Electrochemiluminescence-Based Immunoassays For Cytokines

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Abstract

Immunoassays for human cytokines are demonstrated using a new assay detection system from Meso Scale Discovery (MSD). MSD's Multi-Array™ platform combines array technologies and electrochemiluminescence detection to achieve ultra-fast, highly sensitive assays. This system allows electrochemiluminescence assays to be carried out directly in multi-well plates having integrated electrodes. The surface selectivity of the electrochemiluminescence measurement allows assays to be performed without any wash steps.

Data are shown for the simultaneous measurement of four cytokines (TNF-α, IFN-γ, IL1-β and IL-6) in wells containing a patterned array of antibodies. The assays are sensitive, rapid, require minimal sample and have an excellent dynamic range. The assays may be carried out in no-wash with excellent sensitivity (1-10 pg/mL) and dynamic range (3-4 logs). For cytokines requiring ultimate sensitivity, a single wash format offers low end detection of 0.05 pg/mL. Examples are provided showing the application of the assay technology to cell-based assays and drug screening.

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Inflammatory Cytokine Panel

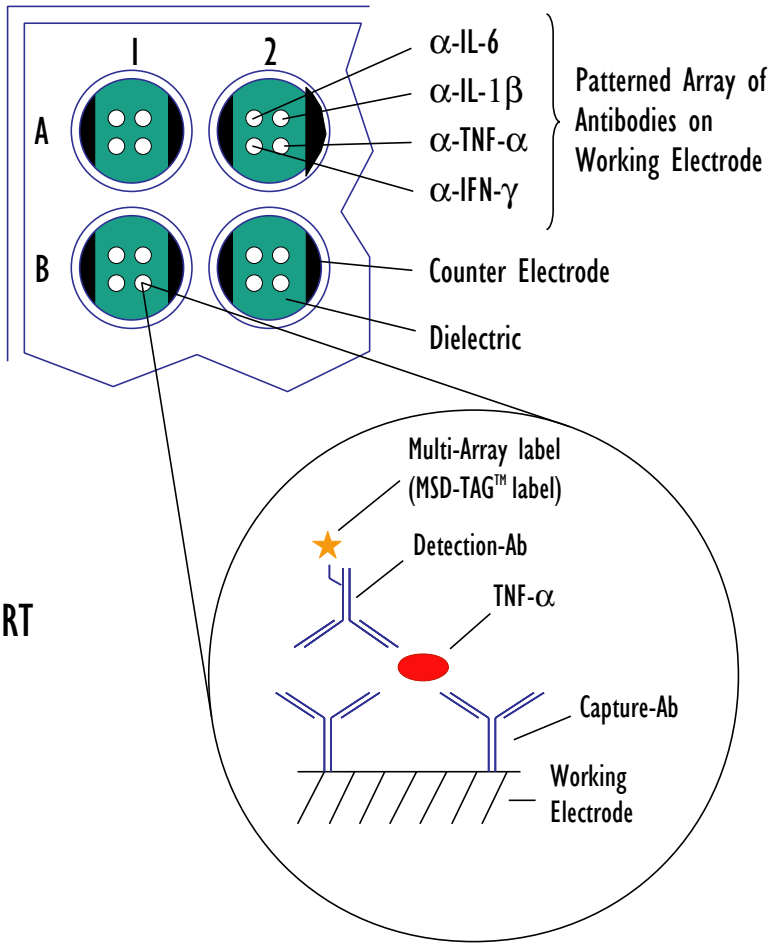
Materials

96-well MSD™ Multi-Spot™ Plate containing patterned array of anti-cytokine capture antibodies

Assay Diluent: Buffered diluent containing blocking agents

TAG-Ab: Mixture of four MSD-TAG™ labeled detection antibodies in Assay Diluent

Assay Buffer: Buffer optimized for electrochemiluminescence measurement



Procedure (One Wash Assay)

To each well of Multi-Array™ Plate :

1) Add 20 uL sample, shake 1h at RT
2) Add 20 uL MSD-TAG mixture, shake 1h at RT
3) Wash 3x with PBS
4) Add 100 uL MSD Assay Buffer T (1x)
5) Analyze plate using Sector HTS Reader

