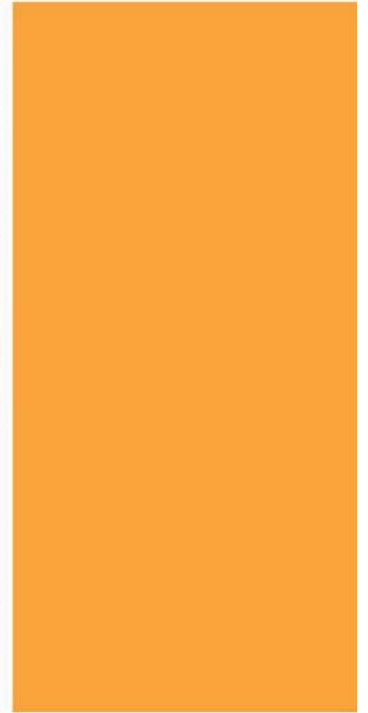


## Multiplex Suspension Array



# Bio-Plex<sup>®</sup> 200 System

Hardware Instruction Manual





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# Section 1

## General Information

### 1.1 About This Manual

A Bio-Rad service engineer will install the Bio-Plex® 200 system. However, the procedure is provided herein as a reference, in addition to instructions for maintaining your Bio-Plex 200 system. This manual uses certain conventions to facilitate understanding of the text material and to assist operators in using the Bio-Plex 200 system.

#### Conventions

Left and right sides of the system components are as viewed from the front (operator's position) unless otherwise stated.

#### Notes, Cautions, and Warnings

Notes, cautions, and warnings are used to highlight certain operating procedures and recommendations.

A note indicates a special procedure, an exception to normal operation, or something else of specific interest to the reader. Notes are preceded by the word "Note" in italics.

The following symbols describe the warning and cautions used in the operation of this instrument.

#### Warning Symbols



General Warning



Puncture Hazard



Pinch Point Hazard

(See manual for specific areas where these symbols may be found.)

### 1.2 Safety Information

Your safety and the safety of others are very important to us. To help you make informed decisions about safety, we have provided comprehensive operating procedures and safety information in this manual and on labels affixed to instrumentation. This information will alert you to any potential hazards. Please review the safety information contained in this manual.

The user should be present during operation of the Bio-Plex 200 system. This system contains electrical, mechanical, and laser components that, if handled improperly, are potentially harmful. In addition, biological hazards may be present during system operation. Therefore, Bio-Rad recommends that all Bio-Plex 200 system users become familiar with the specific safety advisory below, in addition to adherence to standard laboratory safety practices. The protection provided by the equipment may be impaired or the warranty voided if the equipment is used in a manner not specified by Bio-Rad Laboratories, Inc.

### 1.2.1 Electrical Safety Information



**Warning:** This instrument must be connected to an approved power source.



**Warning:** Do not perform any maintenance or cleaning of the electrical components (except for fuses) of this instrument.



**Warning:** This system contains fluidics. In the event of a fluid leak, turn off all power to the system and disconnect all power cords. Contact Bio-Rad Technical Support for further information.

*Note:* Waste levels must be manually monitored. Do not allow the waste container to overflow! Empty the waste container each time sheath fluid is filled. The waste container should not be placed on top of the Bio-Plex array reader.

### 1.2.2 Laser Safety Information



**Caution:** Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous laser radiation exposure.

This instrument and its accessories are certified according to US FDA 21 CFR 1040.10 of the Center for Devices, Radiological Health (CDRH) as a class 1 laser device. The two lasers contained within the array reader produce diode laser energy of up to 10 mW at 532 nm (reporter laser) and 635 nm (classification laser).

The United States and international regulations require the following warnings to appear on the instrument during operation and maintenance. These labels appear on the back panel of the instrument:



**CLASS 1 LASER PRODUCT**  
**IEC 60825-1 SAFETY OF LASER PRODUCTS 1998 - 01**

**Complies with 21 CFR 1040.10 and 1040.11**

**Do not remove cover.**

**No user serviceable parts inside.**

**CAUTION**

Laser radiation when open!

**DO NOT STARE INTO BEAM OR VIEW DIRECTLY  
WITH OPTICAL INSTRUMENTS!**

**NE PAS REGARDER DANS LE FAISCEAU NI A L'OEIL  
NI A L'AIDE D'INSTRUMENTS D'OPTIQUE**

**Caution:** Removal of the array reader cover is intended for trained service personnel only. Do not attempt to operate the instrument with the cover removed. When routine maintenance is performed, power to the instrument must be OFF and the power cord must be disconnected.

This label appears on the back of the instrument:

**CAUTION**

**LASER RADIATION WHEN OPEN  
AVOID EXPOSURE TO BEAM**

**CAUTION**

**RAYONNEMENT LASER EN CAS  
D'OUVERTURE EXPOSITION  
DANGEREUSE AU FAISCEAU**

All laser apertures are located within the instrument and are contained within a protective housing. This label appears next to the laser apertures, located inside the optics enclosure, enclosed in the instrument:

**AVOID EXPOSURE**

**Laser radiation is emitted  
from this aperture**



### 1.2.3 Mechanical Safety Information



**Caution:** During operation, this system contains exposed, moving parts. Risk of personal injury is present. Keep hands and fingers away from the sample probe and the syringe arm, as well as the microplate platform during operation.



*Note:* Access doors must be closed while operating the Bio-Plex 200 system.

### 1.2.4 Biological Safety Information



**Warning:** All human and animal samples may contain hazardous infectious agents. Follow appropriate biosafety procedures when handling these products and any containers.

Observe all local, state, and federal biohazard handling regulations when disposing of biohazardous waste material.

### 1.2.5 Blue Indicator Light

*Note:* The blue lights above the sample arm, on the microplate platform, and on the high-throughput fluidics (HTF) system indicate the on/off status of the respective system components. The blue light emitting diode (LED) does not emit laser light or light in the UV spectrum.

## Section 2 Introduction

### 2.1 The Bio-Plex<sup>®</sup> Suspension Array System and Multiplexing Technology

The Bio-Plex suspension array system is a unique and complete system comprising a 96-well fluorescent microplate reader, Bio-Plex Manager™ software, validation and calibration kits, and assays. The system is designed, manufactured, and tested as a fully integrated system to ensure accurate and reproducible assay results that are comparable across different laboratories. Centered around a flow-based dual laser detector with real-time digital signal processing, the Bio-Plex 200 system is able to distinguish up to 100 different families of color-coded, monodisperse polystyrene beads, each bearing a different homogeneous capture assay (but all using the same signal molecule) in a single 50 µl sample. This high degree of multiplexing dramatically increases the amount of useful information from rare or volume-limited samples, such as mouse and rat serum, and allows you to investigate analyte and biomarker interrelationships that would not have been possible with traditional analysis systems. A microplate platform allows the automated analysis of 96-well plates. The throughput of samples using this system will allow analysis of more than 9,600 assay points in 30 min in a 96-well plate.

The Bio-Plex suspension array system uses up to 100 color-coded bead sets, each of which may be conjugated with a unique specific reactant. Each reactant is specific for a different target analyte. Reactants can include enzyme substrates, receptors, antigens, and antibodies to create, for example, a capture sandwich immunoassay. To perform a multiplex assay, sample and reporter molecules are allowed to react with the conjugated bead mixture in microplate wells. The flow-based Bio-Plex 200 system identifies each specific reaction based on bead color and quantitates it. The magnitude of the reaction is measured using fluorescently labeled reporter molecules also specific for each target analyte. Bio-Plex Manager software automates data analysis and generation of detailed summary reports. With the Bio-Plex suspension array system you can:

- Simultaneously quantitate up to 100 analytes per sample from culture media and serum
- Automatically analyze up to 96 samples in 30 min
- Instantly customize your assay by mixing Bio-Plex assays, or create your own assays
- Dramatically increase the amount of useful data obtained from a single sample

For more specific or updated information, visit us at [www.bio-rad.com/bio-plex/](http://www.bio-rad.com/bio-plex/)

## 2.2 Description of System Components

The Bio-Plex 200 system is comprised of the following components:

- Array reader — combines 2 lasers, fluidics, and real-time digital signal processing to distinguish up to 100 different color-coded different color-coded bead sets, each representing a different assay
- Microplate platform — automates the reading of 96-well plates, yielding up to 9,600 data points in ~ 35 min
- PC and monitor — controls the Bio-Plex suspension array system via Bio-Plex Manager software
- MCV (maintenance, calibration, and validation) plate IV — automates the maintenance, calibration, and validation functions of the array reader
- Bio-Plex reservoir — allows maintenance functions to be seamlessly run before and after a protocol resulting in improved walkaway capability
- Calibration kit — contains beads to standardize daily signal output and ensure unit-to-unit reproducibility of the reader
- Validation kit — contains beads to validate the operational specifications of the reader, including accuracy, linearity, dynamic range, slope, fluidics, and optical alignment
- Optional HTF — delivers up to 20 L of sheath fluid without user intervention
- Sheath fluid cube — contains 20 L of sheath fluid (1x) for the array reader

### 2.2.1 Array Reader

The array reader (Figure 1) is a compact flow analysis unit integrating a dual laser detection system, optics, fluidics, and advanced digital signal processing. When used with the microplate platform, the array reader facilitates the simultaneous analysis of up to 100 different analytes from a single sample. The features of the array reader are outlined in Table 1.



Fig. 1. Array reader – front and side panel features.

**Table 1. Array Reader Front and Side Panel Features.**

<b>Feature</b>	<b>Description</b>
<b>Sample arm</b>	The sample arm transports the sample from the 96-well microtiter plate in the microplate platform to the cuvette. Upon operation, the carriage drops automatically to the microtiter plate for sample retrieval.
<b>Sample needle</b>	A stainless-steel sample needle acquires sample from the 96-well plate in the microplate platform.
<b>Chem inert fitting</b>	Covered by the sample arm cover, this fitting may be disconnected to allow replacement of the sample needle if necessary.
<b>Access doors</b>	There are two access doors on the face of the array reader. The centermost door allows access to the syringe. The left door provides access to the sheath filter.
<b>Air, waste fluid, and sheath fluid connectors</b>	Located on the side of the instrument, these connectors couple directly to the sheath and waste fluid connectors. The air connector is green, the sheath connector is blue, and the waste fluid connector is orange.

The rear panel features of the array reader are shown in Figure 2 and described in Table 2.



**Fig. 2. Array reader – rear panel features.**

**Table 2. Array Reader Rear Panel Features.**

Feature	Description
<b>Communications port P1</b>	The DB9-PIN connector is used to connect the array reader to the computer.
<b>Communications port P2</b>	The DB9-PIN connector is used to connect the array reader to an HTF.
<b>Air filter and access door</b>	A replaceable filter cleans the air used to pressurize sheath fluid. This filter is enclosed behind an access door. Refer to the Care and Maintenance section (page 27) for routine maintenance procedures.
<b>Ventilation filter (not shown)</b>	Located on the bottom of the instrument, the ventilation filter must be checked and cleaned as necessary. Refer to the Care and Maintenance section (page 28) for cleaning procedures.
<b>Power connector</b>	Contains the instrument on/off switch and fuses. Refer to the Care and Maintenance section (Section 4, page 29) for fuse replacement instructions.



