Bio-Plex Pro™ Human Inflammation Panel I Assays

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro™ Human Inflammation Assays and Treg Cytokine	10044282

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, which includes detailed instructions and a list of kit components, 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 diluents, including wash buffer, assay buffer, standard diluent HB, detection antibody diluent HB, and sample diluent HB, 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. Calibrate the Bio-Plex system by following the prompts within the Bio-Plex Manager™ software. This can be done now or during an assay incubation step.

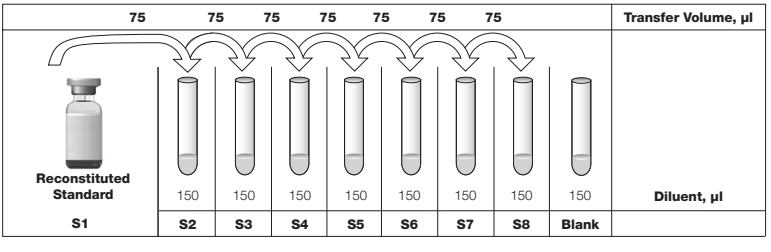
5. Prepare 1x wash buffer. Mix 10x stock by inversion to ensure all salts are in solution. Then dilute 1 part 10x wash buffer with 9 parts dH_20.

6. Reconstitute the vial of standards in standard diluent HB (or a diluent similar to your sample matrix) by adding **781 µl** of diluent. Reconstitute the vial of control in **250 µl** of standard diluent HB, as shown below. **Vortex** at medium speed for **5 sec** and incubate all vials **on ice** for precisely **30 min.**

Sample Type	Diluent for Standards and Controls*	Add BSA
Serum and plasma	Sample diluent HB	None
Culture media, with serum	Culture media	None
Culture media, serum-free	Culture media	To 0.5% final
Lavage, lysate, other fluids	Sample diluent HB	To 0.5% final

* If using diluents other than the standard diluent HB provided, then users must establish their own control ranges.

7. Prepare a **threefold** standard dilution series and blank as shown below. **Vortex** at medium speed for **5 sec** between liquid transfers.
Note: The controls are ready to use after reconstitution. No dilution is needed. Controls are included with the fixed panel only.



8. After thawing samples, prepare according to the guidelines shown below.

Sample Type	Dilution Factor	Diluent
Serum and plasma	1:4	Sample diluent HB
Fluids	User defined	Diluent + 0.5% BSA w/v

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

# of Wells	10x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	575	5,175	5,750

Running the Assay

Note: Make sure all assay components are at RT before pipetting. **Vortex** at medium speed.

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 the plate two times** with **100 µl** Bio-Plex wash buffer.
4. **Vortex** samples, standards, blank, and control. **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** at RT for **1 hr**.
6. With 10 min left in the incubation, **vortex** detection antibodies for **15 sec** and quick-spin to collect liquid. Dilute to 1x as shown below.

# of Wells	10x Detection, µl	Detection Ab Diluent HB, µl	Total Volume, µl
96	300	2,700	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 with aluminum foil and incubate** at **850 ± 50 rpm** in the dark for **30 min** at RT. Meanwhile, prepare Bio-Plex Manager software protocol; enter standard S1 values and units provided in the assay kit.
10. With 10 min left in the incubation, **vortex** 100x SA-PE for **5 sec** and quick-spin to collect liquid. Dilute to 1x as shown below and 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 with aluminum foil and incubate** at **850 ± 50 rpm** in the dark for **10 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** for **30 sec**.
16. Remove the sealing tape and **read plate** using the settings below.

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

* A similar Luminex-based system may be used.

17. Control is included with the fixed panel only. If the control was run, then compare the observed concentration against the ranges provided in the assay kit. Ranges apply only when standards and controls are prepared in Bio-Plex standard diluent HB.

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