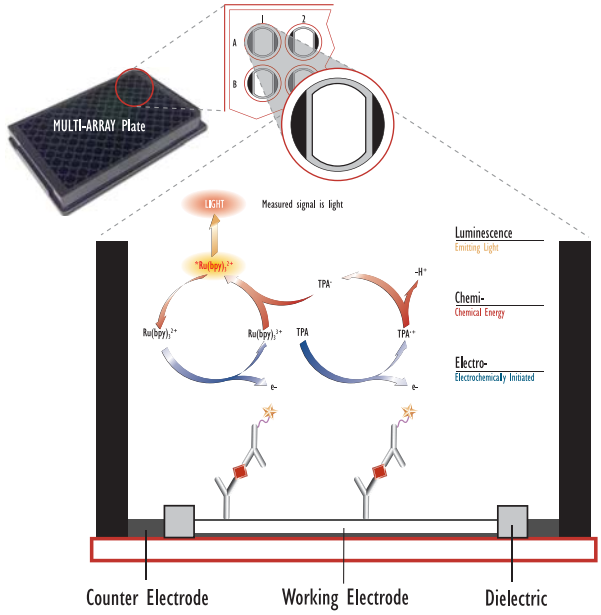
Human Cytokine Assay Products from Meso Scale Discovery

In this poster, we present a collection of products 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. The Human TH1/TH2 10-Plex Ultra-Sensitive Kit is presented here as an illustration of a higher order multiplex product that is available from the MSD catalog. Sample data is given including spot layout, standard curves, and detection limits. Functional performance data is presented (spike recovery, dilution linearity, precision studies) to demonstrate the utility in rigorous applications involving complex biological samples. In addition to cytokine multiplex kits, MSD offers custom cytokine products which enable combinations of cytokine and other assays that can be designed with breadth and flexibility to meet specific customer applications.

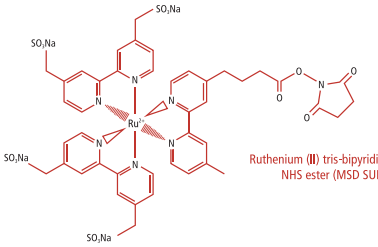
The MSD® Platform

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



Electrochemiluminescence Features:

- Minimal background signals and high signal to background ratios - the stimulation mechanism (electricity) is decoupled from the signal (light)
- Proximity - only labels bound near the electrode surface are detected, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and are conveniently conjugated to biological molecules
- Emission at ~620 nm - eliminating problems with color quenching
- Signal amplification - multiple excitation cycles of each label enhance light levels and improve sensitivity



Kits and Protocols

MSD cytokine kits and protocols are designed to optimize workflow and ease-of-use while maximizing assay performance in terms of sensitivity, dynamic range, and recovery. The products have been used successfully to measure many sample matrices including cell culture supernatants, serum, plasma, sputum, bronchoalveolar lavage, and other bodily fluids. Two standard protocols are given below with the tissue culture protocol recommended for the Tissue Culture Cytokine Kits and the serum / plasma protocol recommended for measurement of complex samples using the Ultra-Sensitive Kits. 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.

Tissue Culture Kit and Protocol

1. Add 25 µL of Sample/Calibrators, incubate 1-2 hours at RT.
2. Add 25 µL of Detection Antibodies, incubate 2 hours at RT.
3. Wash with PBS-T. Add 150 µL /well Read Buffer and read.

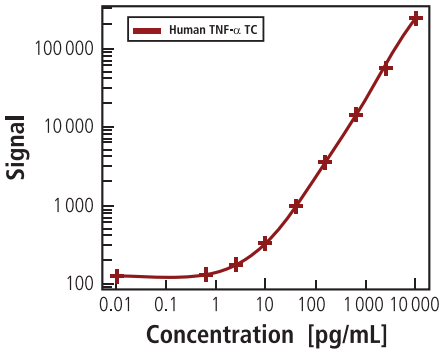
Ultra-Sensitive Kit, Serum/Plasma Protocol

1. Add 25 µL of MSD Assay Diluent, incubate 30 min at RT.
2. Add 25 µL of Sample/Calibrators, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL of Detection Antibodies, Incubate 1-2 hours at RT.
4. Wash with PBS-T. Add 150 µL /well Read Buffer and read.

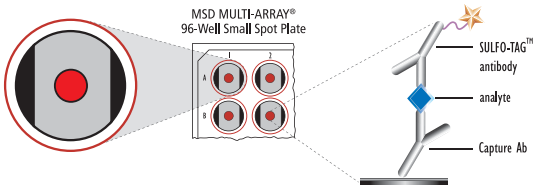
Human TNF-α Assay

Singleplex human cytokine assays from MSD provide a means for rapid measurement of analyte in a simple format. In the example below, Tissue Culture Kits and Ultra-Sensitive Kits for Human TNF-α offer highly sensitive assays with a wide dynamic range. Functional data demonstrating spike recovery and dilution linearity recoveries close to 100% is presented.

