LEGENDplex™, A New Multiplex Solution for Cytokine and Chemokine Quantification in Biological Samples

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Abstract

Current multiplex assay technologies on the market for soluble targets are frequently associated with high reagent and instrument cost, inconsistent assay performance, inaccurate data analysis, and lack of good reproducibility. To address the issues, we developed four multiplexed bead-based assay panels for simultaneous quantification of soluble markers using standard flow cytometers available in many laboratories. Our human and mouse T helper (Th) panels allow quantification of 13 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 and T follicular cells. Our human and mouse proinflammatory chemokine panels detect 13 chemokines, including MCP-1 (CCL2), RANTES (CCL5), IP-10 (CXCL10), Eotaxin (CCL11) , TARC (CCL17), MIP-1α (CCL3), MIP-1β (CCL4), MIG (CXCL9), and MIP-3α (CCL20). The human chemokine panel also includes ENA-78 (CXCL5), GROα (CXCL1), I-TAC (CXCL11), and IL-8 (CXCL8), and the mouse chemokine panel contains LIX (CXCL5), KC (CXCL1), BLC (CXCL13) and MDC (CCL22). Each antibody pair was carefully selected for assay specificity, sensitivity, accuracy and reproducibility. The assay panels have been validated in biological samples, detecting changes as expected. Compared with similar assay products on the market, LEGENDplex™ assays provide equal or better analytical performance with reduced cost, better performance consistency, and greater flexibility. To facilitate analysis of data (FCS 2.0, 3.0, 3.1, and list mode files) from different flow cytometers, a software package with an intuitive interface, accurate curve-fitting, and robust reporting was developed and is offered free of charge. The assays and software can be used for quantitative and qualitative analysis of soluble biomarkers from serum, plasma, cell culture supernatant, and other sample types, offering a more economical alternative for multiplex solutions.

Principle of the assay

On-plate or in-tube assay protocols

25 µL Assay Buffer or serum matrix
25 µL Standard or samples (1:1 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 Compatible FACS Instruments
↓
Data analysis using the Data Analysis Software

Multiplexed Bead-Based Sandwich Immunoassay:

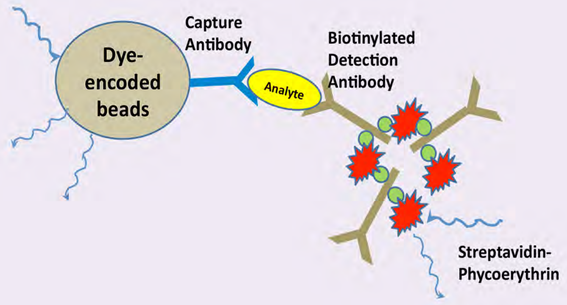


Figure 1. The multiplex assays are available in pre-defined panels, mix & match kits, and cross-panel custom kits.

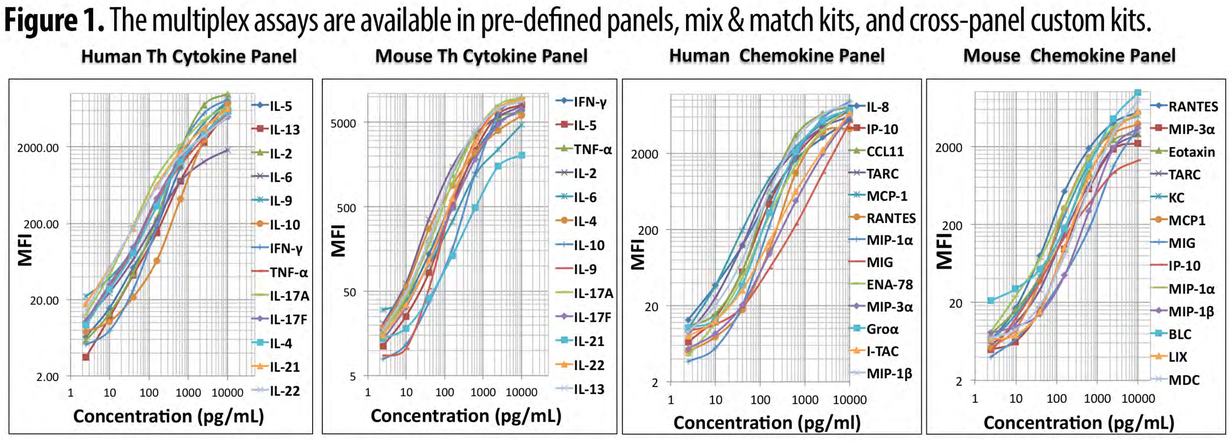


Figure 2. The multiplex assays are analytically robust.

