# Human Cytokine Assay Products from Meso Scale Discovery

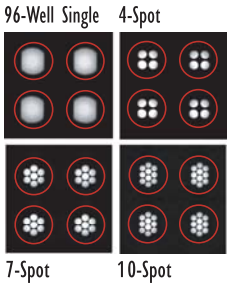
Paul F. Grulich, David H. Stewart, Pankaj Oberoi, Rob Calamunci, George Sigal and Jacob N. Wohlstadter

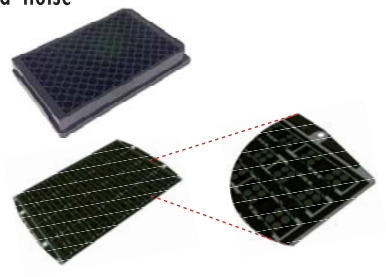
In this poster, we present a collection of assays and applications that demonstrate the power of MSD® technology as a foundation for high performance cytokine assays. Examples of cytokine assays in both singleplex and multiplex formats show that multiple cytokines can be simultaneously measured without compromising assay performance. Two kit types are available depending on the requirements of the particular application. Tissue Culture Kits are recommended for cell culture applications and Ultra-Sensitive Kits are recommended for complex matrices (serum/plasma) and achieving greater sensitivity in cell culture applications. In multiplexed cytokine assays, the preferred combination of cytokines depends on the particular application and system being studied. Several different cytokine arrays are depicted to give an indication of the breadth and flexibility of combinations available. For each of these panels, sample data is given including spot layout, standard curves, detection limits, and spike recoveries in human samples. To demonstrate the robustness of MSD cytokine panels for rigorous applications, data from a validation study is presented including assay performance and repeatability across multiple days and users. The validation data reflects the low variability and excellent recovery of spiked calibrators.

# MSD Technology

## Patterned Surface Arrays

- Patterned electrodes
  - 24-well, 96-well, 384-well
- Multiplexing
- High-throughput
- Sample volumes 10-25 µL for 96-well and 384-well plates
- Reduced noise



## Multiple Instruments

- Two imaging instruments with greater multiplexing capability
- Two Personal Readers for assay development
- Very fast read time

SECTOR™ PR 400 Reader

SECTOR Imager 6000

# Protocols

MSD Cytokine protocols are designed to optimize workflow and ease-of-use while maximizing assay performance in terms of sensitivity, dynamic range, and recovery. The protocols have been used successfully for many sample matrices including cell culture supernatants, serum, plasma, sputum, bronchoalveolar lavage, and other bodily fluids. Two standard protocols are given below with the serum / plasma protocol recommended as a standard protocol for other biological samples. With appropriate validation, these protocols can be modified to improve workflow or performance by eliminating or changing the number of washes, adding blocking steps, or changing the volumes of assay constituents. Samples in complex matrices typically do not require dilution prior to use in MSD cytokine assays although dilution may be required to achieve desired recoveries in some cases.

## Cell Supernatant Protocol:

1. Add 25 µL of Sample / Calibrators; Incubate 1-2 hr at RT
2. Add 25 µL of Detection Antibodies; Incubate 2 hr at RT
3. Wash 3X with PBS
4. Add 150 µL/well Read Buffer and Read

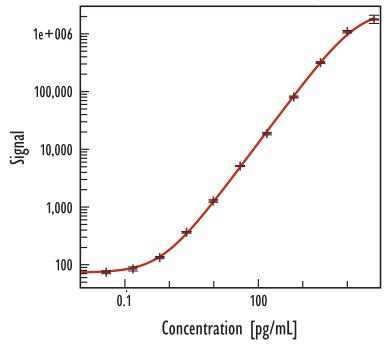
## Human Serum / Plasma Protocol:

1. Add 25 µL of MSD Assay Diluent; Incubate 30 min at RT
2. Add 25 µL of Sample / Calibrators; Incubate 2 hr at RT
3. Wash 3X with PBST
4. Add 25 µL of Detection Antibodies; Incubate 1-2 hr at RT
5. Add 150 µL/well Read Buffer and Read

# Human IL-8 Cytokine Assay (MULTI-ARRAY™ 96-Well Small Spot Plate)

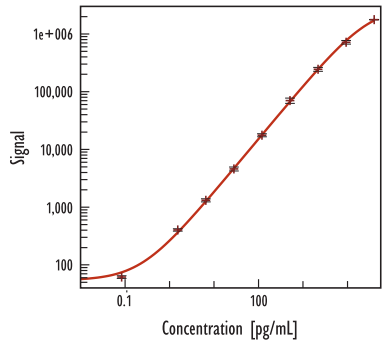
Below are examples of cytokine assay titration data using two different protocols. The data was collected using the Human IL-8 Tissue Culture Kit with the MSD Human Cytokine Reagent Kit.

