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Abstract

Cytokines are important circulating signal proteins. They mediate a wide range of physiological responses including immunity, inflammation, and hematopoiesis. They are also associated with a spectrum of diseases ranging from tumor growth to infection to Parkinson. Cytokines are measured either by immunoblot or immunoassay. Both approaches are time consuming and can only analyze a single cytokine at a time. With the development of Luminex® technology, which uses color-coded microspheres analyzed with a dedicated reader with digital signal processing, simultaneous detection of up to 100 analytes is possible in a single well of a microtiter plate. Here we present data from the Bio-Plex protein array system that demonstrate sensitive, simultaneous quantitation of cytokines in human or mouse sera and in tissue culture media. We can detect all 8 cytokines at a limit of detection less than 10 pg/ml and with inter- and intra-assay CV less than 10%. The advantages of this approach are the standardized plate format, compression of labor, and most importantly, extremely efficient use of sample. Using an 8-plex assay, a single 96-well plate can generate 786 concordant cytokine values from 12 µl of sample/well.

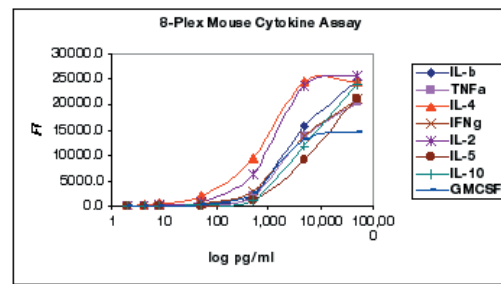
Methods

Assay Protocol
(for Bio-Plex mouse or human cytokine kits)

1. Incubate antigen (standards or unknown samples) with polystyrene beads conjugated with capture antibody
2. Incubate with detection antibody (biotinylated antibody)
3. Incubate with streptavidin-PE
4. Analyze on Bio-Plex protein array reader

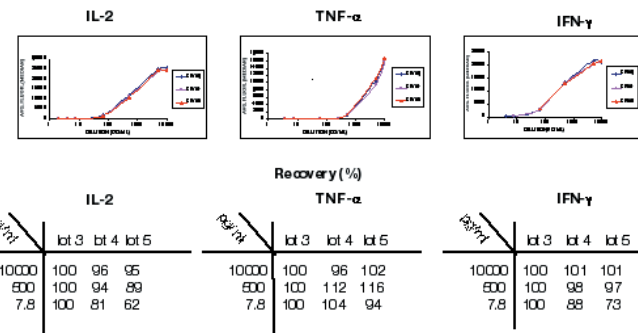
Results — Validations

Standard Curves of 8-Plex Mouse Cytokine Assay



- 0–50,000 pg/ml of antigens were spiked into 50 ml of standard diluent and quantitated using 8-plex mouse cytokine assay.
- Samples were run in duplicate.

Recovery Assay: Bio-Plex Standard Diluent vs Different Pooled Mouse Sera



- 0–50,000 pg/ml of antigens were spiked into 50 µl of standard dilution of 3 different batches of pooled mouse sera.
- Samples were run in duplicate.
- Other cytokines exhibited similar results. Data not shown.

Inter- and Intra-Plate Precision

Inter-Plate Precision (n = 3 Plates)

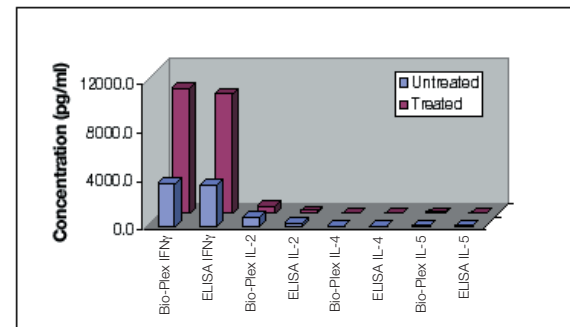
pg/ml	019 IL-6	021 IFN-γ	034 GM-CSF	036 TNF-α	038 IL-2	052 IL-4	054 IL-5	056 IL-10
50,000	3%	3%	1%	2%	3%	2%	3%	4%
15,557	1%	1%	1%	1%	1%	4%	1%	3%
5,556	1%	1%	3%	4%	1%	5%	1%	4%
1,000	4%	3%	4%	8%	2%	5%	4%	9%
250	9%	7%	5%	7%	5%	6%	5%	9%
125	7%	8%	5%	7%	3%	8%	4%	7%
62.5	7%	4%	5%	8%	7%	8%	3%	5%
31.25	5%	8%	5%	6%	10%	4%	7%	3%
15.6	9%	6%	4%	7%	6%	6%	2%	3%
0	11%	7%	11%	6%	9%	5%	6%	3%

