Requires Bio-Plex Manager™ 4.1 software (or later versions)

Bio-Plex® Precision Pro™

Cytokine Assay

Instruction Manual

For technical support, call your local Bio-Rad office or in the US, call 1-800-4BIORAD (1-800-424-6723).

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Section 1
Introduction

Bio-Plex® Precision Pro™ cytokine assays are highly sensitive magnetic bead-based multiplex assays that allow the accurate measurement of low levels of cytokines in diverse matrices including serum, plasma, and culture supernatant. The multiplexing feature makes it possible to quantitate the level of multiple cytokines in a single well of a 96-well microplate in just 3 hr, using as little as 12.5 µl of serum or plasma, or 50 µl of culture supernatant.

As one of the most recent additions to the Bio-Plex suspension array system, these assays incorporate magnetic beads into their design. The magnetic beads allow the use of an assay protocol similar to non-magnetic Bio-Plex cytokine assays, with the option of using magnetic separation of wash steps instead of vacuum filtration (and allows automation of many of the steps). The 25-bead map in Bio-Plex Manager™ 4.1 software (or later versions) is required for data acquisition.

These assays are offered in a convenient kit format that includes assay, reagent, and diluent components in a single box. Standard diluents for serum and plasma are included, as are additional lylophilized cytokines which can be used to prepare user-specified quality controls.

For a current listing of Bio-Plex Precision Pro cytokine assays, visit us on the Web at **www.bio-rad.com/bio-plex/**

Section 2
Principle

Technology

The Bio-Plex® suspension array system is built around three core technologies. The first is a novel technology that uses up to 100 unique fluorescently dyed beads (xMAP technology) that permit the simultaneous detection of up to 100 different types of molecules in a single well of a 96-well microplate. The second is a flow cytometer with two lasers and associated optics to measure the different molecules bound to the surface of the beads. The third is a high-speed digital signal processor that efficiently manages the fluorescent output.

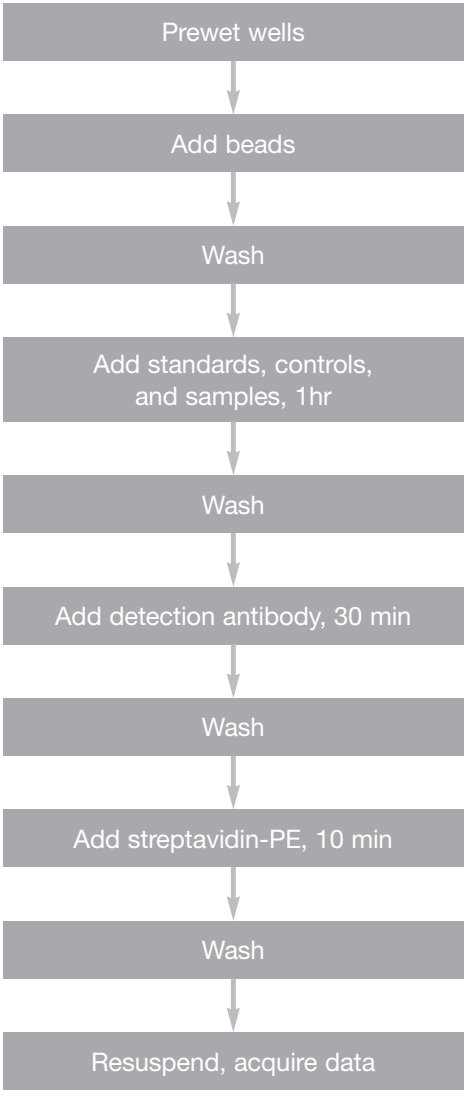
Assay Format

The principle of these 96-well plate-formatted, bead-based assays is similar to a capture sandwich immunoassay. An antibody directed against the desired target cytokine is covalently coupled to internally dyed beads. The coupled beads are allowed to react with a sample containing the target cytokine. After a series of washes to remove unbound protein, a biotinylated detection antibody specific for a different epitope is added to the reaction. The result is the formation of a sandwich of antibodies around the target cytokine. Streptavidin-phycoerythrin (streptavidin-PE) is then added to bind to the biotinylated detection antibodies on the bead surface.