Procedure (No Wash Assay)

Same as above, except:

- Omit step 3
- In step 4, use MSD Assay Buffer P (2X)

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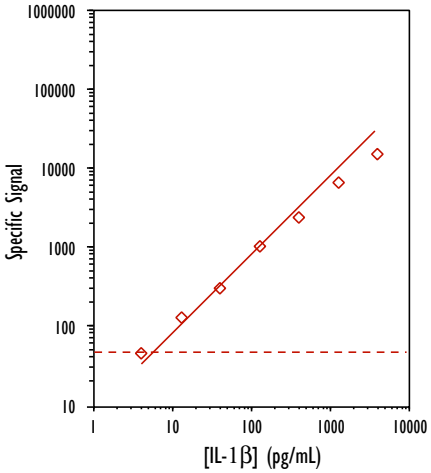
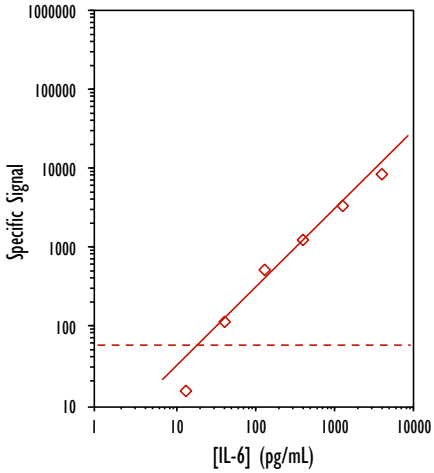
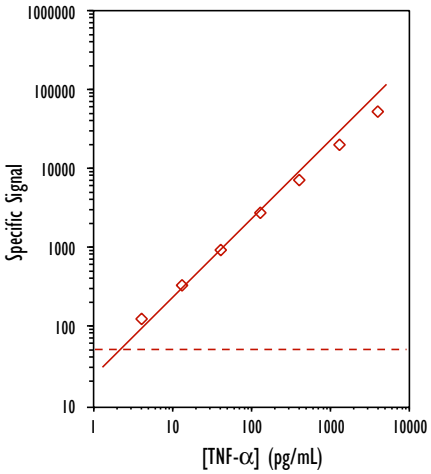
Cytokine Panel Performance: No Wash Format

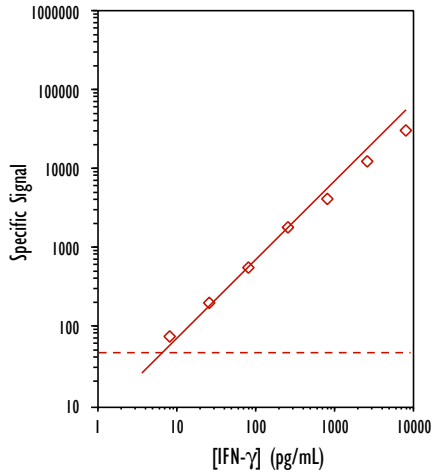




Calibrators are in RPMI media containing 10 % human serum

No Wash
25% above background signal





- Detection Limits: ~10 pg/mL (No Wash)
- Linear Range Extends to >3000 pg/mL
- Method is scalable to larger arrays

Cytokines measured in non-wash formats have excellent sensitivity

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Measurement of Cytokine Production in Whole Blood

Procedure:

1) Dilute whole blood 1:10 in RPMI-1640 medium in a multi-well plate.

2) Add LPS* (lipopolysaccharide) or LPS + PHA* (phytohemaglutinin).

