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™ technology combines array technologies and electrochemiluminescence detection to achieve ultra-fast, highly sensitive assays in a convenient format. 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 is shown for i) an IL1β assay carried out on an avidin-coated plate and ii) a four cytokine panel (IL1β, IL6, TNF-α and IFN-γ) carried out using wells containing a patterned array of antibodies. Data is shown for both washed and unwashed formats. The assay format is useful for measuring cytokines in cell culture media and serum. The results demonstrate the Multi-Array platform provides sensitive, robust assays in a simple format amenable to high-throughput screening.

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Electrochemiluminescence-Based Immunoassays for Cytokines

Multi-Array™ Technology

Unified technology platform with instruments, plates and reagents for drug discovery.

Combines the power of microarrays with the sensitivity of electrochemiluminescence.

96-, 384- and 1536 microplate formats.

Multi-Spot™ plates with high density arrays for multiplexing.

Sector HTS™ Instrument: High resolution imaging detection and robotic integration for HTS and large-scale proteomics.

Sector PR™ Instrument: Medium throughput benchtop reader for assay development, cellular and molecular biology, research in therapeutic areas, secondary screening, QC. Assays developed on Sector PR port to Sector HTS.

Sector HTS

Sector PR

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IL1β IMMUNOASSAY

Materials

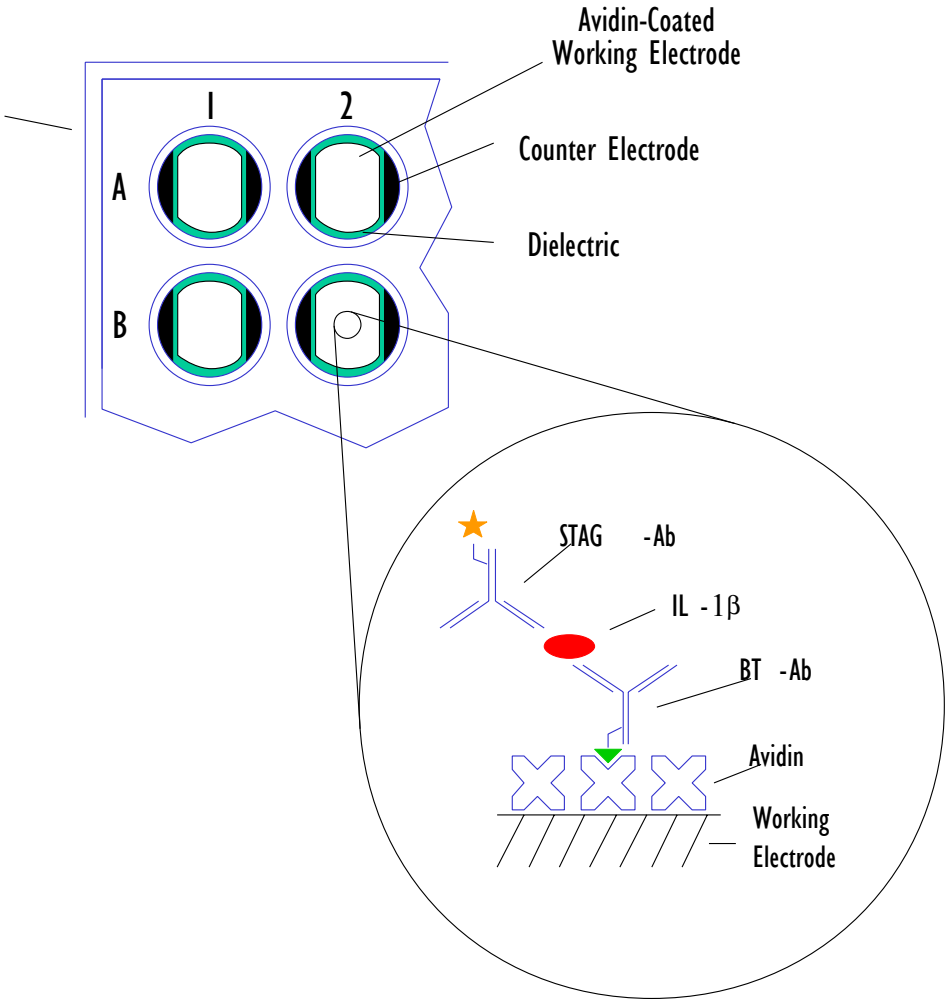
96 Well Plate: Avidin-Coated Multi-Array Plate (Meso Scale Discovery)
Assay Diluent: Buffered diluent containing blocking agents
BT-Ab: Biotin-labeled capture antibody in Assay Diluent
STAG-Ab: Detection antibody labeled with a sulfonated derivative of Ruthenium(II) tris-bipyridine (STAG) in Assay Diluent
Read Buffer: Buffer optimized for electrochemiluminescence measurement

