New LEGENDplex™ Multi-Analyte Flow Assay Panels for Simultaneous Quantification of 13 Proinflammatory Chemokines in Human and Mouse Samples

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Abstract

Chemotactic cytokines or chemokines play pivotal roles in various processes such as immune surveillance, organ development, angiogenesis, and immune responses. Expression profiling of chemokines, especially those involved in inflammation and immune disorders, is important in achieving a deeper understanding of disease states. Using fluorescence-encoded beads, we have developed two multiplex immunoassay panels to simultaneously detect 13 human or mouse chemokines, respectively. Both human and mouse panels include MCP-1 (CCL2), RANTES (CCL5), IP-10 (CXCL10), Eotaxin (CCL11), TARC (CCL17), MIP-1α (CCL3), MIP-1β (CCL4), MIG (CXCL9), and MIP-3α (CCL20). In addition, the human panel contains ENA-78 (CXCL5), GROα (CXCL1), I-TAC (CXCL11) and IL-8 (CXCL8), and the mouse panel contains LIX (CXCL5), KC (CXCL1), BLC (CXCL13) and MDC (CCL22). Each antibody pair was selected for its sensitivity, specificity, accuracy and reproducibility. Suitable for use on various flow cytometers, the assays have higher sensitivity and wider dynamic range than typical ELISA methods. Further advantages include small sample volume requirement, flexible assay configurations and time- and cost-effectiveness. In addition, the assays have been validated with expected changes in relevant biological samples. The chemokine panels can be used for serum, plasma, cell culture supernatant, and other sample types, offering useful tools for biomedical research and drug discovery.

MATERIALS AND METHODS

- **Multiplexed Bead-based Sandwich Immunoassays** *see Assay Protocols for details*.
- **Instruments:** BD FACScan™, BD FACSCalibur™, BD FACSCanto™, FACSCanto™ II, BD™ LSR, BD™ LSR II, BD LSRFortessa™, BD FACSAria™, BD FACSArray™.
- 96-well microtiter filter plates, vacuum pump and filtration manifold.
- Capture antibody immobilized beads, biotinylated detection antibody cocktail, streptavidin-phycoerythrin conjugate, assay buffer, and wash buffer.
- **Biological Sample Preparation:**

    Human PBMC from healthy donors were isolated using Ficoll-Paque (GE Healthcare) and seeded at 10^6 cells /mL into 48-well plates with the appropriate stimulations as indicated.

    Mouse splenocytes were isolated and seeded at 10^6 cells /mL into 48-well plates with the appropriate stimulations as indicated.

    Cell culture supernatants were collected after 2, 4 or 5 days.

Assay Protocol

25 μL Matrix or Assay Buffer
25 μL Standard or Samples (diluted or neat)
25 μL beads

Incubate with shaking for 2h at RT or O/N at 4°C
Vacuum and wash twice

25 μL Detection Antibody

Incubate with shaking for 1h at RT
No vacuum and no wash

25 μL Streptavidin-Phycoerythrin

Incubate with shaking for 30 min at RT
Vacuum and wash twice

Read on a flow cytometer

Assay Principle

Capture Antibody
Biotinylated Detection Antibody
Bead
Analyte
Streptavidin-Phycoerythrin

Standard Curves

Human Chemokine Panel — IL-8, IP-10, CCL11, TARC, MCP-1, RANTES, MIP-1α, MIG, ENA-78, MIP-3α, Groα, I-TAC, MIP-1β

Mouse Chemokine Panel — RANTES, MIP-3α, Eotaxin, TARC, KC, MCP1, MIG, IP-10, MIP-1α, MIP-1β, BLC, LIX, MDC

Assay Characteristics

Human Chemokine Panel

Analyte	MDC in Buffer (pg/mL)	MDC in Serum (pg/mL)	Spike Recovery in Serum	Dilution Linearity in Serum	Intra-Assay CV
IL-8	1.0	1.4	103%	102%	7%
IP-10	1.4	1.1	95%	115%	5%
CCL11	2.6	1.4	102%	113%	5%
TARC	1.5	0.8	90%	107%	4%
MCP-1	1.0	0.9	94%	102%	6%
RANTES	1.9	4.3	80%	105%	5%
MIP-1α	1.6	2.1	104%	101%	5%
MIG	2.3	9.4	96%	96%	9%
ENA-78	1.2	1.1	73%	117%	7%
MIP-3α	1.4	2.5	85%	114%	4%
Groα	2.7	6.7	81%	96%	3%
I-TAC	1.1	1.1	78%	94%	6%
MIP-1β	1.6	1.4	92%	103%	4%