Data Acquisition and Analysis

Data from the reaction are then acquired using the Bio-Plex suspension array system (or Luminex system), a dual-laser, flow-based microplate reader system. The contents of the well are drawn up into the reader. The lasers and associated optics detect the internal fluorescence of the individual dyed beads as well as the fluorescent signal on the bead surface. This identifies each assay and reports the level of target protein in the well. Intensity of fluorescence detected on the beads indicates the relative quantity of targeted molecules. A high speed-digital processor efficiently manages the data output, which is further analyzed and presented as fluorescence intensity on Bio-Plex Manager™ software, the accompanying software package.

Assay Workflow



Section 3
Required Materials

Bio-Plex® Precision Pro™ assays are offered in a convenient kit format that includes assay, reagent, and diluent components all in a single box (does not require separate reagent and diluent kits). These assays require the use of Bio-Plex Manager™ software version 4.1 or higher.

Component	Units
Coupled magnetic beads (25x)	1 vial
Detection antibodies (10x)	1 vial
Standard	2 vials
Control	1 vial
Standard diluent (serum)	10 ml
Standard diluent (plasma)	10 ml
Sample diluent	15 ml
Assay buffer	75 ml
Wash buffer	150 ml
Detection antibody diluent	15 ml
Streptavidin-PE (100x)	1 vial
Sterile filter plate (96-well)	1 plate
Sealing tape	1 pack of 4

Storage and Stability

Kit components should be stored at 4ºC and should never be frozen. Coupled magnetic beads and streptavidin-PE should be stored in the dark. All components are guaranteed for up to 6 months from the date of purchase when stored as specified in this manual.

Section 4
Recommended Materials

For optimal results, the use of the items below is recommended.

Item	Ordering Information
Bio-Plex® Suspension Array System (or Luminex System)	Bio-Rad catalog #171-000205
Bio-Plex Validation Kit	Bio-Rad catalog #171-203001
Bio-Plex Calibration Kit	Bio-Rad catalog #171-203060
Microtiter Plate Shaker IKA-Schuttler MTS-4 shaker for 4 microplates or Lab-Line Model 4625 Plate Shaker (or equivalent, capable of 300–1,100 rpm)	IKA catalog #3208000 VWR catalog #57019-600
Filter Plate Vacuum Apparatus Millipore MultiScreen vacuum manifold or Bio-Rad Aurum™ vacuum manifold **IMPORTANT**: The use of filter plate manifolds other than the one specified may result in diminished assay performance; see section 8 for instructions specific to this assay	Millipore catalog #MAVM0960R Bio-Rad catalog #732-6470
Vortexer VWR brand mini-vortexer Scientific Instruments Vortex-Genie 2 mixer	VWR catalog #58816-121 VWR catalog #58815-234
Reagent Reservoir Corning, Inc. Costar 50 ml reagent reservoir 4870	Bio-Rad catalog #224-4872
Other Materials Pipets and pipet tips, sterile distilled water, aluminum foil, absorbent paper towels, 1.5 ml microcentrifuge tubes, 15 ml culture tubes	

Section 5
Sample Preparation

This section provides instructions for preparing samples derived from serum, plasma, and culture supernatant. For sample preparations not mentioned here, consult the publications listed in Bio-Rad bulletin 5297, available for download at **discover.bio-rad.com**

Serum and Plasma Samples

Note that for plasma samples, EDTA tubes are recommended; however, sodium citrate tubes are acceptable. Extremely lipemic samples may be filtered with a 0.22 µm filter to prevent clogging. Hemolyzed samples are not suitable for Bio-Plex® Precision Pro™ cytokine assays.