Procedure (One Wash Assay)

To well of Plate:
1) Add 20uL BT-Ab, shake 1 hr. at RT
2) Add 20uL STAG-Ab+ 20 uL Sample (in cell culture media), shake 1 hr. at RT
3) Wash 3x with PBS
4) Add 100 uL Read Buffer
5) Analyze plate using Sector HTS Instrument

Procedure (No Wash Assay)

Same as above but omit step (3)



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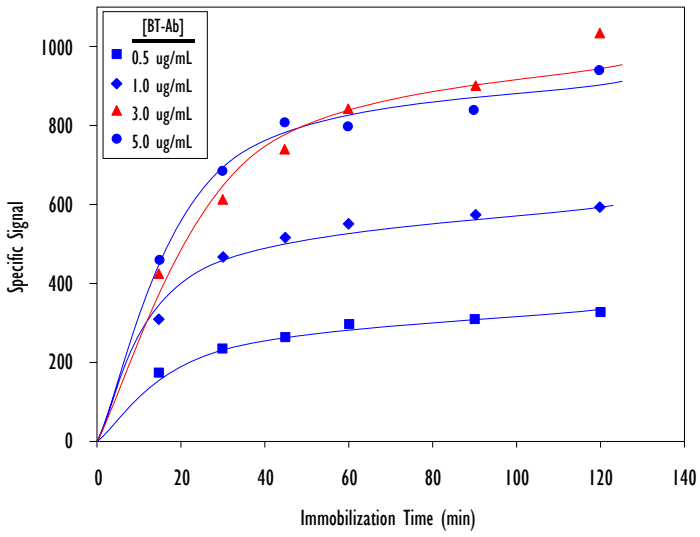
IL-1β ASSAY OPTIMIZATION

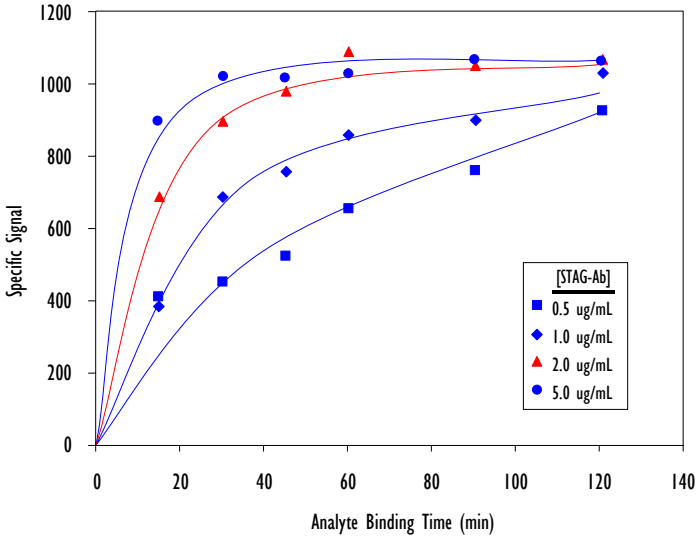
Optimization of Capture Antibody Immobilization (Step 1)

[STAG-Ab] = 2ug/mL, [IL1β] = 50 pg/mL
Analyte Binding Time = 1 hr



Optimization of Analyte Binding Step (Step 2)