Intra-Plate Precision (n = 2 plates)

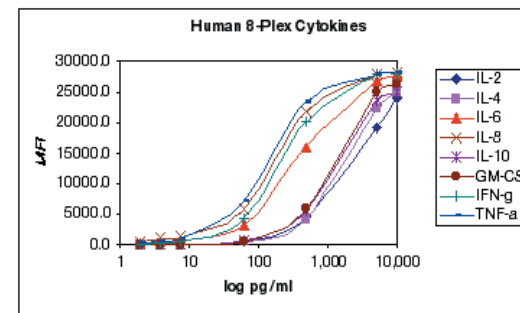
pg/ml	019 IL-6	021 IFN-γ	034 GM-CSF	036 TNF-α	038 IL-2	052 IL-4	054 IL-5	056 IL-10
5,000	2%	2%	3%	3%	2%	7%	2%	4%

- Intra-plate precision: average of 2 runs, 96 replicates per run.
- Inter-plate precision: average of 3 runs, triplicate samples per concentration.

Bio-Plex Cytokine Kits vs ELISA

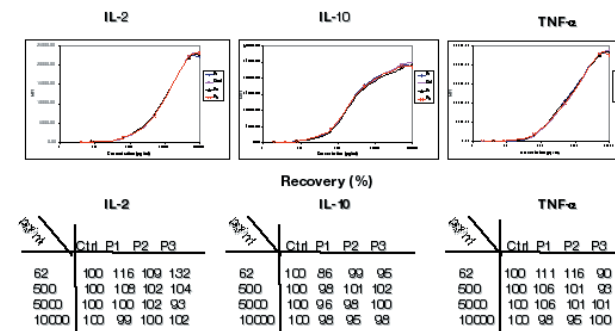


Standard Curves of 8-Plex Human Cytokine Assay



- 0–10,000 pg/ml of antigens were spiked into 50 ml of standard diluent and quantitated using 8-plex human cytokine assay.
- Samples were run in duplicate.

Recovery Assay: Bio-Plex Standard Diluent vs Different Human Patient Serum



- 0–10,000 pg/ml of antigens were spiked into 50 ml of standard diluent and 1:4 dilution of 3 different patient sera.
- Samples were run in duplicate.
- Control is standard diluent; P1, P2 and P3 are from patient 1, 2 and 3 respectively. Other cytokines exhibited similar results. Data not shown.

Sensitivity of Bio-Plex Human 8-Plex Cytokine Assay (pg/ml)

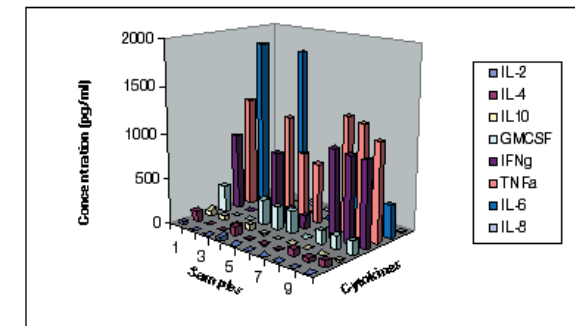
	IL-2	IL-4	IL-6	IL-8	IL-10	GM-CSF	IFN-γ	TNF-α
LOD	1.95	1.95	1.95	1.95	1.95	1.95	3.9	1.95

Sensitivity of Bio-Plex Mouse 8-Plex Cytokine Assay (pg/ml)

	IL-2	IL-4	IL-5	IL-10	IL-1β	GM-CSF	IFN-γ	TNF-α
LOD	1.95	1.95	1.95	1.95	3.9	1.95	1.95	1.95

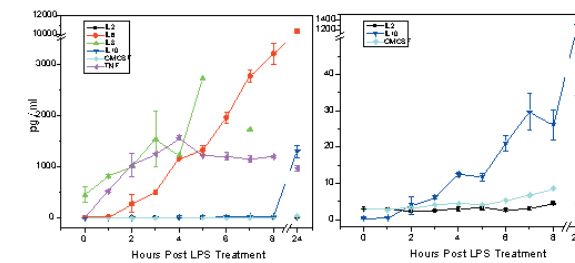
- Sensitivity is for serum and cell supernatant matrices.
- Sensitivity is defined as detectable signal greater than the sum of background + 2 x standard deviation.