**Fig. 3A. Sheath filter internal fluidics features.**



**Fig. 3B. Internal fluidics features.**

**Table 3. Array Reader Internal Fluidics Features.**

Feature	Description
<b>Syringe</b>	Located behind the center door immediately to the left of the sample needle assembly, the syringe delivers sample from the 96-well microplate to the cuvette via an intermediate sample loop.
<b>Sample loop (not shown)</b>	The sample is drawn into the sample loop by the syringe pump and injected into the cuvette for analysis.
<b>Sheath filter</b>	This filter removes particles greater than 5 µm in diameter from the sheath fluid. Refer to the Care and Maintenance section (page 28) for routine maintenance instructions.

## 2.2.2 Microplate Platform

The microplate platform (Figures 4 and 5) allows the automated processing of samples from a 96-well microplate. The features of the microplate platform are outlined in Table 4.

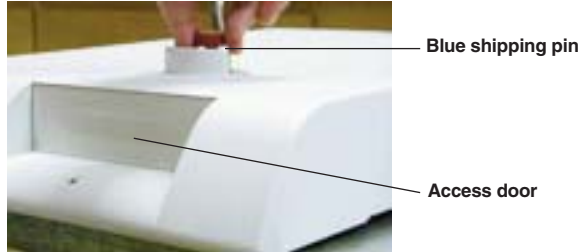


Fig. 4. Microplate platform – front view.



Fig. 5. Microplate platform – back panel view.

**Table 4. Microplate Platform Features.**

Feature	Description
<b>Access door</b>	This door provides access to the assay plate holder. Operation of the access door is controlled through the system software.
<b>Blue shipping pin</b>	A temporary fitting for shipping purposes.
<b>Communications port</b>	A DB9-PIN connector used to connect the microplate platform to the computer.
<b>Power connector</b>	Contains the instrument on/off switch and fuses. Refer to the Care and Maintenance section (page 29) for fuse replacement instructions.

### 2.2.3 High-Throughput Fluidics (HTF)

The Bio-Plex HTF (high-throughput fluidics) is designed to automate the introduction of sheath fluid into the array reader (Figures 6 and 7). With the HTF, you can run samples continuously without the need to replenish the sheath supply. The HTF automatically draws sheath fluid from a nonpressurized bulk container to constantly maintain a reservoir of pressurized sheath fluid. A single 20 L container provides enough sheath fluid for 48 hr or more of normal operation, or forty 96-well assay plates. The features of the HTF are outlined in Table 5.

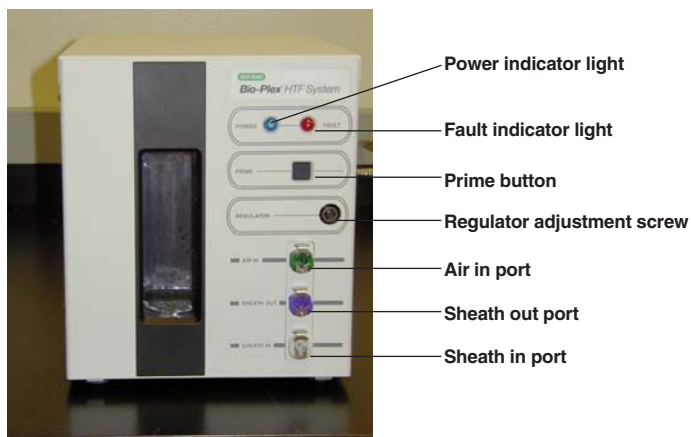


Fig. 6. HTF – front view.



Fig. 7. HTF – rear panel view with connection to Bio-Plex 200 system.

**Table 5. HTF Features.**

Feature	Description
Power indicator light	Indicates that the power to the system is on
Fault indicator light	Indicates when a fault has occurred with the system
Prime button	Primes the HTF
Regulator adjustment screw	Adjusts the sheath pressure of the array reader
Air in port	Port where air line from array reader is connected
Sheath out port	Port where sheath line from array reader is connected
Sheath in port	Port where sheath supply (sheath cube) is connected to the HTF
Communications port	A DB9-PIN connector used to connect the HTF to the reader

## 2.2.4 Computer and Monitor

The Bio-Plex 200 system may be supplied with a computer. If so, please transfer the computer's registration to your company's name following unpacking.

## 2.2.5 Maintenance, Calibration, and Validation Plate

The Bio-Plex maintenance, calibration, and validation (MCV) plate IV (Figure 8) is a specially designed accessory to facilitate automated system startup, calibration and shut-down procedures, as well as validation routines used to qualify the performance of the array reader. It is designed for use with the Bio-Plex validation kit to verify the performance of the instrument. Sized like a 96-well microplate, it contains labeled wells for bead solutions as well as larger reservoirs for system wash and sterilization solutions.

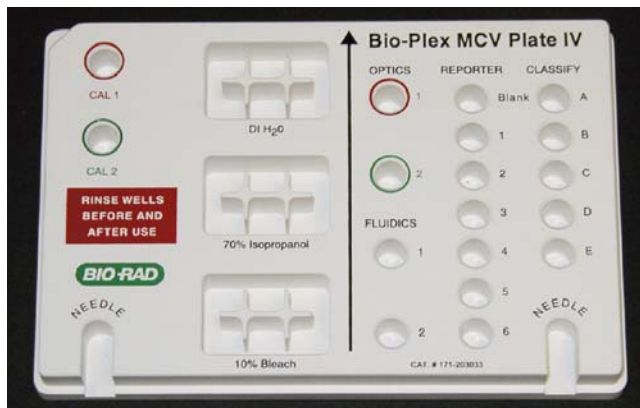


Fig. 8. Maintenance, calibration, and validation (MCV) plate IV.

## 2.2.6 Bio-Plex Reservoir

The Bio-Plex Reservoir (Figure 9) is a specially designed accessory that enables “walk away” capabilities and greatly simplifies maintenance functions. The reservoir is used in conjunction with a protocol that allows the user to perform maintenance functions either before starting a run or at the completion of a run without manual intervention.



Fig. 9. Bio-Plex reservoir.

## 2.3 Recommended Additional Equipment Not Provided

### Surge protector

We recommend the use of a 6-outlet surge protector, with a minimum surge current of 12,000 A; power, 1,500 W; clamping voltage, 336 V; clamping response, <500 psec; maximum leakage current, <50  $\mu$ A. UL-listed (for USA user), CSA-certified, CE-marked (for use outside USA). See [www.alliedelec.com](http://www.alliedelec.com) and part number 575-9715.

### Uninterruptible Power Supply (UPS)

We recommend the use of an uninterruptible power supply (UPS) to protect your system from a power outage. Choose a supply that can provide 1,050 W for at least 45 min. The UPS should be UL-listed, CSA-certified, and CE-marked when used internationally.

## 2.4 Bio-Plex Assays

The Bio-Plex assays have been carefully integrated into the Bio-Plex 200 system to ensure seamless operation and accurate, reproducible results. For more specific information, consult Bio-Plex assay manuals or visit our web site at [www.bio-rad.com/bio-plex/](http://www.bio-rad.com/bio-plex/)

Bio-Rad offers a series of preformatted kits for your convenience. Other xMAP assays that utilize the Luminex technology can be run on the system. Bio-Rad also offers mixed-to-order assay panels of any combination of available analytes. Visit [www.bio-rad.com/bio-plex/x-plex/](http://www.bio-rad.com/bio-plex/x-plex/) for details.

## Section 3 Installation

An authorized Bio-Rad service representative will set up the Bio-Plex<sup>®</sup> 200 system in your laboratory. The following general setup procedure is provided here for reference.

### 3.1 Unpacking

An authorized Bio-Rad service representative will install your Bio-Plex 200 system in your laboratory. If, upon inspection of the shipping containers, you suspect that damage to the contents may have occurred, contact Bio-Rad Technical Support.



**Warning:** Due to the weight of the array reader, it is recommended that two people, one on each side of the instrument, lift the system from the bottom.



**Warning:** The array reader contains sensitive optics. Do not jar the instrument when unpacking.

### 3.2 System Location

Selection of an appropriate location for your Bio-Plex 200 system is critical for optimal performance. Following is a list of recommended placement conditions.

1. Place on a clean, flat, and stable surface free of excessive dust or moisture. This surface must be free of other instrumentation that may cause vibration.
2. Do not obstruct the area below the array reader, and allow at least 2" of clearance around the machine.
3. The ambient temperature should be stable and within the range of 15–30°C (21°C is optimal), and the relative humidity should not exceed 80%, noncondensing. It is preferable to place the instrument in a location where the temperature does not deviate by more than  $\pm 2^\circ\text{C}$ . Avoid drafty locations as this may contribute to excessive temperature fluctuation.



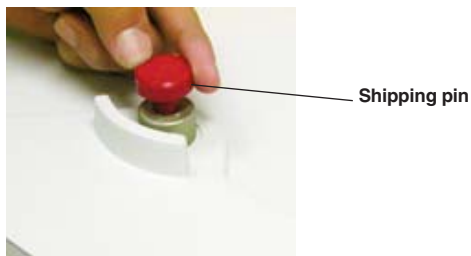
4. The maximum distance between the computer and the microplate platform and array reader should be 1.5 m (5 ft), the length of the communications cable supplied with the instruments.
5. Do not place any items on top of the array reader. The cover is not designed to support objects and thus the optics could be damaged.
6. If installing the HTF, allow an area ~3 ft below the array reader for the 20 L sheath fluid cube.

*Note:* The array reader contains sensitive optics that can be forced out of alignment through improper handling and unnecessary moving. It is recommended that an authorized service representative move your system. Following any system moves, it is necessary to validate the optical alignment and report any changes. Refer to the Bio-Plex validation kit manual for validation of the optical alignment.

### 3.3 Microplate Platform Setup

The microplate platform should be shipped with the following items:

- Microplate platform
  - Power cord
  - Communication cable
  - Needle guide
  - 2 sample needles (11.6 cm/4.6 in)
  - Shield
1. Unpack all components and ensure that all accessories are supplied.
  2. Place the microplate platform on a clean, flat, and stable surface.
  3. Unscrew and remove the shipping pin (Figure 10).



**Fig. 10. Remove shipping pin.**

4. Make sure that the power switch to the microplate platform is off, and connect the communications cable to the microplate platform's communication cable port. Attach a power cord to the power connector of the microplate platform, but do not plug it into an outlet until the array reader and microplate platform are aligned (Figure 11).
5. Position the microplate platform in the location where you want it to be used.
6. Connect the communication cable to communication port 1 on the rear of the computer.

