Bio-Plex Pro™ TGF-β Assays

Instruction Manual

For technical support, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.
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Introduction

Transforming Growth Factor (TGF-β) Assays

The TGF-β family of proteins plays an important role in a wide range of cellular functions including proliferation, differentiation, migration, and apoptosis. There are three known isoforms of TGF-β with overlapping functions in normal physiology and in disease states such as cancer. TGF-β1 promotes TH17 immune cell development and bone growth and remodeling. TGF-β2 plays a vital role in embryonic development and has been shown to suppress IL-2 dependent T-cell tumors. TGF-β3 regulates cell differentiation, adhesion, and extracellular matrix formation in embryogenesis and wound healing.

Bio-Plex Pro™ TGF-β assays are magnetic bead–based multiplex assays designed to measure TGF-β1, TGF-β2, and TGF-β3 in human, mouse, and rat sample matrices such as serum, plasma, urine, tissue culture supernatant, and milk.

Multiplexing with Bio-Plex Pro Assays

Bio-Plex Pro assays enable researchers to quantify multiple protein biomarkers in a single well of a 96-well plate in just 3 to 4 hours. These robust immunoassays require as little as 12.5 µl serum or plasma, or 50 µl cell culture supernatant or other biological fluid. The use of magnetic (MagPlex) beads allows researchers to automate wash steps on a Bio-Plex Pro (or similar) wash station. Magnetic separation offers greater convenience, productivity, and reproducibility compared to vacuum filtration.

For more information please visit **www.bio-rad.com/bio-plex**.

Principle

Technology

The Bio-Plex® multiplex system is built upon the three core elements of xMAP technology:

- Fluorescently dyed microspheres (also called beads), each with a distinct color code or spectral address to permit discrimination of individual tests within a multiplex suspension. This allows simultaneous detection of up to 500 different types of molecules in a single well of the 96-well microplate on the Bio-Plex® 3D system, up to 100 different types of molecules on the Bio-Plex® 200 system, and up to 50 different types of molecules on the Bio-Plex® MAGPIX™ system
- On the Bio-Plex 200 and Bio-Plex 3D systems, a dedicated flow cytometer with two lasers and associated optics to measure the different molecules bound to the surface of the beads. In the Bio-Plex MAGPIX, the entire sample load volume is injected into a chamber where the beads are imaged using LED and CCD technology
- A high-speed digital signal processor that efficiently manages the fluorescence data

Assay Format

Bio-Plex Pro™ assays are essentially immunoassays formatted on magnetic beads. The assay principle is similar to that of a sandwich ELISA (Figure 1). Capture antibodies directed against the desired biomarker are covalently coupled to the beads. Coupled beads react with the sample containing the biomarker of interest. After a series of washes to remove unbound protein, a biotinylated detection antibody is added to create a sandwich complex. The final detection complex is formed with the addition of streptavidin-phycoerythrin (SA-PE) conjugate. Phycoerythrin serves as a fluorescent indicator or reporter.

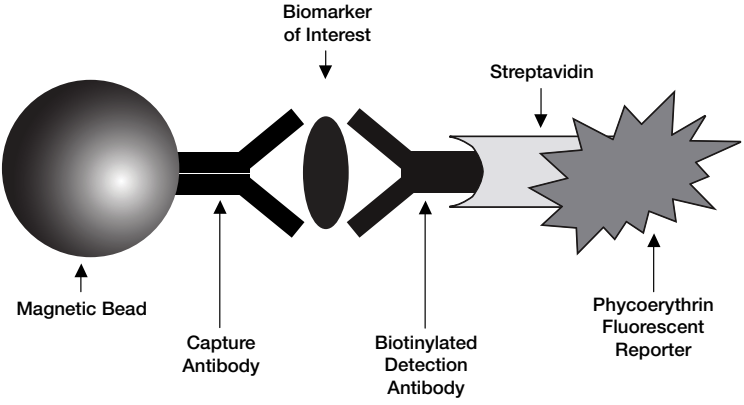


Fig. 1. Bio-Plex sandwich immunoassay.

Data Acquisition and Analysis

Data from the reactions are acquired using a Bio-Plex system or similar Luminex-based reader. When a multiplex assay suspension is drawn into the Bio-Plex 200 reader, for example, a red (635 nm) laser illuminates the fluorescent dyes within each bead to provide bead classification and thus assay identification. At the same time, a green (532 nm) laser excites PE to generate a reporter signal, which is detected by a photomultiplier tube (PMT). A high-speed digital processor manages data output, and Bio-Plex Manager™ software presents data as median fluorescence intensity (MFI) as well as concentration (pg/ml). The concentration of analyte bound to each bead is proportional to the MFI of reporter signal.

Using Bio-Plex Data Pro™ software, data from multiple instrument runs can be combined into a single project for easy data management, quick visualization of results, and simple statistical analysis.

Kit Contents and Storage

Reagents Supplied

TGF-β1, TGF-β2, and TGF-β3 assays are available in a convenient kit format that includes assay, reagent, and diluent components in a single box (Table 1).

Table 1. Contents of Bio-Plex Pro™ TGF-β assays.*

Component	1 x 96-Well Format
Standard diluent	1 bottle, 10 ml
Sample diluent	1 bottle, 40 ml
Assay buffer	1 bottle, 50 ml
Wash buffer	1 bottle, 200 ml
Detection antibody diluent	1 bottle, 5 ml
Streptavidin-PE (100x)	1 tube
Filter and/or flat bottom plate (96-well)	1 plate
Sealing tape	1 pack of 4
Assay Quick Guide	1 booklet
Coupled magnetic beads (20x)	1 tube
Detection antibodies (20x)	1 tube
Standard	1 vial

* Volumes shown are approximate.

Storage and Stability

Kit contents should be stored at 4°C and never frozen. Coupled magnetic beads and streptavidin-PE should be stored in the dark. All components are guaranteed for a minimum of 6 months from the date of purchase when stored as specified.

Reagents Required but Not Supplied

TGF-β is secreted as part of a complex that causes it to be inactive. It is necessary to expose samples to acidic conditions in order to activate TGF-β. The following reagents are required:

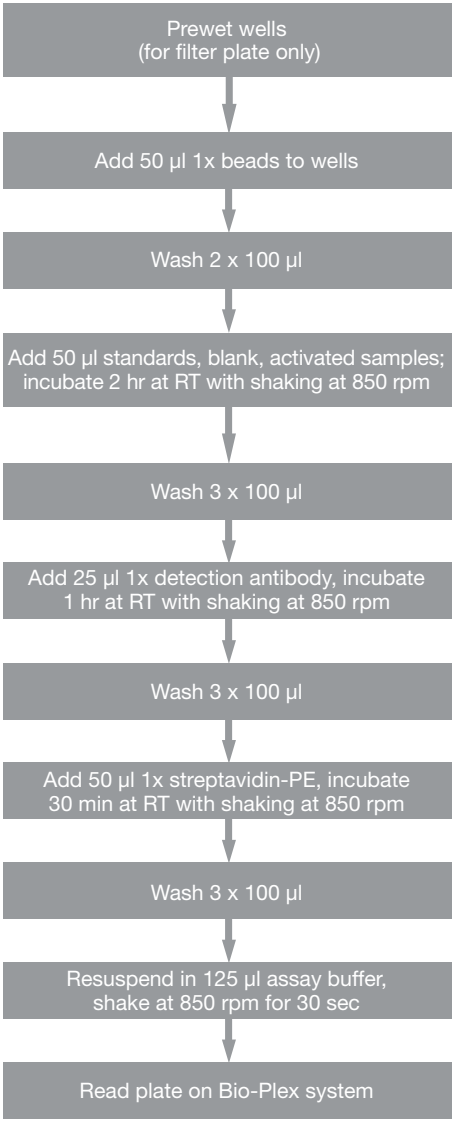
- 1 N hydrochloric acid: To make 100 ml of 1 N HCl, add 8.33 ml of 12 N HCl slowly to 91.67 ml of deionized water, and mix well
- 1.2 N sodium hydroxide/0.5 M HEPES: To make 100 ml of 1.2 NaOH/0.5 M HEPES, add 12 ml of 10 N NaOH to 75 ml of deionized water, and mix well. Add 11.9 g of HEPES (free acid, MW 238.3), mix well, and bring the final volume to 100 ml with deionized water