[BT-Ab] = 3ug/mL, [IL1β] = 50 pg/mL
BT-Ab Immobilization Time = 1 hr



Binding Reactions Approach Completion in Less Than 1 Hour

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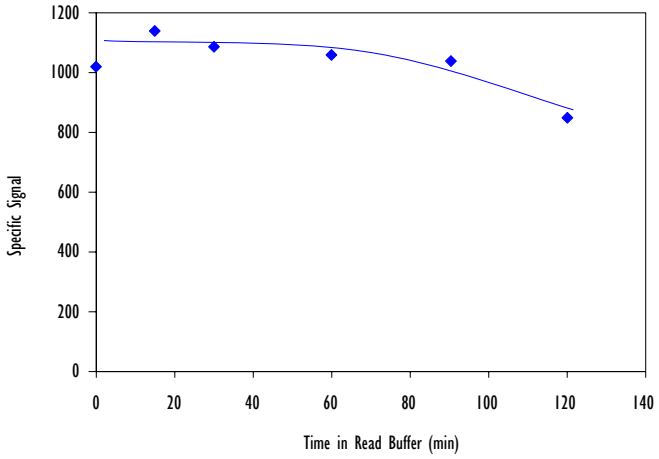
IL-1β ASSAY ROBUSTNESS

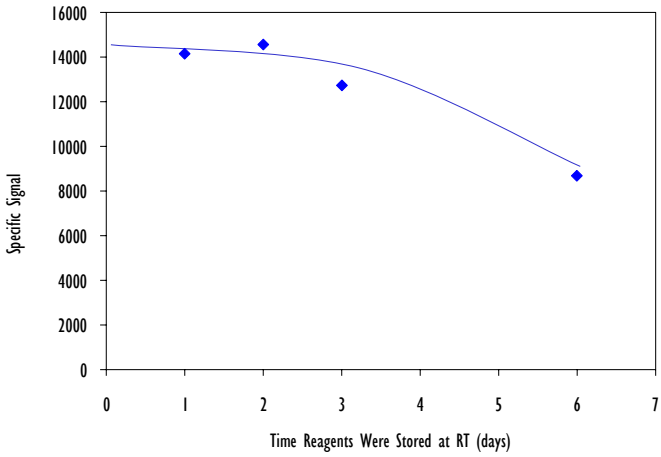
Stability of Sandwich Complex in Read Buffer

[BT-Ab] = 3 ug/mL, [STAG-Ab] = 2ug/mL
[Analyte] = 50 pg/mL
Incubation Times = 1 hr



Stability of Reagents at Room Temperature

[BT-Ab] = 3 ug/mL, [STAG-Ab] = 2ug/mL
[Analyte] = 1000 pg/mL
Incubation Times = 1 hr
Working Antibody Solutions and Calibrators Stored at RT for Specified Time