## Cell Supernatant Assay




| Human IL-8 | | |
|---|---|---|
| Concentration (pg/mL) | Signal | |
| | Mean | %CV |
| 0 | 78 | 14.7 |
| 0.010 | 67 | 11.7 |
| 0.038 | 71 | 7.1 |
| 0.15 | 68 | 9.7 |
| 0.61 | 118 | 4.4 |
| 2.4 | 322 | 2.5 |
| 9.8 | 1,083 | 1.1 |
| 39 | 3,869 | 2.9 |
| 156 | 14,962 | 4.2 |
| 625 | 58,406 | 8.8 |
| 2,500 | 236,433 | 2.2 |
| 10,000 | 809,925 | 10.1 |
| 40,000 | 1,394,565 | 16.1 |

## Serum / Plasma Assay




| Human IL-8 | | |
|---|---|---|
| Concentration (pg/mL) | Signal | |
| | Mean | %CV |
| 0 | 53 | 9.4 |
| 0.15 | 47 | 5.3 |
| 2.4 | 322 | 4.9 |
| 9.8 | 1,083 | 6.3 |
| 39 | 3,915 | 8.1 |
| 156 | 15,579 | 6.2 |
| 625 | 64,351 | 10.7 |
| 2,500 | 231,900 | 8.0 |
| 10,000 | 701,686 | 7.4 |
| 40,000 | 1,771,058 | 0.6 |

## Detection Limits

| Sample Type | Detection Limit (pg/mL) |
|---|---|
| Cell Supernatant | 0.3 |
| Serum | 0.3 |
| Plasma | 0.6 |

Detection Limits were determined across multiple runs using 2.5 standard deviations above the background.

## Recoveries

| Sample Type | Average % Recovery |
|---|---|
| Cell Supernatant | 90 |
| Serum | 87 |
| EDTA Plasma | 85 |
| Heparin Plasma | 93 |

Plasma data was collected using a modified plasma protocol with a reduced sample volume of 10 µL.

Spike recoveries were determined in each matrix over a range of spike levels from 9.8 to 313 pg/mL. Each spike was tested in triplicate.

## Endogenous Levels

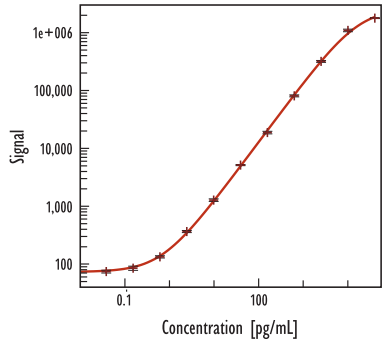
| Sample Type (# of unique samples) | Samples Below Detectable Range | Range |
|---|---|---|
| Serum (n=9) | 0 | 4.2 - 42.6 |
| EDTA Plasma (n=5) | 0 | 0.9 - 2.1 |
| Heparin Plasma (n=5) | 0 | 1.8 - 5.2 |

Endogenous cytokine levels (pg/mL) were determined for different sample types across multiple samples (n/d indicates cytokine level below the detection limit).

# Human TNF-α Ultra-Sensitive Cytokine Assay (MULTI-ARRAY 96-Well Small Spot Plate)

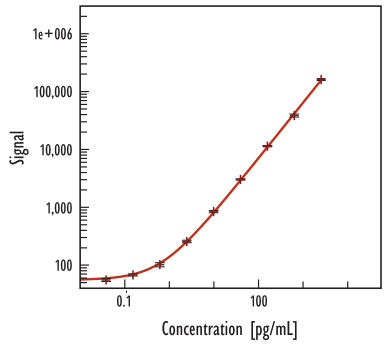
Below are examples of cytokine assay titration data using two different protocols. The data was collected using the Human TNF-α Ultra-Sensitive Kit.

## Cell Supernatant Assay




| Human TNF-α US | | |
|---|---|---|
| Concentration (pg/mL) | Signal | |
| | Mean | %CV |
| 0 | 81 | 7.4 |
| 0.010 | 74 | 4.5 |
| 0.038 | 74 | 5.5 |
| 0.15 | 85 | 8.2 |
| 0.61 | 134 | 4.4 |
| 2.4 | 365 | 4.2 |
| 9.8 | 1,275 | 5.1 |
| 39 | 5,130 | 2.1 |
| 156 | 18,732 | 5.0 |
| 625 | 80,597 | 3.9 |
| 2,500 | 317,523 | 3.7 |
| 10,000 | 1,089,784 | 5.1 |
| 40,000 | 1,776,323 | 1.6 |

## Serum / Plasma Assay




| Human TNF-α US | | |
|---|---|---|
| Concentration (pg/mL) | Signal | |
| | Mean | %CV |
| 0 | 61 | 10.0 |
| 0.15 | 59 | 11.3 |
| 0.61 | 56 | 5.6 |
| 2.4 | 68 | 4.3 |
| 9.8 | 102 | 8.2 |
| 39 | 258 | 5.0 |
| 156 | 847 | 4.7 |
| 625 | 3,036 | 2.1 |
| 2,500 | 11,396 | 2.5 |
| 10,000 | 38,595 | 6.0 |
| 40,000 | 161,589 | 3.5 |

## Detection Limits

| Sample Type | Detection Limit (pg/mL) |
|---|---|
| Cell Supernatant | 0.2 |
| Serum | 0.3 |
| Plasma | 0.7 |

Detection Limits were determined across multiple runs using 2.5 standard deviations above the background.