### 3.4 Array Reader Setup

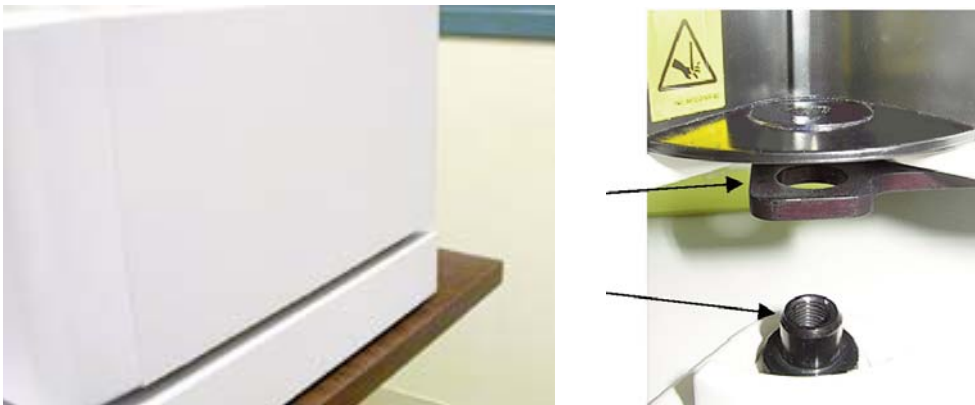
The array reader should be packaged with the following items:

- Array reader
- Power cord

- Communication cable
  - Sheath fluid bottle
  - Sheath fluid waste bottle
  - 1 sample needle (11.6 cm/4.6 in)
1. Unpack all components, being careful not to jar the array reader. Ensure that all accessories are supplied.
  2. Carefully place the array reader onto the microplate platform so that the back edges and corners of both instruments are in alignment and the black alignment plate on the reader (top arrow, Figure 11) fits over the black knob on the top of the microplate platform (bottom arrow, Figure 11).



**Warning:** Get a helper. One person should not attempt to lift the array reader. To avoid back injury, always bend your knees and keep a straight back when lifting heavy objects.



**Fig. 11. Reader aligned on top of microplate platform.**

3. Plug the microplate platform power cord into an approved outlet. A surge protector is recommended to protect the microplate platform from power fluctuations. See Recommended Additional Equipment Not Provided on page 11.
4. A communication cable is supplied to connect into communication port 1 (P1) at the rear of the array reader and to a USB port at the back of the computer. **However, do not make this connection until after the software is installed.** Connect a power cord to the array reader's power connector.
5. Connect the communication cable in the microplate platform communication port to communication port 1 (COM1) of the computer (Figure 12).
6. Plug the array reader and microplate platform power cords into a properly grounded electrical outlet.



Fig. 12. Completed connections of computer and monitor to the Bio-Plex suspension array system.

### 3.5 Connecting the Sheath Fluid and Waste Containers

1. Attach the 1.5 L waste bottle (orange-ringed cap) to the orange **Waste** connector on the left side of the array reader. An audible click indicates proper connection. Lubricating the rubber O-rings with water can facilitate attaching these connectors. The waste bottle should not be placed on top of the instrument.



**Warning:** Waste levels must be monitored. Do not allow the waste bottle to overflow! Empty the waste bottle each time the sheath fluid container is filled.

2. Attach the 1.0 L sheath bottle (blue-ringed cap) to the array reader as follows:
  - a) Connect the air line (uppermost tube) to the green connector on the array reader.
  - b) Connect the sheath fluid line to the blue connector.

An audible click will be heard when the hoses are properly connected. For proper operation, the sheath bottle must be placed at the same level as the Bio-Plex suspension array system, and the cap tightened.

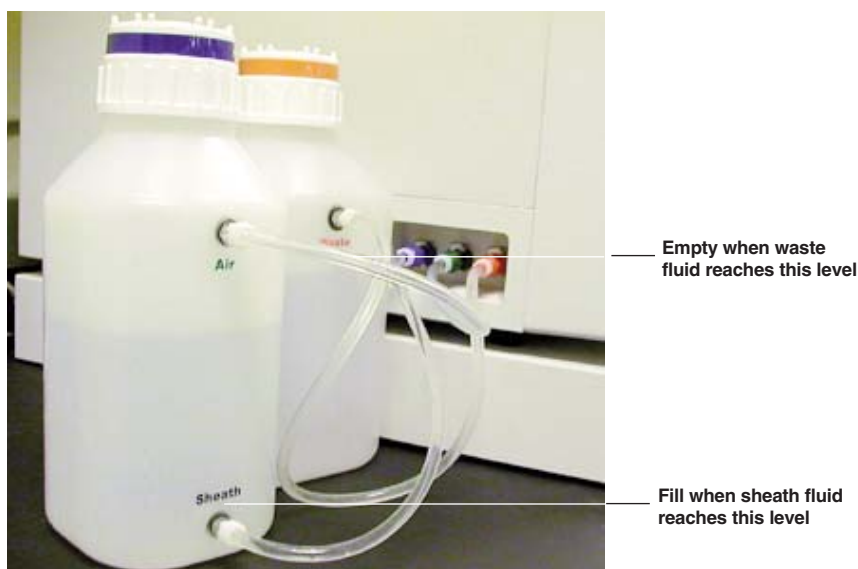


**Warning:** Do not switch the caps on the waste and sheath bottles. The orange-ringed cap must go on the waste bottle and the blue-ringed cap must go on the sheath bottle for the reader to function properly.

3. Fill the sheath fluid bottle with sheath fluid supplied in the 20 L cube container (catalog #171-000055) to just below the air intake and tighten the cap. (Figure 13)



**Warning:** In order to maintain system pressure, the sheath fluid bottle cap must be tight. Do not overtighten or seal will be lost.



**Fig. 13. Waste and sheath fluid bottle connections.**

*Note:* To set up the 20 L sheath fluid cube container for use, remove tape from box and lift off the round white cover. Pull spout out of the box. Unscrew cap and replace it with the spigot cap included in the box.

*Note:* The waste bottle must be emptied and the sheath bottle must be refilled after reading two assay plates.

*Note:* If you have an HTF it should be set up after the initial reader setup is complete.

### **3.6 Computer and Monitor Connections**

When you receive the Bio-Plex 200 system, please transfer the computer's registration to your company's name.

1. Unpack the computer and place it on a bench adjacent to the array reader. Typical computer placement is to the right of the array reader since sheath and waste fluid bottles are located on the instrument's left side. **DO NOT** place the computer on top of the array reader.
2. Unpack the monitor and place it on top of the computer, or in another suitable place. **DO NOT** place the monitor on top of the array reader.
3. Connect the monitor to the computer, install the power cords into the respective power connectors, and plug into an approved outlet.
4. Connect the keyboard and mouse.
5. Your computer will be loaded with Bio-Plex Manager™ software during installation. See Section 3.7 for software loading requirements.

### **3.7 Software Installation**

#### **3.7.1 System Software Loading**

Your computer will be loaded with Bio-Plex Manager™ software during installation. However, in the event that it is necessary to reload the software, proceed as follows:

1. Disconnect the communication cable that connects the computer to the array reader at the computer (USB port), if not already done.

2. Insure that the array reader, microplate platform, and HTF unit are turned off.
3. Insert the Bio-Plex Manager CD-ROM into the CD drive of the computer.
4. Select **Install Bio-Plex Manager**.
5. After installation, remove the installation disk.
6. Attach the HASP key.
7. Reconnect the USB communication cable and turn on the array reader, microplate platform, and HTF unit.

*Note:* Please refer to the Bio-Plex Manager software manual for detailed installation instructions.

### 3.7.2 Communication Ports

Bio-Plex Manager will automatically detect the port configuration.

## 3.8 Installing or Changing the Sample Needle

### 3.8.1 Installing/Changing the Long Sample Needle



**Warning:** Turn the power to the array reader off before installing or changing the sample needle.

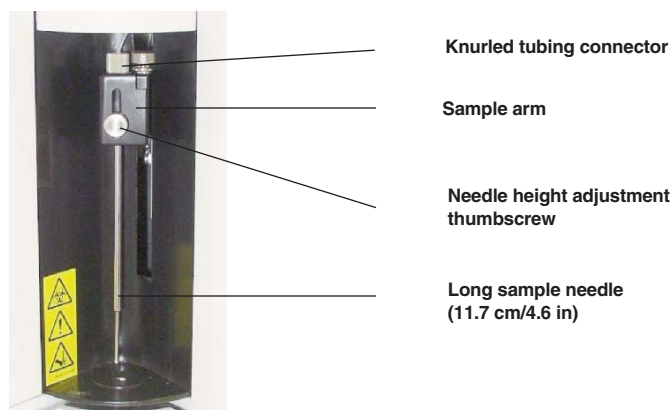
For use with the microplate platform, a long sample needle (11.7 cm/4.6 in) must be installed. A spare long needle is shipped with the microplate platform.

1. Make sure that the power to the array reader is switched off. Make sure the power cord is unplugged from the outlet.
2. Remove the light housing directly above the sample arm by grasping and firmly pulling out (Figure 14). The housing remains attached by a wire. Place the housing on top of the array reader, out of the way.



**Fig. 14. Removing the light housing.**

3. Remove the knurled tubing connector (Cheminert fitting) atop the sample arm by grasping the sample arm and turning the connector counterclockwise (Figure 15). If the connector is difficult to remove, push up gently on the sampling needle.



**Fig. 15. Sample needle assembly.**

4. Remove the sample needle by grasping the needle and gently pushing up.
5. Insert the new needle into the sample arm, making sure it aligns with the needle guide. If the needle is not aligned into the needle guide, carefully reposition the array reader to align the needle and the needle guide. Replace the tubing connector, and tighten by grasping the sample arm and turning the connector clockwise, being careful that the threads are correctly aligned. Hand-tighten only.
6. Reinstall the light housing by pushing until it snaps into place. Take care not to pinch the clear sample tubing.
7. Install the shield to cover the sampling needle area. This shield can be removed for making adjustments to the needle.

### 3.8.2 Adjusting Sample Needle Height



**Warning:** Keep hands and fingers out of the microplate platform when performing this procedure!

The height of the sample needle must be adjusted when (1) the style of microplate has changed, and (2) when the sample needle is replaced. The MCV plate included with your system provides a method for adjusting sample needle height for standard flat-bottom or filter plates (Millipore catalog #MSBVS1210) and for PCR plates.

