Bio-Plex™ Cytokine Assay Protocol

Quick Guide

Preparation

1. Power up Bio-Plex system. Warm up and calibrate.
2. Reconstitute and dilute standard.*
3. Perform bead dilution (see Table 1).
4. Prewet wells with assay buffer at 150 µl/well. Vacuum filter.

* **Standard dilution**
Quick-spin standard. Reconstitute in appropriate sample matrix. Let tube sit 30 min on ice. Perform serial dilution in appropriate sample matrix. Do not use assay buffer. Refer to the cytokine assay instruction manual.

Table 1. Conjugated bead dilution (light sensitive).
Vortex bead vial. Dilute beads with assay buffer to 1x.

Wells	25x Stock Beads (µl)	Assay Buffer (µl)	Total Volume (µl)
96	240	5,760	6,000
48	120	2,880	3,000
32	80	1,920	2,000
24	60	1,440	1,500

Add beads, standards, and samples

5. Vortex diluted beads. Add 50 µl/well. Filter-wash 2x with 100 µl/well wash buffer.
6. Add standards and samples at 50 µl/well.
7. Seal plate. Shake at 1,100 rpm for 30 sec, then 300 rpm for 30 min **in the dark**.
8. Perform detection antibody dilution (see Table 2).

Table 2. Detection antibody dilution. Quick-spin the vial. Dilute with detection antibody diluent.

Wells	Stock Detection Antibody (µl)				Final Volume (µl)
	10x	25x	50x	100x	
96	300	120	60	30	3,000
48	150	60	30	15	1,500
32	100	40	20	10	1,000
24	75	30	15	7.5	750

Add detection antibodies

9. Filter-wash 3x with 100 µl/well wash buffer.
10. Vortex diluted detection antibodies. Add 25 µl/well.
11. Seal plate. Shake at 1,100 rpm for 30 sec, then 300 rpm for 30 min **in the dark**.
12. Perform streptavidin-PE dilution (see Table 3).

Table 3. Streptavidin-PE dilution. Quick-spin the vial. Dilute stock (100x) with assay buffer to 1x. **Store in the dark.**

Wells	Streptavidin-PE (100x) (µl)	Assay Buffer (µl)	Total Volume (µl)
96	60	5,940	6,000
48	30	2,970	3,000
32	20	1,980	2,000
24	15	1,485	1,500

Add streptavidin-PE

13. Filter-wash 3x with 100 µl/well wash buffer.
14. Vortex diluted streptavidin-PE and add 50 µl/well.
15. Seal plate. Shake at 1,100 rpm for 30 sec, then 300 rpm for 10 min **in the dark**.
16. Filter-wash 3x with 100 µl/well wash buffer.
17. Resuspend beads with 125 µl/well assay buffer.
18. Seal plate.

Read plate

19. Prior to reading the plate, shake at 1,100 rpm for 30 sec.
20. Remove seal. Read plate.

BIO-RAD

Cytokine Assay Methodology