## Recoveries

| Sample Type | Average % Recovery |
|---|---|
| Cell Supernatant | 100 |
| Serum | 88 |
| EDTA Plasma | 106 |
| Heparin Plasma | 114 |

Plasma data was collected using a modified plasma protocol with a reduced sample volume of 10 µL.

Spike recoveries were determined in each matrix over a range of spike levels from 9.8 to 313 pg/mL. Each spike was tested in triplicate.

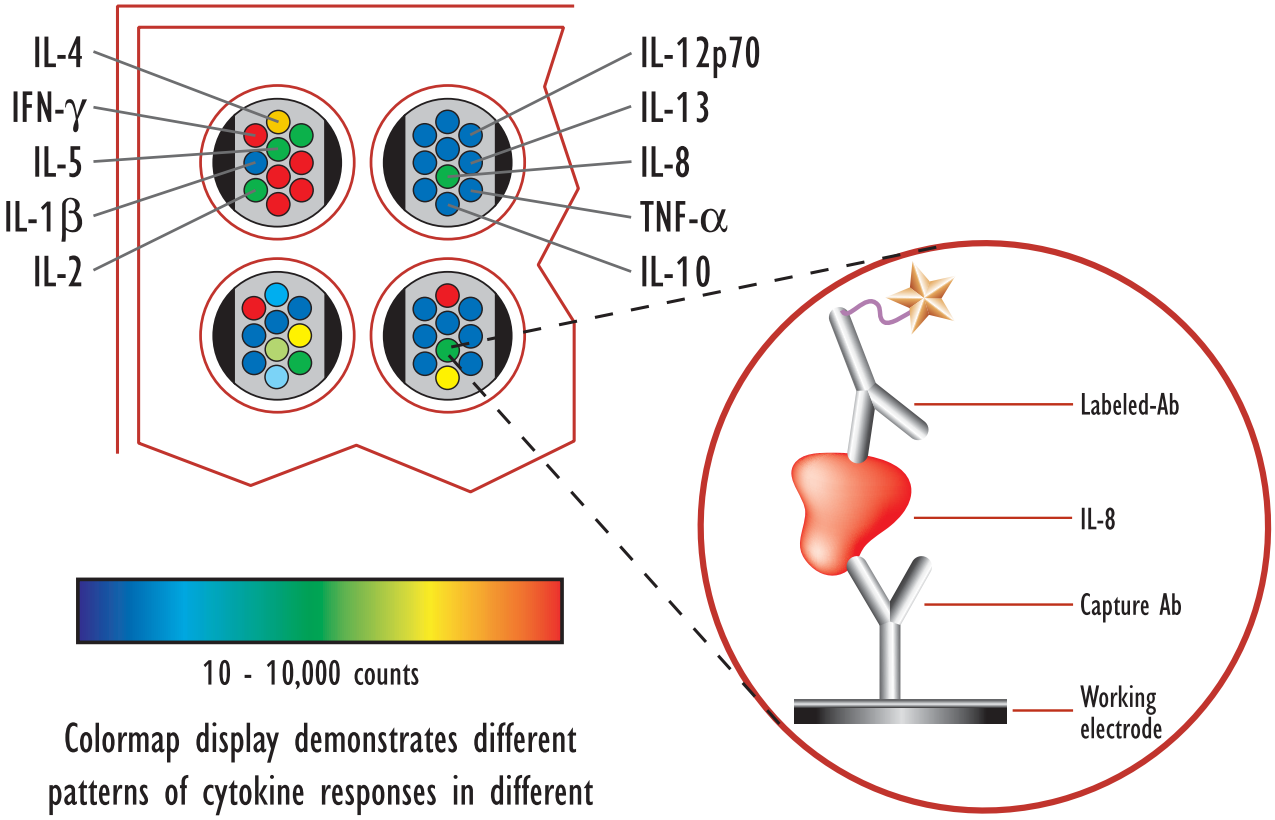
## Endogenous Levels

| Sample Type (# of unique samples) | Samples Below Detectable Range | Range |
|---|---|---|
| Serum (n=10) | 0 | 1.9 - 5.3 |
| EDTA Plasma (n=5) | 1 | n/d - 2.2 |
| Heparin Plasma (n=5) | 0 | 1.6 - 3.8 |

Endogenous cytokine levels (pg/mL) were determined for different sample types across multiple samples (n/d indicates cytokine level below the detection limit).