1. Turn on the array reader and microplate platform.
2. Launch the Bio-Plex Manager software.
3. Click on **Instrument** in the menu bar of the software.
4. Choose **Setup**. Choose **Adjust Needle** from the pull-down menu. The following dialog box appears (Figure 16):

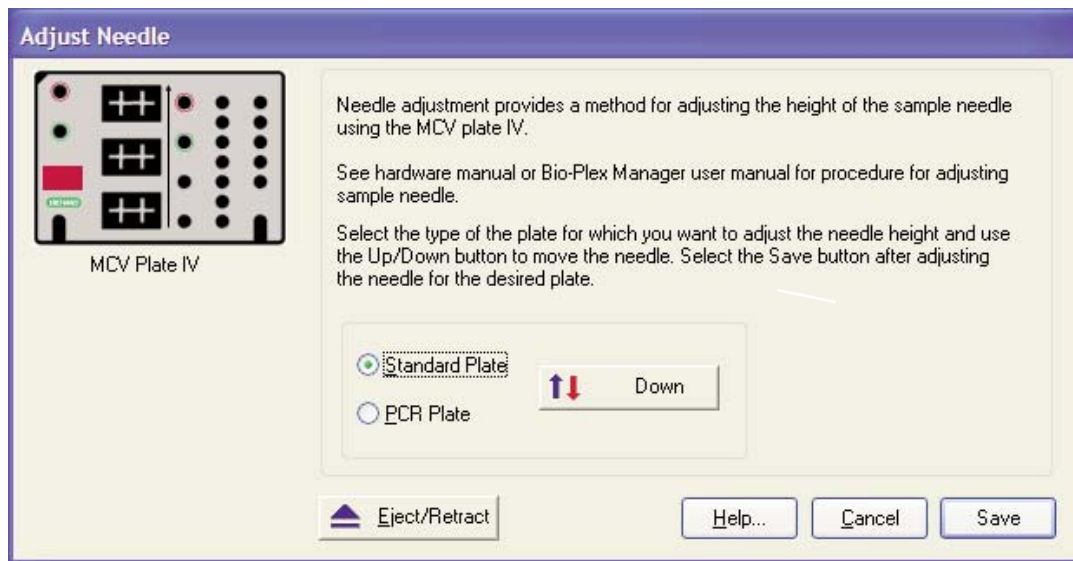


Fig. 16. Sample needle dialog.

5. Select the plate type being used.
6. Click **Eject/Retract** to eject the plate holder.
7. Place the MCV plate on the microplate platform with the black arrow facing toward the array reader.
8. Click on the **Eject/Retract** button to retract the plate.
9. Tape the access door of the microplate platform open. It will be necessary to be able to see inside the access door.
10. In the **Adjust Needle** window, click on the **Down** button. The needle will move to the down position. **Note:** This button is labeled **Up** or **Down** dependent on it's current state.
11. With the needle in the down position, loosen the needle height adjustment thumbscrew at the top of the needle so that the needle housing can move up and down freely (Figure 17).

*Note:* All adjustments to the needle height must be made when the needle is in the down position.

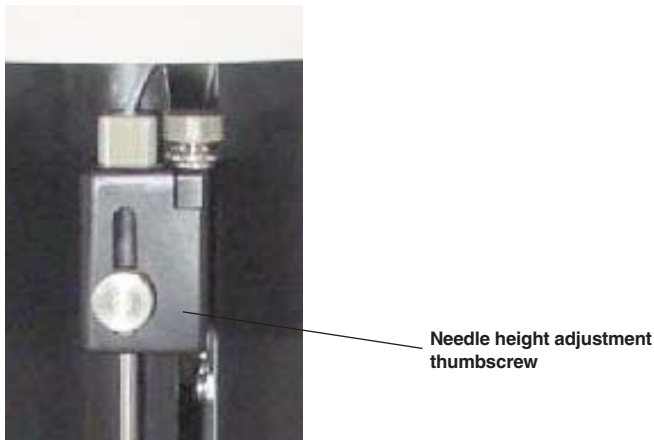


Fig. 17. Sample needle assembly.

12. By holding onto the needle height adjustment thumbscrew on the needle arm, manually move the needle so that it just touches the bottom of the needle adjustment well of the MCV plate. Move the needle up and down gently a couple of times to verify that the needle is barely touching the bottom of the well.
13. Tighten the needle height adjustment thumbscrew so that it is no longer possible to manually move the needle up and down. Take care to ensure that the needle does not move while you are tightening the screw. Do not overtighten.
14. In the **Adjust Needle** window, click on the **Up/Down** button to move the needle up and down. Look inside the microplate platform at the MCV plate. The needle should just touch the MCV plate at the bottom of the cutout (use flashlight for better viewing). Readjust the needle height if necessary.
15. When the needle is adjusted properly, click the **Eject/Retract** button.
16. Remove the MCV plate from the microplate platform and click **Eject/Retract**.
17. Click on the **Save** button in the **Adjust Needle** window.
18. Perform a **Wash Between Plates** step to remove any air introduced into the lines.

### 3.9 Initial System Priming

This procedure is to be performed only during the initial installation of the array reader.

1. Fill the maintenance, calibration, and validation (MCV) plate (Figure 18) with deionized water and 70% isopropyl alcohol in the appropriate wells.

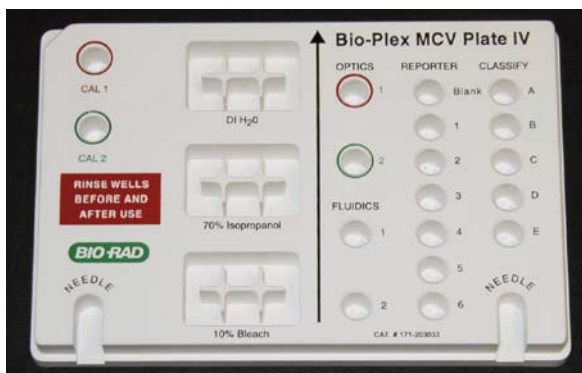


Fig. 18. MCV plate IV.



2. Click the **Eject** icon. Insert the MCV plate into the microplate platform. Click **Retract**.
3. Choose **Instrument** from the main menu bar. Choose **Additional Functions**, followed by **Prime**.
4. Inspect the waste line outside the instrument for air pockets. Repeat the priming procedure until no air pockets are observed in the waste line outside of the array reader. This may require a few priming cycles.
5. Choose **Instrument** from the main menu bar. Choose **Additional Functions**. Choose **Alcohol Flush**. Wait for function to complete.
6. Choose **Instrument** from the main menu bar. Choose **Additional Functions**. Click **Wash**. Wait for function to complete.
7. Repeat **Wash** two more times.

### 3.10 Resetting Instrument Pressure Settings

1. After the system has been primed, it is necessary to run the **Reset Instrument Pressure Settings** utility for optimal instrument performance. The utility initially sets the **Calibration Pressure** and the **Manufacturing Pressure** to the same value. Subsequently, whenever a calibration is performed the **Calibration Pressure** will be updated, but it should not diverge very far from the original **Manufacturing Pressure** setting unless there is a system problem. The settings are displayed in the **Instrument Info** window under the **Device Status** tab.
2. The **Reset Instrument Pressure Settings** utility should be performed first using the sheath fluid bottle attached to the system. If a HTF is included with the system, this utility will be rerun in the **HTF Setup** section which follows.
3. With the array reader off and Bio-Plex Manager software closed, go to the Utilities folder in Bio-Plex Manager 5.0 (go to Start > Programs > Bio-Plex Manager 5.0 > Utilities). Click on **Reset Instrument Pressure Settings**. This will set up the system to set the instrument pressures the next time Bio-Plex Manager is launched.
4. Power on the array reader and start Bio-Plex Manager software. As the program opens, you will see a message that the system is **Updating system settings**. A message noting that the settings were successfully updated will be displayed when the process is complete.
5. Restart the array reader and Bio-Plex Manager software.

### 3.11 HTF Setup

1. Unpack all components. Ensure that all accessories are supplied.
2. Start with the original sheath fluid and waste containers connected to the array reader. Check that the sheath fluid container is filled just below the air intake valve.
3. Make sure the array reader is turned on, and start the Bio-Plex Manager software.
4. From the **Instrument** menu, select **Additional Functions**, then select **Prime**.
5. Open the **Instrument Information** window and select the **Device Status** tab (Figure 19).

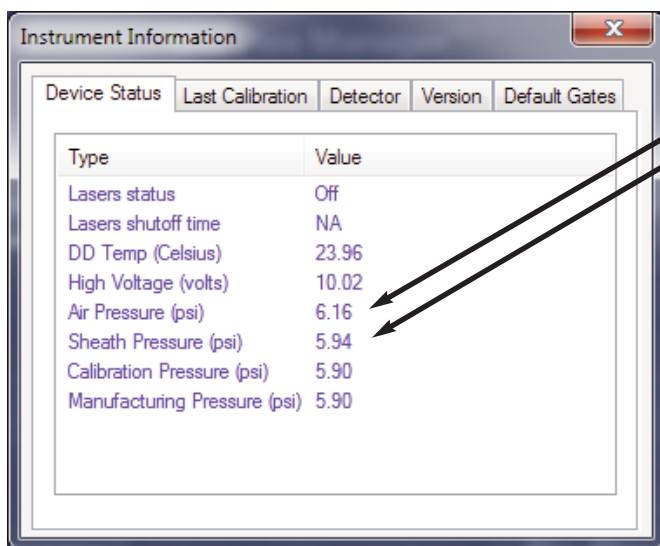


Fig. 19. Device status window.

- After the pressure has stabilized, record the air and sheath pressure (Figure 19, arrows).

Air pressure: \_\_\_\_\_ psi

Sheath pressure \_\_\_\_\_ psi

Save this information. You will need it later in the installation procedure, and you will also need to refer to it if you use the array reader with the original sheath fluid container again.

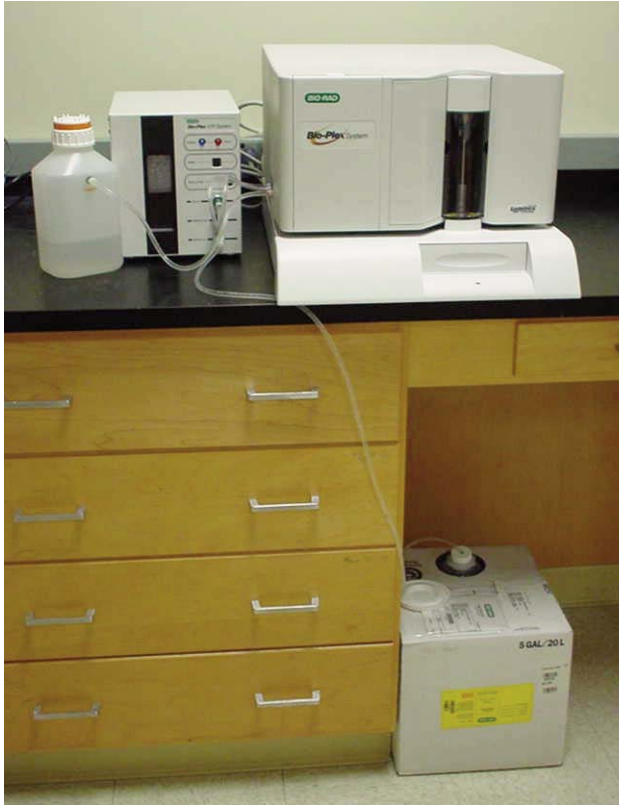
- At the end of the prime cycle, disconnect the sheath fluid container. Store it in a safe place. If you plan to use the HTF waste line, disconnect the waste container as well.

