Bio-Plex Protein Array System

Hardware Instruction Manual

Catalog Numbers
171-000001
171-000003
171-000005
171-000007

BIO-RAD

Table of Contents

Section 1
General Information

1.1 About this Manual

A Bio-Rad service engineer will install the Bio-Plex protein array system. However, the procedure is provided herein as a reference, in addition to instructions for maintaining your Bio-Plex protein array system. This manual uses certain conventions to facilitate understanding of the text material and to assist operators in using the Bio-Plex protein array 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 protein array system. This system contains electrical, mechanical, and laser components, which, if handled improperly, are potentially harmful. In addition, biological hazards may be present during system operation. Therefore, Bio-Rad recommends that all Bio-Plex protein array 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 warrant voided if the equipment is used in a manner not specified by Bio-Rad Laboratories.

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 Laboratories for further information.

Note: Waste levels must be manually monitored–do not allow the waste container to overflow! Empty 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 21 CFR 1040.10 of the Center for Devices, Radiological Health (CDRH) as a class XX laser device. The two lasers contained within the array reader produce diode laser energy up to 10mW 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.

This label appears on the back panel of the instrument:

This label appears 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
NU NI A L'AIDE D'INSTRUMENTS D'OPTIQUE

Caution: Removal of the Bio-Plex 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 protein array system.

1.2.4 Biological Safety Information

Warning: All human/animal samples may contain hazardous infectious agents. Follow appropriate biosafety procedures when handling these products and 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 and in the microplate platform indicate the on/off status of the System. The blue light emitting diode (LED) does not emit light in the UV spectrum.

Section 2
Introduction

2.1 The Bio-Plex Protein Array System and Multiplexing Technology

The Bio-Plex protein array system is a unique and complete system of 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 insure 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 protein array system is able to distinguish up to 100 different families of color-coded, monodisperse polystyrene beads, each bearing a different homogenous capture assay (but all using the same signal molecule) in a single 50 ul 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 for the automated analysis of 96-well plates. The throughput of samples using this system will allow for analysis of more than 18,000 assay points per hour in a 96-well plate.

The Bio-Plex protein 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 protein. 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 microtiter plate wells. The flow-based Bio-Plex protein array system identifies each specific reaction based on bead color, and quantitates it. The magnitude of the reaction is measured using fluorescent-labeled reporter molecules also specific for each target protein. Bio-Plex Manager software automates data analysis and generation of detailed summary reports. With the Bio-Plex protein array system you can

- Simultaneously quantitate up to 100 of protein analytes from culture media and serum
- Automatically analyze up to 96 samples in 30 minutes
- 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/bioplexsystem.

2.2 Description of System Components

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

- Bio-Plex protein array reader—instrument that sorts and measures signal on the surface of Bio-Plex assay beads
- Bio-Plex microplate platform—automates the reading of 96-well assay plates
- Computer and monitor—controls the Bio-Plex protein array system via Bio-Plex Manager software
- MCV (maintenance, calibration and validation) plate—automates the maintenance, calibration and validation functions of the Bio-Plex array reader
- Calibration kit—kit containing beads to standardize daily signal output and ensure unit to unit reproducibility of the reader
- Validation kit—kit containing beads to validate the primary performance parameters of the reader, including linearity, sensitivity, and reproducibility
- Sheath fluid cube—contains the sheath fluid for the array reader

2.2.1 Bio-Plex Array Reader

The Bio-Plex array reader is a compact flow analysis unit integrating a dual laser detection system, optics, fluidics, and advanced digital signal processing. When used with the Bio-Plex microplate platform, the array reader facilitates the simultaneous analysis of up to 100 different protein analytes from a single sample.

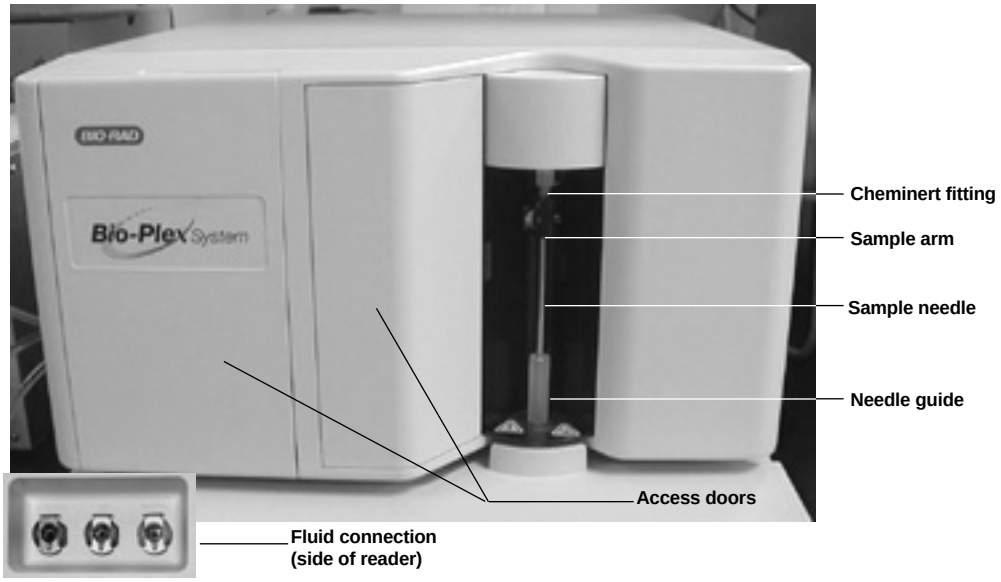

Fig. 1. Bio-Plex Array Reader–front and side panel features.