# Multiplexed MSD Cytokine Assays

## MSD Human TH1/TH2 Cytokine Assay

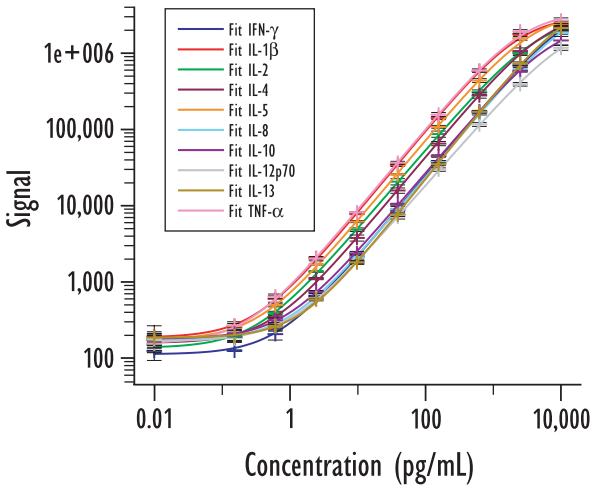



Colormap display demonstrates different patterns of cytokine responses in different wells of a 96-well 10-spot assay plate.

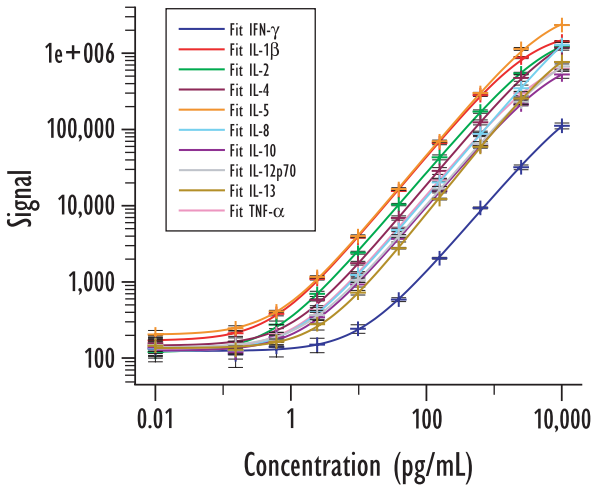
Each well in an MSD MULTI-SPOT® plate contains multiple spots, each with a capture antibody for a particular biological assay. The assays are independent of one another and each is optimized for maximum performance in detecting its particular analyte.

# Human TH1/TH2 Panel - Cell Culture Applications

## Ultra-Sensitive Kit




## Tissue Culture Kit




## Detection Parameters (pg/mL)

| | | IFN-γ | IL-1β | IL-2 | IL-4 | IL-5 | IL-8 | IL-10 | IL-12p70 | IL-13 | TNF-α |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ultrasensitive Assay | LLOD | 0.5 | 0.3 | 0.2 | 0.3 | 0.2 | 0.4 | 0.5 | 0.5 | 0.6 | 0.1 |
| | LLOQ | 0.9 | 0.3 | 0.2 | 0.6 | 0.1 | 1.2 | 0.5 | 0.7 | 0.3 | 0.1 |
| High Bind Assay | LLOD | 6.6 | 0.2 | 0.4 | 0.6 | 0.2 | 0.7 | 1.2 | 1.0 | 1.5 | 0.9 |
| | LLOQ | 12.4 | 0.3 | 0.6 | 1.1 | 0.3 | 1.4 | 2.3 | 2.4 | 2.3 | 0.7 |

MSD has developed two cytokine kits that can be used for cell culture applications. The detection limits achieved with the Ultra-Sensitive Kits are as much as 2-10 times lower than the standard Tissue Culture Kit and can be used to detect cytokines that are present at very low levels (less than 1 pg/mL). Lower limit of detection (LLOD) is calculated using 2.5 standard deviations above the background. Lower limit of quantification (LLOQ) is defined as the lowest concentration at which the CV of the concentration is less than 20% and the recovery of spiked calibrator is within 20% of the expected value.

