Bio-Plex Human 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 human 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

Every Bio-Plex singleplex assay, multiplex panel, or x-Plex™ multiplex panel requires a cytokine reagent kit. 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 human cytokine 27-plex panel
- Bio-Plex human 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 human cytokine assays at the standard (low) PMT setting (in serum). StatLIA five-parameter logistic weighting (available in Bio-Plex Manager 3.0 or later) was used to fit the curves (Gottschalk and Dunn 2004).

Overall Performance

Overall assay performance is summarized in Table 1.

Applications

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

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

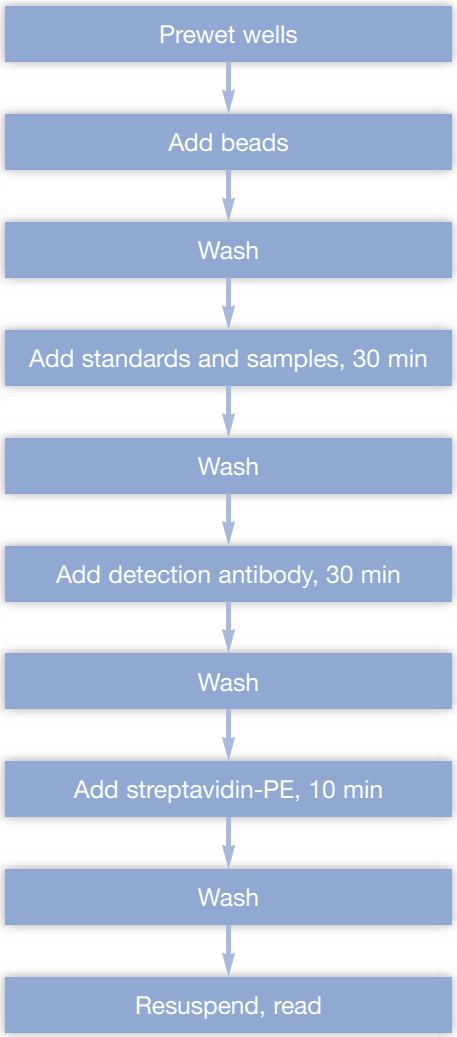

Fig. 1. Bio-Plex cytokine assay workflow.

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)

Information in this tech note was current as of the date of writing (2005) and not necessarily the date this version (rev A, 2006) was published.

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

	IL-1β	IL-1ra	IL-2	IL-4	IL-5	IL-6	IL-7	IL-8	IL-9	IL-10	IL-12 (p70)	IL-13	IL-15	IL-17
Bead region	32	25	38	52	33	19	74	54	77	56	75	51	73	76
Limit of detection (pg/ml)**	0.8	1.4	1.1	0.5	0.8	1.1	0.5	0.5	0.7	0.9	0.5	2.1	4.2	0.2
% Recovery at ~8,000 pg/ml***	105	102	97	96	99	91	97	92	99	99	102	102	103	100
Intra-assay %CV†														
@ ~500 pg/ml	4.0	4.0	4.0	3.0	5.0	5.0	5.0	4.0	10.0	2.0	3.0	5.0	3.0	3.0
@ ~125 pg/ml	2.0	5.0	3.0	3.0	3.0	6.0	2.0	4.0	2.0	5.0	3.0	10.0	3.0	6.0
Inter-assay %CV††														
@ ~925 pg/ml	14.7	7.6	6.5	5.3	8.4	16.6	16.3	11.6	7.9	10.4	9.6	7.3	21.5	5.2
@ ~300 pg/ml	8.6	4.6	5.2	6.7	6.8	7.2	6.0	6.6	4.7	5.7	8.2	7.2	6.0	4.3
Dynamic range (pg/ml)	2.19–35,952	2.49–40,806	1.37–22,515	0.31–5,050	2.42–39,714	2.41–39,525	3.24–53,093	1.93–31,592	2.08–34,000	1.62–26,600	2.76–45,254	0.56–9,112	1.96–32,111	1.26–20,616
1,000 pg = x IU	82.6	N/A	10.4	15.3	9.2	9.7	83.7	2.1	N/A	2.6	N/A	0.4	N/A	N/A
WHO standard product #	86/680		86/504	88/656	90/586	89/548	90/530	89/520		92/516		94/622		

