BIO-RAD

Bio-Plex™ Pro Human Cytokine, Chemokine, and Growth Factor Assays

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Human Cytokine, Chemokine, and Growth Factor Assays	10000111560

This guide can be used to prepare and run a full 1 x 96-well assay plate. New users can go to **bio-rad.com/HCS** and download the 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 Multiplex Immunoassay 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 the other items on ice until needed
     - Mix by inversion to ensure all salts are in solution
     - Prepare 1x wash buffer: dilute **1 part** 10x wash buffer (60 ml) with **9 parts** distilled water (540 ml)
   - Begin to thaw frozen samples
3. Prepare the sample dilution according to the guidelines provided in the following table. It is important to centrifuge serum or plasma samples at **1,000 x g** for **15 min** at **4°C** to remove particulates from all samples prior to use.

Sample Type	Recommended Sample Dilution	Diluent
Serum and plasma	1:4	Sample diluent HB
Culture media and fluids	User defined	Diluent + 0.5% bovine serum albumin (w/v)

Note: ICAM-1 and VCAM-1 require higher dilution for serum and plasma (100-fold recommended). Refer to the Bio-Plex Pro Human Cytokine, Chemokine, and Growth Factor Assays Instruction Manual (10000111560) for detailed sample preparation recommendations.

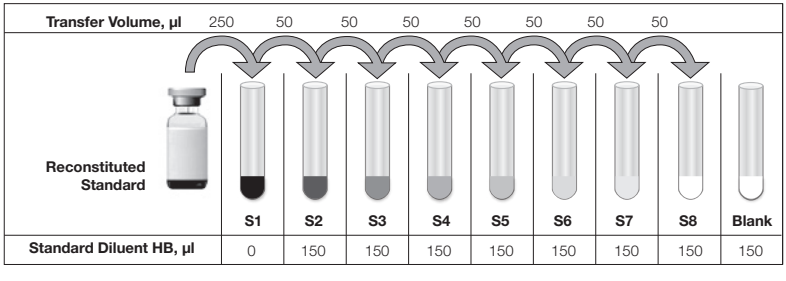
4. Prime the wash station for a flat bottom plate or set a vacuum manifold to –1 to –3" Hg for filter plate.

5. Calibrate the Bio-Plex System by following the prompts in Bio-Plex Manager Software.

6. Reconstitute the standards and control by adding 250 µl of standard diluent HB to each. **Vortex** at medium speed for **5 sec** and incubate all vials on ice for precisely **30 min**.

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

   Note: Standards are at S1 concentration after reconstitution. Controls are ready to use after reconstitution and no dilution is needed. Controls are included with the fixed panel only.



8. **Vortex** the coupled beads at medium speed for **30 sec** and **dilute to 1x** in Bio-Plex Assay Buffer as shown. Protect from light.

Premixed Panels

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

Singleplex Assays

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

Note: 20x singleplex beads allow multiplexing up to 20 analytes.

Running the Assay

1. **Vortex** the diluted (1x) beads. **Add 50 µl** to each well of the assay plate.
2. **Wash the plate two times** with **100 µl** Bio-Plex Wash Buffer.
3. **Vortex** the samples, standards, blank, and controls. **Add 50 µl** to each well.
4. Cover the plate with sealing tape. Incubate on shaker at **850 ± 50 rpm** at RT for **30 min**.
5. With 10 min left in the incubation, **vortex** the detection antibodies for **5 sec** and quick-spin to collect liquid. **Dilute to 1x** as shown.
6. **Wash the plate three times** with **100 µl** wash buffer.

Premixed Panels

Number of Wells	10x Detection Antibodies, µl	10x Detection Antibody Diluent HB, µl	Total Volume, µl
96	300	2,700	3,000

Singleplex Assays

Number of Wells	Singleplex #1 20x Detection Antibodies, µl	Singleplex #2 20x Detection Antibodies, µl	Detection Antibody Diluent HB, µl	Total Volume, µl
96	150	150	2,700	3,000

Note: 20x singleplex beads allow multiplexing up to 20 analytes.

7. **Vortex** the diluted (1x) detection antibodies. **Add 25 µl** to each well.
8. Cover the plate with sealing tape and incubate at **850 ± 50 rpm** for **30 min** at RT. Meanwhile, prepare the Bio-Plex Manager Software protocol; enter standard S1 values and units provided in the assay kit.
9. With 10 min left in the incubation, **vortex** 100x streptavidin-phycoerythrin (SA-PE) for **5 sec** and quick-spin to collect liquid. **Dilute to 1x** as shown and protect from light.

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 at **850 ± 50 rpm** for **10 min** at RT.

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

14. Resuspend the beads in **125 µl** assay buffer. Cover and shake at **850 ± 50 rpm** for **30 sec**.

15. Remove the sealing tape and **read plate** using the following settings:

Instrument	RP1 (PMT)	DD Gates	Bead Events
Bio-Plex 3D*	Standard	Select MagPlex Beads	50
Bio-Plex 100, 200*	Low	5,000–25,000	50
Luminex MAGPIX	N/A, use default instrument settings		

* Or similar Luminex System.

16. Controls are included with the fixed panel only. If the control was run, 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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