

Bio-Plex Mouse Cytokine Assays

Introduction

Bio-Plex cytokine assays are multiplex bead-based assays (xMAP technology) designed to quantitate multiple cytokines in diverse matrices. They can be used to analyze tissue and cell culture supernatants, serum, and plasma. This technical information sheet outlines several performance characteristics of the mouse cytokine assays, including detection range, sensitivity, recovery, and precision. Bio-Plex cytokine assays are for research use only and are not to be used in diagnostic procedures. Bio-Rad selects antibodies from numerous sources to generate the best capture/detection pair.

Methods

Instruments and Reagents

Bio-Plex cytokine reagent kits must be used with a singleplex assay, a multiplex panel, or an x-Plex™ multiplex panel. For serum or plasma samples, Bio-Rad recommends species-specific diluent kits for optimum recovery. For tissue culture standards and samples, simply dilute in tissue culture medium.* The following instruments and reagents were used to generate the data in this document:

- Bio-Plex mouse cytokine 23-plex panel
- Bio-Plex mouse serum diluent kit
- Bio-Plex cytokine reagent kit
- Bio-Plex validation kit
- Bio-Plex calibration kit
- Bio-Plex Manager™ software, version 4.0
- Bio-Plex suspension array system

Protocol

Assays were performed according to the flowchart in Figure 1. Refer to the Bio-Plex cytokine assay instruction manual for the detailed protocol.

Performance Characteristics

Standard Photomultiplier Tube (PMT) Setting

Table 1 shows representative raw data, and Figure 2 shows the corresponding standard curves for the Bio-Plex mouse cytokine assays at the standard (low) PMT setting in serum. StatLIA five-parameter logistic weighting was used to fit the curve (Gottschalk and Dunn 2004).

Overall Performance

Overall assay performance is summarized in Table 1.

Applications

For a list of publications using Bio-Plex mouse cytokine assays, refer to bulletin 5297.

* Add carrier protein, such as 0.1–0.5% BSA, to RPMI.

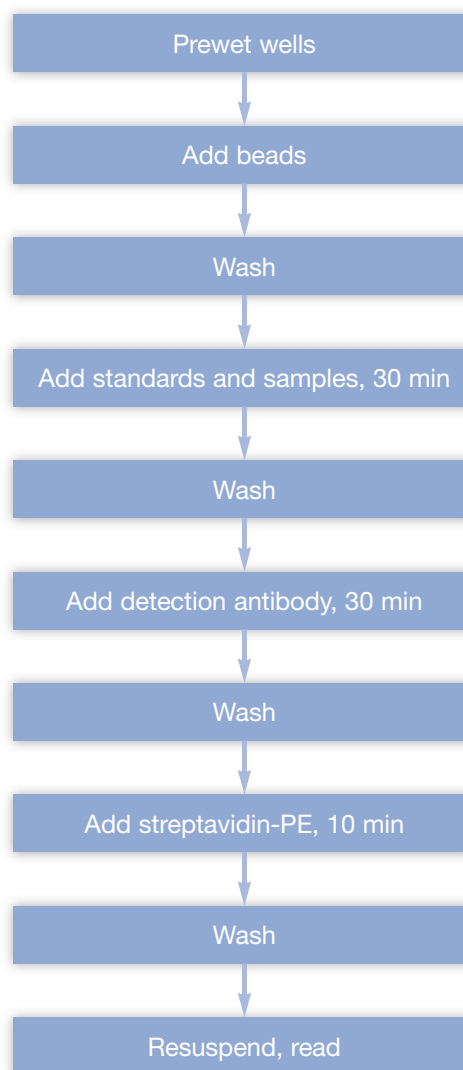


Fig. 1. Bio-Plex cytokine assay workflow.

References

Gottschalk PG and Dunn JR II, Fitting Brendan's five-parameter logistic curve, Bio-Rad bulletin 3022 (2004)

Bio-Plex cytokine assay references, Bio-Rad bulletin 5297 (2005)

The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation. xMAP is a trademark of the Luminex Corporation. StatLIA is a trademark of Brendan Scientific Corporation.

Table 1. Overall performance of Bio-Plex mouse cytokine assays at the standard PMT setting.*

	IL-1 α	IL-1 β	IL-2	IL-3	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 (p40)	IL-12 (p70)	IL-13
Bead region	53	19	36	18	32	52	38	33	56	76	58	37
Limit of detection (pg/ml)	2	7	3	2	3	2	2	15	2	2	4	9
% Recovery at dilution 2 or 3**	100	97	102	98	92	105	99	93	98	101	98	96
Intra-assay %CV***												
Dilution 4 (~500 pg/ml)	3.4	4.4	3.3	2.7	3.2	2.3	4.2	6.7	2.8	2.7	2.7	2.2
Dilution 5 (~100 pg/ml)	7.2	5.9	3.2	3.5	2.9	7.7	9.0	7.7	6.8	3.7	3.8	9.9
Inter-assay %CV†												
Dilution 4 (~500 pg/ml)	4.0	4.4	4.8	4.2	12.7	11.6	8.5	4.4	3.8	3.3	5.7	8.7
Dilution 5 (~100 pg/ml)	3.5	3.7	3.3	5.3	8.4	6.1	7.1	9.0	3.5	4.6	2.1	22.7
Dynamic range (pg/ml)	1.79–29,248	1.95–31,872	3.22–52,736	1.99–32,640	4.33–70,912	1.94–31,744	1.21–19,776	1.12–18,304	2.29–37,586	1.02–16,768	1.88–30,720	2.20–25,968