1. Collect and process the serum or plasma samples and assay immediately or freeze at –20ºC. Avoid repeat freezing and thawing.
2. Centrifuge the samples at 13,200 rpm for 10 min at 4ºC to clear the samples of precipitate. Alternatively, carefully filter the samples with a 0.22 µm filter to prevent instrument clogging.
3. Immediately dilute 1 volume of sample with 3 volumes of sample diluent. Keep the samples on ice until ready for use.

Culture Supernatant Samples

1. Collect and process the culture supernatant samples and assay immediately or freeze at –20ºC. Avoid repeat freezing and thawing.
2. If required, dilute the culture supernatant with culture medium. Serum-free culture medium should contain carrier protein (such as BSA) at a concentration of at least 0.5%. Keep the samples on ice until ready for use.

Section 6
Standard Preparation

Two tubes of lyophilized cytokine standard are provided in each Bio-Plex® Precision Pro™ cytokine assay. However, only one of the tubes is required per 96-well plate. The product insert provided with the assay lists the concentration of the reconstituted standard. This procedure will prepare enough standard to run each dilution in duplicate.

Reconstitute Standards

1. Gently tap the glass vial containing the lyophilized cytokine standard on a solid surface to ensure the pellet is at the bottom.
2. Reconstitute 1 vial of lyophilized standard with 500 µl of the appropriate standard diluent. Do not use assay buffer to dilute standards.

Sample	Standard Diluent
Serum	Serum standard diluent
Plasma	Plasma standard diluent
Culture supernatant	Same culture medium used to prepare samples

3. Gently vortex 1–3 sec and incubate on ice for 30 min. Be consistent with the incubation time for optimal assay performance.

Prepare Standard Dilution Series

The cytokine concentrations specified for the 8-point standard dilution set have been selected for optimized curve fitting using the 5-parameter logistic (5PL) or 4-parameter logistic (4PL) regression in Bio-Plex Manager™ software. Results generated using dilution points other than those listed in this manual have not been optimized.

1. Label a set of 1.5 ml Eppendorf tubes as shown in the diagram on the next page.

2. Pipet the appropriate volume of standard diluent into the tubes (see diagram below). Use serum standard diluent for serum samples, plasma standard diluent for plasma samples, and culture medium for culture samples.

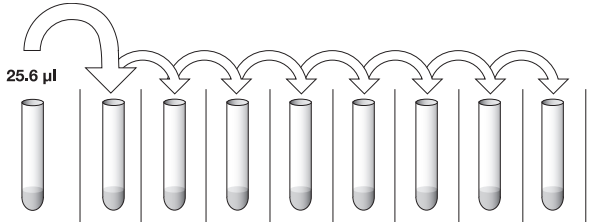
3. Add 25.6 µl of the reconstituted standard to the first 1.5 ml tube containing 374.4 µl of standard diluent. Vortex gently. This is identified as S1 in the diagram below and in the product insert provided with assay.

4. Continue making serial dilutions of the standard as shown. After making each dilution, vortex gently and change the pipet tip after every transfer.

   NOTE: Running an additional two 0 pg/ml blanks is strongly recommended. Use 50 µl of the appropriate standard diluent as the blank sample. The 0 pg/ml points should be formatted as blanks, not as points in the curve, when using Bio-Plex Manager software. The blank wells are also useful for troubleshooting and determining LOD.

5. Keep the standards on ice until ready for use. Standards should be used immediately and should not be frozen for future use.

Standard Dilution Series



Reconstituted standard								
Transfer volume (µl)	25.6	100	100	100	100	100	100	100
Standard diluent or medium (µl)	374.4	300	300	300	300	300	300	300
Concentration (pg/ml)*	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**

* Refer to the product insert for the S1 value for each analyte.
Each standard is a 4-fold dilution of the preceding one.

Section 7
Control Preparation (Optional)

One tube of lyophilized cytokine control is provided in each Bio-Plex® Precision Pro™ cytokine assay. The preparation of high, medium, and low controls is optional to monitor plate-to-plate variations. This section provides instructions on how to reconstitute the lyophilized control. The product insert provided with the assay lists the concentration of the reconstituted control. The reconstituted control can then be further diluted to prepare any concentration of user-specified quality controls. To ensure optimal assay performance, the cytokine controls should be prepared in a manner consistent as that used to prepare the cytokine standards.

