Bio-Plex Pro Mouse Th17 Cytokine Assays

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Cytokine, Chemokine, and Growth Factor Assays	10000111560

This guide can be used to prepare and run a full 1 x 96-well assay plate. New users should 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, detection antibody diluent HP, and sample diluent, 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.

	Serum and Plasma		Culture Supernatant and Other Fluids		Cell and Tissue Lysate	
Assay	Dilution	Diluent	Dilution	Diluent	Dilution	Diluent
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
Mouse ICAM-1	1:100	Bio-Plex Standard and Sample Diluents	User optimized			

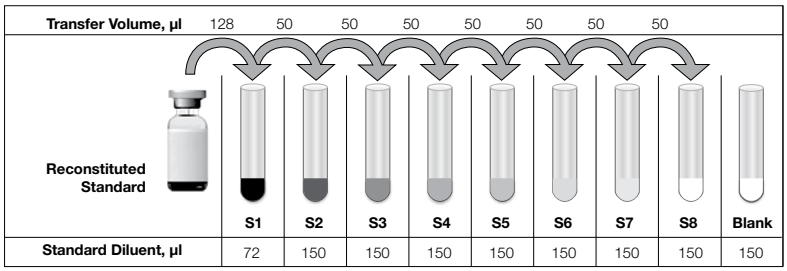
* If samples are serum-free, add bovine serum albumin (BSA) to 0.5% final w/v.

For example:

- For serum or plasma cytokine assays, dilute samples 1:4 by adding 40 µl sample + 120 µl Bio-Plex Sample Diluent
- For serum or plasma ICAM-1 assays, dilute samples 1:100
  - First dilution (1:4): 10 µl sample + 30 µl Bio-Plex **Sample Diluent**
  - Second dilution (1:25): 5 µl from the first dilution + 120 µl Bio-Plex **Standard Diluent**

4. Calibrate the Bio-Plex System in Bio-Plex Manager Software.
5. Reconstitute the standards and control by adding **500 µl** of standard diluent 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: The control is 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

Number of Wells	Singleplex #1 10x Beads, µl	Singleplex #2 10x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	570	570	4,560	5,700

Note: 10x singleplex beads allow multiplexing up to ten 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 HP, µl	Total Volume, µl
96	300	2,700	3,000

Singleplex Assays

Number of Wells	Singleplex #1 10x Detection Antibodies, µl	Singleplex #2 10x Detection Antibodies, µl	Detection Antibody Diluent HP, µl	Total Volume, µl
96	300	300	2,400	3,000

Note: 10x singleplex beads allow multiplexing up to ten 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.

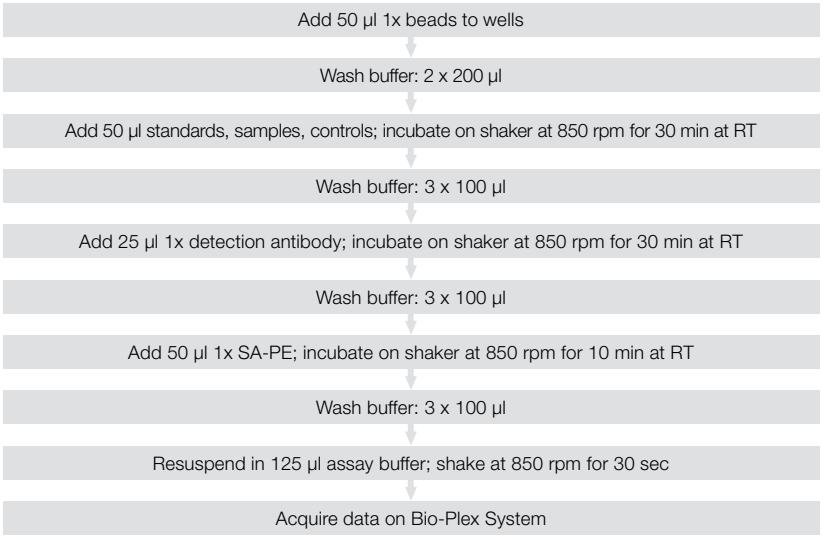
Number of Wells	100x SA-PE, µl	Assay Buffer, µl	Total Volume, µl
96	60	5,940	6,000

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.

Assay Workflow



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