*Note:* It is important to document your instrument's original sheath pressure in case bottles are used.

- Place the HTF next to the sheath fluid connection on the array reader. Do not place the HTF on top of the array reader. Place the sheath cube 3–4 ft below the level of the array reader and HTF. Figure 20 shows a typical setup.



**Warning:** The Sheath container **must** be below the level of the Bio-Plex reader. Placing the sheath container on the same level or higher than the Bio-Plex reader can draw sheath fluid into the array reader and damage the system.



**Fig. 20. HTF setup.**

9. Make the following connections to connect the HTF to the array reader:
- Connect the sheath fluid line (blue fitting) to the **Sheath Out** connector on the front of the HTF. Lubricating the rubber O-rings with water facilitates attaching these connectors
  - Connect the air line (green fitting) to the **Air In** connector on the front of the HTF
  - Connect the sheath fluid line to the sheath fluid connector on the side of the array reader (blue fitting)
  - Connect the air line to the air connector on the side of the array reader (green fitting)
  - If you are using the HTF waste line, connect the waste line tubing to the waste connector on the side of the reader, and run the other end of the waste line into an appropriate biohazard receptacle. Cut off excess tubing, and make sure the waste receptacle is level with the array reader or no more than 3 ft below it.

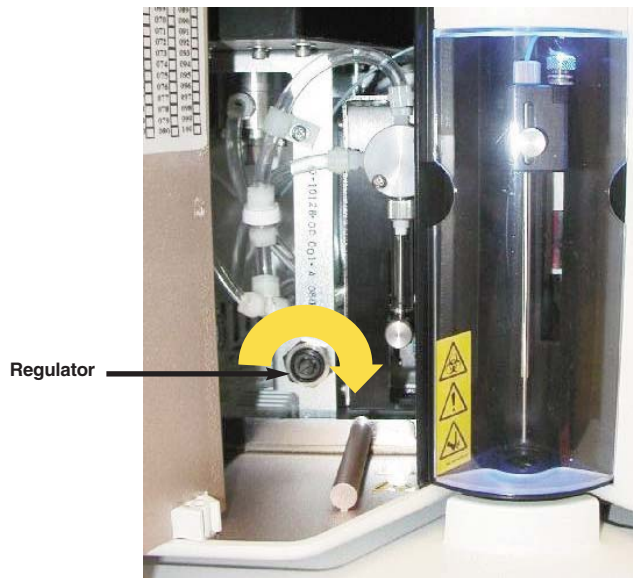
*Note:* The waste container must be vented.

- Connect the sheath fluid intake line (white fitting) to the **Sheath In** connector in the front of the HTF
- Connect the communication cable to the DB9-PIN connector on the back of the HTF system. Connect the other end to communication port 2 (P2) on the back of the Bio-Plex array reader
- Connect the power cord to the back of the HTF and plug the other end into an approved outlet

*Note:* If you are using the 1.5 L waste bottle included with the Bio-Plex system, be sure to empty it after every two plates are read.

**Note:** You may use the additional waste line provided with the system to drain to a larger waste container. The large waste container waste must be positioned so that it is no more than 3 or 4 ft below the array reader. Please note that the instrument flow rate is influenced by waste container placement, which may affect performance.

10. Lower the stainless-steel filter end of the sheath fluid line to the bottom of a full box of sheath fluid. Secure the cap on the sheath fluid box. Position the sheath fluid container on the floor so that the cap is on the top.
11. Turn on the power to the HTF; the HTF should automatically prime itself. You will hear the HTF pump turn on. When the HTF reservoir is about 2/3 full, it will stop priming automatically.
12. Open the center access door on the array reader. Use a screwdriver to turn the regulator screw fully clockwise (this may require several full turns), then one half-turn back.



**Fig. 21. Regulator screw adjustment.**

13. From the **Instrument** menu select **Additional Functions**, and then **Prime**. During this prime cycle use a screwdriver to adjust the regulator on the front of the HTF system (not shown). Adjust it until the sheath pressure displayed in the **Information Box** reads the same as the sheath pressure you recorded in step 6. This may take many turns. The system should stabilize at this sheath pressure. The air pressure should be the same as you recorded in step 6, within 0.1 psi.  
  
If the **Prime** cycle ends before you have completed the adjustment, select **Prime** again and continue to adjust the regulator.
14. When the pressure has been set it is critical that the flow rate be verified. Collect the flowthrough to waste for 2 min in a graduated container capable of estimating volume to 0.5 ml. The volume collected should be 10.5 to 11.5 ml. Use universal precautions to avoid contamination if the system has been used with human blood, body fluids or tissues. To minimize the biological hazard, use the flow rate test kit available from Bio-Rad (catalog #800-0502). If the flow rate is too low, increase the pressure by turning the regulator screw on the HTF unit clockwise. If the rate is too high, decrease the pressure by turning the regulator screw counterclockwise. Start by turning the regulator screw a quarter-turn in the appropriate direction then retesting the flow rate. Continue until the flow rate is within the specification.

15. Because the flow rate in step 14 is adjusted by altering the system pressure, it is necessary to repeat steps 3 to 5 of Section 3.10 (Resetting Instrument Pressure Settings).

*Note:* It is necessary to run the **Reset Instrument Pressure Settings** utility whenever the flow rate (or system pressure) is changed. This is a rare occurrence in normal operation.

### 3.12 Vacuum Manifold Setup

Bio-Rad recommends the use of the Millipore MultiScreen separations system (Millipore catalog #MAVM0960R) for preparing Bio-Plex assays. A setup and validation procedure for this apparatus is included here. More specific instructions for the setup of the apparatus as well as specific product information may be found in the Millipore MultiScreen separations system user guide. Note that depending on the type of samples used in Bio-Plex assays, the pressure necessary to achieve optimal results may be different. If you choose to use your laboratory (house) vacuum system, be aware that fluctuations in vacuum pressure may be extreme enough so that you may need to purchase and integrate a pressure regulator. Alternatively, you may also purchase a vacuum pump to ensure optimal steady-state vacuum pressure. Finally, once you have calibrated your manifold, it is important to validate that this pressure is optimal for performing Bio-Plex assays. Follow the verification procedure in this section closely for optimal results using Bio-Plex assays.

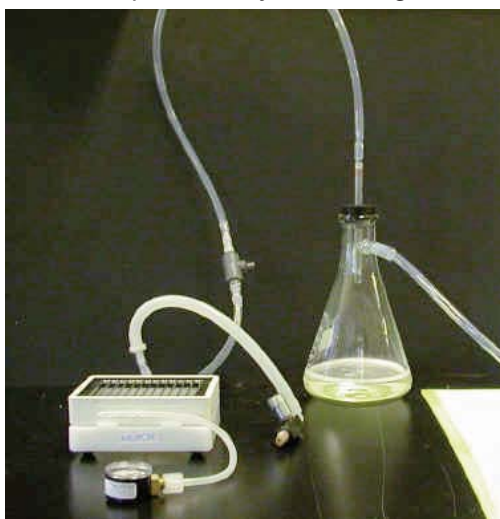
#### Equipment: Required

- Vacuum source — laboratory vacuum or vacuum pump
- Pressure regulator (if extreme fluctuations in house vacuum are a problem)
- Millipore MultiScreen separations system (Millipore catalog #MAVM0960R)
- Flat-bottom microplate (not a filter plate)
- Millipore 96-well filtration plate (Millipore catalog #MSBVS1210)
- Phosphate buffered saline
- 8-channel pipet

#### 3.12.1 System Setup

##### Setup of the MultiScreen Vacuum Manifold

Figure 22 illustrates the vacuum manifold setup and its attachment to a laboratory vacuum source. For more specific details regarding the setup of the manifold, refer to the Millipore MultiScreen separations system user guide.



**Fig. 22. Vacuum manifold and vacuum source setup.**

1. Make sure that the system has been set up according to the directions in the Millipore user guide.  
Hint: Make sure that the connector leading to the vacuum control knob is perpendicular to the manifold. This will ensure that no buffer travels to the vacuum control knob.
2. Place a 96-well flat-bottom microplate (not a filter plate) on the vacuum apparatus.
3. Make sure the **gold** vacuum control valve (Figure 23) is completely open (all colors showing).
4. Make sure the **gray** ON/OFF valve (Figure 24) is completely off (the knob should be perpendicular to the direction of the tubing attached to it).



Fig. 23. Gold vacuum control valve.

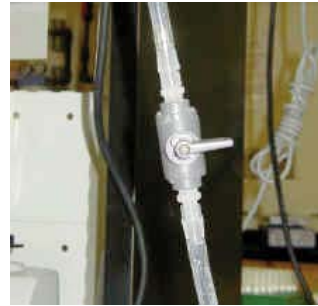


Fig. 24. Gray ON/OFF valve.

5. Turn on the lab vacuum to maximum level and note this setting for performance of assays. In common lab vacuum systems, the lever should be turned so that it is parallel to the vacuum port. Alternatively, turn on the vacuum pump.
6. Slowly open the ON/OFF valve on the manifold to the ON position (parallel to the tubing).
7. Slowly turn the vacuum control valve clockwise until a slight vacuum sound is heard.
8. Press firmly on the 96-well plate (all four corners) until it is sealed securely by the vacuum on the apparatus.
9. Observe the pressure reading on the attached gauge (see below).
10. Adjust the ON/OFF valve and turn the calibration knob so that the gauge reads approximately 1–2.5" Hg.
11. Close the lab vacuum until the 96-well plate is released from the apparatus.
12. Turn on the lab vacuum again, press on the 96-well plate, and look at the indicated pressure on the gauge again.
13. If the pressure is still approximately 1–2.5" Hg, the vacuum apparatus has been correctly calibrated. If not, repeat the steps of the calibration procedure until the desired result is achieved.
14. Turn off the vacuum apparatus and proceed to Section 3.12.2, Validation of Vacuum Pressure.

*Note:* Do not make any further adjustments to the vacuum control valve.

### 3.12.2 Validation of Vacuum Pressure

#### Validating the Vacuum Manifold Pressure for a Bio-Plex Assay

1. Prewet all of the wells of a Millipore 96-well filter plate with 100  $\mu$ l PBS.
2. Place the prewetted filter plate on the calibrated vacuum apparatus.

3. Turn on the laboratory vacuum to maximum level (the same level that was used in the preceding calibration procedure).
4. Press on the filter plate and note the time required to evacuate the solution from the wells. The time required should be 2–5 sec.