# Multi-Day Validation Study

## Detection Limits in pg/mL from Multiple Plates

| | Plate Number | IFN-γ | IL-10 | IL-12p70 | IL-13 | IL-1β | IL-2 | IL-4 | IL-5 | IL-8 | TNF-α |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DAY 1 | 1 | 9.5 | 5.0 | 1.5 | 1.7 | 0.4 | 1.4 | 2.5 | 0.2 | 3.4 | 0.9 |
| | 2 | 8.3 | 9.7 | 1.8 | 1.8 | 0.4 | 1.5 | 2.3 | 0.2 | 2.6 | 0.7 |
| DAY 2 | 3 | 6.0 | 3.4 | 1.2 | 1.2 | 0.2 | 1.2 | 1.9 | 0.2 | 2.5 | 0.7 |
| | 4 | 6.0 | 2.8 | 2.1 | 1.3 | 0.2 | 1.1 | 1.6 | 0.2 | 2.3 | 0.7 |
| DAY 3 | 5 | 6.0 | 3.4 | 1.3 | 1.2 | 0.3 | 0.9 | 1.4 | 0.2 | 2.3 | 0.8 |
| | 6 | 6.5 | 3.3 | 1.3 | 1.5 | 0.3 | 1.0 | 1.9 | 0.2 | 2.1 | 0.8 |
| DAY 4 | 7 | 2.5 | 2.1 | 1.2 | 1.3 | 0.3 | 1.3 | 1.2 | 0.2 | 3.4 | 1.1 |
| | 8 | 2.4 | 2.2 | 1.3 | 1.2 | 0.2 | 0.7 | 2.3 | 0.2 | 3.2 | 1.4 |
| | 9 | 3.1 | 2.1 | 1.3 | 1.4 | 0.3 | 0.6 | 2.0 | 0.2 | 3.1 | 1.2 |

The Human TH1/TH2 10-plex Tissue Culture Kit was tested in a multi-day, multi-user study to assess the repeatability of assay performance in cell culture medium. Detection limits were found to be stable across 9 plates which were run on 4 different days by 2 different users. Detection limits were calculated using 2.5 standard deviations above the background.

### Criteria for Passing:

| | |
|---|---|
| 1) CVs for the standards must be less than 15% at concentrations above the detection limit | PASSED |
| 2) Intraplate CVs for the spike controls must be less than 20% | PASSED |
| 3) Interplate CVs for the spike controls must be less than 20% | PASSED |
| 4) Detection limits must be below 10 pg/mL for all cytokines | PASSED |
| 5) Spike Recoveries for the controls must be within 25% of the spiked values | PASSED |

