	X	X
Set/Clear Outliers		X	X			
Functions that Apply to Results File, Report Data View						
Set/Clear Outliers		X	X			
Show/Hide Outliers		X	X	X	X	X
Report Table Options		X	X	X	X	X
Organize by Type		X	X	X	X	X
Organize by Group		X	X	X	X	X
Show Replicates		X	X	X	X	X
Sort		X	X	X	X	X
Show Help for Table Legend		X	X	X	X	X
Select Analytes to View		X	X	X	X	X
Functions that Apply to Results File, Standard Curve View						
Regression Type		X	X			
Same Regression Type for All Analytes		X	X			
Logistic Weighting Options		X	X			
Labels		X	X	X	X	X
Error Bars		X	X	X	X	X

Function	Admin	Supervisor	Service	Clinician 2	Clinician 1	Reviewer
Analyte		X	X	X	X	X
Axis Transformation		X	X	X	X	X
Swap XY Axes		X	X	X	X	X
Turning On and Off Secure Mode						
Turn On/Off Secure Mode	X					
Unlock Application Override		X				

Note: System Administrator Creates Groups And Assign Users To Groups

Basic Concepts

The following is a brief overview of the basic principles of the Bio-Plex suspension array system. For more information, see *Practical Flow Cytometry*, 3rd edition, by Howard M. Shapiro, M.D. (New York: Wiley-Liss Inc., 1995).

Microspheres

xMAP microspheres are highly uniform, 5.5-micron polystyrene beads that have been crosslinked during polymerization for physical and thermal stability. Microspheres are grouped into sets; each set is then embedded with specific quantities of two fluorescent dyes. The ratio of these two dyes gives each set of microspheres a unique spectral address.

Each set of microspheres is then conjugated with a different reactant specific for a particular target analyte. Reactants can include enzyme substrates, receptors, antigens, and antibodies. A conjugated bead-reactant mixture can be mixed with a sample to create, for example, a capture sandwich immunoassay.

➢ To ensure the stability of the spectral address, it is essential to protect the microspheres from light. Do not subject microspheres to prolonged high temperatures, and protect them from freeze/thaw manipulations.

Reporter fluorochromes

When you mix your samples with the microspheres, the target analytes bind to the reactants on the surface of the beads. The amount of analyte that binds to the beads is then quantitated through the use of a fluorochrome, R-phycoerythrin. The intensity of this fluorochrome as detected by the array reader is directly proportional to the amount of analyte present. The excitation wavelength of the array reader reporter channel is 532 nm, while the emission wavelength is 575 ± 12nm. Specific information on R-phycoerythrin is shown in the following table:

	R-Phycoerythrin
Formula weight (Daltons)	240,000
Absorbance max (nm)	480, 546, 565
Extinction max (M-1cm-1)	1,960,000
Emission max (nm)	578
Quantum yield	0.82

Fluidics

There are two fluidic paths in the array reader. The first path includes a syringe-driven mechanism that controls the sample uptake. This mechanism permits small sample uptake volumes from small reaction volumes. The syringe-driven system transports a user-specified volume of sample from a microplate well to the cuvette. The sample is injected into the cuvette at a steady rate, and the microspheres in the sample are aligned in a single file through the path of the two lasers.

Following analysis, the first sample path is automatically purged with sheath buffer fluid via the second fluidics path. This process effectively removes residual sample in the tubing, valves, and sample needle.

Excitation

There are two lasers in the array reader: one for classifying each microsphere and its associated analyte, and the other for quantitating the amount of analyte bound to each microsphere. The first laser, called the classification or "red" laser, excites the dyes embedded in each microsphere; the fluorescent signal is discriminated with selective emission filters and converted into intensity units by fluorescence detectors and a digital signal processor, and the microsphere is classified. The second laser, called the reporter or "green" laser, excites the fluorochromes bound to the target analytes on the surface of the microspheres; again, the fluorescent signal is discriminated with selective emission filters and converted into intensity units by fluorescence detectors and a digital signal processor, and the amount of analyte is quantitated.

Microsphere Handling

Microsphere Dispersion

xMAP microspheres will settle and aggregate if left undisturbed. You should always ensure that they are evenly distributed before dispensing. Gentle vortexing is the most effective method of mixing for most coated microsphere preparations; however, sonication can also be used to separate aggregated microspheres prior to coupling. After sonication, microspheres generally remain dispersed for about 1 hour. Sonication is most effective if applied to a centrifuged pellet of microspheres. In order to maintain bead concentration, we suggest that you centrifuge your stock beads before sonication.