*Note:* It is important to perform this step exactly as you would perform a wash step in a Bio-Plex assay.

5. If the time required to evacuate the solution from the wells was less than 2 sec, the calibrated pressure is too high. If the time required is greater than 5 sec, the vacuum pressure is too low.
6. If the pressure is too high (evacuation occurs too quickly), open the vacuum control valve slightly (so that fewer colors are showing) and repeat steps 1 through 5 until the evacuation time is 2–5 sec.
7. If the pressure is too low, (evacuation occurs too slowly), close the vacuum control valve slightly (so that fewer colors are showing) and repeat steps 1 through 5 until the evacuation time is 2–5 sec.

*Note:* It is a good practice to validate the vacuum pressure each day during the prewetting step of the Bio-Plex assays. When you have added the assay buffer to the appropriate wells and evacuated the plate on the manifold, ensure that the time required to evacuate the plate is 2–5 sec. This will verify that the manifold is calibrated correctly.

### **Tips**

Listed below are a few key hints and recommendations for using the vacuum manifold. For a more complete listing of potential problems and solutions, consult the troubleshooting guide at the end of this manual.

1. If you notice a large amount of fluctuation in your pressure using laboratory vacuum, you may need to purchase and attach a pressure regulator to the vacuum line. Alternatively, you may purchase a vacuum pump to ensure optimal steady-state pressure. In either case, it is critical to correctly calibrate and validate the vacuum pressure before performing Bio-Plex assays.
2. If you notice that the plates are taking an extended amount of time to evacuate, you may need to replace the gaskets. See the Millipore multiscreen separations system user guide for instructions on replacing the gaskets.
3. Do not allow the laboratory vacuum to continue aspirating the filter plate for more than 10 sec after the solutions are completely gone from the wells. This will result in a significant loss of beads.
4. It is recommended that you clean the manifold regularly. The frequency depends on the reagents you use and how often you use the manifold. Use mild soap or standard laboratory detergent, bleach, or alcohol to clean all surfaces.

### **3.13 Performing System Validation**

Prior to performing analyses with the Bio-Plex 200 system the instrument must be calibrated. Calibration should be done each day after startup is complete and the system is warmed up. You should also recalibrate if the instrument temperature changes by more than 2°C.

The user should verify that the system is performing according to specifications using the Bio-Plex validation kit. Validation should be performed monthly. Additionally, the user should revalidate each time the array reader is moved or if there are problems with the array reader that can't be solved by other procedures. See the validation kit manual for complete system validation instructions.

## Section 4 Care and Maintenance

**Table 6. Summary of Care and Maintenance.**

<b>Daily</b>	Startup, calibrate, wash between plates, shut down
<b>Weekly</b>	Sonicate needle, unclog, check for leaks
<b>Monthly</b>	Validation, clean exterior surface
<b>6 months</b>	Replace syringe seal, clean ventilation filter
<b>Yearly</b>	Replace sheath filter, replace air intake filter

*Note:* Two long needles are included with the Bio-Plex® 200 system. While one is being cleaned, the other can be installed to prevent any downtime from this weekly maintenance.

Proper maintenance and cleaning should be performed in order to preserve the longevity and reliability of the system.

### Regularly As Required

#### Preventing clogs in the fluidics system

The **Start Up**, **Shut Down**, and **Wash Between Plates** procedures must be strictly followed to prevent clogging of the fluidics system.

#### Sheath fluid and waste fluids

Replace the sheath fluid and empty the waste container as required. You must manually monitor the waste container level. Release the instrument's pressure by removing the lid from the sheath fluid container. Add sheath fluid, filling the sheath fluid container to just below the air intake. Discard waste fluid by appropriate means. After closing containers, remove air from the lines by performing a **Start Up**.

One must refill sheath and empty the waste after two full plates are run.

Run a **Wash Between Plates** function after each plate is run.

#### Check for leaks

Open all instrument doors and visually inspect for leaks. If a leak or spill is observed, verify whether it is coming from the sample needle assembly. Check to see that the knurled tubing connector is tightened properly (see Section 3.8). If this is not the source of the leak, turn off all power to the system, disconnect all power cords, and contact Bio-Rad Technical Support for further information.

#### Sanitize

Fill MCV plate with a solution of 10% household bleach. Place MCV plate in microplate platform. Choose **Shut Down**.



## Every Month

### Clean exterior surfaces

Disconnect the instrument from AC power by turning off the power switch on the rear of the array reader and microplate platforms. Unplug both instrument power cords from the wall source. Wipe all exterior surfaces with mild germicidal detergent, followed by a 10% bleach solution. Open both front doors of the array reader and clean all accessible surfaces with detergent followed by a 10% bleach solution. Dry the sheet metal surfaces to prevent corrosion.

## Every 6 Months

### Syringe seal

#### **Warning!**

Turn the unit OFF and unplug the power cord before replacing the syringe plunger! The syringe arm does not deactivate when changing the plunger; injury could result if the system is not unplugged.

Replace the syringe plunger's seal every 6 months. Open the centermost door on the front of the array reader. Locate the syringe (a glass cylinder with a metal rod). Unscrew the knob on the syringe arm (at the bottom of the syringe), and forcefully push the syringe arm down. Unscrew the syringe from the top of its housing and pull the plunger out of the syringe. Remove and replace the plunger seal, and return the plunger to the syringe. Screw the syringe back into the top of its housing, return the syringe arm to its original position, and hand-tighten the screw on the syringe arm. Plug in the power cord and turn the array reader on. Prime the instrument until any bubbles in the syringe seal are eliminated, watching for any leaks in the syringe area. When finished, close the center door. See Table 2 and Figure 3B (page 7).

### Instrument ventilation filter

Check the instrument ventilation filter every 6 months. Clean the filter only when soiled. Disconnect the array reader from AC power by turning off the power switch. Unplug the power cord from the wall source. On the bottom-left side of the array reader, push the clip in and gently slide the filter out. Clean the filter with a vacuum or by placing it under running distilled water. Stand it upright to air-dry. Reinstall it with the arrows facing up.

## Every Year

### Sheath filter

Change the sheath filter once a year. Disconnect the sheath fluid bottle before changing the filter. Separate the filter and tubing from the retaining clips. Cut the tubing close to the filter on both sides of the filter. Connect the tubing to the new filter and return the filter and tubing to the retaining clips. Reconnect the sheath fluid bottle.

### **Air intake filter**

*Note:* **Hold on to the tubing!** Do not allow the tubing to fall inside the instrument.

Replace the air intake filter every year. Disconnect the instrument from AC power by turning off the power switch on the rear of the array reader. Unplug both instrument power cords from the wall source. Looking at the back of the array reader, locate the panel at the top left. Remove the screw at the top of the panel and open the panel door. Pull the filter 3–4 in from the unit. Grasp the tubing. Remove the filter with one hand, and hold the tubing with the other hand. Connect a new filter to the tubing, position the filter inside the panel, and reattach the panel door to the unit.

### **As Required**

#### **Fuse replacement**

To replace the fuses, disconnect the array reader from AC power by turning off the power switch on the rear of the instrument. Unplug the instrument power cord from the wall source. With a small, flathead screwdriver, open the module door and remove the red cartridge. Check both fuses for damage. Replace damaged fuses with the type specified on the sticker to the right of the power connector.

#### **Sample arm vertical height**

The vertical height determines how far into the sample well or tube the sample needle goes when aspirating a sample. It was set during installation of your system. To adjust sample needle height, see Section 3.8.2, Adjusting Sample Needle Height.

### **Shutting Down the Bio-Plex 200 System for Extended Periods of Nonuse**

#### **Thoroughly flush with DI H<sub>2</sub>O**

Place the MCV plate in the instrument with DI H<sub>2</sub>O in the reservoir labeled “DI H<sub>2</sub>O”. Replace the sheath fluid in the bottle or HTF with DI H<sub>2</sub>O. Perform 4 wash cycles (for each cycle go to Instrument > Additional Functions > Wash, then click OK). Next, perform 10 prime cycles (for each cycle go to Instrument > Additional Functions > Prime, then click OK). Before using the Bio-Plex again you must flush out the DI H<sub>2</sub>O by reintroducing sheath fluid into the sheath bottle or HTF unit and performing 10 prime cycles.

## Section 5 Troubleshooting

### 5.1 Troubleshooting Guide for Bio-Plex® 200 System

Message/Problem	Causes	Solution
<b>Bio-Plex Manager™</b> has detected a problem with low bead number.	<b>Most Likely:</b>	
	Too few beads in the assay	Check bead number calculations.
	Plate not shaken 10 min before analysis	Remove plate from array reader and shake for 10 sec.
	Buffer volume in wells is too low (must be at least 125 µl)	Resuspend in 125 µl. Perform <b>Remove Bubbles</b> .
	Microbubble in cuvette	Perform <b>Remove Bubbles</b> . Perform <b>Unclog</b> to verify fluidics integrity.
	Low/no sheath fluid	Refill sheath fluid, check sheath connections. Perform <b>Start Up</b> .
	Possible clog	Perform <b>Unclog</b> and rerun. If unsuccessful, repeat. Remove needle (Figure 15) and sonicate in cleaning solution or 10% bleach for 15 min. If still unsuccessful, contact Technical Support.
	<b>Less Likely:</b>	
	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.
	Red laser failure	Contact technical service.
	Filter plate not flat	Check filter plate flatness.
	Leaky filter plate	1. Check for liquid under plate on microplate platform. 2. Check vacuum apparatus used to prepare sample plate. Make sure vacuum is below 5 in Hg. 3. Check needle height.
Incompatible suspension	Check buffer compatibility.	