Table 1. Bio-Plex Array Reader Front and Side Panel Features

Feature	Description
Sample Arm	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.
Sample Needle	A stainless steel sample needle acquires sample from the 96-well plate in the microplate platform.
Cheminert® Fitting	Covered by the sample arm cover, this fitting may be disconnected to allow replacement of the sample needle if necessary.
Needle Guide	Translucent plastic sleeve that assists in guiding the sample needle into the microplate housed in the microplate platform.
Access Doors	There are two access doors on the face of the array reader. The centermost door allows access to the syringe. The left door provides service panel access
Air, Waste Fluid, and Sheath Fluid Connectors	Located on the side of the instrument, these connectors couple directly to the sheath and wastefluid connectors. The air connector is blue, the sheath connector is green, and the waste fluid connector is orange.

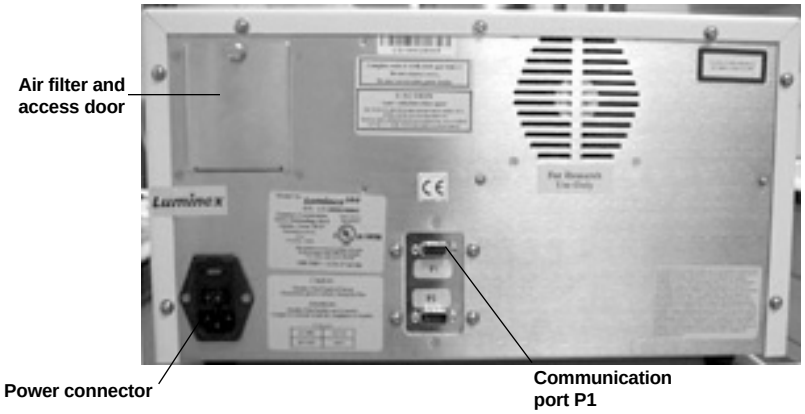

Fig. 2. Bio-Plex Array Reader - rear panel features.

Table 2. Bio-Plex Array Reader Rear Panel Features

Feature	Description
Communications Port P1	A DB9-PIN connector used to connect the Bio-Plex array reader to the computer.
Air Filter and Access Door	A replaceable filter cleans the air used to pressurize sheath fluid. This filter is enclosed behind an access door. Refer to the Maintenance section beginning on page 19 for routine maintenance procedures.
Instrument	Located on the bottom of the instrument, the filter Ventilation Filtermust be checked and cleaned as necessary. Refer to the Maintenance section beginning on page 19 for cleaning procedures.
Power Connector	Contains the instrument on/off switch and fuses. Refer to the Maintenance section beginning on page 19 for fuse replacement instructions.

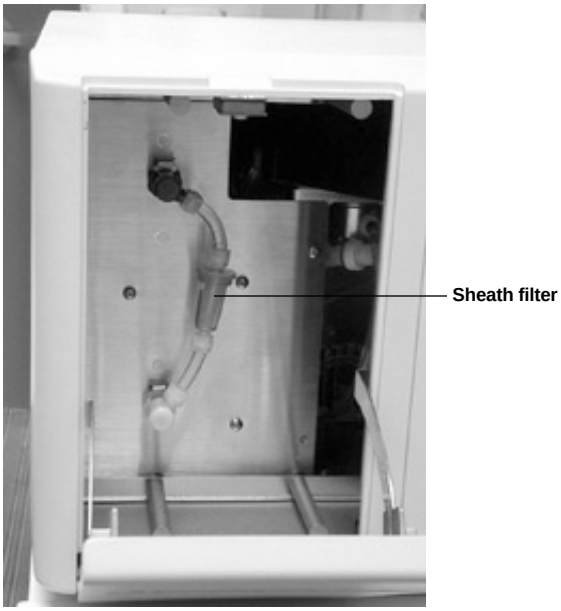

Figure 3. Bio-Plex array reader - internal fluidics features.

Table 3. Bio-Plex Protein Array Reader Internal Fluidics Features

Feature	Description
Syringe	Located behind the center door immediately to the left of the sample needle assembly, the syringe delivers sample from the 96-well microtiter plate to the cuvette via an intermediate sample loop.
Sample Loop (not shown)	The sample is drawn into the sample loop by the syringe pump and injected into the cuvette for analysis.
Sheath Filter	This filter removes particles greater than 5 microns in diameter from the sheath fluid. Refer to the Maintenance section beginning on page 19 for routine maintenance instructions.

2.2.2 Bio-Plex Microplate Platform

The Bio-Plex microplate platform allows the automated processing of samples from a 96-well microtiter plate.

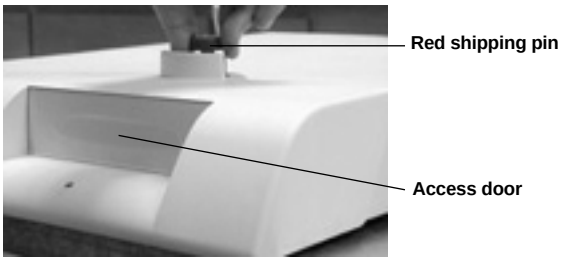

Fig. 4. Bio-Plex microplate Platform–front view.

Fig. 5. Microplate platform–back panel view.