Analyte	MDC** in Buffer (pg/mL)	MDC** in Serum (pg/mL)	Serum Level (pg/mL)	Spike Recovery (Serum)	Linearity of Dilution	Intra-Assay CVs
Hu IL-5	1.12	1.29	ND***	125%	111%	7%
Hu IL-13	0.83	1.12	ND	88%	113%	9%
Hu IL-2	0.96	1.25	ND ~ 34	99%	113%	9%
Hu IL-6	1.10	1.14	ND ~ 1585	116%	117%	12%
Hu IL-9	0.99	1.16	ND ~ 10	91%	101%	8%
Hu IL-10	1.09	0.90	ND	100%	126%	7%
Hu IFNγ	1.04	1.38	ND ~ 6	82%	127%	10%
Hu TNFα	0.96	0.91	ND ~ 40	83%	118%	9%
Hu IL-17A	1.48	1.83	ND	72%	115%	5%
Hu IL-17F	1.07	1.26	ND ~ 40	86%	113%	12%
Hu IL-4	0.68	1.13	ND	86%	120%	6%
Hu IL-21	1.42	2.29	ND ~ 44	89%	101%	8%
Hu IL-22	1.97	2.22	ND ~ 28	89%	108%	7%

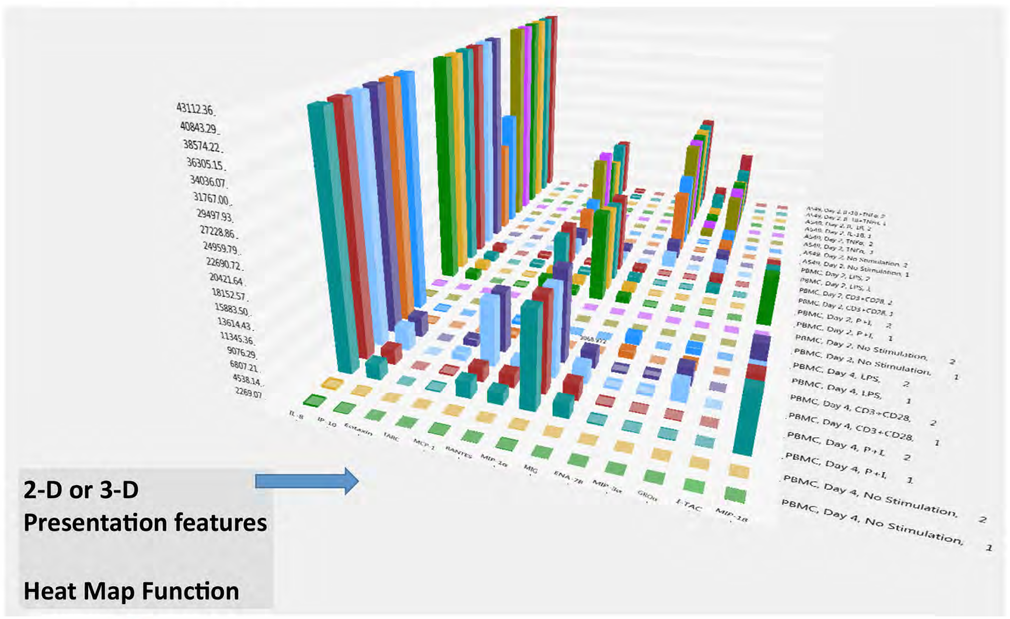
Analyte	MDC in Buffer (pg/mL)	MDC in Serum (pg/mL)	Serum Level (pg/mL)	Spike Recovery (Serum)	Linearity of Dilution	Intra-Assay CVs
Mu IFNγ	1.0	1.1	ND - 44.3	87%	113%	7%
Mu IL-5	1.6	2.0	ND - 4.3	91%	112%	10%
Mu TNFα	2.3	2.0	ND - 8.7	91%	124%	10%
Mu IL-2	1.2	1.1	ND	77%	110%	9%
Mu IL-6	1.7	1.6	5- 37.5	86%	115%	9%
Mu IL-4	0.9	0.9	ND	85%	122%	11%
Mu IL-10	2.2	1.9	ND	98%	129%	7%
Mu IL-9	2.4	2.4	ND - 5.6	87%	128%	5%
Mu IL-17A	2.0	1.8	ND - 11.1	87%	112%	8%
Mu IL-17F	0.8	1.4	ND	87%	108%	6%
Mu IL-21	2.3	5.4	ND - 28.4	116%	97%	5%
Mu IL-22	1.3	1.6	ND - 12.3	92%	106%	8%
Mu IL-13	2.4	2.1	ND	110%	117%	10%