Reconstitute Cytokine Controls

1. Gently tap the glass vial containing the lyophilized cytokine control on a solid surface to ensure the pellet is at the bottom.
2. Reconstitute 1 vial of lyophilized control with 500 µl of the appropriate diluent. Do not use assay buffer to dilute controls. This is identified as C0 in the product insert provided with the assay.

Sample	Diluent
Serum	Serum standard diluent
Plasma	Plasma standard diluent
Culture supernatant	Same culture medium used to prepare samples

3. Gently vortex 1 – 3 sec and incubate on ice for 30 min. Be consistent with the incubation time to ensure optimal assay performance.
4. The reconstituted cytokine control should be further diluted to create the desired QC samples in the same diluents specified in the table above. To obtain the concentration of each reconstituted cytokine control, refer to C0 in the product insert provided with the assay.

Section 8
Assay Instructions

The following instructions apply to Bio-Plex® Precision Pro™ cytokine assays. All of the necessary components are provided premixed for ease of use.

Plan Experiment

1. Assign which wells of a 96-well plate will be used for each standard, control, and sample (see the example below).
2. Determine the total number of wells that will be used in the assay. Include a 25% excess (or add 2 wells for every 8 wells used) to ensure that enough diluted coupled beads, detection antibodies, and streptavidin-PE are prepared.

Example Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	B	B	5	5	13	13	21	21	29	29
B	2	2	1	1	6	6	14	14	22	22	30	30
C	3	3	2	2	7	7	15	15	23	23	31	31
D	4	4	3	3	8	8	16	16	24	24	32	32
E	5	5	1	1	9	9	17	17	25	25	33	33
F	6	6	2	2	10	10	18	18	26	26	34	34
G	7	7	3	3	11	11	19	19	27	27	35	35
H	8	8	4	4	12	12	20	20	28	28	36	36

Prepare Coupled Magnetic Beads

Protect the beads from light by covering the tubes with aluminum foil. Keep all tubes on ice until ready to use.

1. Vortex the coupled beads (25x) at medium speed for 15–20 sec.
2. Prepare a sufficient volume of coupled beads (1x) using assay buffer. Each well requires 2 µl of coupled beads (25x) adjusted to a final volume of 50 µl with assay buffer (refer to the example below).

Example Bead Calculations

# of Wells	25x Beads (µl)	Assay Buffer (µl)	Total Volume (µl)
96	240	5,760	6,000
48	120	2,880	3,000
32	80	1,920	2,000
24	60	1,440	1,500

Calibrate Vacuum Apparatus

The vacuum apparatus must be calibrated at the beginning of the assay to ensure an optimal bead yield. For more detailed instructions, refer to the Bio-Plex suspension array system hardware instruction manual.

1. Prewet all the wells of a 96-well filter plate with 100 µl of assay buffer.
2. Place the filter plate on the vacuum apparatus and turn on the vacuum to the maximum level.
3. Press on the filter plate and note the time required to remove the buffer from the wells by vacuum filtration. The evacuation time should be 2–5 sec.

   If the evacuation time is <2 sec, the pressure is too high. Open the vacuum control valve slightly and repeat steps 1–3.

   If the evacuation time is >5 sec, the pressure is too low. Close the vacuum control valve slightly and repeat steps 1–3.

Assay Procedure

Bring all buffers to room temperature. Avoid bubbles when pipetting.

Assay Key – The following terms are repeated throughout the assay procedure. Refer to these detailed instructions when wash, incubate, and vacuum-filter are shown in bold.