	IL-17	Eotaxin	G-CSF	GM-CSF	IFN- γ	KC	MCP-1 (MCAF)	MIP-1 α	MIP-1 β	RANTES	TNF- α
Bead region	72	74	54	73	34	57	51	77	75	55	21
Limit of detection (pg/ml)	1	148	1	7	6	3	14	24	2	5	6
% Recovery at dilution 2 or 3**	102	97	98	104	99	101	97	103	98	101	98
Intra-assay %CV***											
Dilution 4 (~500 pg/ml)	3.2	3.6	2.3	5.0	5.4	2.7	4.5	3.2	3.8	3.6	5.1
Dilution 5 (~100 pg/ml)	5.5	7.6	4.9	7.6	5.1	3.9	9.7	5.3	6.7	4.6	3.8
Inter-assay %CV†											
Dilution 4 (~500 pg/ml)	7.7	15.7	4.4	2.9	3.5	4.7	5.2	5.8	4.9	2.9	3.9
Dilution 5 (~100 pg/ml)	4.5	27.1	4.6	6.1	7.0	6.0	5.9	5.9	5.8	3.4	4.8
Dynamic range (pg/ml)	1.71–28,096	1.46–23,920	1.94–31,808	2.14–35,008	1.55–25,472	1.91–31,360	2.02–33,152	1.97–32,320	1.68–27,456	1.43–23,424	2.28–37,312

* This data was generated using the mouse cytokine 23-plex panel, catalog #171-F11241, lot #5001592; other lots may vary slightly. Cross-reactivity was negligible.
 ** Relative to expected value.
 *** Intra-assay coefficient of variation (CV) was calculated from three samples within a single plate.
 † Inter-assay CV was calculated from three samples each from five plates.

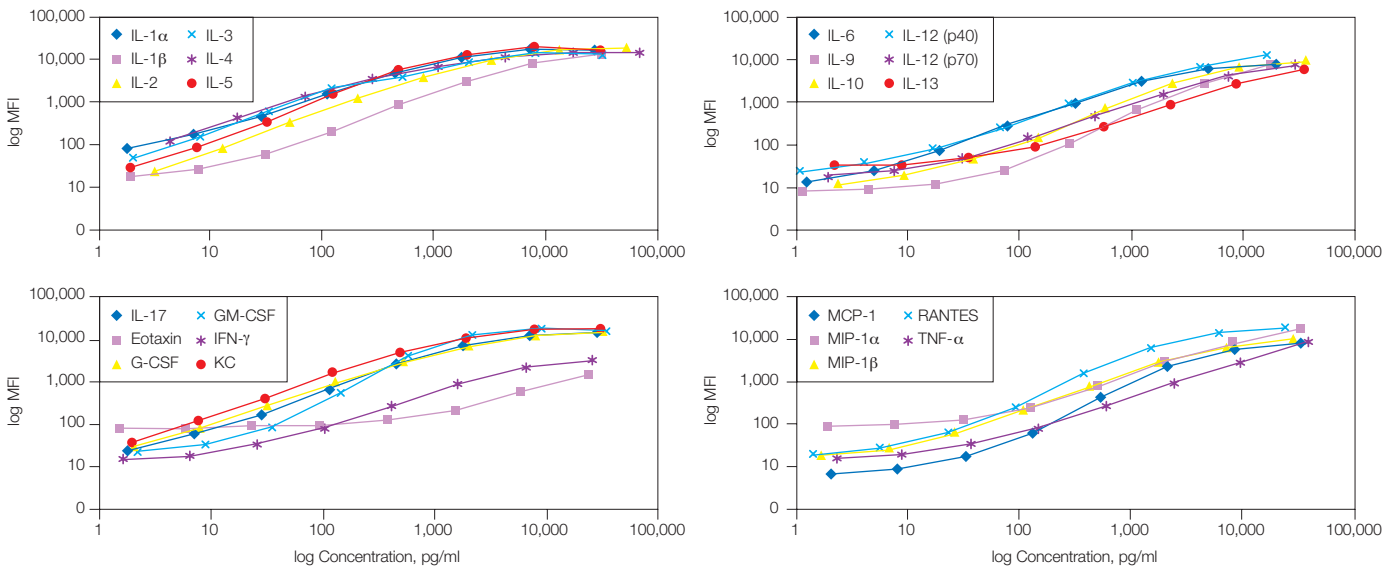


Fig. 2. Standard curves for mouse cytokine assays at the standard PMT setting.



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