Stable Reagents, Stable End Point

Amenable to Automation

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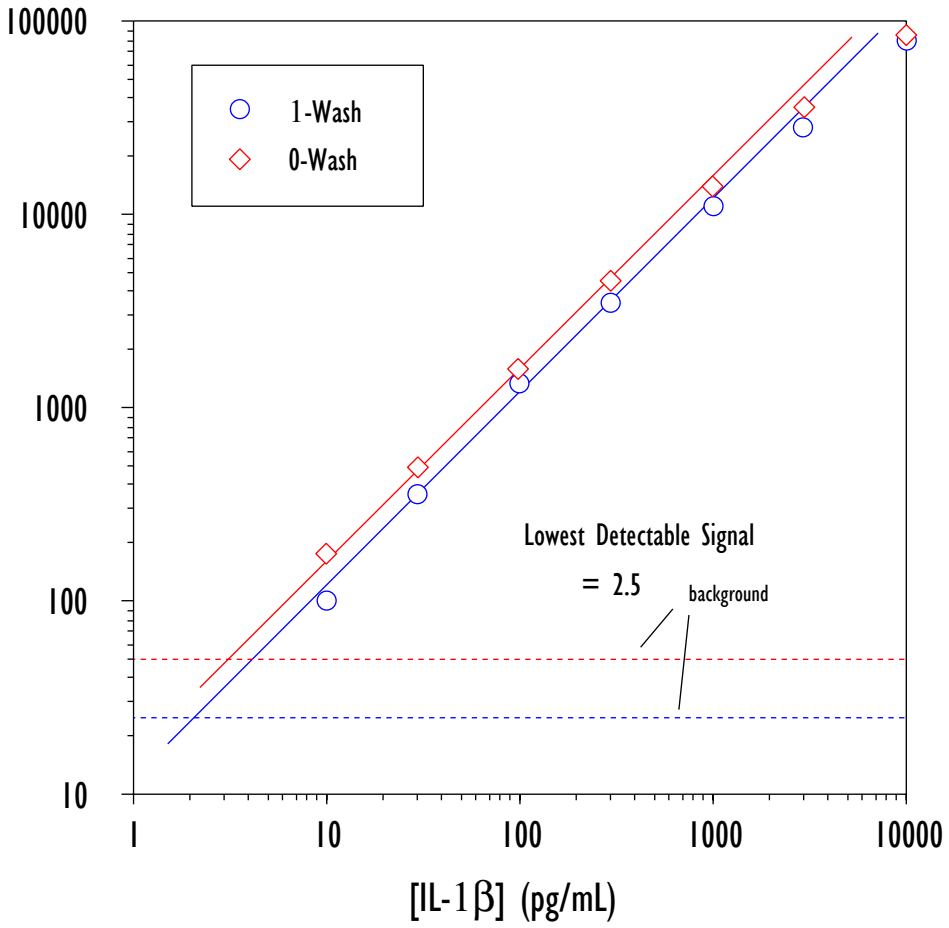
IL-1β ASSAY PERFORMANCE



IL-1β calibrators prepared in cell culture media: RPMI + 10% fetal calf serum.

- Assay tolerant of biotin in sample
- Assay tolerant of serum in sample
- Small sample size (20 uL)
- Detection Limits:
  2 pg/mL (1-Wash)
  5 pg/mL (0-Wash)

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INFLAMMATORY CYTOKINE PANEL

Materials

96 Well Plate: Multi-Spot™ Plate (MSD) having patterned array of anti-cytokine capture antibodies

Assay Diluent: Buffered diluent containing blocking agents

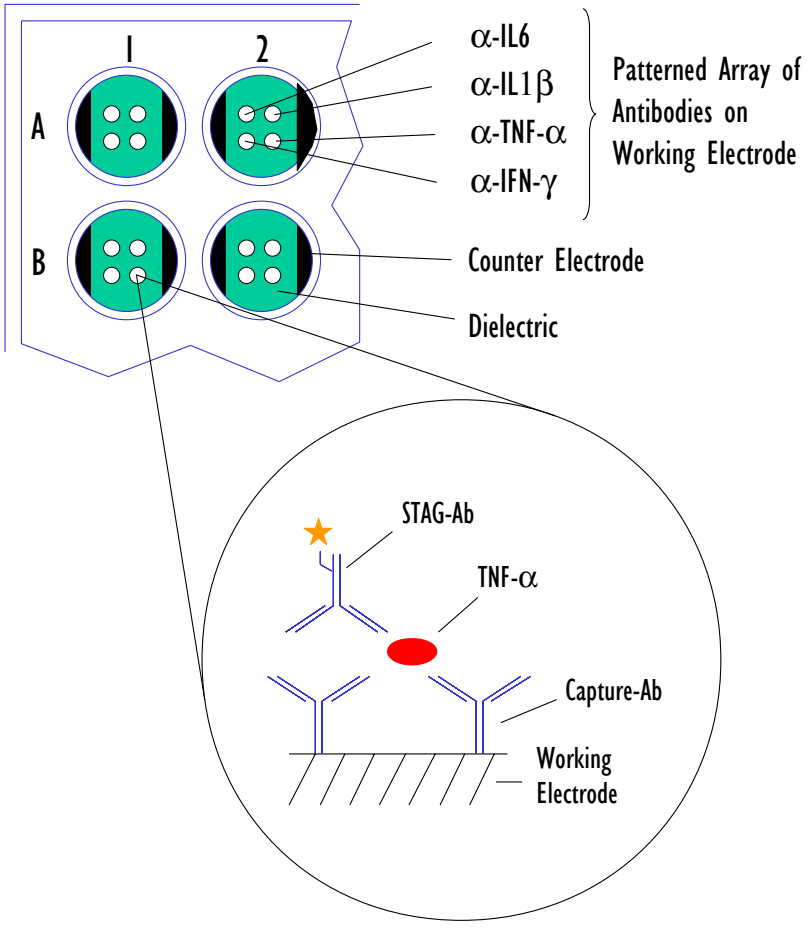
STAG-Ab: Mixture of four Ru-labeled detection antibodies labeled with a sulfonated derivative of Ruthenium(II) tris-bipyridine (STAG) in Assay Diluent

