Bio-Plex Pro™ Assays

Quick Guide 6

For use with	Instruction Manual #
Bio-Plex Pro Human Isotyping Assays	10028370

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 the 10x wash buffer, assay buffer, and isotyping diluent 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.

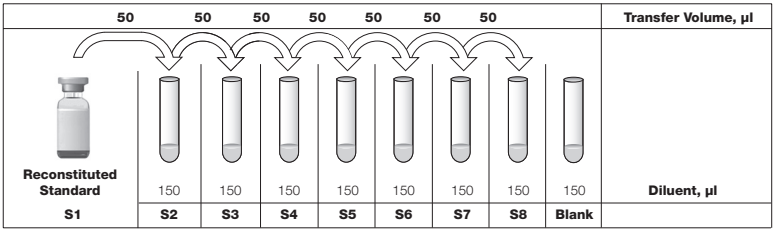
6. Reconstitute the vial of standards in **781 µl** of a diluent similar to your final sample type or matrix. Reconstitute the vial of quality controls in **250 µl** of the same diluent, as shown below. **Vortex** for **5 sec** and incubate all vials at once **on ice** for **30 min**.

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

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

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

Note: The quality controls are ready to use after reconstitution. No dilution is needed.



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

Sample Type	Diluent	Add BSA	Recommended Sample Dilution
Serum and plasma	Isotyping diluent	None	1:40,000 for IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgM 1:500 for IgE 1:20,000 for IgG total
Culture media, with serum	Culture media	None	User defined
Culture media, serum-free	Culture media	To 0.5% final	User defined

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

# 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 pipetting.

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 controls. **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 **1hr**.
6. 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.

# of Wells	20x Detection Ab, µl	Detection Ab Diluent, µl	Total Volume, µl
96	150	2,850	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 and incubate** at **850 ± 50 rpm**, as described above, 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 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

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 and incubate** at **850 ± 50 rpm**, as described above, 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 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		

* A similar Luminex-based system may be used.

17. If the quality controls were run, compare the observed concentrations against the ranges provided in the assay kit. Ranges apply only when standard and controls are prepared in Bio-Plex isotyping diluent.

P10028208

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723*
Australia *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11*
Brazil *55 11 5044 5699* ***Canada*** *905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *420 241 430 532* ***Denmark*** *44 52 10 00*
Finland *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 31 884 0*
Greece *30 210 9532 220* ***Hong Kong*** *852 2789 3300*
Hungary *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050*
Italy *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460*
Mexico *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *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 *27 861 246 723* ***Spain*** *34 91 590 5200*
Sweden *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189* ***Thailand*** *800 88 22 88*
United Kingdom *020 8328 2000*