Table 4. Bio-Plex Microplate Platform Features

Feature	Description
Access Door	This door provides access to the assay plate holder. Operation of the access door is controlled through the system software.
Red Shipping Pin	A temporary fitting that is replaced with the Needle Guide
Communications Port	A DB9-PIN connector used to connect the Bio-Plex microplate platform to the computer.
Power Connector	Contains the instrument on/off switch and fuses. Refer to the Maintenance section beginning on page 19 for fuse replacement instructions.

2.2.3 Computer and Monitor

The Bio-Plex protein array system may be supplied with a Dell computer. If this is the case, please transfer the computer's registration to your company's name following unpacking. The Dell Customer support number in the U.S. is 1-800-624-9897. You can also reach Dell through the Internet at www.dell.com.

2.2.4 Maintenance, Calibration, and Validation Plate

The Bio-Plex maintenance, calibration and validation (MCV) plate is a specially designed accessory to facilitate automated Bio-Plex protein array system start up, 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 microtiter plate, it contains labeled wells for bead solutions as well as larger reservoirs for system wash and sterilization solutions.

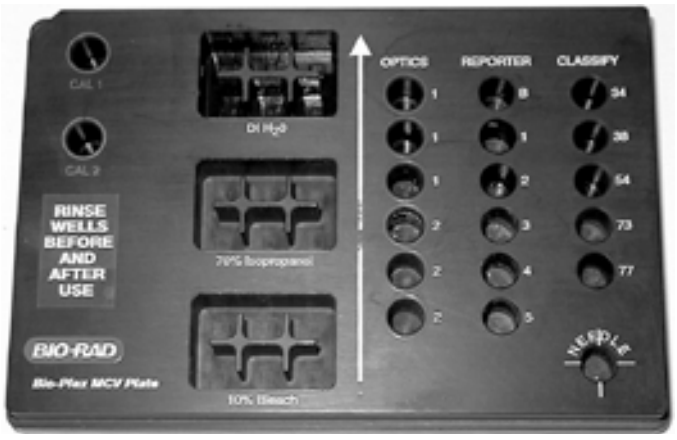

Fig. 6. Maintenance, calibration and validation (MCV) plate.

2.3 Additional Equipment Required (not provided)

For optimal results, we recommend the use of the following:

Microtiter plate shaker
VWR MTS4 shaker for 4 plates, catalog # 33994-220

Vortex mixer
VWR mini-vortexer, catalog #58816-121

Filter plate vacuum apparatus
Millipore 96-well filter plate manifold, catalog # MAVM0960R. Refer to Bio-Plex assay manual for set up and usage instructions specific to Bio-Plex assays.

Filter plate
We recommend the use of Millipore filter plates (catalog #MABVS1210) for all Bio-Plex assays.

Sonicator bath

Surge protector
We recommend the use of a 6-outlet surge protector, with minimum surge current of 12,000 amps; watts: 1500; clamping voltaqge: 336 volts; clamping response: <500 picoseconds; maximum leakage current: <50 microamps

2.4 Bio-Plex Assays

The Bio-Plex assays have been carefully integrated into the Bio-Plex system to ensure seamless operation and accurate, reproducible results. Some general information regarding Bio-Plex assays is listed below. For more specific information, consult Bio-Plex assay manuals or visit our web site at www.bio-rad.com/bioplexsystem.

2.4.1 Assay Beads

Bio-Plex assays utilize 5.5um uniform polystyrene microspheres. The reaction occurs on the surface of each microsphere, as opposed to the bottom of a microtiter plate in traditional immunoassays resulting in more efficient kinetics and increased sensitivity.

A total of 100 color-coded beads are available for use in the Bio-Plex protein array system and are named from 1 to 100. It is possible to multiplex 100 analytes within a single sample. Bio-Plex Manager permits simple protocol set-up for each multiplex assay. See Bio-Plex Manager software manual for more details.

For convenience, 96-well filter plates are used to perform assays with beads. Incubation and washing steps can be performed in a single plate without transfer of beads.

Bio-Rad offers a series of preformatted kits for your convenience. However, if you choose to develop a custom assay, Bio-Rad offers a protein coupling kit that allows you to create your own set of unique analytes using the Bio-Plex protein array system.

2.4.2 Sample Types Compatible with the Bio-Plex Assay

Sample types that are compatible with the Bio-Plex system include serum, plasma and cell lysate preparations. Listed below are a few tips to maximize performance of the Bio-Plex protein array system:

- Pre-filter samples with a 0.45 micron, hydrophilic, low protein-binding filter to minimize the incidence of fluidics clogging problems.
- Always resuspend samples in a PBS-based buffer before reading on the Bio-Plex array reader.
- Follow closely the instruction described in the preformatted kit.

Section 3
Installing the Bio-Plex System

An authorized Bio-Rad service representative will set up the Bio-Plex protein array system in your laboratory. The following general set up procedure is provided here for reference.

3.1 Unpacking

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

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

Warning: The Bio-Plex 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 protein array 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.
2. Do not obstruct the area below the Bio-Plex array reader, and allow at least 2" of clearance around the machine.
3. Avoid locating the instrument where drafts are present.

4. The ambient temperature should be stable and within the range of 15°C to 30°C (21°C is optimal) and that the relative humidity not exceed 80%, non-condensing. It is preferable to place the instrument in a location where the temperature does not deviate by more than +/- 2°C. Avoid drafty locations as this may contribute to excessive temperature fluctuation.
5. The maximum distance between the computer and the microplate platform and array reader should be 1.5 meters (5 ft.), the length of the communications cable supplied with the instruments.

Note: The Bio-Plex 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 optical alignment procedure.