Message/Problem	Causes Cause	Solution
<b>Bio-Plex Manager has detected a problem with bead selection.</b>	<p>Incorrect bead regions selected in the protocol</p> <p>Incorrect regions selected when preparing the assay</p> <p>Too few beads in the assay</p>	<p>Compare bead regions in the assay with those selected in the protocol.</p> <p>Verify regions chosen during assay preparation.</p> <p>Verify that the correct number of beads in one or more regions were used.</p>
<b>Bio-Plex Manager has detected a problem with assignment of beads into regions.</b>	<p><b>Most likely:</b></p> <p>Calibration performed before the array reader was warmed up</p> <p>Improper calibration</p> <p>Microbubbles present in cuvette.</p> <p><b>Less likely:</b></p> <p>Calibration beads are photobleached (do not expose to light for more than 1 hr)</p> <p>Array reader was calibrated with a dirty MCV plate</p> <p>Misalignment of optics</p>	<p>Perform 30 min <b>Warm Up</b> and recalibrate.</p> <p>Check that the target values of the CAL beads match values entered in the software, then recalibrate.</p> <p>Perform <b>Remove Bubbles</b>.</p> <p>Recalibrate with new Cal1 beads.</p> <p>Clean MCV plate and recalibrate.</p> <p>Perform <b>Optical Validation</b>. Contact Technical Support if values are not within range.</p>
<b>Bio-Plex Manager has detected a problem with aggregated beads.</b>	<p>Clumped beads present</p> <p>Sheath reservoir is empty</p> <p>Waste reservoir is overfilled</p> <p>Problem with doublet discrimination (DD)</p> <p>Incompatible suspension buffer used</p>	<p>Vortex plate at 900 rpm for 1 min.</p> <p>Refill sheath reservoir. Perform <b>Start Up</b>.</p> <p>Empty waste and reconnect.</p> <p>Use default DD gate setting. Run Classify Validation to check DD efficiency.</p> <p>Check hardware manual for buffer compatibility.</p>
<b>Check Link in status bar of software.</b>	<p>Array reader or microplate platform not turned on</p> <p>Software is not communicating with array reader</p> <p>Cables from computer to array reader or microplate platform are loose/not connected</p>	<p>Turn on array reader and microplate platform.</p> <p>Close and restart Bio-Plex Manager software.</p> <p>Check cables for proper connections.</p>

Message/Problem	Causes	Solution
<b>Pressurizing in status bar of software.</b>	<p>Leak in sheath bottle or cap</p> <p>Sheath and waste bottle caps are reversed</p> <p>System pressure settings incorrectly set</p>	<p>Tighten sheath cap or replace sheath bottle.</p> <p>Make sure that the blue-ringed cap is on the sheath bottle and the orange-ringed cap is on the waste bottle.</p> <p>Update pressure settings. Contact Technical Support for instructions.</p>
<b>Needle stuck in down position.</b>	<p>Protective assay plate covering was not removed</p> <p>Needle guide is not screwed all the way in</p> <p>Sample needle is bent</p>	<p>See hardware manual for procedure for raising needle stuck in down position. Then remove cover from assay plate.</p> <p>Tighten needle guide by turning tube clockwise until tight.</p> <p>Replace bent needle with a new needle (Section 3.8.1, p. 16).</p>
<b>No assay signal detected.</b>	<p><b>Most likely:</b></p> <p>Error in assay preparation</p> <p>Incorrect target values used in calibration</p> <p>Waste container overfilled</p> <p>Sheath reservoir low or empty</p> <p><b>Less likely:</b></p> <p>Waste line not connected properly</p> <p>Laser not functioning</p>	<p>Make sure that streptavidin-PE was added. See the assay kit manual.</p> <p>Check that target values in calibration dialog box match the values on Cal1 and Cal2 bottles.</p> <p>Empty waste. Reconnect waste. Perform <b>Unclog</b>.</p> <p>Refill sheath reservoir. Perform <b>Start Up</b>.</p> <p>Check waste line connection. Make sure that it clicks into place. Make sure cap is vented and there is no pressure inside the waste bottle.</p> <p>Perform <b>Calibrate</b> using calibration kit. Call Technical Support for further assistance.</p>

Message/Problem	Causes	Solution
<b>Bio-Plex Manager has detected a change in sheath pressure.</b>	<p><b>Most likely:</b></p> <p>Sheath reservoir cap not on securely</p> <p>Sheath bottle lines are not connected properly</p> <p><b>Less likely:</b></p> <p>Sheath fluid level above the AIR port on the sheath container</p> <p>Sheath and waste bottle caps are reversed</p> <p>Sheath bottle has a leak</p> <p>HTF unit not turned on</p> <p>Air compressor not working</p>	<p>Tighten sheath cap. Click <b>OK</b>. Message should disappear within 2 min.</p> <p>Make sure that all hoses are connected to the appropriate ports, and that they clicked into place.</p> <p>Adjust sheath fluid level so that sheath fluid is below the AIR port of sheath bottle.</p> <p>Make sure that the blue-ringed cap is on the sheath bottle and the orange-ringed cap is on the waste.</p> <p>Try new sheath bottle. Call technical service for further assistance.</p> <p>Turn on HTF unit and ensure that message disappears.</p> <p>Listen for air pump to turn on when <b>Warm Up</b> is selected. Contact Technical Support for further assistance.</p>
<b>Bio-Plex Manager has detected a change in the temperature of the array reader. Please calibrate before running on assay to ensure accurate results.</b>	Room temperature has changed	Calibrate array reader.
<b>The calibration was unsuccessful. Please repeat calibration. If calibration fails a second time, consult Troubleshooting Guide.</b>	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. Make sure you are using a clean MCV plate.
<b>The calibration was unsuccessful. Bio-Plex Manager has detected a problem with low bead number. Please repeat calibration.</b>	Calibration procedure failed due to low bead number	Run <b>Unclog</b> procedure, then repeat calibration. If problem persists, contact Technical Support. See "low bead number" in troubleshooting guide.
<b>Optical Validation Procedure shows value(s) outside of acceptable range(s).</b>	Problem with optical component of array reader	Recalibrate the array reader and then repeat validation procedure. If values are still out of range, contact Technical Support.
<b>Reporter Validation Procedure shows value(s) outside of acceptable range(s).</b>	Problem with optical component of reader	Recalibrate the array reader and then repeat validation procedure. If values are still out of range, contact Technical Support.
<b>Classify Validation Procedure shows value(s) outside of acceptable range(s).</b>	Problem with calibration or optical component of reader	Recalibrate the array reader and then repeat validation procedure. If values are still out of range, contact Technical Support.

Message/Problem	Causes	Solution
<b>HTF sheath reservoir does not refill.</b>	<p>Power not on</p> <p>Lines not connected</p> <p>Sheath cube more than 3 ft below HTF</p>	<p>Press prime button on front of HTF.</p> <p>Turn power off then on again on HTF.</p> <p>Disconnect air tubing connecting array reader to HTF. HTF should prime and fill.</p>
<b>HTF audible alarm and red fault indicator light on.</b>	<p>Sheath cube is empty</p> <p>HTF not on level surface</p> <p>Filtered end of sheath tube not below level of sheath in cube</p> <p>Sheath container is not below level of HTF</p> <p>Sheath filter clogged</p> <p>HTF may be overfilled</p>	<p>Insert filtered end of sheath intake line into new container and press <b>Prime</b> button on front of HTF.</p> <p>Ensure that HTF system is on flat surface.</p> <p>Ensure that filter is below level of sheath in cube.</p> <p>Place the sheath cube 3–4 ft below the level of the reader and HTF system (Figure 20).</p> <p>Replace sheath filter.</p> <p>Disconnect the Sheath Out fitting at the array reader. Discharge sheath fluid into a waste container by depressing the plunger at the end of the Sheath Out tubing using a screwdriver or other similar object. Drain until HTF tank is about half full.</p> <p>Call Technical Support if all actions above do not resolve problem.</p>

## 5.2 Troubleshooting Guide for Vacuum Manifold

Message/Problem	Causes	Solution
<b>No Flow/no vacuum.</b>	<p>Lid on plate</p> <p>All wells not wet, or unused wells not covered or sealed</p> <p>Poor alignment of plates with gasket</p> <p>Vacuum trap filled</p> <p>Filter on pump clogged</p> <p>Pump not turned on</p> <p>Manifold ON/OFF valve in OFF position</p> <p>Manifold pressure gauge turned to the lowest value</p> <p>Damaged gasket</p> <p>Gauge plug missing</p>	<p>Remove lid.</p> <p>Wet unused wells with Milli-Q water. (You can reuse these wells later.) Or, tape the unused wells with sealing tape. If you need to seal partial rows or columns, seal the unused rows or columns with tape and leave the adjacent unused row partially sealed. Then wet with buffer since it is difficult to make seals at the row edge.</p> <p>Align plates.</p> <p>Empty trap.</p> <p>Replace filter.</p> <p>Turn on pump.</p> <p>Turn to ON position.</p> <p>Turn up to higher value.</p> <p>Replace gasket.</p> <p>Replace gauge plug in side of manifold ring using hex key wrench.</p>
<b>Wells do not empty at the same time/uneven flow.</b>	<p>Lid on plate</p> <p>Vacuum line turned off or clogged</p> <p>Debris in sample</p> <p>Bad plate-to-vacuum manifold seal plate.</p> <p>Too many beads</p>	<p>Remove lid.</p> <p>Clear line and repeat.</p> <p>Remove cellular debris prior to adding to wells.</p> <p>To ensure a good plate-to-manifold seal, put pressure on corners of plate.</p> <p>Check to ensure that proper bead concentration was used.</p>
<b>Leakage during incubation.</b>	<p>High surfactant concentration</p> <p>Failure to blot under-drain after filtration and before incubation</p> <p>Under-drain contacting surface</p> <p>Absorbent material contacting under-drain</p> <p>Excessive agitation or vibration</p>	<p>Lower the concentration.</p> <p>Blot under-drain.</p> <p>Place plate on smooth, flat surface so nothing touches the underdrain spouts.</p> <p>Place on flat, nonabsorbent material (such as lid).</p> <p>Mix using orbital table at a lower volume (maximum 200 µl on shaker, 340 µl without a shaker) or use lower speed.</p>



## 5.3 Technical Support

For technical assistance with the Bio-Plex 200 system, including all hardware and software, contact your local Bio-Rad office or, in the US, call 1-800-424-6723. All accessories and spare parts not listed in this document can be ordered similarly, or write to Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547.

## Section 6 Bio-Plex 200 System Specifications

### General Technical Specifications

#### Environmental conditions

Operating temperature	15–30°C (59–86°F)
Operating humidity	20–80%, noncondensing
Operating altitude	Designed to operate at 2,400 m (7,874 ft) above mean sea level or below
Compensatory range	± 2°C

**UL installation category** UL Installation Category II, as defined in Annex J of UL 3101-1

**Pollution degree** Pollution Degree 2, as defined in Section 3.7.3.2 of UL 3101-1

### Array Reader Specifications

**Input voltage range** 100–240 V, ~1.5 A, 47–63 Hz

**Physical dimensions (W x D x H)** 43 x 51 x 23 cm

**Weight** 23 kg (60 lb)

#### Lasers

Reporter laser	532 nm, 10 mW
Classification laser	635 nm, 8.5 mW, diode

#### Fluidics

Sheath flow rate	90 µl/sec
Cuvette	200 µm square flow channel
Sample injection rate	60 µl/min

#### Electronics

Reporter channel detection	Photomultiplier tube, A/D resolution 14 bits
Classification and doublet discriminator channel detection	Avalanche photodiodes with temperature compensation, A/D resolution 12 bits
Communications interface	RS232 and USB

#### Signal processing

Measurement resolution	15 bits effective
Processor modes	Linear, with logarithmic or linear display option
Dynamic range	70 dB

## Microplate Platform Specifications

<b>Input voltage range</b>	100–240 V, ~2.25 A, 47–63 Hz
<b>Physical dimensions (W x D x H)</b>	44 x 61 x 8 cm (17.3 x 24 x 3 in)
<b>Weight</b>	14.4 kg (32 lb)
<b>Communications interface</b>	RS232
<b>Plate capacity</b>	One 96-well microplate no thicker than 0.75 in

## HTF Specifications

<b>Input voltage range</b>	100–240 V, 1.8 A, 47–63 Hz
<b>Physical dimensions (W x D x H)</b>	20 x 30 x 75 cm, (8 x 12 x 10 in)
<b>Weight</b>	9 kg (20 lb)

## Computer Specifications

<b>Component</b>	<b>Minimum</b>	<b>Recommended</b>
Operating system	Windows XP or XP Professional	Windows XP Professional
Processor	Pentium 4 or equivalent, 2.8 GHz	Core 2, 2.6 GHz or higher
Hard disk space	80 GB	160 GB
System memory	1 GB	2 GB
Screen resolution	1,024 x 768	1,280 x 1,024
Screen colors	256 colors	24-bit True Color
Ports for connecting Bio-Plex instrument (workstation only)	1 RS232 serial port and 1 USB port	1 RS232 serial port and 1 USB 2.0 port
Port for connecting hardware protection key (software license)	1 USB port	1 USB 2.0 port
Other software	Internet Explorer 6.0 or higher Microsoft Excel 2003 or higher	Internet Explorer 8.0 Microsoft Excel 2007

## **Section 7**

### **Warranty Statement**

This warranty statement may vary outside of the continental United States. Please contact your local Bio-Rad office for the exact terms of your warranty.