# CV Data

| | Concentration (pg/mL) | DAY 1 | | | | | | | | | | DAY 2 | | | | | | | | | | DAY 3 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | IFN-γ | IL-1β | IL-2 | IL-4 | IL-5 | IL-8 | IL-10 | IL-12p70 | IL-13 | TNF-α | IFN-γ | IL-1β | IL-2 | IL-4 | IL-5 | IL-8 | IL-10 | IL-12p70 | IL-13 | TNF-α | IFN-γ | IL-1β | IL-2 | IL-4 | IL-5 | IL-8 | IL-10 | IL-12p70 | IL-13 | TNF-α |
| Plate 1 | 0 | 14.2 | 10.7 | 10.6 | 15.7 | 7.4 | 10.4 | 12.8 | 9.6 | 8.0 | 7.6 | 10.6 | 9.4 | 12.1 | 14.8 | 5.9 | 8.6 | 13.0 | 10.5 | 9.5 | 9.6 | 12.1 | 8.8 | 12.2 | 14.3 | 5.9 | 9.2 | 14.8 | 14.5 | 10.4 | 11.4 |
| | 2 | 9.7 | 3.0 | 6.8 | 10.6 | 1.1 | 6.7 | 2.2 | 8.2 | 4.1 | 3.9 | 13.1 | 4.1 | 8.7 | 12.7 | 2.2 | 3.5 | 11.2 | 6.3 | 2.9 | 3.9 | 8.2 | 6.2 | 7.5 | 9.0 | 1.6 | 6.2 | 9.6 | 11.7 | 5.4 | 9.9 |
| | 10 | 16.3 | 4.1 | 7.6 | 6.9 | 3.8 | 5.3 | 5.9 | 3.7 | 6.9 | 4.5 | 8.1 | 3.9 | 5.3 | 5.0 | 2.4 | 5.8 | 4.6 | 3.8 | 2.1 | 4.4 | 6.1 | 2.1 | 5.1 | 2.0 | 1.7 | 3.4 | 4.2 | 2.9 | 2.3 | 5.2 |
| | 39 | 6.0 | 4.5 | 4.6 | 1.3 | 4.3 | 3.3 | 4.5 | 1.0 | 0.8 | 7.2 | 4.2 | 5.4 | 4.0 | 2.7 | 2.6 | 2.1 | 4.1 | 2.3 | 2.0 | 1.2 | 3.8 | 2.1 | 4.1 | 2.8 | 1.8 | 4.1 | 1.3 | 1.8 | 2.6 | 6.4 |
| | 156 | 4.9 | 3.8 | 5.6 | 1.9 | 3.2 | 1.7 | 6.3 | 1.5 | 2.4 | 7.5 | 4.1 | 3.9 | 3.6 | 2.0 | 2.4 | 3.2 | 6.0 | 3.8 | 4.4 | 5.9 | 7.0 | 6.9 | 7.8 | 7.5 | 5.8 | 6.3 | 6.1 | 9.1 | 10.0 | 14.0 |
| | 625 | 4.7 | 3.1 | 2.7 | 3.1 | 1.8 | 0.6 | 8.2 | 0.9 | 3.0 | 3.8 | 3.5 | 3.6 | 4.2 | 2.9 | 2.5 | 3.6 | 2.7 | 1.3 | 2.3 | 2.1 | 3.6 | 0.9 | 1.8 | 5.1 | 2.0 | 4.1 | 2.8 | 4.4 | 5.0 | 10.4 |
| | 2,500 | 5.6 | 5.1 | 4.6 | 4.5 | 2.7 | 4.4 | 6.1 | 3.5 | 3.5 | 2.9 | 4.3 | 5.2 | 4.8 | 3.8 | 3.3 | 4.8 | 2.2 | 4.2 | 2.8 | 3.0 | 3.4 | 3.1 | 4.0 | 4.1 | 2.3 | 7.5 | 5.0 | 5.1 | 5.7 | 11.2 |
| | 10,000 | 6.7 | 4.4 | 3.0 | 2.9 | 0.6 | 2.7 | 1.6 | 3.5 | 2.0 | 2.5 | 5.2 | 5.8 | 5.6 | 4.0 | 0.8 | 3.8 | 2.0 | 1.5 | 1.2 | 3.9 | 3.8 | 2.8 | 2.0 | 3.1 | 1.2 | 5.8 | 5.2 | 4.0 | 2.8 | 7.8 |
| Plate 2 | 0 | 14.5 | 10.6 | 10.3 | 19.1 | 7.2 | 8.6 | 25.1 | 16.9 | 7.3 | 9.4 | 13.0 | 6.8 | 13.5 | 15.7 | 6.7 | 10.1 | 13.6 | 18.7 | 8.5 | 11.0 | 13.2 | 9.2 | 12.2 | 18.4 | 6.0 | 9.3 | 13.5 | 9.9 | 12.0 | 11.9 |
| | 2 | 11.6 | 3.6 | 6.1 | 9.6 | 1.4 | 4.0 | 10.0 | 4.2 | 4.8 | 5.3 | 8.9 | 2.2 | 5.8 | 9.4 | 2.3 | 5.9 | 15.0 | 7.8 | 5.6 | 4.3 | 5.2 | 3.1 | 4.9 | 11.2 | 3.6 | 8.0 | 7.1 | 9.6 | 3.0 | 3.6 |
| | 10 | 10.3 | 1.5 | 1.7 | 3.0 | 0.8 | 4.8 | 4.9 | 4.7 | 4.9 | 4.5 | 10.0 | 1.7 | 6.4 | 4.1 | 1.4 | 5.2 | 6.9 | 5.4 | 3.8 | 2.2 | 12.9 | 2.6 | 6.4 | 3.1 | 4.7 | 3.7 | 3.7 | 5.8 | 3.6 | 4.8 |
| | 39 | 2.5 | 4.3 | 3.9 | 1.7 | 2.0 | 3.7 | 5.1 | 2.9 | 4.4 | 5.6 | 4.2 | 4.8 | 3.1 | 2.6 | 1.3 | 1.9 | 4.4 | 3.3 | 3.4 | 4.8 | 5.4 | 6.2 | 7.8 | 2.7 | 4.7 | 4.9 | 3.9 | 2.8 | 2.2 | 2.8 |
| | 156 | 1.4 | 2.9 | 4.3 | 2.5 | 3.1 | 3.8 | 5.4 | 4.8 | 6.3 | 6.5 | 1.8 | 3.3 | 3.3 | 4.4 | 3.7 | 5.9 | 5.8 | 5.5 | 6.0 | 6.3 | 3.8 | 2.9 | 6.4 | 2.1 | 2.8 | 3.2 | 2.7 | 3.7 | 4.6 | 7.1 |
| | 625 | 2.7 | 3.1 | 1.3 | 2.0 | 1.1 | 8.2 | 2.3 | 2.6 | 3.1 | 9.4 | 3.2 | 1.6 | 1.3 | 1.2 | 0.8 | 4.8 | 3.5 | 3.5 | 4.5 | 4.1 | 4.0 | 2.5 | 3.8 | 4.8 | 2.4 | 3.2 | 4.0 | 2.4 | 3.7 | 4.5 |
| | 2,500 | 3.1 | 3.5 | 4.6 | 3.7 | 2.5 | 6.8 | 5.3 | 4.2 | 5.2 | 7.0 | 4.3 | 1.2 | 2.7 | 2.9 | 1.5 | 3.4 | 7.8 | 4.1 | 4.0 | 5.3 | 6.6 | 1.2 | 3.5 | 5.0 | 2.6 | 2.5 | 3.1 | 2.1 | 1.5 | 6.0 |
| | 10,000 | 3.9 | 3.9 | 3.7 | 4.1 | 2.1 | 9.8 | 2.6 | 6.7 | 5.5 | 8.0 | 2.7 | 3.1 | 3.6 | 2.2 | 1.0 | 3.0 | 1.8 | 3.9 | 3.6 | 6.9 | 6.5 | 5.5 | 5.7 | 6.8 | 1.9 | 3.6 | 3.0 | 4.3 | 2.0 | 2.9 |