3.3 Bio-Plex Microplate Platform Setup

The Bio-Plex microplate platform should be shipped with the following items:

- Bio-Plex microplate platform
- Power cord
- Communication cable
- Needle guide
- Sample needle (11.7 cm/4.6 in.)
- Needle adjustment tool (hex key wrench)
- 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 the red shipping pin.

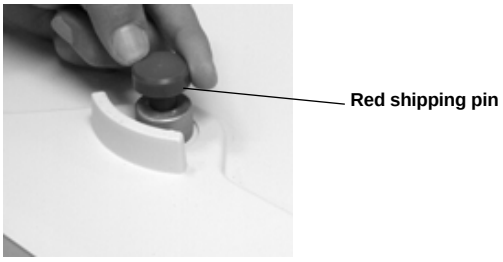

Figure 7. Remove shipping pin.

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 yet until the array reader and microplate platform are aligned (Figure 8).
5. Position the microplate platform in the location where you want it to be used.
6. Connect the communication cable to Port 2 on the rear of the computer.

3.4 Bio-Plex Array Reader Setup

The Bio-Plex array reader should be shipped with the following items:

- Bio-Plex array reader
- Power cord
- Communication cable
- Sheath fluid bottle
- Sheath waste bottle
- Sample tube holders (2; for 1.2 ml and 1.5 ml tubes)

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. Slide the array reader forward until the reader's sample port is aligned over the microplate platform sample port.

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.

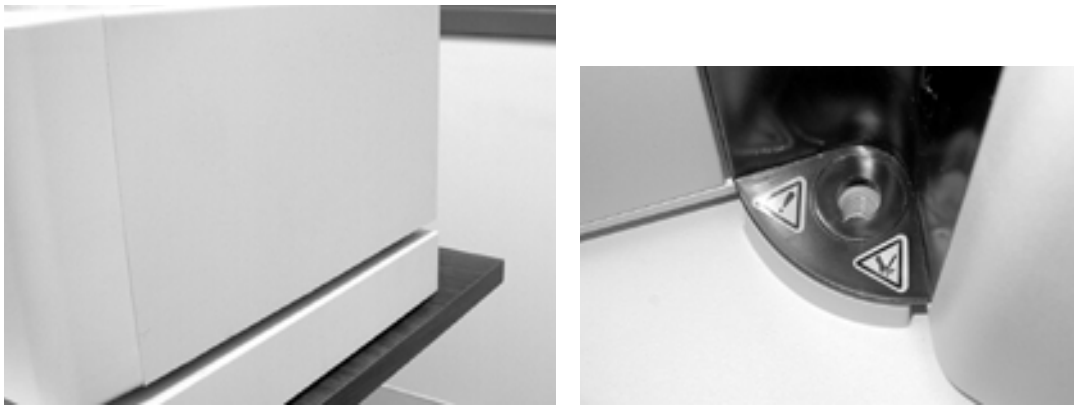

Fig. 8. Reader aligned on top of microplate platform.

3. Install the needle guide (translucent plastic tube shipped with the microplate platform) by screwing it into the microplate platform as shown in Figure 9. It may be necessary to slightly move the position of the array reader to allow for complete installation of the needle guide.

Note: Make sure the needle guide is completely screwed in. Failure to completely screw in the needle guide may prevent sample needle from moving to the down position.

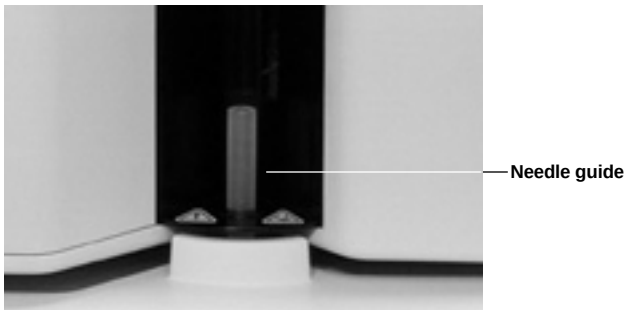

Fig. 9. Needle guide positioning.

4. Plug the microplate platform power cord into an approved outlet. A surge protector (UPS) is recommended to protect the microplate platform from power fluctuations. See "Additional Equipment Required" on page 10.
5. Connect a communication cable into Port 1 (P1) at the rear of the array reader, and to communication port 1 at the back of the computer. Connect a power cord to the array reader's power connector (Figure 11).
6. Connect the communication cable in the microplate platform communication port to communication port 2 of the computer (Figure 11).
7. Plug the array reader and microplate platform power cords into approved electrical outlet.

3.5 Sheath and Waste Fluid Container Connections

1. Attach the waste container (orange-ringed cap) to the orange "Waste" connector on the left side of the array reader. An audible "click" indicates proper connection. The waste container should not be placed on top of the instrument.

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

2. Attach the sheath container (blue-ringed cap) to the array reader as follows:
   1) connect the air line (uppermost tube) to the green connector on the array reader
   2) 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 container must be placed at the same level as the Bio-Plex protein array system.

