Human Cytokine Assay Products from Meso Scale Discovery

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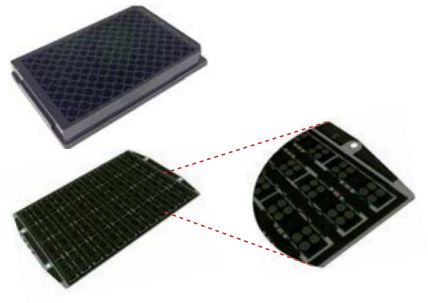
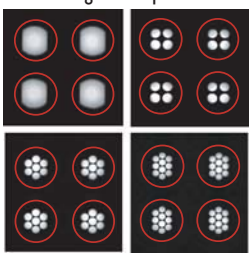
In this poster, we present a collection of assays and applications that demonstrate the power of MSD® technology as a foundation for high performance cytokine assays. Examples of cytokine assays in both single-plex and multiplex formats show that multiple cytokines can be simultaneously measured without compromising assay performance. Different plate types are available depending on the requirements of the particular application; both high bind and ultrasensitive assay formats are shown here. Ultrasensitive assays have 2-10 fold greater sensitivity depending on the particular cytokine. 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. To demonstrate the robustness of MSD cytokine panels for rigorous applications such clinical and GLP work, 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 10-20 µL for 96-well and 384-well Plates
- Reduced Noise





SECTOR™ PR 400 Reader

Multiple Instruments

- Two Imaging Instruments for HTS
- Two Personal Readers for Assay Development
- Very Fast Read Time

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, BAL, and other bodily fluids. Three standard protocols are given below. 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.

Cell Supernatant Protocol:

1. Add 20 uL of Sample / Calibrators; Incubate 1-2 hr at RT
2. Add 20 uL of Detection Antibodies; Incubate 1-2 hr at RT
3. Wash 3X with PBS
4. Add 150 uL/well Read Buffer and Read

Human Serum Protocol:

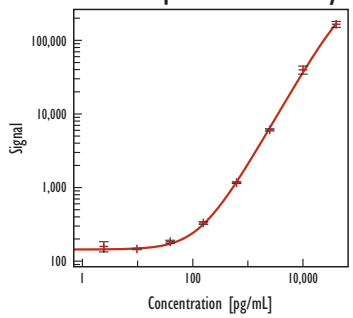
1. Add 20 uL of MSD Assay Diluent; Incubate 30 min at RT
2. Add 20 ul of Sample / Calibrators; Incubate 1-2 hr at RT
3. Add 20 uL of Detection Antibodies; Incubate 1-2 hr at RT
4. Wash 3X with PBS
5. Add 150 uL/well Read Buffer and Read

Human Plasma Protocol:

1. Add 30 uL of MSD Assay Diluent; Incubate 30 min at RT
2. Add 10 ul of Sample / Calibrators; Incubate 1-2 hr at RT
3. Wash 3X with PBS
4. Add 20 uL of Detection Antibodies; Incubate 1-2 hr at RT
5. Wash 3X with PBS
6. Add 150 uL/well Read Buffer and Read

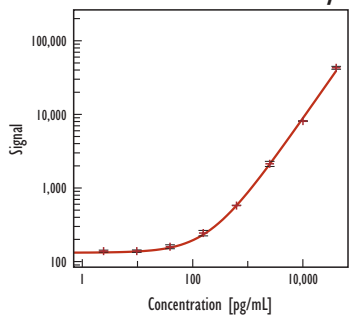
Human IL-8 Cytokine Assay (MULTI-ARRAY™ 96-Well Small Spot Plate)

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

Above is example data from cytokine assays using standard protocols.

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	85
EDTA Plasma	84
Heparin Plasma	92

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.

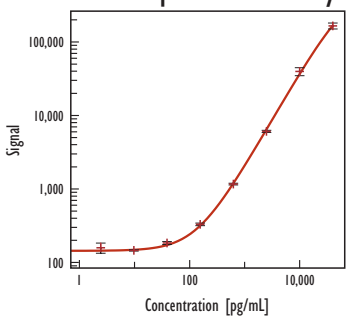
Endogenous Levels

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).

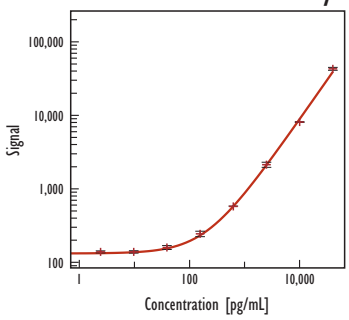
Human TNF-α Ultrasensitive Cytokine Assay (MULTI-ARRAY™ 96-Well Small Spot Plate)

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

Above is example data from cytokine assays using standard protocols.

