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

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Abstract

T helper cells (Th cells) play important roles in regulating the responses of the immune system. They secrete cytokines to stimulate various effector cells, such as cytotoxic T cells, B cells and macrophages. Accurate measurement of Th cytokine expression is critical to identify the corresponding Th cell responses and to develop in depth understanding of the immune responses. We have developed two multiplexed assay panels, i.e., the human and mouse Th panels, using fluorescence–encoded beads that are suitable for use on various flow cytometers. Both panels allow simultaneous quantification of 13 human or mouse cytokines including interleukins (IL-2, 4, 5, 6, 9, 10, 13, 17A, 17F, 21, 22), IFN-γ and TNF-α, which are collectively secreted by Th1, Th2, Th9, Th17, Th22 or T follicular cells. Each antibody pair was carefully selected for assay specificity, sensitivity, accuracy and reproducibility. The assays provide higher detection sensitivity and broader dynamic ranges than traditional ELISA methods. The panels have been validated by detecting expected changes in biological samples. Further advantages include small sample volume, flexible assay configurations, and time- and cost-effectiveness. The Th cytokine 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

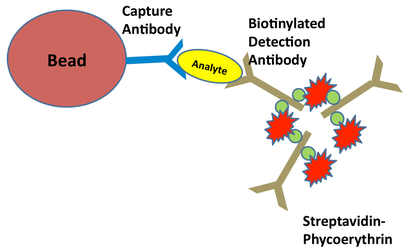
25 µL Matrix or Assay Buffer
25 µL Standard or Samples (diluted or neat)
25 µL beads
↓ Incubate with shaking for 2h at RT or O/N at 4°C
Vacuum and wash twice

25 µL Detection Antibody
↓ Incubate with shaking for 1h at RT
No vacuum and no wash

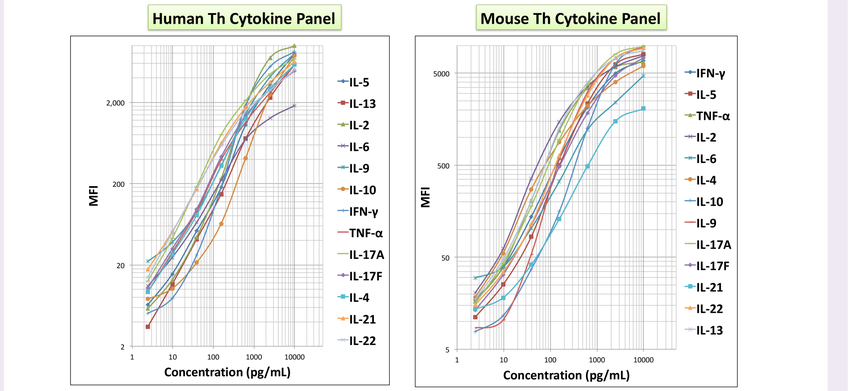
25 µL Streptavidin-Phycoerythrin
↓ Incubate with shaking for 30 min at RT
Vacuum and wash twice

Read on a flow cytometer

Assay Principle



Standard Curves



Assay Characteristics

Human Th Cytokine Panel

Analyte	MDC in culture medium (pg/mL)	MDC in serum (pg/mL)	Spike recovery	Linearity of Dilution in serum	Intra assay CV
IL-5	1.12	1.29	125%	104%	6%
IL-13	0.83	1.12	88%	122%	6%
IL-2	0.96	1.25	99%	116%	9%
IL-6	1.1	1.14	116%	96%	10%
IL-9	0.99	1.16	91%	114%	8%
IL-10	1.09	0.9	100%	122%	7%
IFN-γ	1.04	1.38	82%	109%	10%
TNF-α	0.96	0.91	83%	108%	8%
IL-17A	1.48	1.83	72%	105%	5%
IL-17F	1.07	1.26	86%	117%	8%
IL-4	0.68	1.13	86%	125%	6%
IL-21	1.42	2.29	89%	98%	8%
IL-22	1.97	2.22	89%	96%	5%

Mouse Th Cytokine Panel

Analyte	MDC in culture medium (pg/mL)	MDC in serum (pg/mL)	Spike recovery	Linearity of Dilution in serum	Intra assay CV
IL-5	1.6	2.0	91%	112%	8%
IL-13	2.4	2.1	110%	117%	7%
IL-2	1.2	1.1	77%	110%	9%
IL-6	1.7	1.6	86%	115%	10%
IL-9	2.4	2.4	87%	128%	5%
IL-10	2.2	1.9	98%	129%	7%
IFN-γ	1.0	1.1	87%	113%	7%
TNF-α	2.3	2.0	91%	124%	9%
IL-17A	2.0	1.8	87%	112%	5%
IL-17F	0.8	1.4	87%	108%	6%
IL-4	0.9	0.9	85%	122%	11%
IL-21	2.3	5.4	116%	97%	5%
IL-22	1.3	1.6	92%	106%	8%

*MDC: Minimum Detectable Concentration

Figure 1. Human Th Cytokine Panel Biological Sample Test Results (pg/mL).

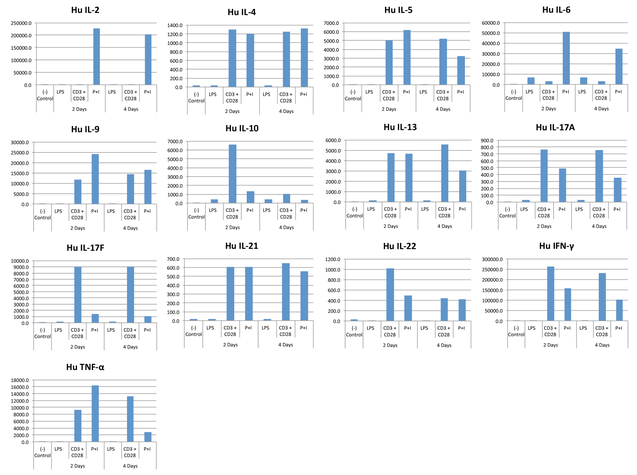
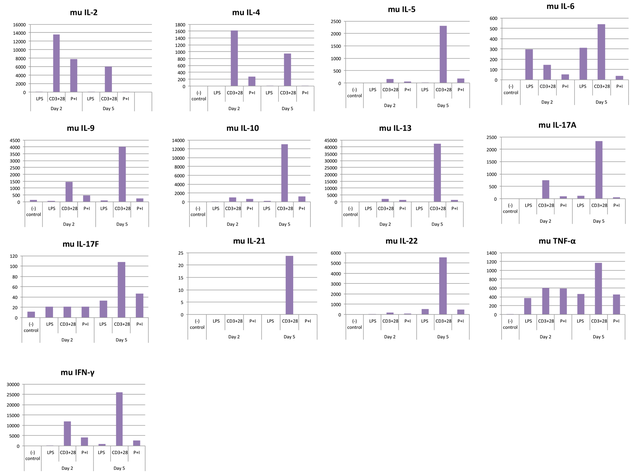


Figure 2. Mouse Th Cytokine Panel Biological Sample Test Results (pg/mL).



CONCLUSIONS

We have developed bead-based multiplex assays for simultaneous quantification of cytokines related to T helper cell responses for human and mouse samples.

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

The utility of the multiplex assay panels was further validated by using relevant biological samples and in laboratories studying T helper cell biology.