Human 8-Plex Cytokine Assay of Human Serum Samples



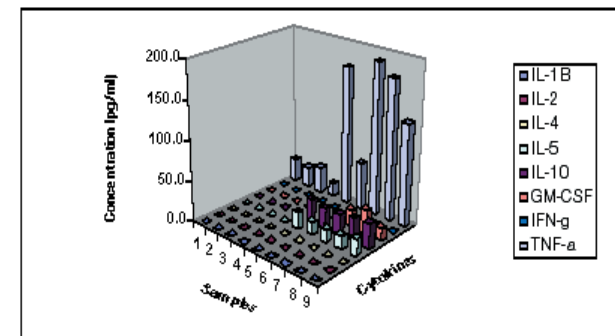
- Human serum samples were diluted 1:4 using Bio-Plex sample diluent A. Assay used 50 µl of 1:4 dilution.
- Samples were run in duplicate.

Cytokine Production by Human PBMCs *in vitro*



Note: IL4 and IFNγ were assayed but fell below the assay limit of detection.
Methods: Human peripheral blood mononuclear cells were isolated from whole blood by Ficoll-gradient centrifugation. Final cell pellets were resuspended in RPMI medium supplemented with 10% fetal bovine serum. Cells were plated in poly-lysine 96-well plates at a density of 10⁵ cells per well in a final volume of 200 µl. Lipopolysaccharide (LPS) was added to a final concentration of 1 ng/ml. At the indicated times, 100 µl cell-conditioned medium was removed for processing for Bio-Plex cytokine 8-plex analysis.

Results — Applications

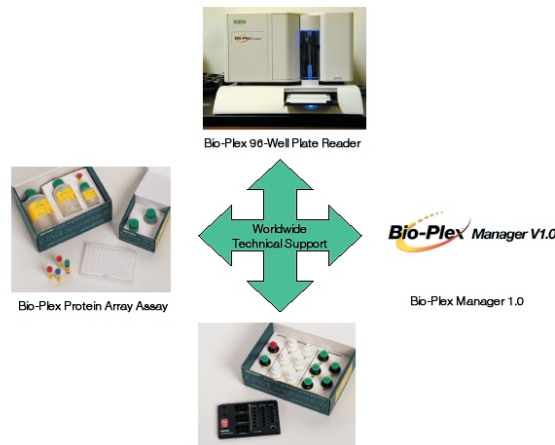


- Mouse serum samples were diluted 1:4 using Bio-Plex sample diluent B. Assay used 50 µl of 1:4 dilution.
- Samples were run in duplicate.

Conclusions

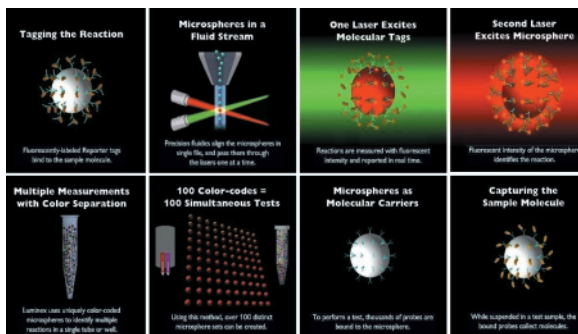
1. The Bio-Plex protein array system powered by Luminex technology performs highly multiplexed assays for analysis of specific protein-protein interactions.
2. The system reduces assay time and sample volume by performing multiple analyses simultaneously rather than sequentially.
3. The Bio-Plex cytokine assay (multiplexed) provides a much clearer picture of the cytokines and chemokines produced during any cellular response.
4. The assay using the Bio-Plex protein array system is not limited to cytokines. It can be applied to any soluble factor, such as immunoglobulin epitopes, cardiac markers, hormones, tumor markers, allergy testing, and others.
5. In general, Bio-Plex 8-plex human or mouse cytokine kits and the Bio-Plex protein array system have been demonstrated to be remarkably robust with better reproducibility, more accuracy, better sensitivity, and better cost- and time-efficiency.

Bio-Plex Protein Array System



Introduction

Bio-Plex System Using Luminex Technology Allows Multiple Measurements via Color Separation





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