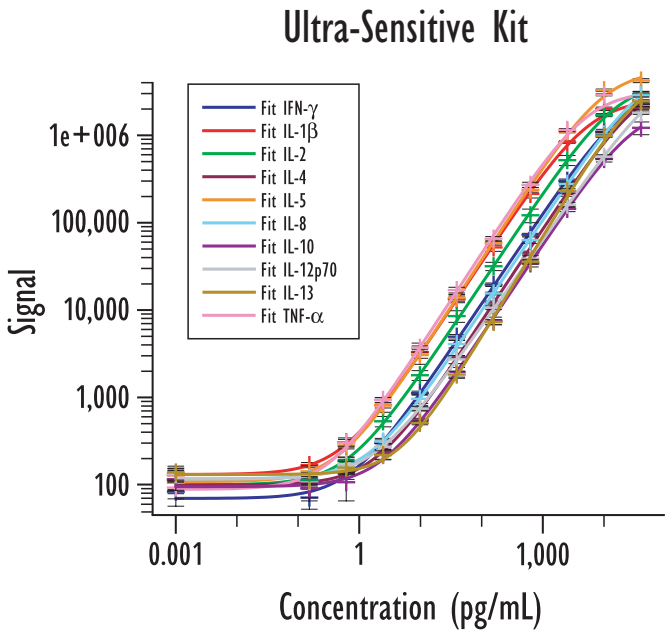
The CVs of the standard curve data (4 replicates for each concentration; 26 zeros) were largely below 5% (color key: 10-15%, 15-20%, >20%). Within the first 6 plates, the CVs of the spiked controls (24 measurements for each control) were less than 10% for all cytokines. The interplate CVs were a little higher than the intraplate CVs. Excellent spike recoveries were observed for all cytokines (within 20% of 100% recovery).

## Spike Recovery Data

| | IFN-γ | | | IL-10 | | | IL-12p70 | | | IL-13 | | | IL-1β | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spike (pg/mL) | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery |
| 16 | 16.3 | 5.5 | 101.7 | 15.7 | 5.8 | 97.9 | 17.8 | 2.0 | 111.5 | 16.6 | 4.4 | 103.6 | 15.6 | 6.0 | 97.8 |
| 63 | 53.3 | 9.7 | 84.6 | 56.8 | 5.5 | 90.2 | 68.1 | 3.3 | 108.2 | 60.7 | 5.1 | 96.4 | 55.5 | 3.6 | 88.0 |
| 250 | 223.6 | 9.2 | 89.4 | 221.8 | 3.8 | 88.7 | 271.4 | 4.8 | 108.5 | 236.0 | 5.9 | 94.4 | 233.9 | 1.4 | 93.6 |

| | IL-2 | | | IL-4 | | | IL-5 | | | IL-8 | | | TNF-α | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spike (pg/mL) | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery | Calc Conc | %CV | %Recovery |
| 16 | 16.5 | 5.5 | 103.2 | 18.6 | 4.2 | 116.0 | 16.0 | 2.5 | 100.3 | 15.6 | 2.2 | 97.3 | 15.5 | 2.3 | 97.0 |
| 63 | 59.3 | 6.0 | 94.2 | 63.5 | 5.0 | 100.8 | 61.1 | 1.2 | 96.9 | 58.4 | 6.3 | 92.7 | 60.6 | 4.6 | 96.2 |
| 250 | 243.5 | 8.3 | 97.4 | 252.6 | 5.5 | 101.0 | 241.6 | 1.7 | 96.6 | 217.8 | 2.8 | 87.1 | 231.6 | 4.6 | 92.7 |

# Human TH1/TH2 Panel - Serum and Plasma Applications



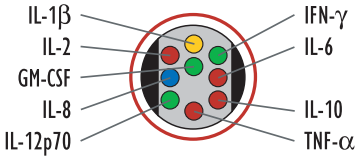

## Performance

| | | IFN-γ | IL-1β | IL-2 | IL-4 | IL-5 | IL-8 | IL-10 | IL-12p70 | IL-13 | TNF-α |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Detection Parameters (pg/mL) | LLOD | 0.3 | 0.5 | 0.4 | 1.1 | 0.1 | 0.4 | 1.2 | 0.7 | 3.3 | 0.2 |
| | LLOQ | 0.4 | 1.0 | 1.3 | 3.2 | 0.1 | 0.7 | 1.9 | 1.6 | 2.6 | 0.4 |
| Percent Recovery (%) | | 87 | 109 | 105 | 101 | 104 | 103 | 82 | 119 | 86 | 125 |
| Endogenous Levels (pg/mL) | | 0.7 -3.2 | 0.2 - 1.1 | 0.8 - 5.0 | 2.0 - 9.7 | 0.3 - 1.5 | 3.2 - 30 | 1.4 - 20 | 1.2 - 11.5 | 4.5 - 42 | 1.8 - 5.2 |

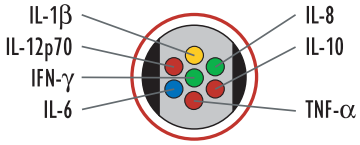
Purified cytokines were spiked into human serum at multiple levels, and the recovery of the spiked cytokine was measured in triplicate using the Human TH1/TH2 10-plex Ultra-Sensitive Kit. The table below summarizes the average calculated recoveries, detection parameters, and endogenous cytokine levels measured in eight individual sera. LLOD and LLOQ were determined as described previously.