Table 2. Recommended materials.

Item	Ordering Information
Bio-Plex Pro™ Assays Quick Guide 5	Bulletin #10024986 (download at **www.bio-rad.com/bio-plex**)
Bio-Plex® 200 system or Luminex system with HTF	Bio-Rad catalog #171-000205
Bio-Plex validation kit Run the validation kit monthly to ensure optimal performance of fluidics and optics systems	Bio-Rad catalog #171-203001
Bio-Plex calibration kit Run the calibration kit daily to standardize fluorescence signal	Bio-Rad catalog #171-203060
Bio-Plex Pro wash station For use with magnetic bead–based assays only	Bio-Rad catalog #300-34376
Bio-Plex Pro II wash station For use with both polystyrene (nonmagnetic) and magnetic bead–based assays	Bio-Rad catalog #300-34377
Bio-Plex handheld magnetic washer For use with magnetic bead–based assays only	Bio-Rad catalog #170-020100
Bio-Plex Pro flat bottom plates, 40 x 96-well For magnetic separation on the Bio-Plex Pro wash station	Bio-Rad catalog #171-025001
Microtiter plate shaker IKA MTS 2/4 shaker for 2 or 4 microplates or Barnstead/Lab-Line Model 4625 plate shaker (or equivalent capable of 300–1,100 rpm)	IKA catalog #320-8000 VWR catalog #57019-600
Bio-Rad® Aurum™ vacuum manifold For vacuum filtration	Bio-Rad catalog #732-6470
BR-2000 vortexer	Bio-Rad catalog #166-0610
Reagent reservoirs, 25 ml For capture beads and detection antibodies	VistaLab catalog #3054-1002 or VistaLab catalog #3054-1004
Reagent reservoir, 50 ml (for reagents and buffers)	VistaLab catalog #3054-1006
Pall Life Science Acrodisc, 25 mm PF syringe filter (0.8/0.2 µm Supor membrane)	Pall Life Sciences catalog #4187
Filter plate, 1 x 96-well, clear plastic lid and tray	Bio-Rad catalog #171-304502
Titertube® micro test tubes For preparing replicate standards, samples, and controls prior to loading the plate	Bio-Rad catalog #223-9390

Other: 15 ml polypropylene tubes for reagent dilutions, calibrated pipets, pipet tips, sterile distilled water, aluminum foil, absorbent paper towels, 1.5 or 2 ml microcentrifuge tubes, and standard flat bottom microplate (for calibrating vacuum manifold).

Assay Workflow



Important Considerations

Instruments and Software

The assays described in this manual are compatible with all currently available Luminex-based life science research instruments. Assays can be read and analyzed with either Bio-Plex Manager™ software or Luminex xPONENT software.

Assay Procedures

Pay close attention to vortexing, shaking, and incubation times and to Bio-Plex® reader PMT (RP1) setting, as these have been optimized specifically for each assay panel.

Assay Quick Guide

Each assay kit includes a printed Bio-Plex Pro™ Assay Quick Guide (bulletin #10024986), which can be used to prepare and run a full 1 x 96-well assay plate. Users can also download a copy at **www.bio-rad.com/bio-plex**.

Bead Regions

Bead regions for all analytes are listed in the Read Plate section.

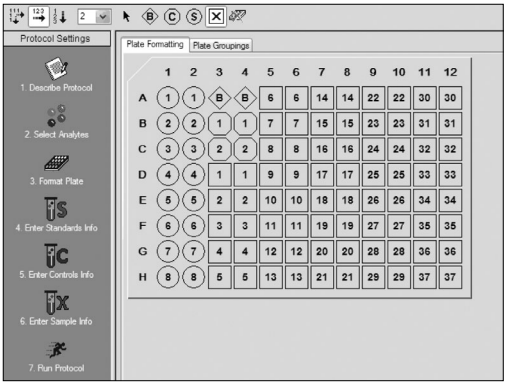
Multiplexing Compatibility

Do not mix TGF-β assays with other Bio-Plex assay panels or reagent kits. Protocols, reagents, and sample treatment conditions are not compatible.

1. Plan Plate Layout

Prior to running the assay, determine the total number of wells in the experiment using the Plate Layout Template on page 36 or the Plate Formatting tab in Bio-Plex Manager™ software. A suggested plate layout is shown in Figure 2, with all conditions in duplicate.

1. Assign standards to columns 1 and 2, with the highest concentration in row A and the lowest concentration in row H.
2. Assign the blank to wells A3 and A4. The blank should consist of your chosen standard diluent and be processed in the same manner as sample and standard wells. Note that Bio-Plex Manager automatically subtracts the blank (B) MFI value from all other assay wells.
3. User-defined controls are assigned to wells in columns 3 and 4.
4. The remainder of the plate is available for samples.
5. Once the total number of wells is known, calculate the required volumes of beads, detection antibody, and streptavidin-PE needed. Use Tables 7–8, 10–11, and 12, respectively, or the Calculation Worksheet on pages 37–38.



Legend

S	Standard
B	Blank
X	Samples
C	Controls

Fig. 2. Suggested plate layout. For detailed instructions on plate formatting in Bio-Plex Manager, see section 8, Read Plate.

2. Prepare Instrument

Start up and calibrate the Bio-Plex® 100/200 or similar system with Bio-Plex Manager™ software prior to setting up the assay. The calibration kit should be run daily or before each use of the instrument to standardize the fluorescent signal. To prepare either a Bio-Plex® 3D or Bio-Plex® MAGPIX™ reader, consult its respective user manual.

The validation kit should be run monthly to ensure performance of fluidics and optics systems. Refer to either the software manual or online Help for directions on how to conduct validation.

Start Up System (Bio-Plex 100, 200, or Similar)

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

2. Turn on the reader, XY platform, and HTF (if included). Allow the system to warm up for 30 min (if not already done).

3. Select Start up  and follow the instructions. If the system is idle for 4 hr without acquiring data, the lasers will automatically turn off. To reset the 4-hr countdown, select Warm up  and wait for the lasers/optics to reach operational temperature.

Calibrate System

1. Select Calibrate  and confirm that the default values for CAL1 and CAL2 are the same as the values printed on the bottle of Bio-Plex calibration beads. Use the Bio-Plex system low RP1 target value even if assays will be run at high RP1.