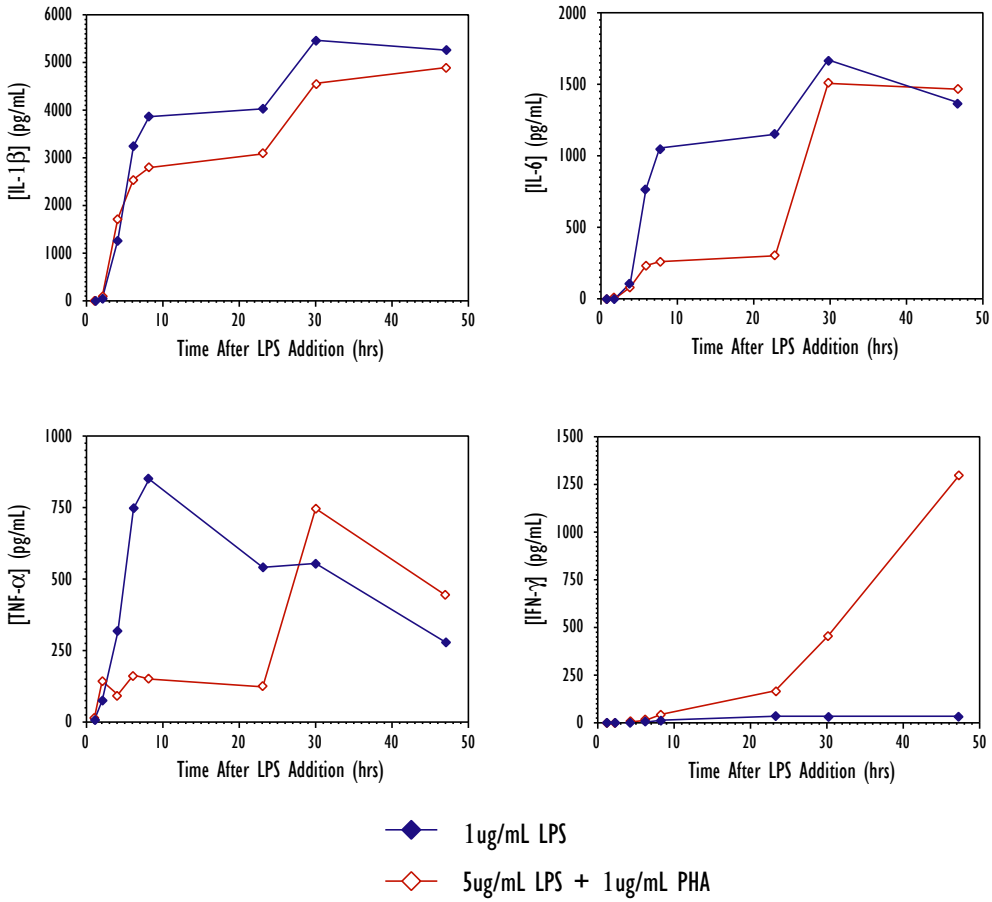
3) Incubate cells at 37° C in CO_2 incubator.

4) Remove 20 uL sample, transfer to MSD Multi-Spot™ Plate and assay for cytokine levels in washed format.

* LPS mimics bacterial response

* PHA mimics viral response

Electrochemiluminescence from a section of a Multi-Spot Plate, measured using a Sector HTS Reader