Term	Detailed Directions
Wash	Add 100 µl of wash buffer to each well. Place the filter plate on a calibrated vacuum apparatus and remove the buffer by vacuum filtration. Blot the bottom of the filter plate with a clean paper towel. Repeat as specified.
Incubate	Gently cover the filter plate with a new sheet of sealing tape. Place the filter plate on a microplate shaker and then cover with aluminum foil. Shake the filter plate at room temperature at 1,100 rpm for 30 sec, then at 300 rpm for the specified incubation time.
Vacuum-filter	Place the filter plate on a calibrated vacuum apparatus and remove the buffer by vacuum filtration. Blot the bottom of the filter plate with a clean paper towel.

1. Equilibrate the diluted standards, samples, and controls at room temperature for 20 min prior to use.
2. Prewet and block the desired number of wells in a 96-well filter plate with 100 µl of assay buffer and **vacuum-filter**. If fewer than 96 wells are required, mark the plate to identify the unused wells for later use and cover the unused wells with sealing tape.
3. Vortex the coupled magnetic beads (1x) for 15–20 sec at medium speed. Add 50 µl to each well and immediately **vacuum-filter**.
4. **Wash** twice.
5. Gently vortex the diluted standards, controls, and samples for 1–3 sec. Add 50 µl of standard, control, or sample to each well, changing the pipet tip after every volume transfer. **Incubate** for 1 hr.

6. While the samples are incubating, perform a 30 sec quick-spin centrifugation of the detection antibody (10x) prior to pipetting to collect the entire volume at the bottom of the vial.

7. Prepare a sufficient volume of detection antibodies (1x) using detection antibody diluent. Each well requires 2.5 µl of detection antibodies (10x) adjusted to a final volume of 25 µl with detection antibody diluent (refer to the example below).

Example Detection Antibody Calculations

# of Wells	10x Detection Antibody (µl)	Detection Antibody Diluent (µl)	Total Volume (µl)
96	300	2,700	3,000
48	150	1,350	1,500
32	100	900	1,000
24	75	675	750

8. After incubating the samples, slowly remove and discard the sealing tape, then **vacuum-filter**.

9. **Wash** 3 times.

10. Vortex the detection antibodies gently and add 25 µl to each well. **Incubate** for 30 min.

11. While the detection antibodies are incubating, perform a 30 sec quick-spin centrifugation of the streptavidin-PE (100x) prior to pipetting to collect the entire volume at the bottom of the vial.

12. Prepare a sufficient volume of streptavidin-PE (1x) using assay buffer. Each well requires 0.5 µl of streptavidin-PE (100x) adjusted to a final volume of 50 µl with assay buffer (refer to the example on the following page).

Example Streptavidin-PE Calculations

# of Wells	100x Streptavidin-PE (µl)	Assay Buffer (µl)	Total Volume (µl)
96	60	5,940	6,000
48	30	2,970	3,000
32	20	1,980	2,000
24	15	1,485	1,500

13. After the detection antibody incubation, slowly remove and discard the sealing tape, then **vacuum-filter**.
14. **Wash** 3 times.
15. Vortex the streptavidin-PE (1x) vigorously and add 50 µl to each well. **Incubate** for 10 min.
16. After the streptavidin-PE incubation, slowly remove and discard the sealing tape, then **vacuum-filter**.
17. **Wash** 3 times.
18. Add 125 µl of assay buffer to each well. **Incubate** for 30 sec to resuspend the beads. Acquire the data immediately as described in Section 9.

Section 9
Data Acquisition

Bio-Plex® Precision Pro™ cytokine assays require the use of Bio-Plex Manager™ software version 4.1 or higher. Recommendations for acquiring data using the Bio-Plex suspension array system are listed below. Alternatively, refer to the Bio-Plex Manager™ software user guide or the instructions provided with the Luminex instrument.

Prepare System

1. Empty the waste bottle and fill the sheath fluid bottle before starting (if HTF not present). This will prevent fluidic system backup and potential data loss.

2. Turn on the reader and microplate platform (and HTF if present). Allow the system to warm up for 30 min.

3. Select Start up  and follow the instructions to prepare the reader to acquire data. If the system is idle for 4 hr, the lasers will automatically turn off and a 30 min warm-up period will again be required prior to acquiring data. Select Warm up  and wait for the optics to reach operational temperature.