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	87
EDTA Plasma	106
Heparin Plasma	115

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.

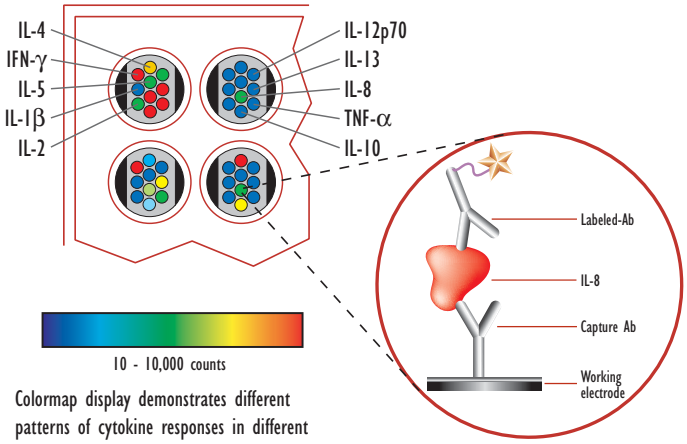
Endogenous Levels

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 Assay

MSD Human TH1/TH2 Cytokine Array

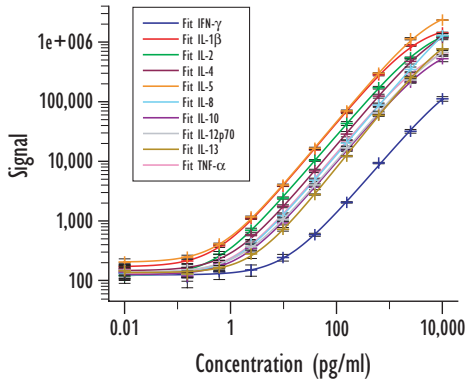


Colormap display demonstrates different patterns of cytokine responses in different wells of a 96-well 10-spot assay plate.

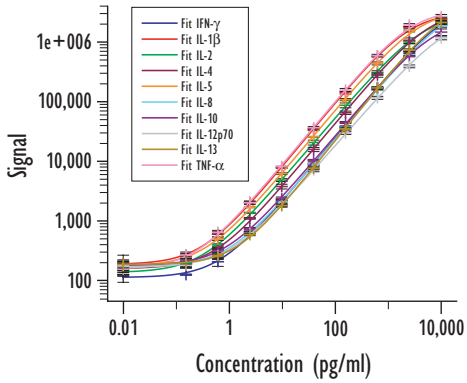
Each well in an MSD MULTI-SPOT® plate contains multiple spots, each with a capture antibody for a particular biological assay. The assays are independent of one another and each is optimized for maximum performance in detecting its particular analyte.

Human TH1/TH2 Cytokine Array - High Bind & Ultrasensitive Assays for Cell Culture Supernatants

High Bind Assay



Ultrasensitive Assay



Detection limits (pg/mL)

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

MSD has developed cytokine assays for both standard and ultrasensitive applications. The detection limits on ultrasensitive assay plates are typically 2-10 times lower than the standard assays and can be used to detect cytokines that are present at very low endogenous levels (less than 1 pg/mL). Detection limits were calculated using 2.5 standard deviations above the background.

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	9.45	4.95	1.47	1.69	0.43	1.44	2.47	0.21	3.35	0.93
	2	8.30	9.74	1.77	1.77	0.40	1.45	2.25	0.19	2.64	0.69
DAY 2	3	5.99	3.40	1.23	1.16	0.24	1.15	1.90	0.16	2.52	0.73
	4	6.08	2.77	2.06	1.29	0.22	1.10	1.60	0.16	2.25	0.66
DAY 3	5	5.96	3.35	1.30	1.21	0.25	0.89	1.36	0.16	2.26	0.82
	6	6.54	3.29	1.30	1.52	0.26	0.95	1.87	0.16	2.05	0.82
DAY 4	7	2.52	2.07	1.22	1.26	0.25	1.25	1.22	0.19	3.44	1.10
	8	2.41	2.21	1.32	1.15	0.23	0.70	2.27	0.20	3.20	1.38
	9	3.12	2.07	1.32	1.38	0.26	0.62	1.96	0.20	3.08	1.23

The human TH1/TH2 Cytokine Array 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 (+/- 20%).