Multiple cytokines measured in a complex matrix

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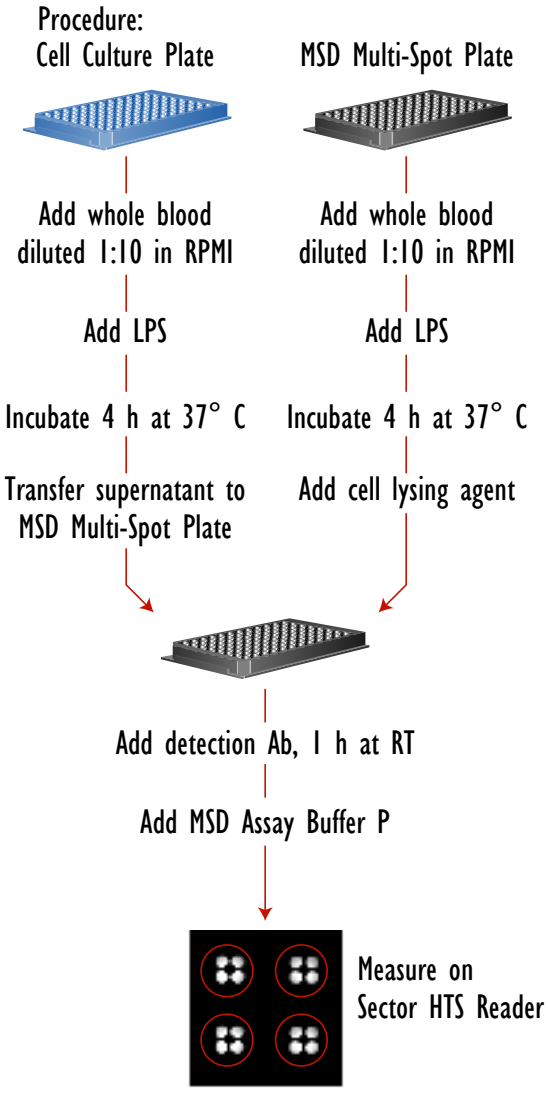
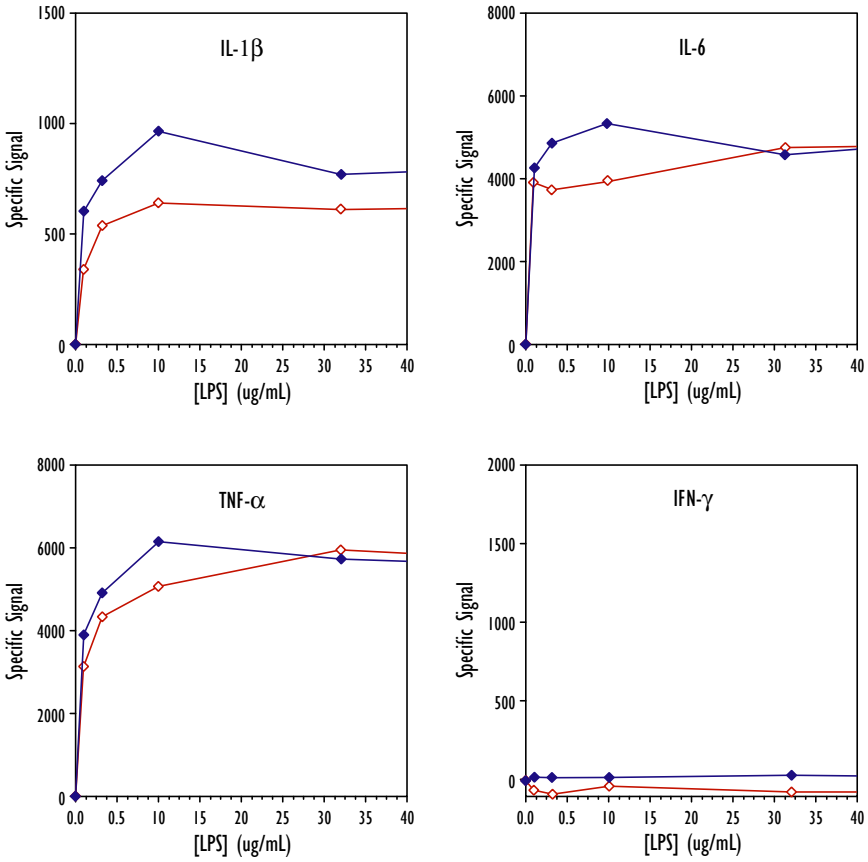
Cell Activation and Cytokine Measurement in Multi-Spot Plates