Read Buffer: Buffer optimized for electrochemiluminescence measurement

Procedure

To well of Plate:

1)Add 20 uL sample, shake 1 hr. at RT
2)Add 20 uL STAG-Ab mixture, shake 1 hr. at RT
3)Wash 3x with PBS
4) Add 100 uL Read Buffer
5) Analyze plate using Sector HTS Instrument



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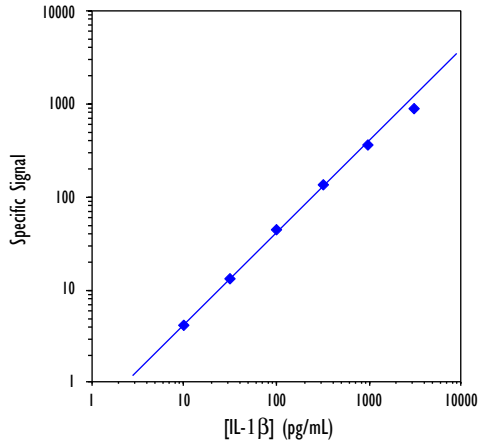
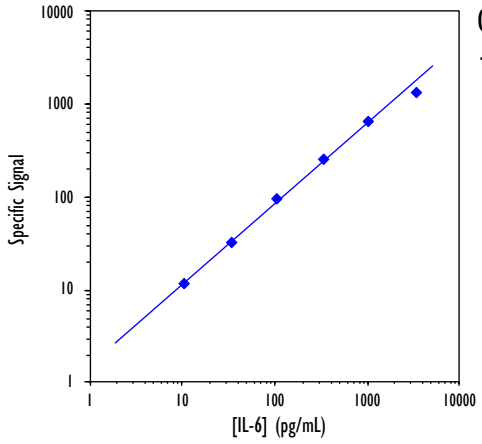
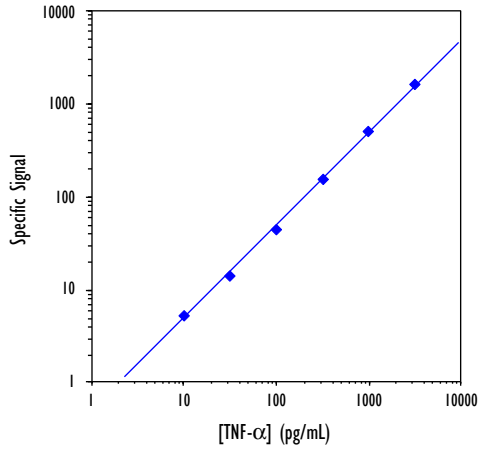
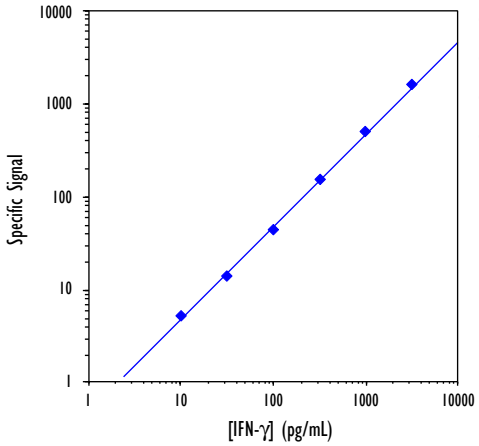
CYTOKINE PANEL PERFORMANCE