Bio-Rad Laboratories, Inc. warrants to the customer that the Bio-Plex® 200 system (catalog #171-000201, 171-000203, 171-000205, and 171-000207) will be free from defects in materials and workmanship, and will meet all performance specifications for the period of 1 year from the date of shipment. If such defects appear within this period, the defective part(s) will be replaced or the entire unit will be replaced, at Bio-Rad's option, free of any charges to the buyer other than expenses incurred in returning the unit to the factory. Bio-Rad's obligation under this warranty is specifically limited to the aforementioned replacement or repairs. However, the following defects are specifically excluded:









1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories, Inc. or their agent.
3. Damage due to use of sheath fluid not specified by Bio-Rad Laboratories, Inc.
4. Damage due to use with bead-based assay reagents not specified by Bio-Rad Laboratories, Inc.
5. Damage due to use with calibration and validation reagents not specified by Bio-Rad Laboratories, Inc.
6. Damage caused by deliberate or accidental misuse.
7. Damage caused by disaster.
8. Damage resulting from facility problems such as power surges.

The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties, of merchantability, fitness for a particular purpose or otherwise, expressed or implied in fact or by law, and state Bio-Rad's entire and exclusive liability and Buyer's exclusive remedy for any claims or damages in connection with the furnishing of goods or parts, their design, suitability for use, installation or operation, Bio-Rad will in no event be liable for any special, incidental or consequential damages whatsoever, and Bio-Rad's liability under no circumstances will exceed the contract price for the goods for which liability is claimed.





No rights or licenses under any of Luminex Corporation's patents are granted by or shall be implied from the sale or acquisition of this Bio-Plex 200 system containing Luminex technology (the "System") to you, the end-user. By using this System, you agree that (i) the System is sold only for use with fluorescently labeled microsphere beads authorized by Luminex ("Beads"), and (ii) you obtain rights under Luminex's patents to use this System by registering this System with Bio-Rad in accordance with the instructions accompanying this System and purchasing a kit containing Beads.

Computer equipment is supplied by an independent vendor. Should you encounter any problem with the computer equipment within 30 days, Bio-Rad will assume responsibility for replacement. Should the problem occur after 30 days, you will be covered by the normal warranty terms and will be put in direct contact with the vendor.

## Section 8 Ordering Information — System Accessories

Catalog #	Description	
<b>General System Accessories</b>		
171-000050	<b>HTF</b>	
171-000055	<b>Sheath Fluid, 20 L</b>	
171-002001	<b>Communication Cable, 5 ft, DB9</b>	
171-002003	<b>Communication Cable, 5 ft, USB</b>	
171-002002	<b>Communication Cable, 3 ft, CAN BUS</b>	
<b>Array Reader Accessories</b>		
171-002010	<b>Sheath Fluid Bottle, 1 L, polypropylene, includes 2 ports and tubing</b>	
171-002012	<b>Sheath Waste Bottle, 2 L, polypropylene</b>	
171-002020	<b>Sample Needle, long, (11.6 cm/4.6 in)</b>	

Catalog Number	Product Description	
171-002030	<b>Protective Shield for Sample Needle</b>	
<b>Preventative Maintenance Items</b>		
171-002032	<b>Air Intake Filter</b> (accessed through back of array reader)	
171-002034	<b>Syringe Seal with Cylinder</b>	
171-002056	<b>HTF System Tubing</b>	
171-002040	<b>Sheath Cube Filter, 10 µm</b>	
171-002038	<b>Sheath Fluid Filter With Quick Connect Tubing</b>	
171-002033	<b>Syringe Seal, (4 per/pack)</b>	
<b>Microplate Platform Accessories</b>		
171-002024	<b>Alignment Guide</b>	
<b>Validation and Calibration Accessories</b>		
171-203060	<b>Bio-Plex Calibration Kit</b> , includes Cal1 and Cal2 calibration beads for approximately 50 daily calibration routines	

Catalog Number	Product Description	
171-203032	<b>MCV plate IV</b> , for use with Bio-Plex Manager 5.0 and 4.1 Software	
171-203050	<b>Bio-Plex Reservoir</b> , for use with Bio-Plex Manager 5.0 Software	
171-203001	<b>Bio-Plex Validation Kit 4.0</b> , includes optics validation, fluidics validation, reporter validation, and classify validation bead sets for approximately 50 validation routines	
931-0350	<b>Bio-Plex Preventative Maintenance Kit</b> , includes all parts required for annual preventative maintenance	

## Section 9 Decontamination Information

Before return shipment of Bio-Plex® 200 system equipment, the accessible surfaces and the internal fluidics system must be sanitized and decontaminated. Before Bio-Rad can accept this equipment, you must certify that it is NOT CONTAMINATED with chemical, radioactive, or biological materials or hazards. Make a copy of these two pages and follow the steps below to complete the decontamination certification on page 42. Place the decontamination certificate in a sturdy envelope and tape to the top of the corrugated shipping box.

If the equipment was used in a class 2, 3, or 4 biohazard work area, or if the equipment was exposed to known carcinogens or teratogens, or exposed to radioisotopes other than those listed on the decontamination certificate, we will not accept it for repair. If you have any questions, contact your local Bio-Rad office or, in the US, call 1-800-4BIO-RAD.

The following checklist is provided for your convenience. Please complete and return with the signed decontamination form.

1. Replace the fluid in the sheath bottle with a solution of 10% household bleach and DI water. Fill the 10% bleach reservoir of the MCV plate with 10% bleach. Fill the DI H<sub>2</sub>O reservoir of the MCV plate with distilled water and place the MCV plate in the microplate platform.
2. Turn on the reader and microplate platform. Open Bio-Plex Manager software. Select **Instrument**, then select **Shut down**.
3. When **Shut down** is complete, close Bio-Plex Manager™ software and turn off the power to the array reader and microplate platform. Disconnect the instrument from AC power by turning off the power switch on the rear of the instrument. Unplug the instrument power cord from the wall source.
4. Disconnect the sheath fluid and waste containers.

5. Drain the sheath fluid and waste containers.
6. Rinse the waste container with 10% household bleach solution and drain.
7. Remove all specimens, disposables, and reagents from the instrument.
8. Wash all exterior surfaces with a mild germicidal detergent, followed by a 10% bleach solution.
9. Open both front doors of the instrument and clean all accessible surfaces with detergent followed by a 10% bleach solution.

Any issues related to decontamination or aerosolization are available separately. Contact Bio-Rad Technical Support.

## **Section 10**

### **Legal Notices**

#### **Trademarks**

xMAP is a trademark of Luminex Corporation. The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corp. Cheminert is a trademark of Valco Instruments Co., Inc. HASP is a trademark of Aladdin Knowledge Systems Ltd. Millipore, Milli-Q, and MultiScreen are trademarks of Millipore Corporation. Pentium is a trademark of Intel Corporation. Teflon is a trademark of E.I. duPont de Nemours & Co. Windows 2000, XP, XP Professional, Excel, and Internet Explorer are trademarks of Microsoft Corporation.

**Equipment Return Information Label and  
DECONTAMINATION CERTIFICATE**

Before Bio-Rad can accept this equipment, you must certify that it is NOT CONTAMINATED with chemical, radioactive, or biological materials or hazards. Please indicate if any of the following potential hazards may have come in contact with the equipment, and the steps you have taken to decontaminate the equipment by marking the appropriate box ().

If the equipment was used in a class 2, 3, or 4 biohazard work area, or if the equipment was exposed to known carcinogens or teratogens, or exposed to radioisotopes other than those listed below, we will not accept it for repair in-house. If you need service for such instruments, or if you have any questions, please call 1-800-4BIO-RAD.

1. Chemicals  
 Strong acids or bases (names and concentrations) \_\_\_\_\_  
 Solvent(s) (name) \_\_\_\_\_  
 Other \_\_\_\_\_  
 No hazardous chemicals came in contact with this equipment.
2. Radioactive Materials (Have any of the following isotopes been used with the equipment?)  
 P32       I 125       S 35       C 14      H 3       N 15  
 Other isotopes \_\_\_\_\_  
If so, were these:     Beta emitters     Gamma emitters     Alpha emitters  
Equipment has been surveyed by (method) \_\_\_\_\_ prior to shipment.  
Reading \_\_\_\_\_  
 No radioactive materials came in contact with this equipment.
3. Biological Hazards (Are any of the following applicable to this equipment?)  
 The equipment contained live microorganisms (for example, bacteria) or live virus.  
 The equipment contained live bacteria other than *E. coli*.  
If so, name of bacteria: \_\_\_\_\_  
 Equipment was used in a class 2, 3, or 4 biohazard work area. (We will not accept it for repair. Please call to make other arrangements.)  
 The equipment contained or was exposed to blood, serum, blood products, or other bodily fluids.  
 No biological hazards have come in contact with this equipment.
4. Decontamination This equipment has been decontaminated with:  
\_\_\_\_\_  
\_\_\_\_\_
5.     This instrument has never been used and is in new condition.

I certify that this instrument has been cleaned and decontaminated of any chemical, radioactive, or biological materials or hazards that may have come in contact with the equipment during the equipment's use and operation.

Signed \_\_\_\_\_ Title \_\_\_\_\_

Date \_\_\_\_\_ Printed Name \_\_\_\_\_

Institution \_\_\_\_\_ Phone \_\_\_\_\_

Address \_\_\_\_\_ Fax \_\_\_\_\_







**Bio-Rad  
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Life Science  
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