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

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Abstract
Chemokines and chemokine-like proteins 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 these states. Using fluorescent-encoded beads, we have developed two multiplex immunoassays to simultaneously detect 13 human or mouse chemokines, respectively. Both human and mouse panels include MCP-1 (CCL2), IL-8 (CXCL8), IL-10 (CCL10), IL-16 (CCL16), TNF-alpha (CCL2), IL-6 (CXCL8), IL-1alpha (CCL2), IL-1beta (CCL2), IL-22 (CCL2), and IL-23 (CCL22). Each antibody pair was selected for its specificity, sensitivity, 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 supernatants, and other sample types, offering useful tools for biomedical research and drug discovery.

MATERIALS AND METHODS
- Multiplexed Bead-based Sandwich Immunoassays: assay protocols for details. In instruments: BD FACScan®/BD FACScalibur®, BD FACSAria™/BD FACSAria™ II. 96-well microplates (filters; vacuum pump and filtration system).
- Capture antibody immobilized beads, biotinylated detection antibody cocktail, streptavidin-phycocyanin conjugate, assay buffer, and wash buffer.
- Biological Sample Preparations: human PBMCs from healthy donors were isolated using Ficoll Paque (GE Healthcare) and seeded at 10^6 cells/ml into 48-well plates with appropriate stimulations as indicated. Mouse splenocytes were isolated and seeded at 10^6 cells/ml into 48-well plates with appropriate stimulations as indicated.
- Cell culture supernatants were collected after 2, 4, or 5 days.

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

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

**Figure 3. Human Proinflammatory Chemokine Panel Biological Sample Test Results (pg/ml)**

**Figure 4. Mouse Proinflammatory Chemokine Panel Biological Sample Test Results (pg/ml)**

**CONCLUSIONS**
We have developed bead-based multiplex assays for simultaneous quantification of proinflammatory chemokines for human and mouse samples. These assays are useful tools for high-throughput, wide dynamic range, excellent analytical reproducibility, and drug discovery. The utility of these new bead-based multiplex assays was further validated by using relevant biological samples. The chemokine chemokine panels can be used for serum, plasma, cell culture supernatants, and other sample types, offering a more accurate alternative to other multiplex assays for biomedical research.

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