Multiplex cytokine assay with whole cells in a single well

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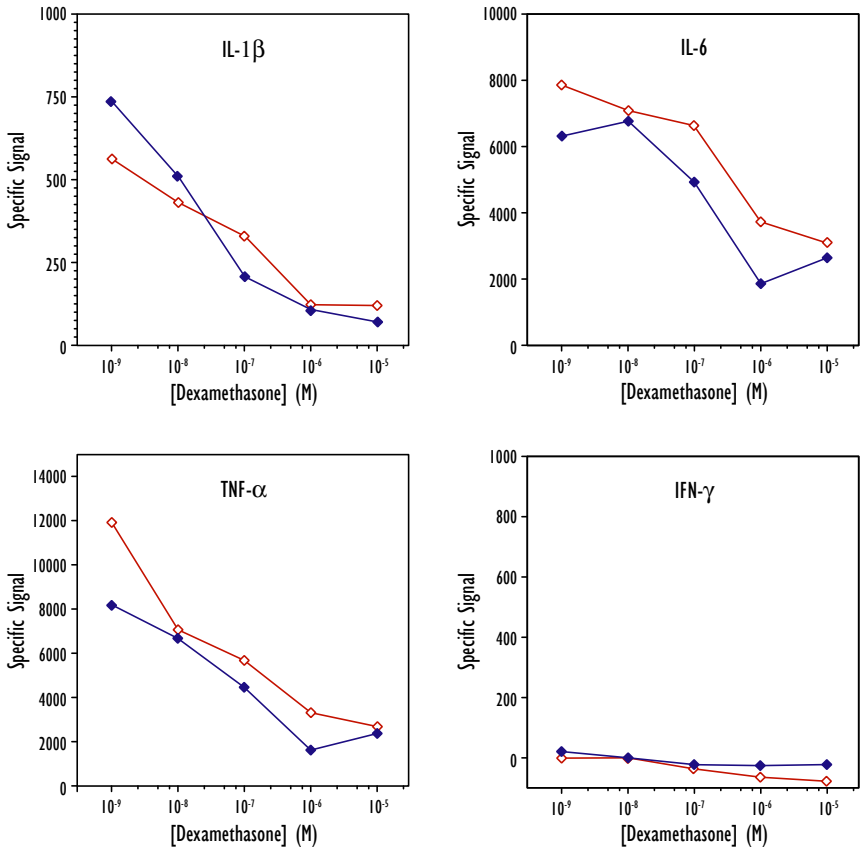
Inhibition of LPS Response

Blood cells (1:10 dilution in RPMI) were treated with 1 uM LPS + varying concentrations of dexamethasone (an inhibitor of LPS response).

Specific signal is corrected for signal observed in the absence of LPS.

No wash assay in lysed blood.

Cells activated in MSD Multi-Spot plates correlate to cells activated in standard (polystyrene) plates.



Cell-based assays in Multi-Spot Plates with no wash steps

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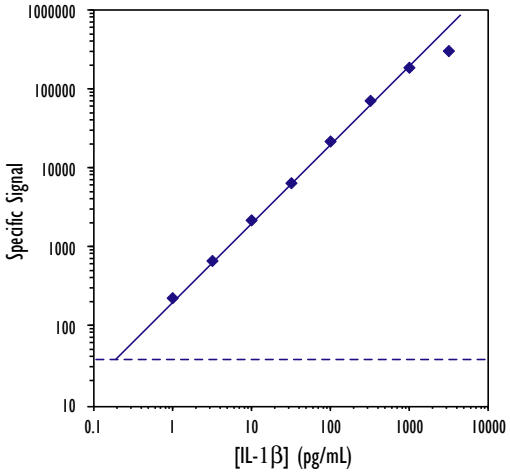
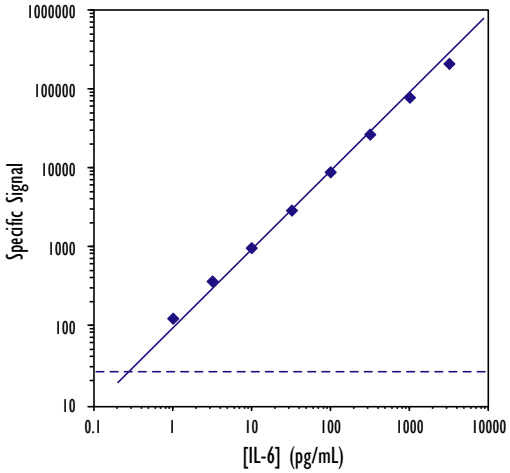
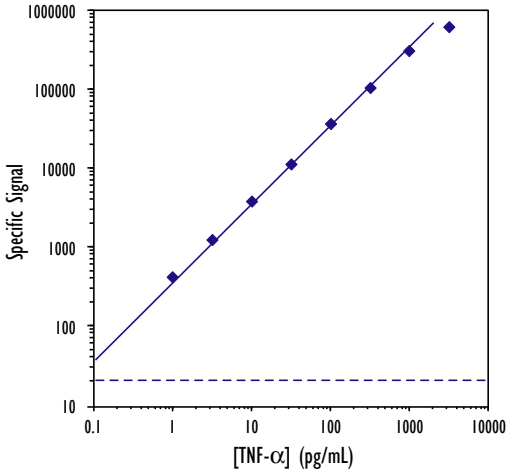
Cytokine Panel Performance: One Wash Format