Mouse Chemokine Panel

Analyte	MDC in Buffer (pg/mL)	MDC in Serum (pg/mL)	Spike Recovery in Serum	Dilution Linearity in Serum	Intra-Assay CV
RANTES	1.28	0.72	84%	101%	4%
MIP-3α	2.25	2.24	87%	88%	5%
Eotaxin	0.99	1.13	102%	95%	6%
TARC	0.85	0.74	77%	118%	6%
KC	0.93	0.93	80%	112%	8%
MCP1	1.23	1.46	88%	110%	4%
MIG	1.32	1.24	131%	83%	8%
IP-10	1.14	1.28	98%	105%	9%
MIP-1α	1.03	1.11	92%	99%	5%
MIP-1β	1.54	1.08	92%	92%	6%
BLC	1.20	1.51	120%	103%	5%
LIX	1.69	1.02	124%	118%	7%
MDC	0.89	0.89	97%	105%	5%

*MDC: Minimum Detectable Concentration

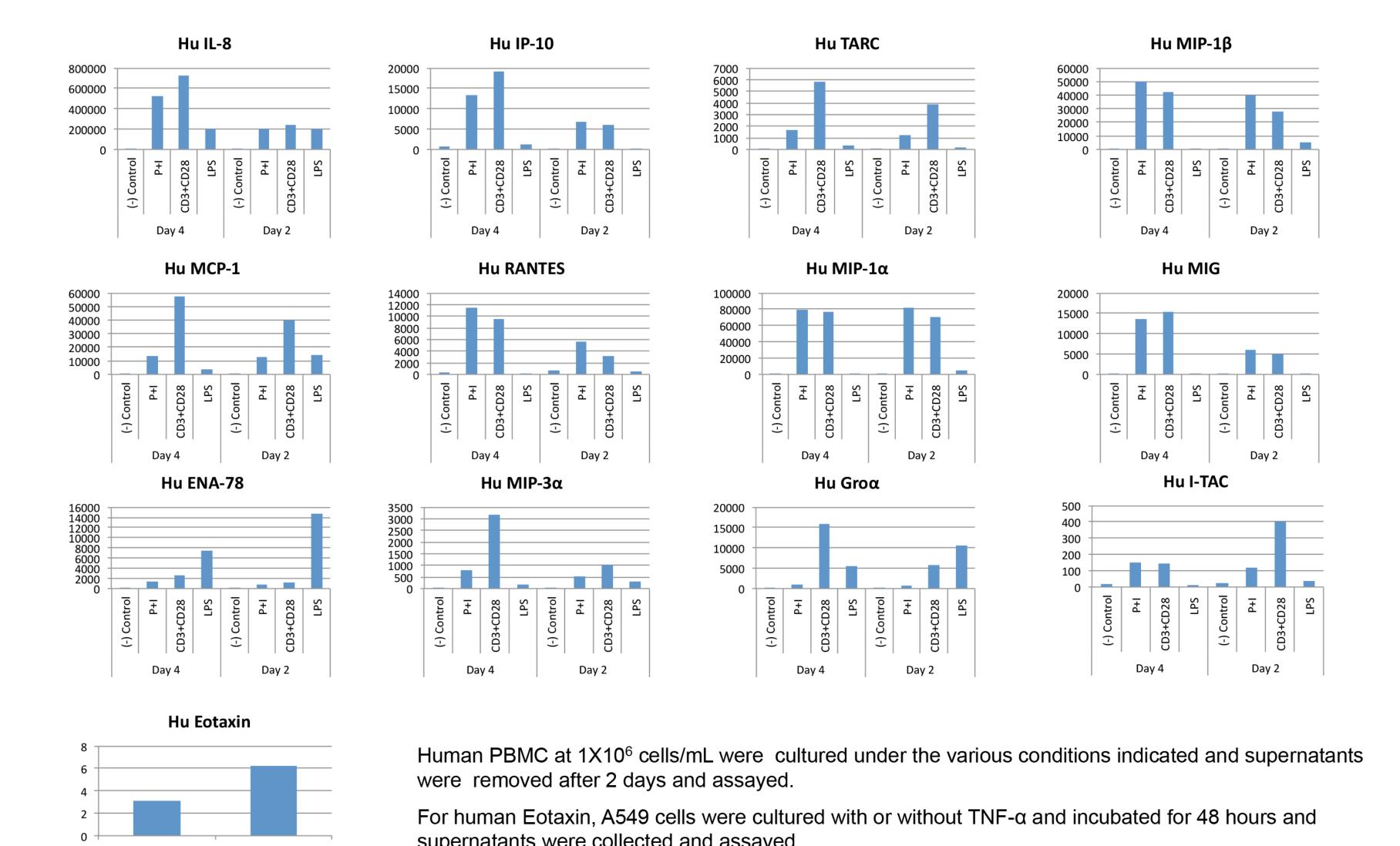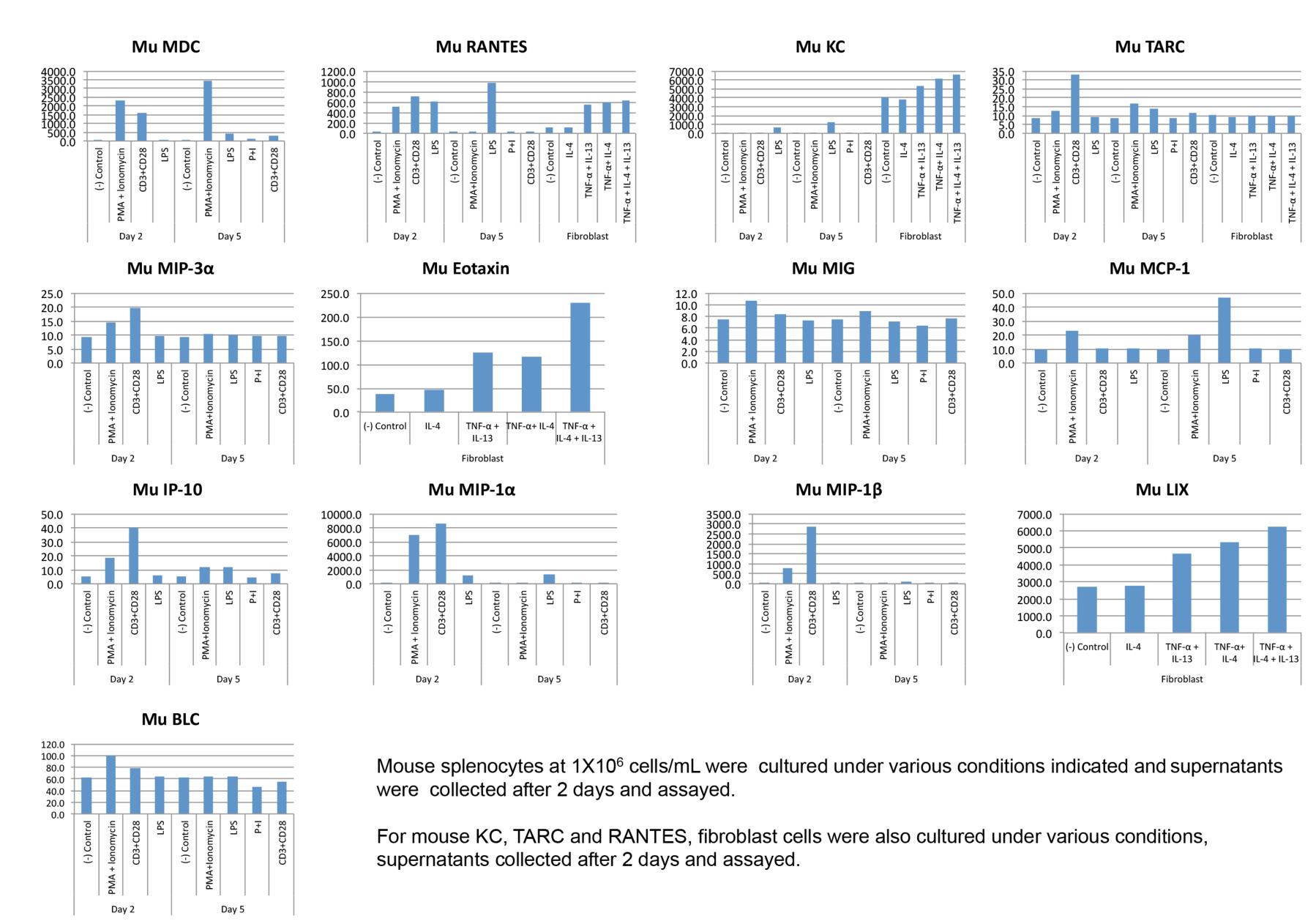**Figure 1.** Human Proinflammatory Chemokine Panel Biological Sample Test Results (pg/mL).

Human PBMC at 1×10^6 cells/mL were cultured under the various conditions indicated and supernatants were removed after 2 days and assayed.

For human Eotaxin, A549 cells were cultured with or without TNF-α and incubated for 48 hours and supernatants were collected and assayed.

Figure 2. Mouse Proinflammatory Chemokine Panel Biological Sample Test Results (pg/mL).

Mouse splenocytes at 1×10^6 cells/mL were cultured under various conditions indicated and supernatants were collected after 2 days and assayed.

For mouse KC, TARC and RANTES, fibroblast cells were also cultured under various conditions, supernatants collected after 2 days and assayed.

CONCLUSIONS

We have developed bead-based multiplex assays for simultaneous quantification of proinflammatory chemokines for human and mouse samples.

These assays are analytically robust with high sensitivity, wide dynamic range, excellent analytical accuracy and reproducibility.

The utility of these flow bead-based multiplex assays was further validated by using relevant biological samples. The customizable chemokine panels can be used for serum, plasma, cell culture supernatant, and other sample types, offering a more economic alternative to other multiplex assays for biomedical research.