Calibrate With High RP1 Target Value

Calibrate using Bio-Plex calibration beads and target values. Daily calibration is recommended before acquiring data.

1. Select Calibrate  and confirm that the default values for CAL1 and CAL2 are the same as the values on the Bio-Plex calibration bead labels. Use the Bio-Plex High RP1 target value for CAL2 calibration for Bio-Plex Precision Pro cytokine assays.

   NOTE: When acquiring data for Bio-Plex Precision Pro cytokine assays with a Luminex instrument, Luminex software, and Luminex calibration beads, it is necessary to convert the Luminex CAL2 calibration bead RP1 target value using the following equation:

   Bio-Plex High RP1 target value = (Luminex RP1 target value) x 4.55

Add the new target value to the Luminex software by selecting Calibrate, then New under the Reporter Channel in the Start Calibration dialog. Enter the new target value and save it as a new lot. Then calibrate using the new RP1 target value.

2. Select OK and follow the instructions for CAL1 and CAL 2 calibration.

Prepare Protocol

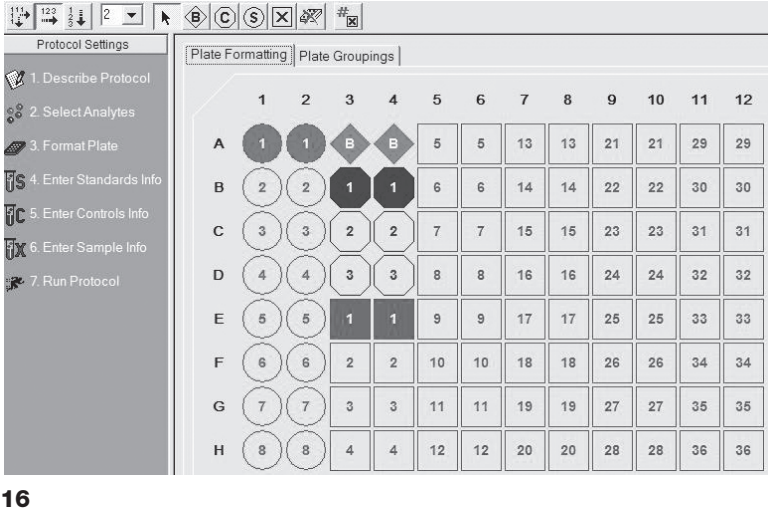
1. Open a new protocol by selecting File, then New from the main menu. Locate the steps at the left of the protocol menu.

   NOTE: To minimize data entry, preset lot-specific Bio-Plex Precision Pro cytokine assay protocols are available for download at **www.bio-rad.com/bio-plex**

2. Select Step 1 (Describe Protocol) and enter information about the assay.

3. Select Step 2 (Select Analytes) and choose the panel for Cytokines. Choose the target proteins for the assays on the plate. Note that this information will already be entered with the preset downloaded protocol.

Plate Formatting Example



4. Select Step 3 (Format Plate) and click on the Plate Formatting tab. Click on (S) and drag the cursor over all the wells that contain standards. Then click on (B) and drag the cursor over the wells that contain blanks. Repeat with (C) to identify all the wells that contain controls and [X] to identify all the wells that contain samples.

   NOTE: If the preset protocol was downloaded, a formatted plate will already be provided. Make any necessary changes to the preset formatted plate to match your plate setup.

5. Select Step 4 (Enter Standards Info) to enter standards information. Note that this information will already be entered with the preset download protocol.

   a) Select each analyte individually from the pull-down cell.

   b) Select the Enter Automatically option and then select the most concentrated value as S1.

   c) Enter the concentration of S1 from the product insert provided with the assay.

   d) Enter the dilution factor as 4 and select Calculate. The standards information will be populated for the selected analyte.

   e) Deselect the box for same concentration values for all analytes. Repeat steps 5a through 5d for each analyte in the assay.