2. Select OK and follow the software prompts for step-by-step instructions for CAL1 and CAL2 calibration.

Note: In Bio-Plex Manager version 6.1 and higher, startup, warm up, and calibration can be performed together by selecting the “Start up and calibrate”  icon.

3. Prepare Wash Method

Bio-Plex Pro™ assays are compatible with both magnetic separation and vacuum filtration methods. However, for best results, we recommend performing the assays in a flat bottom plate with magnetic separation.

Table 3. Summary of compatible wash stations and plate types.

Wash Method	Wash Station	Assay Plate
Magnetic separation	Bio-Plex Pro Bio-Plex Pro II (use MAG programs) Bio-Plex® handheld magnetic washer	Flat bottom plate
Vacuum filtration	Bio-Plex Pro II (use VAC programs) Vacuum manifold (manual)	Filter plate

Setting up the Bio-Plex Pro or Bio-Plex Pro II Wash Station

The wash station does not require calibration; however, it should be primed before use. For more information, refer to the Bio-Plex Pro and Pro II wash station quick guide (bulletin #5826).

1. Install the appropriate plate carrier on the wash station.
2. Use the prime procedure to prime channel 1 with wash buffer.

Setting Up the Bio-Plex Handheld Magnetic Washer

Place an empty flat bottom plate on the magnetic washer by sliding it under the retaining clips. Push the clips inward to secure the plate. Make sure the plate is held securely. If needed, the clips can be adjusted for height and tension. For detailed instructions, refer to the user guide (bulletin #10023087).

Setting up a Vacuum Manifold

Calibrate the vacuum manifold by placing a standard 96-well flat bottom plate on the unit and adjusting the pressure to –1 to –3" Hg. In general, 100 µl liquid should take 3–4 sec to clear the well. For more detailed instructions, refer to bulletin #10005042.

4. Prepare Standards

General Instructions

- It is essential to prepare standards exactly as described in this section. Incorrect preparation may lead to low signal or variable measurements from plate to plate
- The peel-off sticker provided with the standards lists the most concentrated point on the standard curve (S1). Enter this information into Bio-Plex Manager™ software as instructed in section 8

Prepare a Diluent for Standards

1. Refer to Table 4 for recommended diluents based on different sample types. As a general rule, reconstitute and dilute standards in a diluent similar to the final sample type or sample matrix.

2. **If samples are serum or plasma, mix 1 volume of Bio-Plex® standard diluent with 3 volumes of Bio-Plex sample diluent** (each supplied in the kit). The resulting solution is used for reconstitution and subsequent dilution of standards. This results in a serum-matrix based diluent that mimics the matrix in 1:16 diluted serum and plasma samples.

Table 4. Summary of recommended diluents for standards.

Sample Type	Diluent for Standard	Add BSA
Serum and plasma	Standard/sample diluent mix (1:3)	None
Culture media, with serum	Culture media	None
Culture media, serum-free	Culture media	To 0.5% final* (w/v)
Lavage, lysate, other fluids	Sample diluent	To 0.5% final* (w/v)

* At least 0.5% final w/v BSA is recommended to stabilize analytes and reduce adsorption to labware.

Reconstitute a Single Vial of Standards

This procedure prepares enough material to run each dilution in duplicate.

1. Gently tap the vial containing the lyophilized standard.
2. Add **500 µl** of the appropriate standard diluent (see Table 4). Do not use assay buffer to reconstitute the standards.
3. Gently **vortex** the reconstituted standard for **5 sec**, then incubate on ice for **30 min**. Be consistent with the incubation time in every assay to ensure best results.
4. During the incubation period, prepare the samples as instructed in section 5, Prepare Samples.

Prepare the Standard Dilution Series

The following procedure produces an eight-point standard curve with a fourfold dilution between each point. Pipet carefully using calibrated pipets and use new pipet tips for every volume transfer.

1. Label nine 1.5 ml polypropylene tubes S1 through S8 and Blank.
2. Add the specified volume of standard diluent to each tube (Figure 3).
3. **Vortex** the reconstituted standards gently for **5 sec** before removing any volume. Add **128 µl** to the S1 tube containing **72 µl** of standard diluent. **Vortex** at medium speed for **5 sec**, then use a new pipet tip to transfer **50 µl** from S1 tube to S2 tube. **Vortex**.
4. Continue with 1:4 (fourfold) serial dilutions from tube S2 to S8 as shown in Figure 3. Use reconstituted and diluted standards immediately. Do not freeze for future use.
5. Continue with 1:4 serial dilutions as shown in Figure 3. Use reconstituted and diluted standards immediately. Do not freeze for future use.

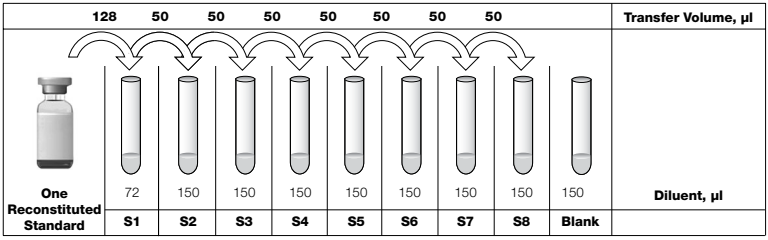


Fig. 3. Preparing a fourfold dilution series with a single reconstituted standard.

RP1 (PMT) Setting for Standard Curves

The Bio-Plex® 200 and 3D systems have two RP1 (PMT or photomultiplier tube) setting options, while the Bio-Plex® MAGPIX™ has no PMT and therefore no PMT setting options. Instead, MAGPIX uses default instrument settings similar to low PMT on the Bio-Plex 200 (Table 5).

Table 5. Overview of PMT setting options on Bio-Plex systems.

Instrument	RP1 (PMT)
Bio-Plex 100, 200*	Low, high
Bio-Plex 3D*	Standard, enhanced
Bio-Plex MAGPIX*	N/A, use default instrument settings

* Or similar Luminex-based system.

The Bio-Plex Pro TGF-β assays were developed on the low PMT setting using the Bio-Plex 200 system. Protocols using alternative standard dilution series or PMT settings should be validated by the end user.

5. Prepare Samples

General guidelines for preparing different sample types are provided here. For more information, contact Bio-Rad Technical Support.

- Once thawed, keep samples on ice. Prepare dilutions just prior to the start of the assay and equilibrate to room temperature before use
- Prepare sample dilutions in 1.5 or 2 ml polypropylene microcentrifuge tubes. If a multichannel pipet will be used to load the plate, then aliquot the required volumes into Titertube® micro test tubes
- Do not freeze diluted samples
- **Important!** before running the assay, samples must be activated and diluted as described on pages 17–18

Serum and Plasma

EDTA or citrate is preferred as an anticoagulant. Heparin-treated plasma, while compatible with Bio-Plex Pro™ assays, may absorb certain soluble proteins of interest. Avoid using hemolyzed samples as this may lead to false positive results.

