Bio-Plex Pro™ Assays

Quick Guide 5

For use with	Instruction Manual #
TGF-β Assays	10024984

This guide can be used to prepare and run a full 1 x 96-well assay plate. For more information on a given step, refer to the complete instruction manual. New users can download the manual at **www.bio-rad.com/bio-plex**.

Important: Pay close attention to **vortexing**, **shaking**, and **incubation instructions**. Deviation from the protocol may result in low assay signal and assay variability.

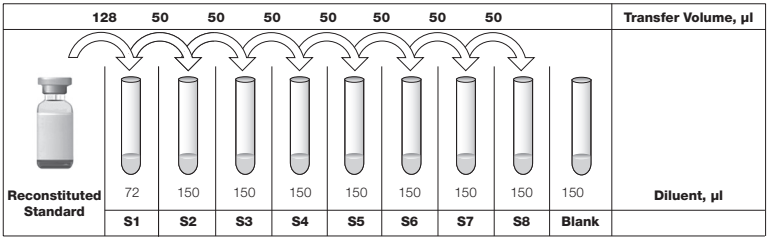
Initial Preparation

1. Plan the plate layout.
2. Start up/warm up the Bio-Plex® system (**30 min**).
   - Bring assay buffer, wash buffer, and sample diluent to room temperature (RT). Keep other items on ice until needed
   - Begin to thaw frozen samples
3. Prime wash station for flat bottom plate or set vacuum manifold to –1 to –3" Hg for filter plate.
4. Follow the prompts in Bio-Plex Manager™ software to calibrate the system. This can be done now or during an incubation step.
5. 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. For samples in serum-free media and other biological fluids, use a diluent that most closely matches the sample matrix. Add carrier protein such as BSA at a final concentration of 0.5% (w/v).

6. Reconstitute a single vial of standards in **500 µl** of a diluent similar to the final sample type or matrix as shown below. **Vortex** for **5 sec** and incubate **on ice** for **30 min**.

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)

7. Prepare a fourfold standard dilution series and blank as shown below. **Vortex** for **5 sec** between liquid transfers.



8. After thawing samples, activate by adding 1 volume of 1 N HCl to 5 volumes of sample. **Vortex**, incubate at RT for **10 min**. Neutralize by adding the same volume of base (1.2 N NaOH/0.5 M HEPES). After treatment, dilute samples as shown below.

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.

9. Vortex the 20x coupled beads for **30 sec** and dilute to 1x in Bio-Plex assay buffer as shown below. Protect from light.

# of Wells	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	288	5,472	5,760

Running the Assay

Note: Make sure all assay components are at RT before proceeding.

1. Prewet filter plate with **100 µl** Bio-Plex assay buffer (skip for flat bottom).
2. **Vortex** the diluted (1x) beads. Add **50 µl** to each well of the assay plate.
3. Wash two times with **100 µl** Bio-Plex wash buffer.
4. **Vortex** samples, standards, blank. Add **50 µl** to each well.
5. Cover plate with sealing tape and protect from light with aluminum foil. Incubate on shaker at **850 ± 50 rpm** for **2 hr** at RT.

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

6. With **10 min** left in the incubation, **vortex** the 20x detection antibodies for **5 sec** and quick-spin to collect liquid. Dilute to 1x in detection antibody diluent as shown below.

# of Wells	20x Detection Ab, µl	Detection Ab Diluent, µl	Total Volume, µl
96	150	2,850	3,000

7. Wash the plate three times with **100 µl** wash buffer.
8. **Vortex** the diluted (1x) detection antibodies. Add **25 µl** to each well.
9. Cover and incubate at **850 ± 50 rpm**, as in Step 5, for **1 hr** at RT. Meanwhile, prepare Bio-Plex Manager software protocol; enter standard S1 values provided in the assay kit.
10. With **10 min** left in the incubation, **vortex** the 100x SA-PE for **5 sec**, quick-spin to collect liquid, and dilute to 1x as shown below. Protect from light.

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

11. Wash the plate three times with **100 µl** wash buffer.
12. **Vortex** the diluted (1x) SA-PE. Add **50 µl** to each well.

13. Cover and incubate at **850 ± 50 rpm**, as in Step 5, for **30 min** at RT.

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

15. Resuspend beads in **125 µl** assay buffer. Cover and shake at **850 ± 50 rpm**, as in Step 5, for **30 sec**.

16. Remove the sealing tape and **read plate** using the settings below.

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.

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