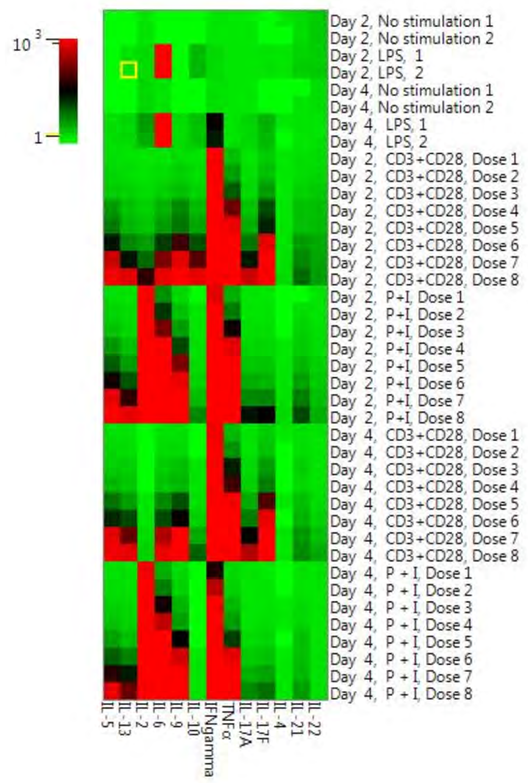
Analyte	MDC in Buffer (pg/mL)	MDC in Serum (pg/mL)	Serum Level (pg/mL)	Spike Recovery (Serum)	Linearity of Dilution	Intra-Assay CVs
Hu IL-8	1.0	1.4	11.5 - 48513	103%	102%	8%
Hu IP-10	1.4	1.1	37.2 - 637	95%	115%	5%
Hu CCL11	2.6	1.4	ND - 379	102%	113%	4%
Hu TARC	1.5	0.8	20.4 - 151	90%	107%	4%
Hu MCP-1	1.0	0.9	159.8 - 3488	94%	102%	6%
Hu RANTES	1.9	4.3	188.2 - 12000	80%	105%	5%
Hu MIP-1α	1.6	2.1	7.0 - 1999	104%	101%	5%
Hu MIG	2.3	9.4	ND - 421	96%	96%	9%
Hu ENA-78	1.2	1.1	12.5 - 935	73%	117%	7%
Hu MIP-3α	1.4	2.5	6.7 - 155	85%	114%	4%
Hu GROα	2.7	6.7	ND - 1551	81%	96%	3%
Hu I-TAC	1.1	1.1	8.1 - 139	78%	94%	6%
Hu MIP-1β	1.6	1.4	6.1 - 195	92%	103%	4%