1. Draw whole blood into collection tubes containing anticoagulant. Invert tubes several times to mix.
2. For serum, allow blood to clot at room temperature for **30–45 min**. For plasma, proceed directly to the centrifugation steps.
3. Perform centrifugation at 1,000 x g for **15 min** at 4°C and transfer the serum or plasma to a clean polypropylene tube.
4. To completely remove platelets and precipitates, centrifuge again at 10,000 x g for **10 min** at 4°C.
5. Activate samples as described below and assay immediately or store untreated/nonactivated samples in single-use aliquots at –70°C. Avoid repeated freeze/thaw cycles.

Cell Culture Supernatant

1. Collect supernatants and perform centrifugation at 1,000 x g for **15 min** at 4°C. For cell lines cultured in serum-free culture media, collect samples and add BSA as a carrier protein to a final concentration of at least 0.5% to stabilize protein analytes and to prevent adsorption to labware.
2. Transfer to a clean polypropylene tube. If cellular debris or precipitates are present, centrifuge again at 10,000 x g for **10 min** at 4°C.
3. Activate samples as described below and assay immediately or store untreated/nonactivated samples in single-use aliquots at –70°C. Avoid repeated freeze-thaw cycles.

Lavage, Sputum, and Other Biological Fluid Samples

Keep all samples on ice until ready for use. The appropriate sample dilution factor should be determined by the user.

1. Centrifuge at 10,000 x g for **10 min** at 4°C to clarify the sample.
2. Activate a portion of the sample as described below and, if needed, dilute in Bio-Plex® sample diluent with BSA added to a final concentration of at least 0.5%.

Lysates

Users will need to optimize the lysis, sample dilution, and sample activation methods to ensure that assay performance is fit for purpose.

1. Prepare the cell or tissue lysates according to the instructions provided with the Bio-Plex cell lysis kit (catalog #171-304011 or 171-304012). The protease inhibitors factor I and factor II are included in the kit. PMSF needs to be added to lysis buffer at a final concentration of 2 mM. The lysates should be free of particulate matter.
2. Determine the protein concentration of the lysate. It may be necessary to test lyse your sample with different volumes of lysing solution to obtain the specified protein concentration range.
3. Activate lysates as described under Sample Activation and Dilution. Dilute the activated sample in sample diluent + 0.5% BSA. The appropriate final lysate protein concentration should be determined by the user. A starting range to try is 50 to 900 ug/ml.

Note: For optimal assay performance, it is important to dilute lysates as much as possible to reduce the detergent concentration.

4. Store untreated/nonactivated lysates at –20°C to –70°C. Avoid multiple freeze-thaw cycles

Sample Activation and Dilution

First, prepare samples as described above. To measure immunoreactive TGF-β, it is necessary to treat all sample types with the following activation procedure. Samples should be assayed immediately after the neutralization step. **Do not activate the TGF-β standards**.

Table 6. Summary of recommended sample diluents and dilution factors.

Sample Type	Diluent	Add BSA	Sample Dilution
Serum and plasma	Sample diluent	None	1:16 final*
Culture media, with serum	Culture media	None	User optimized
Culture media, serum-free	Culture media	To 0.5% final**	User optimized
Lavage, lysate, other fluids	Sample diluent	To 0.5% final**	User optimized

* For example, activate 25 µl sample, neutralize, and bring to a final volume of 400 µl.

** At least 0.5% final w/v BSA is recommended to stabilize analytes and reduce adsorption to labware.

Activation of Serum and Plasma

1. To **activate** the sample, add 1 volume of acid (1 N HCl) to 5 volumes of sample. For example, add 5 µl acid to 25 µl of sample. Mix thoroughly and incubate for 10 min at room temperature.

2. To **neutralize** the sample, add a volume of base (1.2 N NaOH/0.5 M HEPES) equal to the volume of 1 N HCl used. In this example, add 5 µl base and mix thoroughly. Treated sample volume is now 35 µl.

3. The recommended **dilution** is 1:16 of the starting (untreated) sample volume. In this example, starting sample volume was 25 µl and a 1:16 dilution gives 400 µl. To reach a final volume of 400 µl, add 365 µl Bio-Plex sample diluent to 35 µl treated sample.

Note: To achieve neutral pH of a sample (pH 7.2 to 7.6), the actual volume of base required may vary depending on initial sample pH and the buffering capacity of the sample. Verify pH using pH paper before running the assay.

Activation of Cell Culture Supernatant and Other Biological Fluids

Samples may be run "neat" after activation/neutralization or be diluted as required. The appropriate dilution factor should be optimized by the user. Ensure a final sample volume after treatment and dilution of at least 125 µl to allow for duplicate wells on the assay plate.

1. For example, if a 1:4 dilution is desired, **activate** the sample by adding 10 µl acid to 50 µl sample. Mix thoroughly and incubate for 10 min at room temperature.
2. To **neutralize** sample, add 10 µl base. Mix thoroughly. Treated sample volume is now 70 µl.
3. **Dilute** to 1:4 final in the same diluent used to prepare the standards. In this example, starting sample volume was 50 µl and a 1:4 dilution gives 200 µl. To reach a final volume of 200 µl, add 130 µl diluent to 70 µl treated sample

Note: Serum-containing culture medium may contain high concentrations of TGF-β. A preliminary measurement of medium alone is recommended to determine baseline levels.

6. Prepare Coupled Beads

Instructions are provided for diluting the coupled beads to a 1x concentration.

1. Use Tables 7–8 or the Calculation Worksheet on pages 37–38 to calculate the volume of coupled beads and assay buffer needed.
2. Add the required volume of Bio-Plex assay buffer to a 15 ml polypropylene tube.
3. **Vortex** the stock coupled beads at medium speed for **30 sec**. Carefully open the cap and pipet any liquid trapped in the cap back into the tube. This is important to ensure maximum bead recovery. **Do not centrifuge the vial**; doing so will cause the beads to pellet.

4. Dilute coupled beads to 1x by pipetting the required volume into the 15 ml tube. **Vortex**.

   Each well of the assay plate requires 2.5 µl (20x stock) adjusted to a final volume of 50 µl in assay buffer.

5. Protect the beads from light with aluminum foil. Equilibrate to room temperature prior to use.

   Note: To minimize volume loss, use a 200–300 µl capacity pipet to remove beads from the stock tube. If necessary, perform the volume transfer in two steps. Do not use a 1,000 µl capacity pipet and/or a wide bore pipet tip.

Preparing 1x coupled beads from 20x stock (includes 20% excess volume)

Table 7. Premixed panel or one singleplex assay.

# of Wells	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	288	5,472	5,760
48	144	2,736	2,880

Table 8. Mixing singleplex assays.

# of Wells	20x Beads, µl Singleplex #1	20x Beads, µl Singleplex #2	Assay Buffer, µl	Total Volume, µl
96	288	288	5,184	5,760
48	144	144	2,592	2,880

7. Run Assay

Considerations

- Bring all assay components and samples to room temperature before use
- Use calibrated pipets and pipet carefully, avoiding bubbles. Use new pipet tips for every volume transfer
- Pay close attention to vortexing, shaking, and incubation instructions. Deviation from the protocol may result in low assay signal and assay variability
- Assay incubations are carried out in the dark on a shaker at **850 ± 50 rpm**. Cover the plate with sealing tape and protect from light with aluminum foil

Table 9. Summary of wash options and protocols. After each assay step, select the appropriate Bio-Plex Pro™ wash station program or perform the appropriate manual wash step as summarized below.