The following two indirect sonication methods are effective at separating aggregates in closed containers of microsphere preparations.

Inserting a sonicator probe into preparations is not recommended

Probe Sonicator

To disperse a microsphere pellet using a probe sonicator:

1. Place probe tip in a small bath of water.
2. Insert the end of the microsphere tube near the tip of the sonicator probe, without touching it. Do not immerse the tube closure.
3. Adjust sonication for optimal disruption and pulse sonicate until the microsphere pellet is dispersed.

Bath Sonicator

To disperse a microsphere pellet using a bath sonicator:

1. Turn on the bath sonicator and examine the surface for an area of maximum disruption.
2. Insert the end of the tube into the sonicator bath near the area of maximum disruption.
3. Sonicate until the microsphere pellet is dispersed.

Enumeration of Microsphere Suspensions

xMAP microspheres are provided at standard concentrations. The microsphere yield after a coupling process may be less than the starting concentration due to loss during wash steps. The microsphere loss can vary according to operator technique, coupled reactant properties, and scale of coupling.

It is not advisable to construct an assay without defining the concentration of the subject-coupled microsphere preparation. The total surface area (total number of microspheres) represented in an assay is a critical variable, so the optimization and control of this variable begins with manipulation of known microsphere numbers. Microspheres can be counted with a hemocytometer. Please follow counting and calculation methods outlined in the instructions for your hemocytometer.

Microsphere separation methods

Bio-Plex assay development often requires separation of microspheres from an aqueous matrix.

Centrifugation

Microsphere coupling protocols require separation of microspheres from reaction mixtures or wash buffers by means of centrifugation. Generally, a tabletop microcentrifuge that can spin 1.5 ml microcentrifuge tubes can pellet microspheres in 1–2 minutes. The g-forces vary in these instruments from approximately 6,000 to $13,000 \times g$.

Vacuum

A vacuum separation method can be used for routine high throughput washing required for washed assay procedures. In this case, the microsphere reactions are performed in microtiter filter-bottom 96-well plates. A vacuum is applied to the plate allowing the liquid to filter through while retaining the microspheres on the filter. Resuspension is accomplished by adding adequate fluid to each well and by repetitive vigorous pipetting.

Microsphere Agitation During Assay

Agitation of Bio-Plex assays with a plate shaker during incubation steps prevents microspheres from settling during extended incubation periods. The benefit of agitation may not be discernible for some assay conditions and should be evaluated according to the requirements of a given application. It is important to resuspend the beads in an assay for 30 seconds to performing a reading.

Microsphere Stability and Storage

xMAP microspheres are light sensitive and should be protected from light during all stages of storage and usage. In addition, freezing conditions and organic solvents should be avoided.

Reporting Problems

Included in your Bio-Plex Manager installation is a program called Solobug. You can use this program to request features and design changes, or report noncritical problems.

> For critical problems, contact Bio-Rad technical support at the numbers listed on page 12.

To use Solobug, select **Solobug** from the *Start* menu, enter your information and a description of the request in the Solobug window, and click on **Save**. This will create a report that you can attach to an e-mail and send to Bio-Rad.

Please send your Solobug files to Bio-Rad at:

LSG.TechServ.US@Bio-Rad.com (in the U.S.)

LSG.TechServ.Intl@Bio-Rad.com (International)

Software Warranty

Bio-Rad Laboratories warrants that Bio-Plex Manager software shall substantially conform, in all operational features, to Bio-Rad's current specifications as published in Bio-Rad's user and installation guides and that, when properly installed, it will be free of material defects which affect system performance.

The Purchaser must notify Bio-Rad in writing, within 30 days of delivery of the software (not including delivery of any subsequent modifications to the software), of any defect. If the software is found to be defective by Bio-Rad, Bio-Rad's sole obligation under this warranty is to remedy the defect in a manner consistent with Bio-Rad's regular business practices. For a defect which adversely affects the performance of the software, Bio-Rad shall use its best efforts to cure such defect as soon as reasonably practicable after receipt of Purchaser's notice. For minor defects, Bio-Rad shall use its best efforts to correct such minor defects in the next release of its software. If, however, Bio-Rad is unable to cure a major defect within 90 days of receipt of Purchaser's notice, Purchaser shall have the option to cancel this agreement, whereupon Bio-Rad shall refund only the software fees paid.

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