6. Select Step 5 (Enter Controls Info) to enter controls information. This is where the concentration of the user-specified controls is entered into the protocol.

   a) Select each analyte individually from the pull down cell.

   b) Enter the description, concentration, and dilution information for each user-specified control.

   c) Deselect the box for same concentration values for all analytes. Repeat steps 6a and 6b for each analyte in the assay.

7. Select Step 6 (Enter Sample Info) and enter sample information.

Acquire Data

1. Shake the assay plate at 1,100 rpm for 30 sec immediately before acquiring data. Failure to do so will result in increased data acquisition time due to bead settling.

2. Check that the filter plate is flat. While pressing on one end of the plate, observe the distance that the opposite end of the plate is raised off a flat surface. If the distance is >1 mm, transfer all contents to a flat-bottom 96-well plate or another filter plate.

3. Visually inspect the plate and ensure that the assay wells are filled with buffer prior to placing the plate in the Bio-Plex microplate platform.

4. Slowly remove the sealing tape and any plate cover before placing the plate in the reader.

5. Select Step 7 (Run Protocol):

   a) Specify data acquisition for **100 beads per region**.

   b) In Advanced Settings, set the Bead Map to **25 region**.

      NOTE: Bio-Plex Precision Pro cytokine assays contain magnetic beads and require the use of the 25 region map available in Bio-Plex Manager software version 4.1 or higher.

   c) In Advanced Settings, set the sample size to **50 µl**.

   d) In Advanced Settings, confirm that the default DD gate values are set to **5000** (low) and **32000** (high).

      NOTE: When using a Luminex instrument, set the gates according to the Luminex procedure located in the manual.

   e) Select Start and save the .rbx file. Then follow the instructions for data acquisition.

6. If acquiring data from more than one plate, empty the waste bottle and refill the sheath bottle after each plate (if HTF not present). Select Wash Between Plates  and follow the instructions for fluidics maintenance. Then repeat the **Prepare Protocol** and **Acquire Data** steps.

   NOTE: Use the Wash Between Plates command after every plate run to reduce the possibility of clogging the instrument.

7. When data acquisition is complete, select Shut Down  and follow the instructions.

Reacquire Data

It is possible to acquire data from a well or plate a second time using the Rerun/Recovery mode located below Start in Step 7 (Run Protocol).

1. Check the wells where data will be acquired a second time. Any previous data will be overwritten.

2. Remove the buffer by vacuum filtration and add 125 µl of assay buffer to each well. Cover the filter plate with a new sheet of sealing tape.

3. Repeat **Acquire Data** steps 1–6 to acquire data a second time. The data acquired should be similar to the data acquired initially; however, the data acquisition time will be extended since fewer beads are present in each well.

Section 10
Troubleshooting Guides

This troubleshooting guide addresses problems that may be encountered with Bio-Plex® Precision Pro™ cytokine assays. If you experience any of the problems listed below, review the possible causes and solutions provided. This will assist you in resolving problems directly related to how the assay steps should be performed. Poor assay performance may also be due to the Bio-Plex array reader. To eliminate this possibility, we highly recommend use of the Bio-Plex validation kit. This kit will validate all the key functions of the array reader and assist the user in determining whether or not the array reader is functioning properly.

Possible Causes	Possible Solutions
High Inter-Assay CV	
Standards were not reconstituted consistently	Incubate the reconstituted standards for 30 min on ice. Always be consistent with the incubation time and temperature.
Reconstituted standards and diluted samples were not stored properly	Reconstituted standards and diluted samples should be prepared on ice as instructed. Prior to plating, the reconstituted standards and diluted samples should be equilibrated to room temperature.
High Intra-Assay CV	
Bottom of filter plate not dry	Dry the bottom of the filter plate with absorbent paper towel (preferably lint-free) to prevent cross-contamination.