# Human Pro-Inflammatory Panels

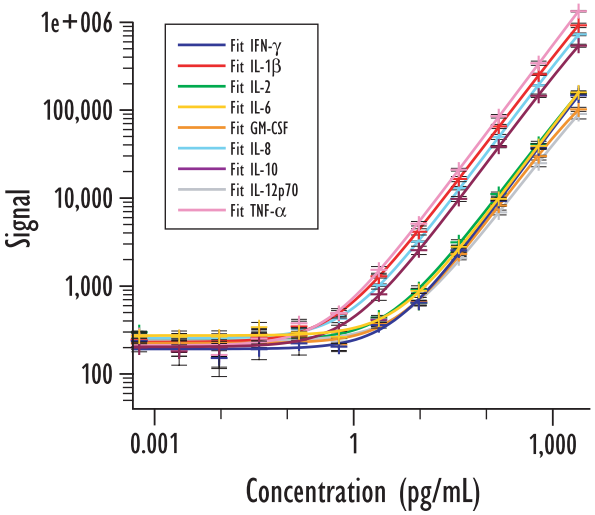
## Human Pro-Inflammatory 9-Plex Cytokine Panel



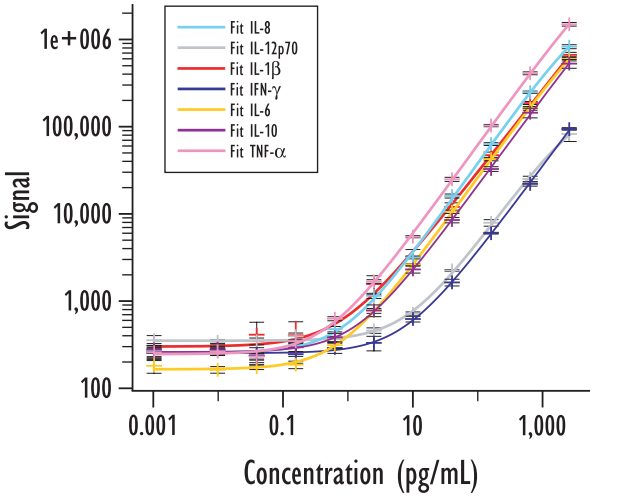

## Human Pro-Inflammatory 7-Plex Cytokine Panel




## Human Pro-Inflammatory 9-Plex Ultra-Sensitive Kit




## Human Pro-Inflammatory 7-Plex Ultra-Sensitive Kit




## Detection Parameters (pg/mL)

| | | IL-2 | IL-8 | IL-12p70 | IL-1β | GM-CSF | IFN-γ | IL-6 | IL-10 | TNF-α |
|---|---|---|---|---|---|---|---|---|---|---|
| 9-Plex | LLOD | 1.0 | 0.2 | 1.6 | 0.2 | 1.6 | 1.5 | 0.3 | 1.3 | 0.1 |
| | LLOQ | 1.5 | 0.2 | 3.2 | 0.3 | 1.4 | 2.1 | 1.7 | 2.3 | 0.5 |
| 7-Plex | LLOD | - | 0.3 | 1.8 | 0.1 | - | 2.1 | 0.3 | 0.4 | 0.1 |
| | LLOQ | - | 0.2 | 2.8 | 2.8 | - | 3.0 | 0.4 | 1.8 | 0 |

## Conclusions

- Diverse product offering including singleplex and multiplex cytokine assays
- Highly sensitive cytokine assays (detection limits ~1-10 pg/mL) demonstrated in Tissue Culture Kits
- Ultra-Sensitive Kits are recommended for complex matrices (serum / plasma) and achieving greater sensitivity in cell culture applications
- Wide dynamic range assays (3-4 logs) enable measurement of low and high level cytokines in the same sample without dilution
- Spiked cytokines in human samples are recovered at the expected levels
- Multi-day validation studies show robust assays suitable for clinical applications (low variability in signals and calculated cytokine levels; consistency across days)
- Assay protocols and diluents have been optimized for cell culture supernatants, serum, plasma, and other human samples
- Simple and rapid workflows / protocols enable more efficient use of time
- Comparable assay performance across the technology platform

Current listing of cytokine offerings can be viewed at:
http://www.mesoscale.com/products/assays/cytokines.htm