	Bio-Plex Pro or Pro II Wash Station	Bio-Plex Pro II Wash Station	Handheld Magnet or Vacuum Manifold
Assay Step	**Magnetic Program**	**Vacuum Program**	**Manual Wash Steps**
Add beads to plate	MAG x2	VAC x2	2 x 100 µl
Sample incubation	MAG x3	VAC x3	3 x 100 µl
Detection Ab incubation	MAG x3	VAC x3	3 x 100 µl
SA-PE incubation	MAG x3	VAC x3	3 x 100 µl

Considerations when Using a Vacuum Manifold

- After each incubation, place the filter plate on a calibrated vacuum apparatus and remove the liquid by vacuum filtration
- To wash, add 100 µl wash buffer to each well and remove the liquid as before. Ensure that all wells are exposed to the vacuum
- Thoroughly blot the bottom of the filter plate with a clean paper towel between each vacuum step to prevent cross contamination
- Place the assay plate on the plastic plate holder/tray as needed
- Before each incubation, gently cover the plate with a new sheet of sealing tape. Avoid pressing down over the wells to prevent leaking from the bottom

Add Coupled Beads, Standards, and Samples

1. Cover unused wells with sealing tape.
2. Prewet the filter plate. Skip this step if using a flat bottom plate.

   Prewet the wells using 100 µl assay buffer and remove the liquid by vacuum filtration. Dry the bottom of the filter plate thoroughly by blotting on a clean paper towel.
3. **Vortex** the diluted (1x) coupled beads for **30 sec** at medium speed. Pour the diluted coupled beads into a reagent reservoir and transfer 50 µl to each well of the assay plate.

   Tip: A multichannel pipet is highly recommended for ease of use and efficiency.
4. **Wash the plate two times** with **100 µl** Bio-Plex® wash buffer using the wash method of choice.
5. **Gently vortex** the diluted standards, blanks, samples, and controls (if applicable) for **5 sec**. Transfer **50 µl** to each well of the assay plate, changing the pipet tip after every volume transfer.
6. **Cover** plate with a new sheet of sealing tape and protect from light with aluminum foil. **Incubate** on shaker at **850 ± 50 rpm** for **2 hr** at room temperature (RT).

Note: 850 rpm provides equivalent performance to shaker settings recommended in previous manuals (1,100 rpm for 30 sec, 300 rpm for incubation).

Prepare and Add Detection Antibodies

Instructions are provided for diluting the detection antibodies to a 1x concentration.

1. While the samples are incubating, use Tables 10–11 or the Calculation Worksheet on pages 37–38 to calculate the volume of detection antibodies and detection antibody diluent needed. Detection antibodies should be prepared **10 min before use**.

2. Add the required volume of Bio-Plex detection antibody diluent to a 15 ml polypropylene tube.

3. **Vortex** the stock detection antibodies for **15–20 sec** at medium speed, then perform a **30 sec** spin to collect the entire volume at the bottom of the tube.

4. Dilute detection antibodies to 1x by pipetting the required volume into the 15 ml tube.

   Each well of the assay requires 1.25 µl (20x stock) adjusted to a final volume of 25 µl in detection antibody diluent.

Preparing 1x detection antibodies from 20x stock (includes 25% excess volume)

Table 10. Premixed panel or one singleplex assay.

# of Wells	20x Detection Antibodies, µl	Detection Antibody Diluent, µl	Total Volume, µl
96	150	2,850	3,000
48	75	1,425	1,500

Table 11. Mixing singleplex assays.

# of Wells	20x Detection Antibodies, µl Singleplex #1	20x Detection Antibodies, µl Singleplex #2	Detection Antibody Diluent, µl	Total Volume, µl
96	150	150	2,700	3,000
48	75	75	1,350	1,500

5. After incubating the beads, samples, standards, and blank, slowly remove and discard the sealing tape.

6. **Wash the plate three times** with **100 µl** wash buffer.

7. **Vortex** the diluted (1x) detection antibodies gently for **5 sec**. Pour into a reagent reservoir and transfer **25 µl** to each well using a multichannel pipet.

8. **Cover** plate with a new sheet of sealing tape and protect from light with aluminum foil. **Incubate** on shaker at **850 ± 50 rpm** for **1 hr** at room temperature.

Prepare and Add Streptavidin-PE (SA-PE)

1. While the detection antibodies are incubating, use Table 12 or the Calculation Worksheet on pages 37–38 to calculate the volume of SA-PE (100x) and assay buffer needed. Streptavidin-PE should be prepared **10 min before use**.

2. Add the required volume of assay buffer to a 15 ml polypropylene tube.

3. **Vortex** the 100x SA-PE for **5 sec** at medium speed. Perform a 30 sec spin to collect the entire volume at the bottom of the tube.

4. Dilute SA-PE to 1x by pipetting the required volume into the 15 ml tube. **Vortex** and protect from light until ready to use.

   Each well of the assay requires 0.5 µl (100x stock) adjusted to a final volume of 50 µl in assay buffer.

Table 12. Preparing 1x SA-PE from 100x stock (includes 25% excess volume).

# of Wells	100x SA-PE, µl	Assay Buffer, µl	Total Volume, µl
96	60	5,940	6,000
48	30	2,970	3,000

5. After the detection antibody incubation, slowly remove and discard the sealing tape.

6. **Wash the plate three times** with **100 µl** wash buffer.

7. **Vortex** the diluted (1x) SA-PE at medium speed for **5 sec**. Pour into a reagent reservoir and transfer **50 µl** to each well using a multichannel pipet.

8. **Cover** plate with a new sheet of sealing tape and protect from light with aluminum foil. **Incubate** on shaker at **850 ± 50 rpm** for **30 min** at room temperature.

9. After the streptavidin-PE incubation step, slowly remove and discard the sealing tape.

10. **Wash the plate three times** with **100 µl** wash buffer.

11. To resuspend beads for plate reading, add **125 µl** of assay buffer to each well. Cover the plate with a new sheet of sealing tape. Shake at room temperature at **850 ± 50 rpm** for **30 sec**, and slowly remove the sealing tape. Ensure that the plate cover has been removed before placing the plate on the reader.

12. Remove the sealing tape and read the plate using the settings below. Refer to section 8, Read Plate, for details.

    Note: Reading at alternative PMT settings on the Bio-Plex 100, Bio-Plex 200, or Bio-Plex 3D requires validation by the end user to ensure that results meet the user's acceptance criteria.

Table 13. Read plate using the appropriate instrument settings.

Instrument	RP1 (PMT)	DD Gates	Bead Events
Bio-Plex 100, 200*	Low	5,000 (low), 25,000 (high)	50
Bio-Plex 3D*	Standard	Select MagPlex beads	50
Bio-Plex® MAGPIX™*	N/A, use default instrument settings		Default

* Or similar Luminex-based system.

8. Read Plate

Bio-Plex Manager™ software is recommended for all Bio-Plex Pro™ assay data acquisition and analysis. Instructions for Luminex xPONENT software are also included. For instructions using other xMAP system software packages, contact Bio-Rad Technical Support or your regional Bio-Rad field applications specialist.

Prepare Protocol in Bio-Plex Manager Software v 6.0 and Higher

The protocol should be prepared in advance so that the plate is read as soon as the experiment is complete.

