

# 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](http://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)

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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.

	128	50	50	50	50	50	50	50		Transfer Volume, $\mu$ l
	72	150	150	150	150	150	150	150	150	
	S1	S2	S3	S4	S5	S6	S7	S8	Blank	Diluent, $\mu$ l

**Note:** Change tips between each dilution.

7. After thawing samples, prepare as shown below.

Assay	Serum and Plasma		Culture Supernatant and Other Fluids		Cell and Tissue Lysates	
	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 $\mu$ g/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, $\mu$ l	Assay Buffer, $\mu$ l	Total Volume, $\mu$ l
96	575	5,175	5,750

### Mouse Cytokine Group III and Rat Cytokine Group I Assays.

# of Wells	20x Beads, $\mu$ l	Assay Buffer, $\mu$ l	Total Volume, $\mu$ l
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

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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™.

The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.



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