	Basic FGF	Eotaxin	G-CSF	GM-CSF	IFN-γ	IP-10	MCP-1 (MCAF)	MIP-1α	MIP-1β	PDGF-BB	RANTES	TNF-α	VEGF
Bead region	44	43	57	34	21	41	53	55	18	47	37	36	45
Limit of detection (pg/ml)**	6.8	14.6	1.1	4.5	19.3	6.5	6.7	2.4	1.1	1.0	1.2	3.0	0.5
% Recovery at ~8,000 pg/ml***	99	93	99	101	96	96	102	104	101	99	104	98	98
Intra-assay %CV†													
@ ~500 pg/ml	2.0	2.0	3.0	4.0	4.0	3.0	13.0	5.0	4.0	4.0	3.0	5.0	4.0
@ ~125 pg/ml	4.0	4.0	4.0	6.0	6.0	4.0	7.0	4.0	4.0	4.0	2.0	5.0	2.0
Inter-assay %CV††													
@ ~925 pg/ml	17.1	8.5	5.3	4.3	10.1	17.8	14.1	15.7	15.9	5.2	17.0	13.4	8.0
@ ~300 pg/ml	4.7	13.3	7.2	3.5	9.7	16.6	7.3	6.8	16.1	6.8	6.9	5.3	7.2
Dynamic range (pg/ml)	2.84–46,542	0.77–12,696	1.98–32,424	1.72–28,165	2.14–35,011	3.11–50,938	1.39–22,802	0.94–15,640	1.24–20,243	2.4–39,334	1.35–22,134	7.74–126,859	2.85–46,712
1,000 pg = x IU	N/A	N/A	107.5	2.9	4.3	N/A	1.0	N/A	N/A	N/A	N/A	56.8	N/A
WHO standard product #			88/502	88/646	88/606		92/794					87/650	

* These data were generated using the human cytokine 27-plex panel, catalog #171-A11127, lot #50002617; other lots may vary slightly. Cross-reactivity was negligible.
** LOD is defined as the value calculated from the standard curve at the point lying 2 standard deviations above the mean background MFI (ten replicates).
*** 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.

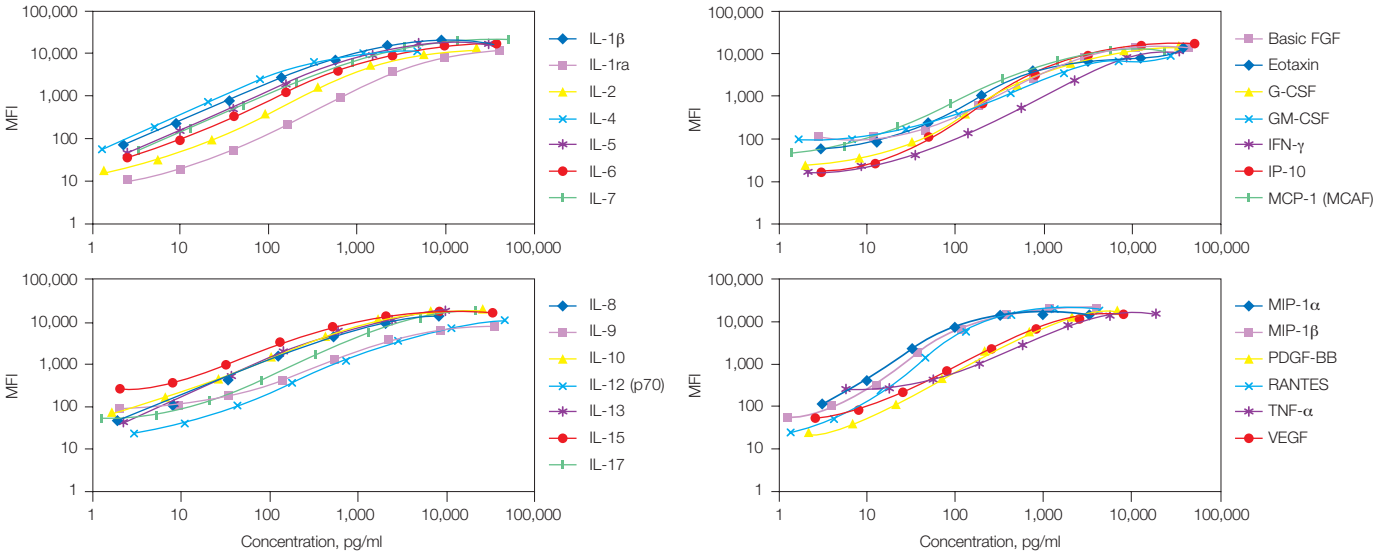

Fig. 2. Standard curves for human cytokine assays at the standard PMT setting.

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