Calibrators prepared in RPMI media + 10 % human serum





- Detection Limits ~ 5 pg/mL
- Linear Range Extends to > 1000 pg/mL
- Development of 0-Wash Assay in Progress
- Method is Scalable to Larger Arrays

Multiple cytokines are measured in a 20 uL sample in one well

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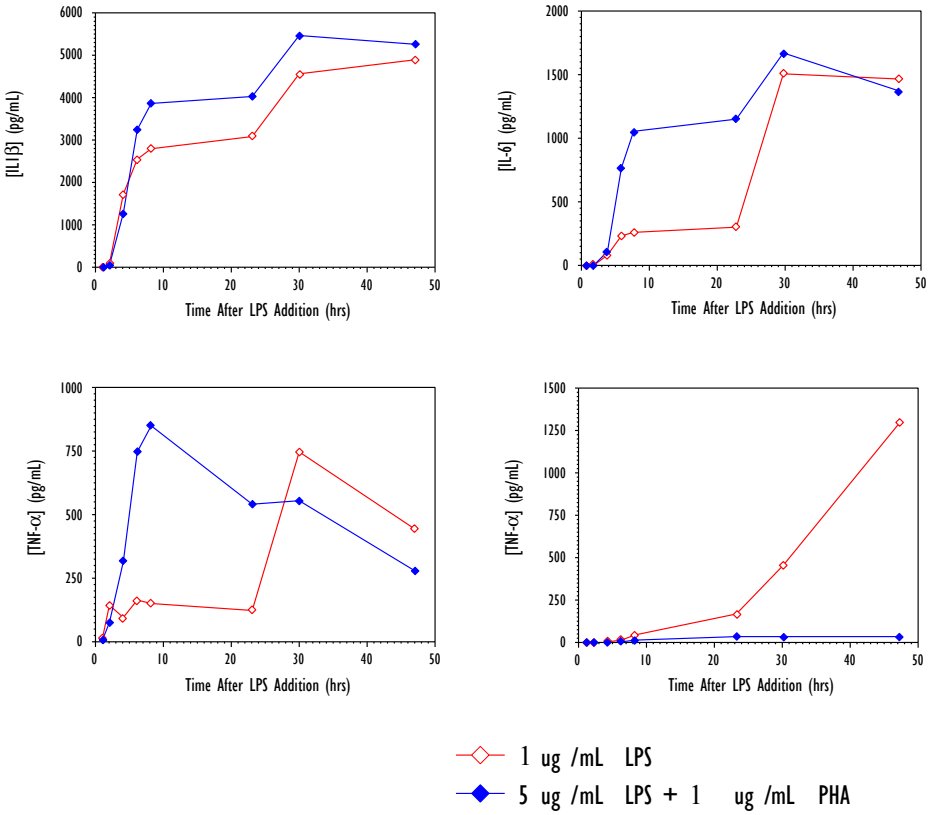
MEASUREMENT OF CYTOKINE PRODUCTION IN WHOLE BLOOD

Procedure:

1) Whole blood diluted 1:10 in RPMI-1640 in multi-well plate.
2) Added LPS (lipopolysaccharide) or LPS + PHA (phytohemaglutinin).
3) Incubate cells at 37° C in CO_2 incubator.
4) Remove 20 uL sample and assay for cytokine levels.

Electrochemi-luminescence from a Multi-Spot Plate sector measured using a Sector HTS Instrument



Multiple cytokines are measured in a complex matrix

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Conclusion

MSD Multi-Array Technology for Cytokine Assays:

- Simple, Robust, Format Amenable to High-throughput Screening
- Fast
- Sensitive
- Wide Dynamic Range
- Tolerant of Complex Sample Matrices
- Amenable to No-wash Formats
- Single- and Multi-Analyte Analysis

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