Analyte	MDC in Buffer (pg/mL)	MDC in Serum (pg/mL)	Serum Level (pg/mL)	Spike Recovery (Serum)	Linearity of Dilution	Intra-Assay CVs
Mu RANTES	1.28	0.72	11.4 -34.9	84%	101%	6%
Mu MIP-3a	2.25	2.24	3.3 - 73.5	87%	88%	7%
Mu Eotaxin	0.99	1.13	3.3 - 323.7	102%	95%	7%
Mu TARC	0.85	0.74	1.0 - 14.4	77%	118%	8%
Mu KC	0.93	0.93	102.1 - 451	80%	112%	6%
Mu MCP1	1.23	1.46	13.9 - 11.6	88%	110%	6%
Mu MIG	1.32	1.24	14.1 - 262.5	131%	83%	9%
Mu IP-10	1.14	1.28	ND - 5.4	98%	105%	8%
Mu MIP-1a	1.03	1.11	2.0 - 23.1	92%	99%	6%
Mu MIP-1b	1.54	1.08	ND	92%	92%	4%
Mu BLC	1.20	1.51	3.4-12398.3	120%	103%	4%
Mu LIX	1.69	1.02	221.1 - 719	124%	118%	6%
Mu MDC	0.89	0.89	42.4 -433.3	97%	105%	5%

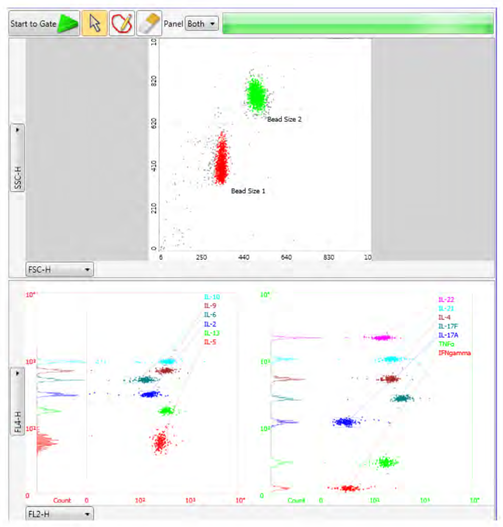
**MDC: Minimum Detectable Concentration ***ND: Non-detectable

Figure 3. The multiplex assay panels are biologically validated.





The free-of-charge LEGENDplex™ data analysis software provides intuitive and user interfaces, automatic gating and analysis functions, unique data presentation features, and one-click data summary and reporting.



Th cytokine panels: Human PBMC or mouse splenocytes at 1X10^6 cells/mL were cultured under various conditions indicated and supernatants were removed after 2 or 4 (or 5 for mouse) days and assayed using the LEGENDplex™ Human or Mouse Th Cytokine Panel, respectively. As shown, human Th cytokines were specifically induced by CD3 plus CD28 and PMA plus Ionomycin stimulations, but not by LPS (except IL-6). Mouse panel data not shown.

Proinflammatory chemokine panels: Human PBMC or mouse splenocyte at 1X10^6 cells/mL were cultured under various conditions indicated and supernatants were removed after 2 or 4 (5 for mouse) days and assayed. For human Eotaxin, A549 cells were cultured with or without TNF-α and incubated for 48 hours and supernatant were collected and assayed.
For mouse Eotaxin, LIX, and KC, mouse fibroblast cells were cultured under various conditions indicated and supernatants were collected after 2 days and assayed. Mouse data not shown.

CONCLUSIONS

We developed new bead-based multiplex assay panels for simultaneous quantification of human or mouse Th cytokines and pro-inflammatory chemokines using various flow cytometers commonly available.

The multiplex assay panels are analytically robust (high assay sensitivities, wide dynamic ranges, great accuracy and reproducibility, 12.5 µL serum/plasma volume requirement) and are biologically validated through in house efforts and world-wide research collaborations.

Free-of-charge data analysis software specifically designed for the multiplex assays offers simple, accurate and fast data analysis, summary, and reporting for FCS and list mode files from flow cytometers.

The multiplex assays are customizable through mix & match selections for within-panel targets and cross-panel selections.

With more panels coming soon, the LEGENDplex ™ multiplex assays offer instrument-independent and more economic solutions that overcome high cost and quality consistency issues associated with existing multiplex assays on the market.

For more information visit: **biolegend.com/legendplex**