Bio-Plex Pro™ Human Cytokine Screening Panel

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Human Cytokine Screening Panel	10000092045

New users can download the complete manual, which includes detailed instructions and a list of kit components, at **bio-rad.com/bio-plex**.

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 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** dH_20 (540 ml)
   - Begin to thaw frozen samples
3. Prepare sample dilution according to the guidelines provided in the table below. 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
Culture media and fluids	User defined	Diluent + 0.5% BSA (w/v)

Note: ICAM-1 and VCAM-1 require higher dilution for serum and plasma (recommended 100-fold).

4. Calibrate the Bio-Plex System within Bio-Plex Manager™ Software.
5. 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.

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

   Note: Standards are at S1 concentration after reconsititution and the controls are ready to use after reconstitution. Controls are included with the fixed panel only.

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

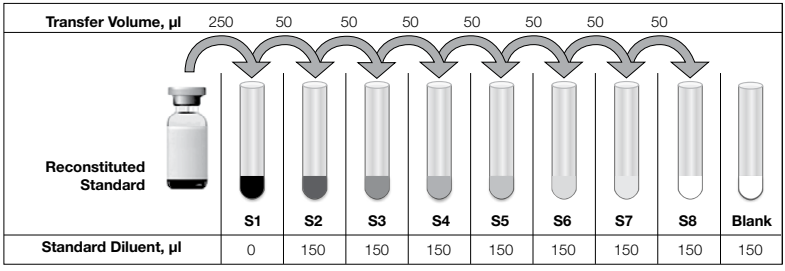
Premixed Panels

# of Wells	10x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	570	5,130	5,700

Singleplex Assays

# of Wells	Singleplex #1 and #2 20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	285	5,130	5,700

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** samples, standards, blank, and control. **Add 50 µl** to each well.
4. Cover 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** detection antibodies for **5 sec** and quick-spin to collect liquid. **Dilute to 1x** as shown below.

Premixed Panels

# of Wells	10x Detection Ab, µl	10x Detection Ab Diluent HB, µl	Total Volume, µl
96	300	2,700	3,000

Singleplex Assays

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

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

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 plate with sealing tape and incubate at **850 ± 50 rpm** for **30 min** at RT. Meanwhile, prepare 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-PE (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

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 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 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 settings below.

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

* Or similar Luminex-based system.

The observed concentration ranges of the control apply only when standards and controls are prepared using the provided Bio-Plex Standard Diluent HB.

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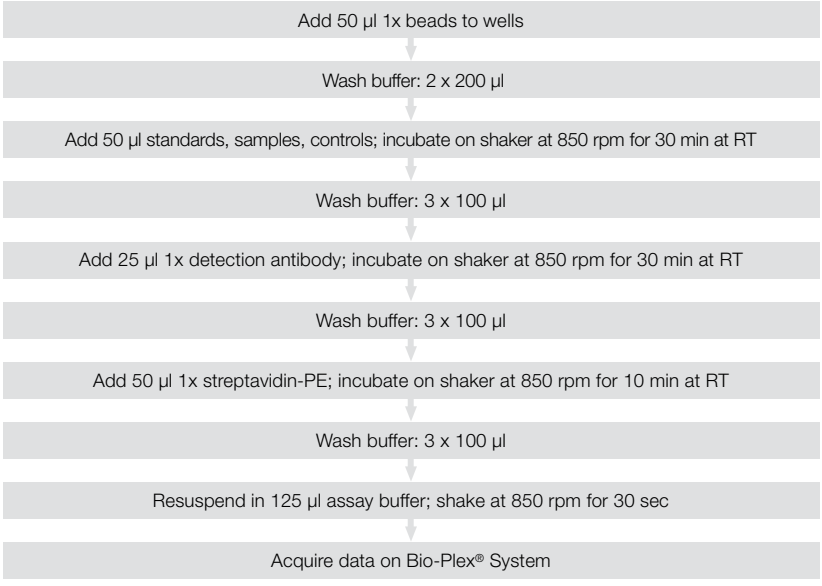
Assay Workflow



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