A protocol file specifies the analytes used in the reading, the plate wells to be read, sample information, the values of standards and controls, and instrument settings.

Bio-Plex Manager software contains protocols for most Bio-Plex® assays. Choose from available protocols or create a new protocol. To create a new protocol, select **File**, then **New** from the main menu. Locate and follow the steps under **Protocol Settings**.

1. Click **Describe Protocol** and enter information about the assay (optional).
2. Click **Select Analytes** and create a new panel. Visually confirm the selected analytes and proceed to step 3.
    a. Click the **Add Panel** button  in the Select Analytes toolbar. Enter a new panel name. Select **Bio-Plex Pro Assay Magnetic** from the assay pull-down menu. If using Bio-Plex Manager version 5.0 or lower, select **MagPlex** from the assay pull-down menu.
    b. Click the **Add** button. Enter the bead region number and name for the first analyte. Click **Add Continue** to repeat for each analyte in the assay.

For reference, bead regions are shown in Table 14.

c. Click the **Add** button when the last analyte has been added and click **OK** to save the new panel.

d. Highlight analytes from the Available list (left) and move to the Selected list (right) using the Add button. To move all analytes at once, simply click the **Add All** button.

e. If some of the analytes need to be removed from the Selected list, highlight them and select **Remove**. If desired, it is possible to rename the panel by clicking on **Rename Panel** and entering a new panel name.

Table 14. TGF-β assay bead regions.

Analyte	Bead Region
TGF-β1	13
TGF-β2	72
TGF-β3	66

3. Click **Format Plate** and format the plate according to the plate layout created in Section 1 (Plan Plate Layout). To modify the plate layout, follow the steps below (see Figure 4).

   a. Select the **Plate Formatting** tab.

   b. Select the standards icon (S) and drag the cursor over all the wells that contain standards. Repeat this process for blanks <B>, controls (C), and samples [X].

4. Click **Enter Standards Info** in the Protocol Settings bar.

   a. Enter the highest concentration of each analyte in the top row (labeled S1) of the table. S1 concentration information is included on the peel-off sticker provided with each vial of standards.

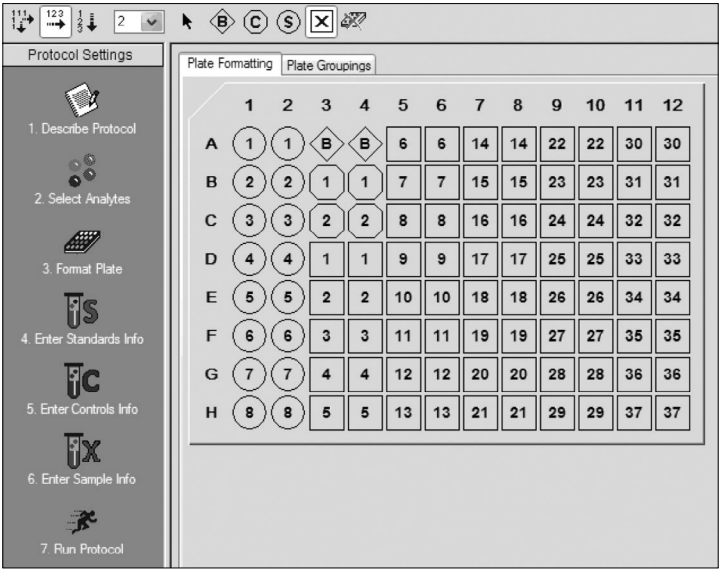


Fig. 4. Plate formatting.

b. Enter a dilution factor of 4 and click **Calculate**. The concentrations for each standard point will be populated for all analytes in the table.

c. Optional: enter the lot number of the vial of standards into the **Standard Lot** box and click **Save**.

5. Click **Enter Controls Info**, and for user-defined controls, select an analyte from the pull-down menu, then enter a description and concentration. Repeat for each additional analyte in the assay.

6. Click **Enter Sample Info** and enter sample information and the appropriate dilution factor.

7. Click **Run Protocol** and confirm that the assay settings are correct.

    a. Refer to Table 13 for the recommended RP1 (PMT) setting. Protocols using alternative PMT settings should be validated by the end user.

    b. Confirm data acquisition is set to **50 beads per region**. In Advanced Settings, confirm that the bead map is set to **100 region**, the sample size is set to **50 µl**, and the DD gates are set to **5,000 (Low)** and **25,000 (High)**. In Bio-Plex Manager software versions 4.0, 4.1, and 4.1.1, check **Override Gates** and set the DD gate values as indicated.

    c. Select **Start**, name and save the .rbx file, and begin data acquisition. The Run Protocol pop-up screen will appear. Click **Eject/Retract** to eject the plate carrier.

Acquire Data

1. Shake the assay plate at **850 ± 50 rpm** for **30 sec** and visually inspect the plate to ensure that the assay wells are filled with buffer. Slowly remove the sealing tape and any plate cover before placing the plate on the plate carrier.

2. Click **Run Protocol** — on the pop-up screen, select **Load Plate** and click **OK** to start acquiring data.

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

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

5. 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 the Run Protocol step. Any previous data will be overwritten.

1. Check the wells from which data will be reacquired.
2. Remove the buffer with the wash method of choice.
3. Add **100 µl** assay buffer to each well. Cover the filter plate with a new sheet of sealing tape. Shake the plate at **850 ± 50 rpm** for **30 sec**. Slowly remove the sealing tape before placing the plate on the plate reader.
4. Repeat the **Acquire Data** steps to reacquire data. The data acquired should be similar to those acquired initially; however, the acquisition time will be extended because the wells have fewer beads.

Data Analysis: Removing Outliers

Outliers are identified as standard data points that do not meet accuracy or precision requirements and should be considered invalid when performing curve fitting. As such, they should be removed to generate a more realistic and accurate standard curve. This may result in an extended assay working range and allow quantitation of samples that might otherwise be considered out of range (OOR).

In Bio-Plex Manager software version 6.0 and higher, outliers can be automatically removed by selecting the **Optimize** button in the Standard Curve window. In Bio-Plex Manager software 6.0 and earlier versions, outliers can be manually selected in the Report Table. Visit online Help to learn more about the standard curve optimizer feature and how outliers are determined.

Previous Versions of Bio-Plex Manager Software

For instructions on using previous versions of Bio-Plex Manager software, please contact Bio-Rad Technical Support.

Luminex xPONENT Software

Although guidelines are provided here, consult the xPONENT software manual for more details. Perform a system initialization with Luminex's calibration and performance verification kit, as directed by Luminex. Select Batches to set up the protocol and follow the information under Settings.

Note: The instrument settings described below apply to Luminex 100/200 and FLEXMAP 3D or Bio-Plex® 3D instruments. For the Bio-Plex® MAGPIX™ reader, use the default instrument settings.

1. Select MagPlex as the bead type for magnetic beads, which automatically sets the DD gates.
2. Volume = 50 µl.
3. Refer to Table 13 to select the appropriate PMT setting for your instrument.
4. Plate name: 96-well plate.
5. Analysis type: Quantitative; 5PL Curve Fit.
6. Number of standards: 8.

Select **Analytes** to set up the panel.

1. Enter **pg/ml** in the Units field.
2. Enter **50** in the Count field.
3. Select the bead region and enter the analyte name.
4. Click **Apply all** for Units and Count.