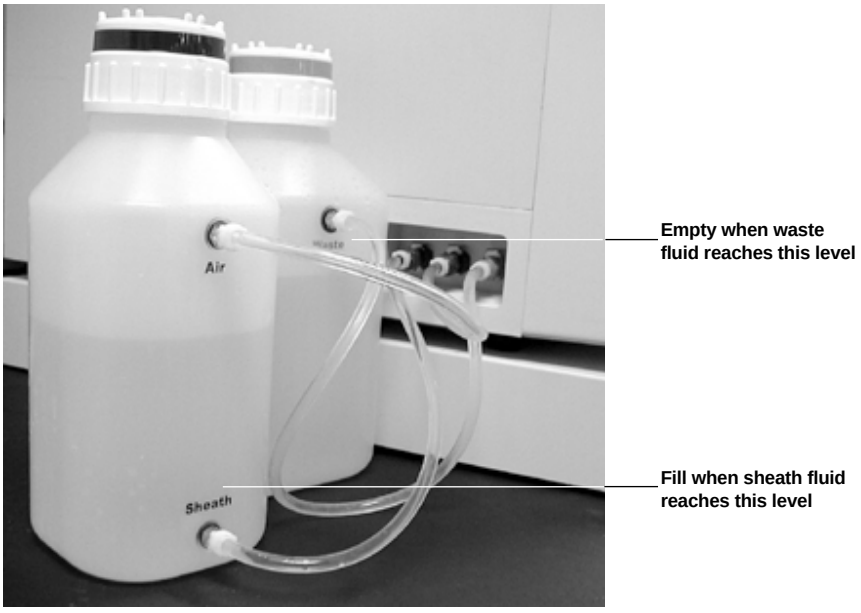

Fig. 10. Waste and sheath fluid bottle connections.

2. Fill the sheath fluid container with sheath fluid supplied in the 20-liter container (catalog # 171-000055) to just below the air intake. Tighten cap (See Figure 10).

Note: To set up the sheath fluid container for use, remove tape from container 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.

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

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

3.6 Computer and Monitor Connections

When you receive the Bio-Plex protein array system, please transfer the computer's registration to your company's name. The Dell customer support number in the U.S. is 1-800-624-9897. You can also reach Dell through the Internet at www.dell.com.

1. Unpack the computer and place on bench adjacent to the Bio-Plex protein array system. Typical computer placement is to the right of the system since sheath and waste fluid bottles are located on the instrument's left side. DO NOT place the computer on top of the Bio-Plex array reader.
2. Unpack the monitor and place on top of computer, or other suitable place.

   DO NOT place the monitor on top of the Bio-Plex 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 4.1 for software loading requirements.

Fig. 11. Completed connections of computer and monitor to the Bio-Plex protein array system.

3.7 Installing/Changing Sample Needle

3.7.1 Installing the long sample needle

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

Your Bio-Plex array reader was shipped with a small sample needle installed. For use with the microplate platform, the long sample needle (11.7 cm/4.6 in.) shipped with the microplate platform must be installed.

1. Make sure that the power to the array read 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 12). The housing remains attached by a wire. Place the housing on top of the array reader, out of the way.

Fig. 12. Remove 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 13). If the tubing is difficult to remove, push up gently on the sampling needle.

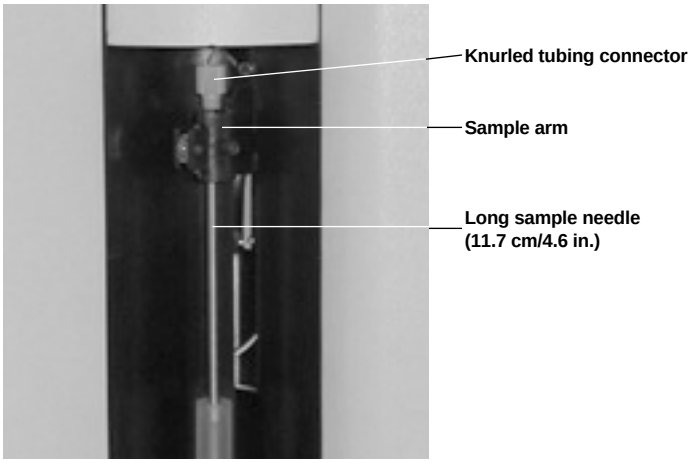

Fig. 13. Sample needle assembly.

4. Remove 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 alignment guide. If the needle is not aligned into the needle alignment guide, carefully reposition the array reader to align the needle and the alignment 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. Re-install 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.

3.7.2 Installing the Short Sample Needle

For troubleshooting fluidics problems, it may be necessary to install the short needle. Follow the steps below to install the short needle and use the Bio-Plex protein array system without the microplate platform.

1. Follow steps 1 through 4 from "Installing the long sample needle".
2. Remove the needle alignment guide by unscrewing the guide counterclockwise.

3. Install the white plastic sample tube holder where the needle alignment guide was previously located. It has a keyhole configuration so that it may only be installed in one direction.

   Note: There are two sample tube holders that were shipped with your system. The larger one accommodates 1.5 ml Eppendorf tubes.

4. Insert the short needle into the sample arm. 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.

5. Re-install the light housing by pushing until it snaps into place.

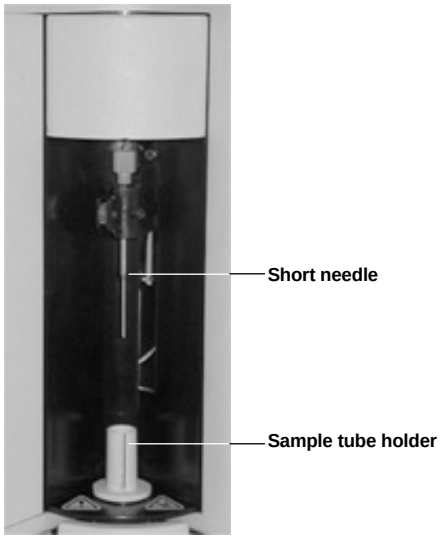

Fig. 14. Short sample needle assembly.