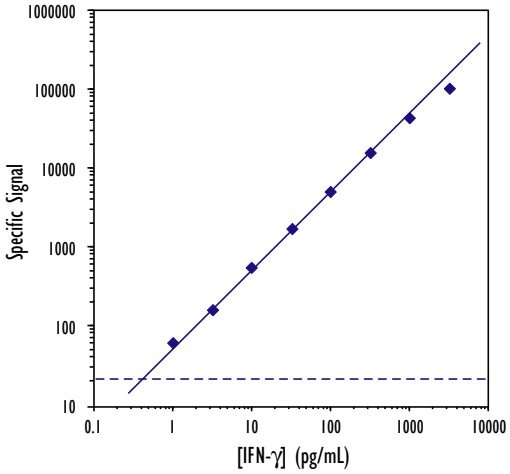




Calibrators are in RPMI media containing 10 % human serum

One Wash
25% above background signal





- Detection Limits: 0.05-0.5 pg/mL (One Wash)
- Linear Range Extends to >3000 pg/mL
- Method is scalable to larger arrays

Cytokines measured in washed formats offer superior sensitivity

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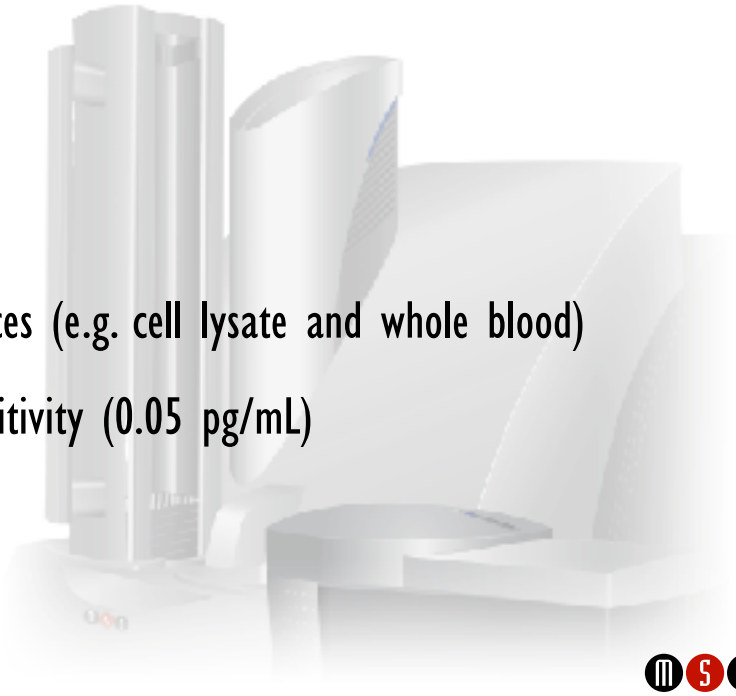
Conclusion

Features of MSD Multi-Array Technology for Cytokine Assays:

- In-well multiplex assays for cytokines
- No-wash formats with excellent sensitivity
- Wide dynamic range (4-5 logs)
- Whole-cell formats
- HTS compatible
  - Simple formats
  - One plate per minute read times
- Tolerant of very complex sample matrices (e.g. cell lysate and whole blood)
- Single wash formats offer ultimate sensitivity (0.05 pg/mL)



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