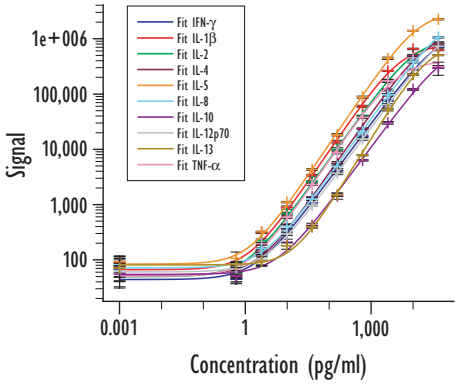
Spike Recovery Data

	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

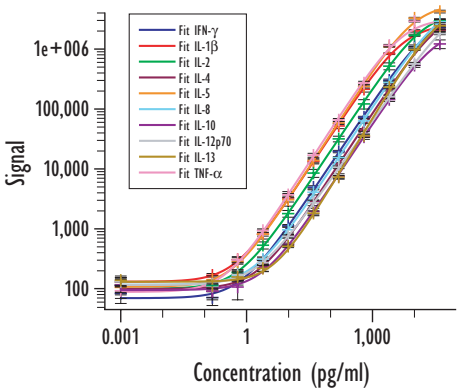
	IL-2			IL-4			IL-5			IL-8			TNF-α		
Spike (pg/mL)	Calc Conc	%CV	%Recovery	Calc Conc	%CV	%Recovery	Calc Conc	%CV	%Recovery	Calc Conc	%CV	%Recovery	Calc Conc	%CV	%Recovery
16	16.5	5.5	103.2	18.6	4.2	116.0	16.0	2.5	100.3	15.6	2.2	97.3	15.5	2.3	97.0
63	59.3	6.0	94.2	63.5	5.0	100.8	61.1	1.2	96.9	58.4	6.3	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 Cytokine Array - Standard & Ultrasensitive Assays for Serum and Plasma

High Bind Assay



Ultrasensitive Assay



Purified cytokines were spiked into human serum, and the recovery of the spiked cytokine was measured in triplicate. The table below represents the average calculated recoveries. Detection limits were calculated using 2.5 standard deviations above the background.

Detection limits (pg/mL)

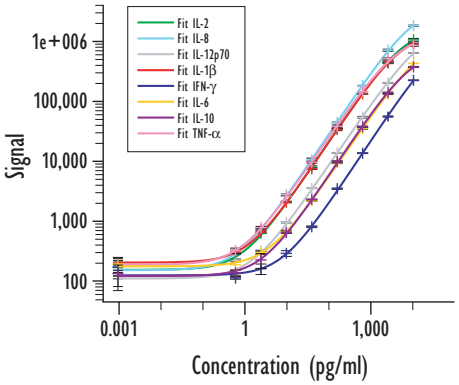
	IFN-γ	IL-1β	IL-2	IL-4	IL-5	IL-8	IL-10	IL-12p70	IL-13	TNF-α
Ultrasensitive Assay	0.8	0.3	0.5	1.3	0.3	1.0	1.9	1.1	2.8	0.3
High Bind Assay	2.9	1.3	1.5	3.1	1.1	3.0	7.9	3.6	13.0	1.7

Percent Recovery of Spiked Cytokines in Human Serum

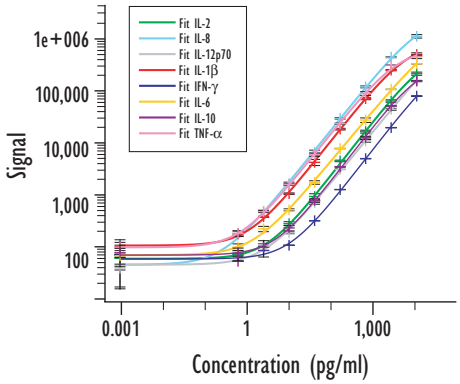
	IFN-γ	IL-1β	IL-2	IL-4	IL-5	IL-8	IL-10	IL-12p70	IL-13	TNF-α
Ultrasensitive Assay	87	109	105	101	104	103	82	119	86	125
High Bind Assay	92	89	82	115	92	93	81	102	109	100

Human Inflammatory Cytokine Array

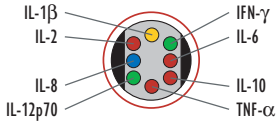
Cell Culture Supernatant Protocol



Serum Protocol



MSD Human Inflammatory Cytokine Array



Detection limits (pg/mL)

	IL-2	IL-8	IL-12p70	IL-1β	IFN-γ	IL-6	IL-10	TNF-α
Cell Supernatant Protocol	0.7	0.5	2.1	0.7	4.4	2.7	2.8	0.6
Serum Protocol	3.7	0.4	4.2	1.0	10.2	1.5	5.0	0.5

Conclusions

- Diverse product offering including single-plex and multi-plex cytokine assays
- Highly sensitive cytokine assays (detection limits ~1-10 pg/mL) in High Bind format
- Ultrasensitive cytokine assays (detection limits <1 pg/mL) are available for applications with very low levels of cytokines
- 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