Section 4
Getting Started

4.1 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. Insert Bio-Plex Manager CD-ROM into the CD drive of the computer.

2. Select Install Bio-Plex Manager

4.2 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 microtiter plate 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 # MABVS1210).

1. Turn on Bio-Plex array reader and microplate platform.

2. Open up the Bio-Plex Manager software.

3. Click on Instrument in the menu bar of the software
4. Choose Set up. Choose Adjust Needle from the pulldown menu. The following dialog box appears (See Figure 15):

Fig. 15. Sample needle dialog.

5. Click *Eject/Retract* to eject the plate holder.
6. Place the MCV plate on the microplate platform with the white arrow facing toward the array reader.
7. Click on the *Eject/Retract* button to retract the plate.
8. Tape the access door of the microplate platform open. It will be necessary to be able to see inside the access door.
9. In the *Adjust Needle* window click on the *Up/Down* button. The needle will move to the down position.
10. Using the hex key needle adjustment tool (supplied with the microplate platform), loosen the screw at the top of the needle so that the needle housing can move up and down freely (See Figure 16).

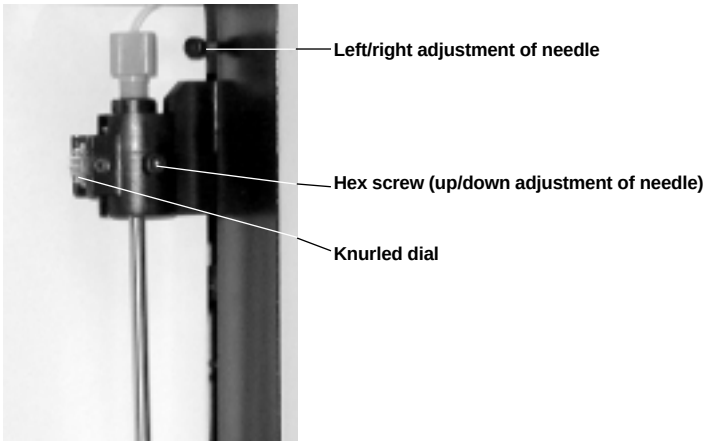

Fig. 16. Sample needle assembly.

11. Manually move the knurled dial on the needle arm downward 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 touching the bottom of the well.
12. Tighten the needle set screw so that it is no longer possible to manually move the needle up and down.

13. 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 not force the MCV plate down when it is in the"down" position. If the MCV plate moves when the needle moves to the down position, readjust the needle height by repeating the previous steps.
14. Check the right-left alignment of the needle by selecting *Up/Down* and checking to ensure that the needle moves up and down freely without rubbing on the sides of the needle alignment guide (Figure 17).
15. To adjust the needle left to right, loosen the left/right adjustment hex screw and adjust needle so that it is centered in the needle alignment guide. Retighten screw.

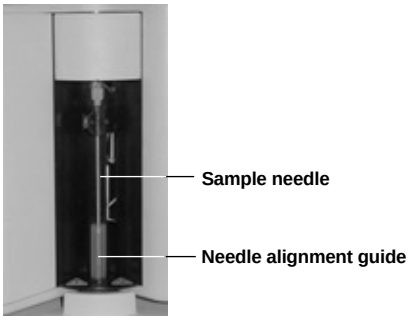

Fig. 17. Sample needle and needle guide.

16. When the needle is adjusted properly, click the Eject button across the top of the title bar.
17. Remove the MCV plate from the microplate platform.
18. Click on the OK button in the Adjust Needle window.

4.3 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.

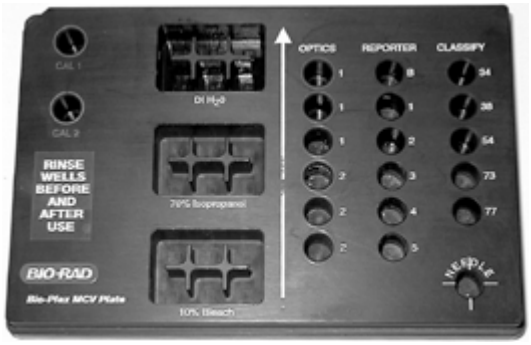

Fig. 18. MCV plate.

2. Click the *Eject* icon. Insert the MCV Plate into the microplate platform. Click *Retract*.
3. Choose Maintenance from the main menu bar. Choose Additional Functions, followed by Prime.
4. Inspect the waste line outside the instrument for air pockets. Repeat the prime procedure until no air pockets are observed in the waste line outside of the array reader. This may require a few prime cycles.

5. Choose *Maintenance* from the main menu bar. Choose *Additional Functions*. Choose *Alcohol Flush*. Wait for function to complete.
6. Choose *Maintenance* from the main menu bar. Choose *Additional Functions*. Click *Wash*. Wait for function to complete.
7. Repeat *Wash* two more times.

4.4 Performing System Validation

Prior to performing analyses with the Bio-Plex protein array system, the user should verify that the system is performing according to specifications using the Bio-Plex validation kit. (Catalog No. 171-203000). See the validation kit manual for complete system validation instructions.

Section 5
Care and Maintenance

Proper maintenance and cleaning of the Bio-Plex protein array system 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 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. *Note*: If a leak or spill is observed, verify whether or not it is coming from the sample needle assembly. Check to see that the knurled tubing connector is tightened properly (see Section 3.7.1). 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 service for further information.