Select **Stds and Ctrls**.

1. Enter standard concentrations, lot number, dilution factor, and other information as applicable.

After the assay is complete, select **Results**, then select **Saved Batches**.

Troubleshooting Guide

This troubleshooting guide addresses problems that may be encountered with Bio-Plex Pro™ assays. If you experience any of the problems listed below, review the possible causes and solutions provided. Poor assay performance may also be due to the Bio-Plex® suspension array reader. To eliminate this possibility, use the validation kit to determine if the array reader is functioning properly.

Possible Causes	Possible Solutions
High Inter-Assay CV	
Standards were not reconstituted consistently between assays	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.
Bottom of filter plate not dry	Dry the bottom of the filter plate with absorbent paper towel (preferably lint-free) to prevent cross-well contamination.

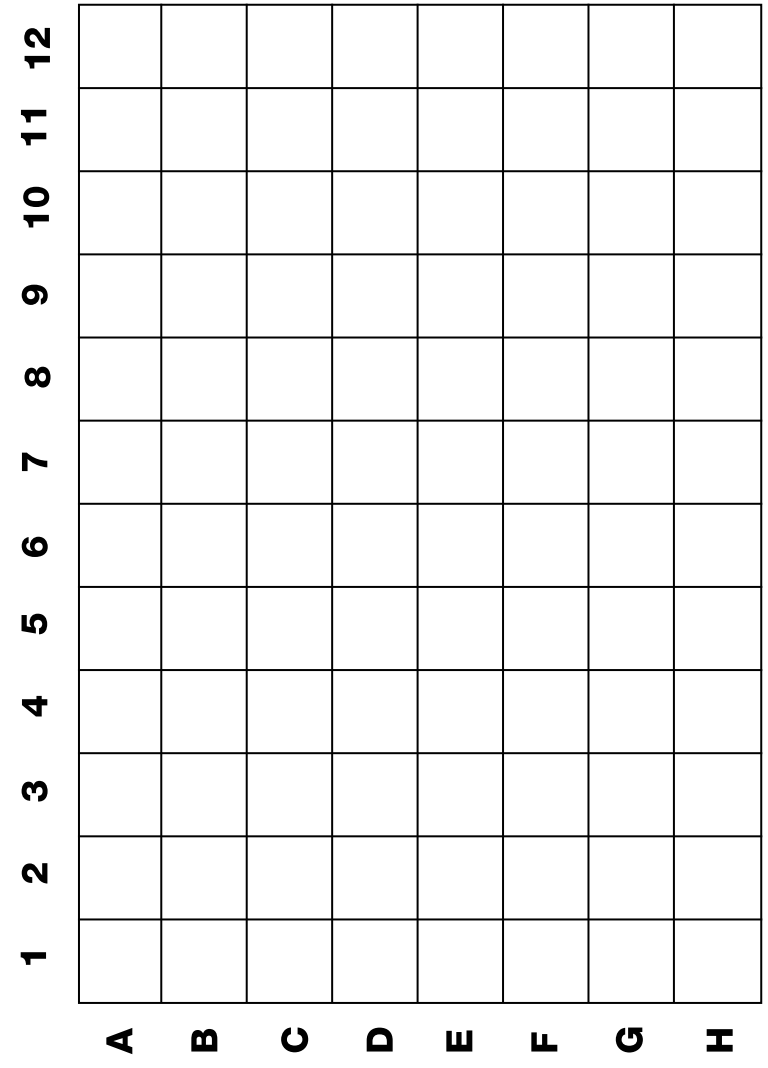
Possible Causes	Possible Solutions
High Intra-Assay CV	
Improper pipetting technique	Pipet carefully when adding standards, samples, detection antibodies, and streptavidin-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer.
Reagents and assay components not equilibrated to room temperature prior to pipetting	All reagents and assay components should be equilibrated to room temperature prior to pipetting.
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. Ensure that the microplate shaker setting is not too high. Reduce the microplate shaker speed to minimize splashing.
Slow pipetting of samples and reagents across the plate	Sample pipetting across the entire plate should take less than 4 min. Reagent pipetting across the entire plate should take less than 1 min.
Bio-Plex wash station: insufficient washing due to clogged pins	Clean dispensing pins with the thicker of the 2 cleaning needles provided with washer. Perform regular rinses to minimize salt buildup.

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 30 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.
Filter plate not shaken enough before incubation steps and prior to reading	Shake the filter plate at 850 ± 50 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 system hardware instruction manual (bulletin #10005042).
Low Signal or Poor Sensitivity	
Standards reconstituted incorrectly	Follow the standard preparation 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 standard diluent or diluent similar to final sample matrix to dilute standards.
Accidentally spiked blank wells	Do not add any antigens to the blank wells.
Detection antibodies or streptavidin-PE incubated too long	Follow the procedure incubation time precisely.
Poor Recovery	
Expired Bio-Plex reagents were used	Check that reagents have not expired. Use new or nonexpired 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.

Possible Causes	Possible Solutions
Poor Recovery	
Improper pipetting technique	Pipet carefully 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.
Impact of Sample Matrix	
Negative MFI values in samples or standards	If samples contain little or no analyte, negative values observed may be due to statistical variation. If assay drift is suspected, retest the samples by positioning them next to the standards. If contamination of standards is suspected, check the standard replicate value and be careful when adding samples to the wells. Matrix effects could also produce negative sample values. Bio-Plex Manager™ software automatically subtracts the blank (B) FI value from all other assay wells. While this has no impact on observed concentrations of samples within the assay working range, it may result in a negative FI value if the blank's FI value is greater than either the standard or sample value. If this is undesirable, then assign wells as a sample (X) or control (C) in the protocol or results file.
Poor precision in serum and plasma sample measurements	Check if any interfering components such as heparin-based anticoagulant, additives, or gel from separators were introduced into the samples. Avoid using hemolyzed and heavily lipemic samples. Remove visible particulate in samples by centrifugation. Avoid multiple freeze/thaw cycles of samples.

Plate Layout Template



Calculation Worksheet

If using either a **premixed panel or one singleplex assay** with 20x stocks of beads and detection antibodies, follow these directions.

