Bio-Plex Pro Human Immunotherapy Panel

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Human Immunotherapy Assays	10000112563

This guide can be used to prepare and run a full 1 x 96-well assay plate. 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 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 the 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.

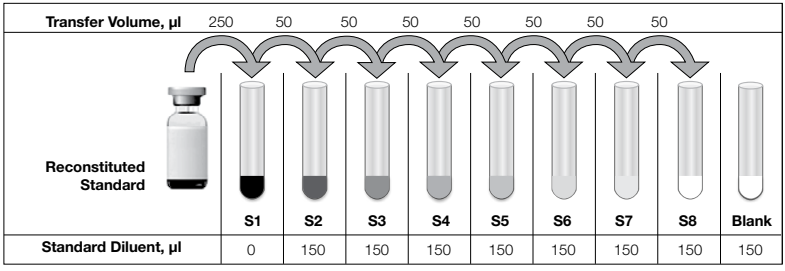
Sample Type	Recommended Sample Dilution	Diluent
Serum and plasma	1:4	Sample diluent
Culture media and fluids	User defined	Diluent + 0.5% bovine serum albumin (BSA) (w/v)

Note: ICAM-1 and VCAM-1 require higher dilution for serum and plasma (recommended 100-fold). Refer to the Bio-Plex Pro Human Immunotherapy Assays Instruction Manual (#10000112563) for detailed sample preparation recommendations.

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. **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** 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	570	5,130	5,700

Singleplex Assays

	Singleplex #1	Singleplex #2		
Number of Wells	20x Beads, µl	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	285	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** the samples, standards, blank, and control. **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.

Premixed Panels

Number of Wells	10x Detection Antibodies, µl	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.

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 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

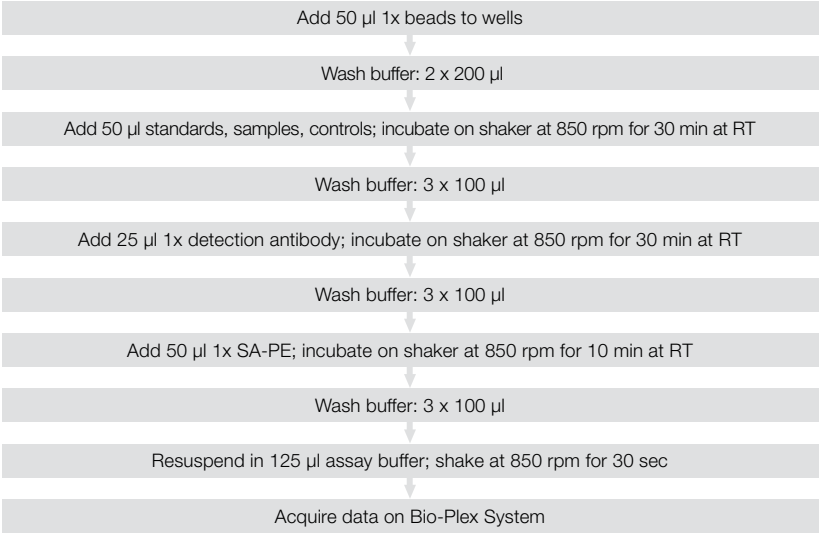
10. **Wash the plate three times** with **100 µl** wash buffer.
11. **Vortex** the diluted (1x) SA-PE. **Add 50 µl** to each well.
12. Cover the plate with sealing tape and incubate at **850 ± 50 rpm** for **10 min** at RT.
13. **Wash the plate three times** with **100 µl** wash buffer.
14. Resuspend 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 (low); 25,000 (high)	50
Luminex MAGPIX	N/A, use default instrument settings		

* Or similar Luminex System.

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

Assay Workflow



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 01 877 89019* ***Belgium*** *32 03 710 53 00* ***Brazil*** *55 11 3065 7550*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *36 01 459 6192*
Denmark *45 04 452 10 00* ***Finland*** *35 08 980 422 00* ***France*** *33 01 479 593 00*
Germany *49 089 3188 4393* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 01 459 6190*
India *91 124 4029300* ***Israel*** *972 03 963 6050* ***Italy*** *39 02 49486600* ***Japan*** *81 3 6361 7000*
Korea *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *310 318 540 666*
New Zealand *64 9 415 2280* ***Norway*** *47 0 233 841 30* ***Poland*** *36 01 459 6191*
Portugal *351 21 4727717* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188*
South Africa *36 01 459 6193* ***Spain*** *34 091 49 06 580* ***Sweden*** *46 08 555 127 00*
Switzerland *41 0617 17 9555* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311*
United Arab Emirates *971 4 8187300* ***United Kingdom*** *44 01923 47 1301*

10000112540 Ver A (12010951) US/EG *19-0219 0419 Sig 0119*