BIO-RAD

Bio-Plex Pro™ Assays

Quick Guide 4

For use with	Instruction Manual #
Human, Mouse, and Rat Cytokine Assays	10014905

This guide can be used to prepare and run a full 1 x 96-well assay plate. Refer to the complete instruction manual for more information on a given step. New users can download the manual, which includes detailed instructions and a list of kit components, at **www.bio-rad.com/bio-plex**.

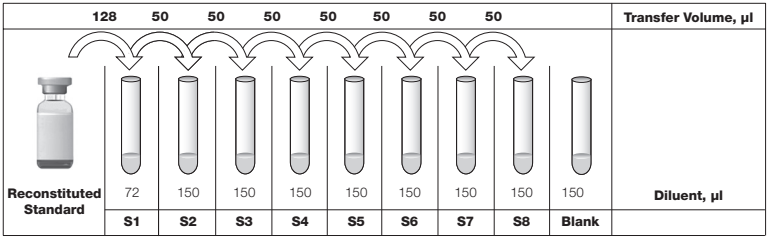
IMPORTANT! Pay close attention to **vortexing**, **shaking**, and **incubation instructions**. Deviation from the protocol may result in low assay signal and assay variability.

Initial Preparation

1. Plan the plate layout.
2. Start up/warm up the Bio-Plex® system (**30 min**).
   - Bring assay buffer, wash buffer, and sample 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 Bio-Plex Manager™ software. This can be done now or during an assay incubation step.
5. Reconstitute a single vial of standards in **500 µl** of a diluent similar to the final sample type or matrix. **Vortex** for **5 sec** and incubate **on ice** for **30 min**.

Sample Type	Diluent for Standards	Add BSA
Serum and plasma	Standard diluent	None
Culture media, with serum	Culture media	None
Culture media, serum-free	Culture media	To 0.5% final (w/v)

6. Prepare a fourfold standard dilution series and blank as shown below. **Vortex** for **5 sec** between liquid transfers. If mixing diabetes assays with cytokine assays, refer to the diabetes instruction manual.



Note: Change tips between each dilution.

7. After thawing samples, prepare as shown below.

	Serum and Plasma		Culture Supernatant and Other Fluids		Cell and Tissue Lysates	
Assay	**Dilution**	**Diluent**	**Dilution**	**Diluent**	**Dilution**	**Diluent**
Human, mouse, and rat cytokines	1:4	Bio-Plex sample diluent	User optimized	Cell culture medium or buffer similar to sample*	User optimized (1:2 of lysates at 200–900 µg/ml protein)	Bio-Plex sample diluent
Human ICAM-1/VCAM-1	1:100	Bio-Plex sample, standard diluent	User optimized			
Mouse ICAM-1	1:200	Bio-Plex serum-based diluent	User optimized			

* If samples are serum-free, add BSA to 0.5% final w/v.

8. Vortex the 10x or 20x coupled beads for **30 sec** and dilute to 1x in Bio-Plex assay buffer as shown. Protect from light.

Human and Mouse Cytokine Group I and II Assays.

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

Mouse Cytokine Group III and Rat Cytokine Group I Assays.

# of Wells	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	288	5,472	5,760

Running the Assay

1. Prewet filter plate with **100 µl** Bio-Plex assay buffer (skip for flat bottom).
2. **Vortex** the diluted (1x) beads for **10–20 sec**. 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. 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. See table for incubation time.

Incubation times for sample, detection Ab, and SA-PE.

Assay	Sample	Detection Ab	SA-PE
Bio-Plex Pro human and mouse cytokine (groups I and II)	30 min	30 min	10 min
Bio-Plex Pro mouse cytokine (group III)	1 hr	30 min	10 min
Bio-Plex Pro rat cytokine (group I)	1 hr	30 min	10 min

Note: 850 rpm provides equivalent performance to recommended shaker settings in previous manuals (**1,100 rpm** for **30 sec**, **300 rpm** for incubation).

6. With 10 min left in the incubation, **vortex** the 10x or 20x detection Abs for **5 sec** and quick-spin to collect liquid. Dilute to 1x as shown below.

# of Wells	Detection Ab, µl		Detection Ab Diluent, µl	Total Volume, µl
	10x	20x		
96	300	—	2,700	3,000
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. Repeat Step 5. See table for incubation time. Meanwhile, prepare Bio-Plex Manager software protocol; enter standard S1 values from the assay kit.
10. With 10 min left in the incubation, **vortex** the 100x SA-PE for **5 sec** and quick-spin to collect liquid. Dilute to 1x as shown 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. Repeat Step 5. See table for incubation time.

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

15. Resuspend beads in **125 µl** assay buffer. Cover plate as in Step 5 and shake the plate at **850 ± 50 rpm** for **30 sec**.

16. Remove sealing tape and **read** the **plate** using the settings below.

Settings for optimal sensitivity on the Bio-Plex 100, 200, 3D or similar system.

Assay	Low PMT, RP1	High PMT, RP1
Bio-Plex Pro human cytokine (group I and II)	•	—
Bio-Plex Pro mouse cytokine (group I, II, and III)	•	—
Bio-Plex Pro rat cytokine (group I)	—	•

Note: Use default instrument settings for Bio-Plex® MAGPIX™.

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