BIO-RAD

Bio-Plex Pro™ TGF-β Assays

Quick Guide

For Use with	Instruction Manual #
TGF-β Assays	10024984

This quick 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 instruction manual. Go to **bio-rad.com/bio-plex** to download the instruction manual, which includes detailed instructions and a list of kit components.

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® Suspension Array 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. After thawing samples, activate them by adding 1 volume of 1 N HCl to 5 volumes of sample. Vortex, then incubate at RT for 10 min. Neutralize the samples by adding the same volume of base (1.2 N NaOH/0.5 M HEPES buffer). After treatment, dilute the samples according to the instructions in Table 1.

Table 1. Dilution of samples.

Sample Type	Diluent	Add Bovine Serum Albumin (BSA)	Sample Dilution
Serum and plasma	Sample diluent HB	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 HB	To 0.5% final	User optimized

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

4. Prime the wash station for a flat-bottom plate. Prepare the 1x wash buffer. Mix the 10x stock by inversion to ensure all salts are in solution, then dilute 1 part 10x wash buffer (60 ml) with 9 parts distilled water (540 ml).

5. Follow the prompts in Bio-Plex Manager™ Software to calibrate the system. This can be done now or during an incubation step.

6. Mix 1 volume of standard diluent HB with 3 volumes of sample diluent HB (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 a carrier protein, such as BSA, at a final concentration of 0.5% (w/v).

7. Reconstitute a single vial of standards in **500 µl** of a diluent similar to the final sample type or matrix as shown in Table 2. **Vortex** for **5 sec** and **incubate on ice** for **30 min**.

Table 2. Reconstitution of standards.

Sample Type	Diluent for Standard	Add BSA
Serum and plasma	Standard/sample diluent HB mix (1:3)	None
Culture media, with serum	Culture media	None
Culture media, serum-free	Culture media	To 0.05% final (w/v)
Lavage, lysate, other fluids	Sample diluent HB	To 0.05% final (w/v)

8. Prepare a fourfold standard dilution series and blank as shown in Figure 1. **Vortex** for **5 sec** between liquid transfers.

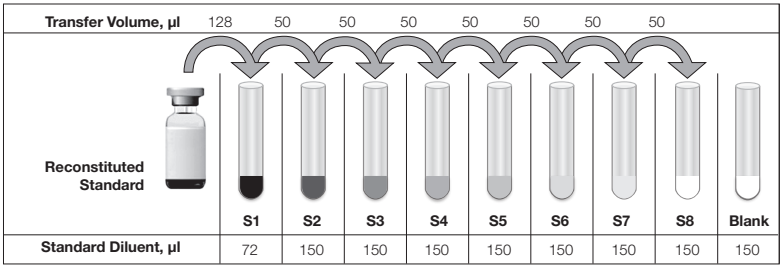

Fig. 1. Schematic showing a fourfold dilution series and blank.

9. **Vortex** the 20x coupled beads for **30 sec** and dilute to 1x in assay buffer (supplied in the kit) as shown in Table 3. Protect from light.

Table 3. Dilution of coupled beads.

Number 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. **Vortex** the diluted (1x) beads. Add **50 µl** to each well of the assay plate.
2. Wash two times with **100 µl** wash buffer (supplied in the kit).
3. **Vortex** samples, standards, and blank. Add **50 µl** to each well.
4. Cover the plate with sealing tape. Incubate on a shaker at **850 ± 50 rpm** for **2 hr** at RT.
5. With **10 min** left in the incubation, **vortex** the 20x detection antibodies for **5 sec** and quick-spin to collect the liquid. Dilute to 1x in detection antibody diluent HB as shown in Table 4.

Table 4. Dilution of detection antibodies.

Number of Wells	20x Detection Antibody, µl	Detection Antibody Diluent HB, µl	Total Volume, µl
96	150	2,850	3,000

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

Table 5. Dilution of SA-PE.

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

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

12. Cover the plate with sealing tape and incubate on a shaker at **850 ± 50 rpm** for **30 min** at RT.

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

14. Resuspend the beads in **125 µl** assay buffer. Cover the plate with sealing tape and incubate on a shaker at **850 ± 50 rpm** for **30 sec**.

15. Remove the sealing tape and read the plate using the settings in Table 6.

Table 6. Bio-Plex instrument settings.

Instrument	RP1 (photomultiplier tube)	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 system.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800*
Austria *43 1 877 89 01 177* ***Belgium*** *32 (0)3 710 53 00* ***Brazil*** *55 11 3065 7550*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *420 241 430 532* ***Denmark*** *45 44 52 10 00*
Finland *358 09 804 22 00* ***France*** *33 01 47 95 69 65* ***Germany*** *49 89 31 884 0*
Hong Kong *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *972 03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000*
Korea *82 2 3473 4460* ***Mexico*** *52 555 488 7670*
The Netherlands *31 (0)318 540 666* ***New Zealand*** *64 9 415 2280*
Norway *47 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 (0) 861 246 723* ***Spain*** *34 91 590 5200*
Sweden *46 08 555 12700* ***Switzerland*** *41 026 674 55 05*
Taiwan *886 2 2578 7189* ***Thailand*** *66 2 651 8311*
United Arab Emirates *971 4 8187300* ***United Kingdom*** *44 020 8328 2000*