BIO-RAD

Bio-Plex Pro™ Mouse Chemokine Assays

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Mouse Chemokine Assays	10000057971

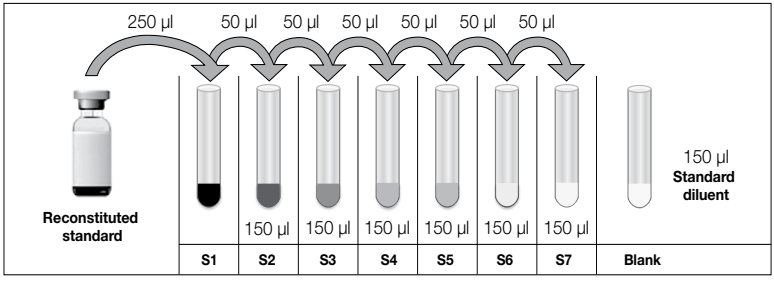
This guide can be used to prepare and run a full 1 x 96-well assay plate. For more information on a given step, refer to the complete instruction manual. New users can download the manual, which includes detailed instructions and a list of kit components, at **bio-rad.com/bio-plex.**

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 diluents, including wash buffer, assay buffer, standard diluent, detection antibody diluent HP, and sample diluent, to room temperature (RT). Keep other items on ice until needed
   - Begin to thaw frozen samples
   - Bring the 10x wash buffer to ambient temperature (RT)
     - Mix by inversion to ensure all salts are into solution
     - Prepare 1x wash buffer. Dilute **1 part** 10x wash buffer (60 ml) with **9 parts** dH_20 (540 ml)
3. 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.
4. Reconstitute the vial of standards in standard diluent (or a diluent similar to your sample matrix) by adding **250 µl** of diluent (this is S1 in step 5). Reconstitute the vial of control in **250 µl** of standard diluent. **Vortex** at medium speed for **5 sec** and incubate all vials on ice for precisely **30 min**. **Note:** If using diluents other than the standard diluent provided, users must establish their own control ranges.

5. Prepare a **fourfold** standard dilution series and blank as shown below. **Vortex** at medium speed for **5 sec** between liquid transfers.
Note: The controls are ready to use after reconstitution. No dilution is needed. Controls are included with the fixed panel only.



6. Prepare sample dilution according to the guidelines provided in the table below. 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.

Sample Type	Dilution Factor	Diluent
Serum or plasma	1:5	Sample diluent
Fluids	User defined	Diluent + 0.5% BSA w/v

Note: MCP-2 requires higher dilution for serum and plasma (recommended is 2,000-fold).

7. **Vortex** coupled beads at medium speed for **30 sec** and dilute to 1x in Bio-Plex Assay Buffer as shown below. Protect from light.

# of Wells	10x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	570	5,130	5,700

Running the Assay

Note: Make sure all assay components are at RT before pipetting. **Vortex** at medium speed.

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** samples, standards, blank, and control. **Add 50 µl** to each well.
4. Cover plate with foil sealing tape. Incubate on shaker at **850 ± 50 rpm** at RT for **30 min**.
5. With 10 min left in the incubation, **vortex** detection antibodies for **15 sec** and quick-spin to collect liquid. Dilute to 1x as shown below.

# of Wells	10x Detection, µl	Detection Ab Diluent HP, µl	Total Volume, µl
96	300	2,700	3,000

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 with foil sealing tape and incubate** at **850 ± 50 rpm** for **30 min** at RT. Meanwhile, prepare 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 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

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 with foil sealing tape and incubate** at **850 ± 50** rpm in the dark for **10 min** at RT.
13. **Wash the plate three times** with **100 µl** wash buffer.
14. Resuspend beads in **125 µl** assay buffer. Cover and shake at **850 ± 50 rpm** for **30 sec**.

15. Remove the foil 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™ (discontinued)	N/A use default instrument settings		

* A similar Luminex-based system may be used.

16. Control is included with the fixed panel only. If the control was run, then compare the observed concentration against the ranges provided in the assay kit. Ranges apply only when standards and controls are prepared in Bio-Plex Standard Diluent.

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