Every month

Sanitize

Fill MCV plate with a solution of 10% bleach/90% water/0.02N NaOH. Place MCV plate in microplate platform. Choose **Shut Down**.

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 six months

We highly suggest that a Bio-Rad field service engineer perform these following tasks. A service contract may be obtained by contacting Bio-Rad Laboratories.

Air intake filter

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

Replace the air intake filter every 6 months. 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 inches 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.

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; then 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 5 times, watching for any leaks in the syringe area. When finished, close the center door. See Table 2 and Figure 3.

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. Re-install it with the arrows facing up.

Pinch valve

The pinch valve squeezes and releases fluidic tubing. Move the tubing every 6 months to avoid crimping of the fluidic line. This service only can be performed by a Bio-Rad Field Service Engineer.

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.

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 4.2, Adjusting Sample Needle Height.

Section 6
Troubleshooting

6.1 Troubleshooting Guide

Message/Problem	Causes	Solution
Message: 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 10 minutes before analysis.	Remove plate from array reader and shake for 10 seconds
	Buffer volume in wells is too low (must be at least 125 µl).	Re-suspend in 125 µl. 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 service.
	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 service.
	Filter plate not flat.	Check filter plate flatness (see hardware manual).
	Incompatible suspension buffer used.	Check buffer compatibility (see hardware manual).
Message: Bio-Plex Manager has detected a problem with bead selection	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 (5,000 per region recommended).	Verify that the correct number of beads were used.

Message/Problem	Causes	Solution
Bio-Plex Manager has detected a problem with assignment of beads into regions	**Most likely:**	
	Calibration was performed before the array reader was warmed up.	Perform 30-minute **WARM UP** and recalibrate.
	Improper calibration	Check that the target values of the CAL beads match values entered in the software, then recalibrate.
	Microbubbles present in cuvettte.	Perform **REMOVE BUBBLES.**
	Less likely:	
	Calibration beads are photobleached (do not expose to light for more than 1 hour).	Recalibrate with new CAL 1 beads.
	Array reader was calibrated with a dirty MCV plate.	Clean MCV plate and recalibrate.
	Misalignment of optics.	Perform **OPTICAL VALIDATION**. Contact technical service if values are not within range.
Bio-Plex Manager has detected a problem with aggregated beads	Clumped beads present	Vortex plate at 900 rpm for 1 minute
	Sheath reservoir is empty	Refill sheath reservoir. Perform **START UP**.
	Waste reservoir is overfilled	Empty waste and reconnect.
	Bio-Plex beads not used in assay.	Use only Bio-Plex assays.
	Incompatible suspension buffer used.	Check hardware manual for buffer compatibility.
Bad 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 assay reader.	Close and restart Bio-Plex Manager.
	Cables from computer to 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	Causes	Solution
No Assay Signal Detected	**Most likely:**	
	Error in assay preparation.	Make sure that streptavidin and cytokine standards have been added. See the Bio-Plex assay kit manual.
	Incorrect target values used in calibration.	Check that target values in calibration dialog box 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**.
	Less likely:	
	Waste line not connected properly.	Check waste line connection. Make sure that it clicks into place.
	Laser not functioning.	Perform **CALIBRATE**. Call technical service for further assistance.
Bio-Plex Manager has detected a change in sheath pressure	**Most likely:**	
	Sheath reservoir cap not on securely.	Tighten sheath cap. Click **OK**. 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 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 service for further assistance.
	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 service for further assistance.
Bio-Plex Manager has detected a change in the temperature of the array reader. Please calibrate before running on assay to ensure accurate results.	Room temperature has changed.	Calibrate array reader.

Problem	Possible Cause	Solution
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.
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 service.
Optical 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 service.
Reporter Validation Procedure shows value(s) outside of acceptable range(s).	Problem with optical component of reader.	Repeat validation procedure. If values are still out of range, contact technical service.
Classify Validation Procedure shows value(s) outside of acceptable range(s)	Problem with calibration or optical component of reader.	Repeat validation procedure. If values are still out of range, contact technical service.

6.2 Technical Service

For technical assistance with the Bio-Plex protein array 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 by contacting your local Bio-Rad office or, in the US, call 1-800-424-6723, or write to Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547.

Section 7
Bio-Plex Protein Array System Specifications

General Technical Specifications

Environmental conditions	
Operating temperature	15 °C to 30 °C (59° to 86° F)
Operating humidity	20% to 80%, non-condensing
Operating altitude	Designed to operate at 2000 m (6600 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

Bio-Plex Array Reader Specifications

Input voltage range	100–240 V~, 1.5 Amps, 47–63 Hz
Physical dimensions	43 cm (17" W) x 51 cm (20" D) x 23 cm (9" H)
Weight	23 kg (60 lbs)
Lasers	
Reporter laser	532nm, >10mW, Frequency-doubled diode; 30 x 60 micron elliptical beam.
Classification laser	635 nm, 10 mW, diode; 30 x 60 micron elliptical beam.
Fluidics	
Sheath flow rate	90 mL/sec
Cuvette	200 mM square flow channel
Sample injection rates	1 mL /sec, 0.75 mL /sec, or 0.5 mL /sec
Electronics	
Reporter channel detection	Photomultiplier tube, A/D resolution 12 bits
Classification and doublet discriminator channel detection	Avalanche photo diodes with temperature compensation, A/D resolution 12 bits
Communications interface	RS 232
Signal processing	
Measurement resolution	15 bits effective
Processor modes	Linear, with logarithmic or linear display option
Dynamic range	70 dB

