Bio-Plex Pro Rat Cytokine Assays

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Rat Cytokine, Chemokine, and Growth Factor Assays	10000142119

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 go to **bio-rad.com/bio-plex** and download the manual, which includes detailed instructions and a list of kit components.

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 Multiplex Immunoassay System (**30 min**). Bring assay buffer, wash buffer, and sample diluent to room temperature (RT) and keep other items on ice until needed. Begin to thaw frozen samples.
3. After thawing samples, prepare them according to the following guidelines.

	Serum and Plasma		Culture Supernatant and Other Fluids		Cell and Tissue Lysate	
Assay	Dilution	Diluent	Dilution	Diluent	Dilution	Diluent
Rat cytokine	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

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

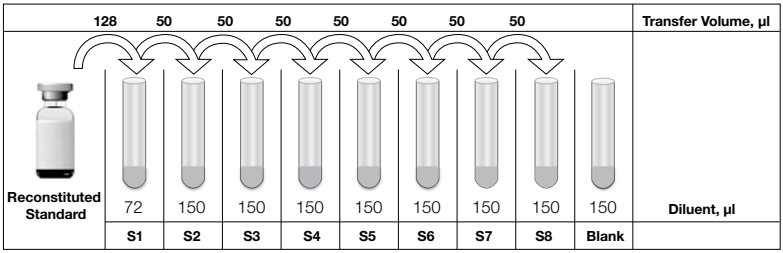
4. Prime the wash station for a flat bottom plate. Prepare 1x wash buffer. Mix 10x stock by inversion to ensure all salts are in solution. Then dilute **1 part** 10x wash buffer (60 ml) with **9 parts** distilled water (540 ml).

5. Calibrate the Bio-Plex System by following the prompts in Bio-Plex Manager Software. This can be done now or during an assay incubation step.

6. 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)

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



Note: Change tips between each dilution.

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

Rat Cytokine Assays

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

Running the Assay

1. **Vortex** the diluted (1x) beads for **10–20 sec**. 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, and blank. Add **50 µl** to each well.
4. Cover the plate with sealing tape and incubate on shaker at **850 ± 50 rpm** at RT. See the following table for incubation times for sample, detection antibody, and streptavidin-phycoerythrin (SA-PE).

	Incubation Time		
Assay	**Sample**	**Detection Antibody**	**SA-PE**
Bio-Plex Pro Rat Cytokine	1 hr	30 min	10 min

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

Number of Wells	20x Detection Antibody, µl	Detection Antibody Diluent HP, µl	Total Volume, µl
96	150	2,850	3,000

6. **Wash the plate three times** with **100 µl** wash buffer.
7. **Vortex** the diluted (1x) detection antibody. Add **25 µl** to each well.
8. Repeat step 4. See table for incubation time. Meanwhile, prepare the Bio-Plex Manager Software protocol; enter standard S1 values provided in the assay kit.
9. 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.

Incubation Time			
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. Repeat step 4. See table for incubation time.

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

14. Resuspend the beads in **125 µl** assay buffer. Cover the plate as in step 4 and shake it at **850 ± 50 rpm** for **30 sec**.

15. Remove the sealing tape and **read plate** using the following settings for optimal sensitivity. Use high photomultiplier tube (PMT) RP1 setting for the Bio-Plex 100, 200, 3D, or similar system. Use default instrument settings for the Bio-Plex MAGPIX Multiplex Reader.

Assay	Low PMT, RP1	High PMT, RP1
Bio-Plex Pro Rat Cytokine	–	•

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