Possible Causes	Possible Solutions
Pipetting technique	Pipet carefully and slowly when adding standards, samples, detection antibodies, and streptavidin-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer.
Reagents and assay components were not equilibrated to room temperature prior to plating	All reagents and assay components should be equilibrated to room temperature prior to plating.
Contamination with wash buffer during wash steps	During the wash steps, be careful not to splash wash buffer from one well to another. Be sure that the wells are filtered completely and that no residual volume remains. Also, be sure that the microplate shaker setting is not too high. Reduce the microplate shaker speed to minimize splashing.
Slow pipeting samples and reagents across the plate	Sample pipeting across the entire plate should take less than 4 min. Reagent pipeting across the entire plate should take less than 1 min.

Possible Causes	Possible Solutions
Low Bead Count	
Miscalculation of bead dilution	Check your calculations and be careful to add the correct volumes.
Beads clumped in multiplex bead stock tube	Vortex for 15–20 sec at medium speed before aliquoting beads.
Vacuum on for too long when aspirating buffer from wells	Do not apply vacuum to the filter plate for longer than 10 sec after the buffer is completely drained from each well.
Did not shake filter plate enough before incubation steps and prior to reading	Shake the filter plate at 1,100 rpm for 30 sec before incubation steps and immediately before reading the plate.
Reader is clogged	Refer to the troubleshooting guide in the Bio-Plex hardware instruction manual.
Low Signal or Poor Sensitivity	
Standards reconstituted incorrectly	Follow the cytokine standard instructions carefully.
Detection antibody or streptavidin-PE diluted incorrectly	Check your calculations and be careful to add the correct volumes.

Possible Causes	Possible Solutions
High Background Signal	
Incorrect buffer was used (for example, assay buffer used to dilute standards)	Use sample matrix or serum standard diluent to dilute cytokine standards.
Spiked “0 pg/ml” wells by mistake	Be careful when spiking standards. Do not add any antigens in the 0 (blank) point.
Streptavidin-PE incubated too long	Follow the procedure incubation time.
Poor Recovery	
Expired Bio-Plex reagents were used	Check that reagents have not expired. Use new or unexpired components.
Incorrect amounts of components were added	Check your calculations and be careful to add the correct volumes.
Microplate shaker set to an incorrect speed	Check the microplate shaker speed and use the recommended setting. Setting the speed too high may cause splashing and contamination. Use the recommended plate shaker.
Pipetting technique	Pipet carefully and slowly when adding standards, samples, detection antibodies, and streptavidin-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer.

Section 11
Safety Considerations

Eye protection and gloves are recommended while using this product. Consult the MSDS for additional information.

Human Source Material. Treat As Potentially Infectious.
The Bio-Plex® Precision Pro™ cytokine assays contain components of human origin. This material should be handled as if capable of transmitting infectious agents. Please use universal precautions. The material has been tested by an FDA approved test and found negative for HBsAg, HIV 1/2 Ab, HIV-1 Ag, and HCV. No test method can provide total assurance that hepatitis B virus, hepatitis C virus, human immunodeficiency virus, or other infectious agents are absent. These components should be handled at Biosafety Level 2 containment [US Government publication: Biosafety in Microbiological and Biomedical Laboratories (CDC, 1999)]. Handle Bio-Plex Precision Pro serum and plasma standard diluents as potentially biohazardous material under at least Biosafety Level 2 containment.

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*Including, but not limited to US patent 5,981,180; 6,046,807; 6,057,107.

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Czech Republic *420 241 430 532* ***Denmark*** *44 52 10 00*
Finland *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0*
Greece *30 210 777 4396* ***Hong Kong*** *852 2789 3300*
Hungary *36 1 455 8800* ***India*** *91 124 4029300/5013478* ***Israel*** *03 963 6050*
Italy *39 02 216091* ***Japan*** *03 5811 6270* ***Korea*** *82 2 3473 4460*
Mexico *55 5200 05 20* ***The Netherlands*** *0318 540666*
New Zealand *64 9415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99*
Portugal *351 21 472 7700* ***Russia*** *7 095 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 0861 246 723*
Spain *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189/2578 7241* ***United Kingdom*** *020 8328 2000*

10008318 US/EG Rev A *06-0143 0305 Sig 1106*