Plan the plate layout and enter the number of wells to be used in the assay:_____ (1)

1. Determine the volume of 1x coupled beads needed.

a. Each well requires 50 µl of coupled beads (1x): _____ (1) x 50 µl = _____ (2) µl

b. Include 20% excess to ensure enough volume: _____ (2) µl x 0.20 = _____ (3) µl

c. Total volume of 1x coupled beads: _____ (2) µl + _____ (3) µl = _____ (4) µl

d. Volume of **20x coupled beads** required: _____ (4) µl /20 = _____ (5) µl

e. Volume of **assay buffer** required: _____ (4) µl – _____ (5) µl = _____ (6)

2. Determine the volume of 1x detection antibody needed.

a. Each well requires 25 µl detection antibodies (1x): _____ (1) x 25 µl = _____ (7) µl

b. Include 25% excess to ensure enough volume: _____ (7) µl x 0.25 = _____ (8) µl

c. Total volume of 1x detection antibodies: _____ (7) µl + _____ (8) µl = _____ (9) µl

d. Volume of **20x detection antibodies** required: _____ (9) µl /20 = _____ (10) µl

e. Volume of **detection antibody diluent** required: _____ (9) µl – _____ (10) µl = _____ (11) µl

3. Determine the volume of 1x streptavidin-PE needed.

a. Each well requires 50 µl streptavidin-PE (1x): _____ (1) x 50 µl = _____ (12) µl

b. Include 25% excess to ensure enough volume: _____ (12) µl x 0.25 = _____ (13) µl

c. Total volume of 1x streptavidin-PE: _____ (12) µl + _____ (13) µl = _____ (14) µl

d. Volume of **100x streptavidin-PE** required: _____ (14) µl / 100 = _____ (15) µl

e. Volume of **assay buffer** required: _____ (14) µl – _____ (15) µl = _____ (16) µl

If **mixing singleplex assays** with 20x stocks of beads and detection antibodies, follow these directions. Enter the number of wells to be used in the assay:_____ 1

1. Determine the volume of 1x coupled beads needed.

a. Each well requires 50 µl coupled beads (1x): _____ 1 x 50 µl = _____ 2 µl

b. Include 20% excess to ensure enough volume: _____ 2 µl x 0.20 = _____ 3 µl

c. Total volume of 1x coupled beads: _____ 2 µl + _____ 3 µl = _____ 4 µl

d. Enter the number of singleplex sets (or analytes) that will be multiplexed = _____ 5

e. Volume of **20x coupled beads** required from **each stock tube**:

_____ 4 µl / 20 = _____ 6 µl

f. Total volume of combined bead stocks: _____ 5 x _____ 6 µl = _____ 7 µl

g. Volume of **assay buffer** required: _____ 4 µl – _____ 7 µl = _____ 8 µl

2. Determine the volume of 1x detection antibody needed.

a. Each well requires 25 µl detection antibodies (1x): _____ 1 x 25 µl = _____ 9 µl

b. Include 25% excess to ensure enough volume: _____ 9 µl x 0.25 = _____ 10 µl

c. Total volume of 1x detection antibodies: _____ 9 µl + _____ 10 µl = _____ 11 µl

d. Enter the number of singleplex sets (or analytes) that will be multiplexed = _____ 5

e. Volume of **20x detection antibodies** required from **each stock tube**:

_____ 11 µl / 20 = _____ 12 µl

f. Total volume of combined detection antibody stock: _____ 12 µl x _____ 5 = _____ 13 µl

g. Volume of **detection antibody diluent** required: _____ 11 µl – _____ 13 µl = _____ 14 µl

3. Determine the volume of 1x streptavidin-PE needed.

a. Each well requires 50 µl streptavidin-PE (1x): _____ 1 x 50 µl = _____ 15 µl

b. Include 25% excess to ensure enough volume: _____ 15 µl x 0.25 = _____ 16 µl

c. Total volume of 1x streptavidin-PE: _____ 15 µl + _____ 16 µl = _____ 17 µl

d. Volume of **100x streptavidin-PE** required: _____ 17 µl / 100 = _____ 18 µl

e. Volume of **assay buffer** required: _____ 17 µl – _____ 18 µl = _____ 19 µl

Safety Considerations

Eye protection and gloves are recommended when using these products. Consult the MSDS for additional information. The Bio-Plex Pro™ assays contain components of animal origin. This material should be handled as if capable of transmitting infectious agents. Use universal precautions. These components should be handled at Biosafety Level 2 containment (U.S. government publication: Biosafety in Microbiological and Biomedical Laboratories (CDC, 1999)).

Legal Notices

Ordering Information

TGF-β Premixed Multiplex Panel	Catalog #
Bio-Plex Pro™ TGF-β 3-plex Panel, 1 x 96	171-W4001M
TGF-β Singleplex Sets*	**Catalog #**
Bio-Plex Pro TGF-β1 Set, 1 x 96	171-V4001M
Bio-Plex Pro TGF-β2 Set, 1 x 96	171-V4002M
Bio-Plex Pro TGF-β3 Set, 1 x 96	171-V4003M
Premixed Cytokine Panels	**Catalog #**
Bio-Plex Pro Human Cytokine 8-Plex Panel, 1 x 96	M50-000007A
Bio-Plex Pro Human Cytokine 17-Plex Panel, 1 x 96	M50-00031YV
Bio-Plex Pro Human Cytokine 21-Plex Panel, 1 x 96	MF0-005KMII
Bio-Plex Pro Human Cytokine 27-Plex Panel, 1 x 96	M50-0KCAF0Y
Bio-Plex Pro Human Cytokine Th1/Th2 Panel, 1 x 96	M50-00005L3
Bio-Plex Pro Mouse Cytokine 8-Plex Panel, 1 x 96	M60-000007A
Bio-Plex Pro Mouse Cytokine 9-Plex Panel, 1 x 96	MD0-00000EL
Bio-Plex Pro Mouse Cytokine 23-Plex Panel, 1 x 96	M60-009RDPD
Bio-Plex Pro Mouse Cytokine Th1/Th2 Panel, 1 x 96	M60-00003J7
Bio-Plex Pro Mouse Th17 Cytokine Panel A 6-Plex, 1 x 96	M60-00007NY
Bio-Plex Pro Mouse Th17 Cytokine Panel B 8-Plex, 1 x 96	171-FA001M
Bio-Plex Pro Mouse Cytokine Th1 Panel, 1 x 96	L60-00004C6
Bio-Plex Pro Mouse Cytokine Th2 Panel, 1 x 96	L60-000UKVT
Bio-Plex Pro Rat Th1/Th2 Panel, 1 x 96	171-K1002M
Bio-Plex Pro Rat Cytokine 24-Plex Panel, 1 x 96	171-K1001M

* Require reagent kit (#171-304070 for vacuum separation or #171-304070M for magnetic separation) and a vial of standards (#171-X40001).

Bio-Plex® x-Plex™ Assays (We Mix)

Premium custom assay service using the Bio-Plex Assay Builder, **www.bio-rad.com/bio-plex/assaybuilder**, to select analytes and plate type of interest. Assays are supplied as premixed coupled beads and detection antibodies in the all-in-one kit format.

Bio-Plex Express Assays (You Mix)

Fast and economical custom assay service using the Bio-Plex Assay Builder, **www.bio-rad.com/bio-plex/assaybuilder**, to select analytes and plate type of interest. Assays are supplied as individual sets of coupled beads and detection antibodies in the all-in-one kit format.

Singleplex Sets and Individual Components

A host of singleplex sets and individual assay components are available. For more information, refer to bulletin 5507 or go to **www.bio-rad.com/bio-plex**.

Bio-Rad Laboratories, Inc.

Life Science Group

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Canada *905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *420 241 430 532* ***Denmark*** *44 52 10 00*
Finland *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 31 884 0*
Greece *30 210 9532 220* ***Hong Kong*** *852 2789 3300*
Hungary *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050*
Italy *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460*
Mexico *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30*
Poland *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 495 721 14 04* ***Singapore*** *65 6415 3188*
South Africa *27 861 246 723* ***Spain*** *34 91 590 5200*
Sweden *08 555 12700* ***Switzerland*** *026 674 55 05*
Taiwan *886 2 2578 7189* ***Thailand*** *800 88 22 88*
United Kingdom *020 8328 2000*

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