Bio-Plex Microplate Platform Specifications

Input voltage range	100–240 V~, 2.25 Amps, 47–63 Hz
Physical dimensions	44 cm (17.3” W) x 61cm (24” D) x 8cm (3“ H)
Weight	14.4 kg (32 lbs)
Communications interface	RS 232
Plate capacity	One 96-well microtiter plate no thicker than 0.75 inches

Section 8
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 warrants to the customer that the Bio-Plex protein array system (catalog numbers 171-000001, 171-000003, 171-000005, and 171-000007) 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 or their agent.
3. Damage due to use of sheath fluid not specified by Bio-Rad Laboratories.
4. Damage due to use with bead-based assay reagents not specified by Bio-Rad Laboratories.
5. Damage due to use with calibration and validation reagents not specified by Bio-Rad Laboratories.
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 thefurnishing 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 protein array 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.

Section 9
Ordering Information–System Accessories

Catalog Number	Product Description
General System Accessories	
171-000055	**Sheath Fluid**, 20 L
171-002001	**Communications Cable**, 5 ft., DB9
Array Reader Accessories	
171-002010	**Sheath Fluid Bottle**, 1 L, polypropylene, including 2 ports and tubing
171-002012	**Sheath Waste Bottle**, 1.2 L, polypropylene, including 1 port and tubing
171-002015	**Replacement Tubing**, sheath and waste bottles
171-002020	**Sample Needle**, 11.7 cm/4.6 in.
171-002026	**Needle Adjustment Tool**
171-002030	**Protective Shield**
171-002032	**Air Intake Filter** (accessed through back of array reader)
171-002036	**Ventilation Filter** (on bottom of array reader)
171-002034	**Syringe Seal**
171-002038	**Sheath Filter and Tubing**
Microplate Platform Accessories	
171-002023	**Needle Guide**
Validation and Calibration Accessories	
171-203030	**Maintenance, Calibration and Validation (MCV) plate**
171-203000	**Bio-Plex Validation Kit**, includes optics validation, reporter validation, and classify validation bead sets for approximately 50 validation routines
171-203060	**Bio-Plex Calibration Kit**, includes CAL1 and CAL2 calibration beads for approximately 50 daily calibration routines

Section 10
Decontamination Information

Before return shipment of Bio-Plex protein array 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. 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 below, we will not accept it for repair. If you have any questions please call 1-800-4BIO-RAD.

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

1. Replace the fluid in the sheath bottle with a solution of 10% household bleach and water. Fill the 10% bleach reservoir of the MCV plate with 10% bleach. Fill the DI H_2O reservoir with distilled water
2. Turn on the reader and microplate platform. Open Bio-Plex Manager. Fill the MCV plate reservoirs with 10% bleach and Di H_2O. Select Instrument, select Shut Down.
3. Close Bio-Plex Manager and turn off the power to the 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.

Was there an internal leak in the instrument?

Yes No

Please indicate if any of the following potential hazards may have come into contact with the equipment and the steps you have taken to decontaminate the equipment by marking the appropriate box ( ).

Instrument Serial Number: _____

1. Chemicals

   Strong Acids or Bases, Concentration and Name _____

   Solvents (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?)

   P 32 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 (e.g. 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:

_____

_____

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 use and operation.

Signed _____ Title: _____

Date: _____ Printed Name: _____

Institution: _____ Phone: _____

Address: _____ Fax: _____

Bio-Rad Laboratories

Life Science Group

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Brazil *Ph. 55 21 507 6191* ***Canada*** *Ph. (905) 712-2771, Fx. (905) 712-2990* ***China*** *Ph. 86-10-8201-1366/68, Fx. 86-10-8201-1367*
Denmark *Ph. 45 44 52-1000, Fx. 45 4452 1001* ***Finland*** *Ph. 358 (0)9 804 2200, Fx. 358 (0)9 804 1100* ***France*** *Ph. 01 47 95 69 65, Fx. 01 47 41 9133*
Germany *Ph. 089 318 84-177, Fx. 089 318 84-123* ***Hong Kong*** *Ph. 852-2789-3300, Fx. 852-2789-1257* ***India*** *Ph. (91-124)-6398112/113/114, Fx. (91-124)-6398115*
Israel *Ph. 03 951 4124, Fx. 03 951 4129* ***Italy*** *Ph. 34 91 590 5200 , Fx. 34 91 590 5211* ***Japan*** *Ph. 03-5811-6270, Fx. 03-5811-6272*
Korea *Ph. 82-2-3473-4460, Fx. 82-2-3472-7003* ***Latin America*** *Ph. 305-894-5950, Fx. 305-894-5960* ***Mexico*** *Ph. 52 5 534 2552 to 54, Fx. 52 5 524 5971*
The Netherlands *Ph. 0318-540666, Fx. 0318-542216* ***New Zealand*** *Ph. 64-9-4152280, Fx. 64-9-443 3097* ***Norway*** *Ph. 47-23-38-41-30, Fx. 47-23-38-41-39*
Russia *Ph. 7 095 979 98 00, Fx. 7 095 979 98 56* ***Singapore*** *Ph. 65-2729877, Fx. 65-2734835* ***Spain*** *Ph. 34-91-590-5200, Fx. 34-91-590-5211*
Sweden *Ph. 46 (0)8-55 51 27 00, Fx. 46 (0)8-55 51 27 80* ***Switzerland*** *Ph. 061-717-9555, Fx. 061-717-9550* ***United Kingdom*** *Ph. 0800-181134, Fx. 01442-259118*

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