Tissue Culture Kit

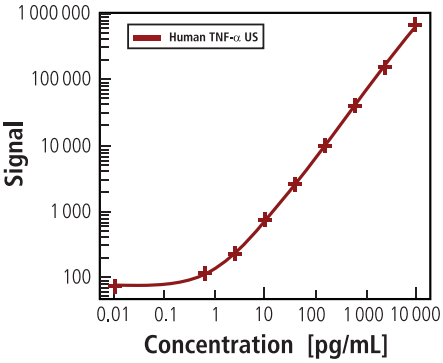


Human TNF-α TC		
Concentration (pg/mL)	Mean	% CV
0	124	9.9
0.61	133	4.4
2	175	2.7
10	334	1.8
39	939	1.4
156	3453	4.5
625	13578	3.7
2500	52668	2.5
10000	229156	3.7



	Human TNF-α TC
LLOD (pg/ml)	1.2

Ultra-Sensitive Kit



Human TNF-α US		
Concentration (pg/mL)	Mean	% CV
0	74	7.5
0.61	113	5.4
2	224	2.6
10	717	3.0
39	2560	2.1
156	10049	1.7
625	39258	3.4
2500	159373	4.9
10000	660990	2.0

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator. The indicated values represent the average LLOD over several kit lots.

	Human TNF-α US
LLOD (pg/ml)	0.5

Spike Recovery

Sample Type	Average % Recovery
Serum	88
EDTA Plasma	106
Heparin Plasma	114

Spike and recovery data is obtained when pooled samples of human serum, heparin plasma, and EDTA plasma were spiked with calibrators at multiple levels throughout the range of the assay. Each spike was done in 3 replicates. An average of pooled samples is shown for each case.

% Recovery = measured / expected * 100

Endogenous Levels

		TNF-α (pg/mL)
Serum	Min	2.8
	Max	6.1
	Median	4.2
EDTA Plasma	Min	4.4
	Max	7.9
	Median	5.8
Heparin Plasma	Min	6.0
	Max	9.7
	Median	7.6

Endogenous levels were measured using 8 sera, EDTA plasma, and heparin plasma from normal human samples. If measured values are below the LLOD, they are indicated as n/d (not detectable).

Dilutional Linearity

	TNF-α US			
	Fold Dilution	Adjusted Conc. (pg/mL)	% CV	% Recovery
Spiked Serum	Neat	645	6.9	
	2	678	5.2	105
	4	660	5.2	97
	8	633	7.8	96
Spiked Plasma	Neat	602	4.6	
	2	646	5.4	107
	4	613	2.3	95
	8	625	1.4	102

To establish dilutional linearity, three pools each of human serum and human heparin plasma were evaluated; a representative pool of each is shown for each case. The pooled samples were spiked at mid level with calibrator and then diluted with Human Serum Cytokine Assay Diluent. Percent recovery is calculated as the measured concentration divided by the concentration measured for the previous dilution (expected).

% Recovery = (measured * dilution factor) / expected * 100

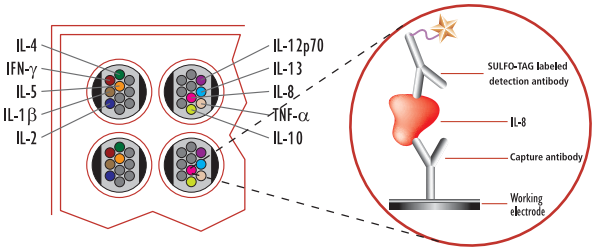
Precision: Multi-Day Study

			Average %CV		
	Control	Runs	Average Conc. (pg/mL)	Intra-Plate	Inter-Plate
TNF-α	High	14	1167	5.3	9.1
	Medium	14	107	4.6	8.0
	Low	14	9.4	3.6	17

Precision data is obtained when control samples containing high, mid, and low levels of each analyte were measured in triplicate on multiple days using multiple plate lots. Each triplicate measurement is defined as a run.

Multiplexed MSD Cytokine Assays

MSD Human TH1/TH2 10-Plex Cytokine Assay

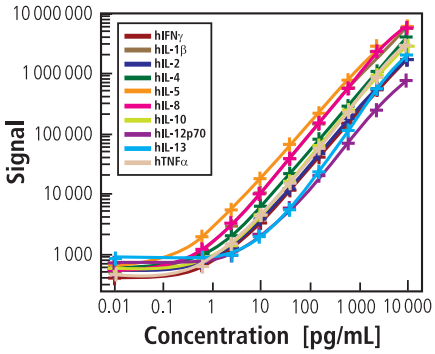


Each well in an MSD MULTI-SPOT® plate contains multiple spots, each with a capture antibody for a particular biological assay. The assays are independent of one another and each is optimized for maximum performance in detecting its particular analyte.

Similar to the singleplex assays, multiplexed cytokine assays offer highly sensitive immunoassays with a very wide dynamic range. This allows for maximum flexibility in measuring samples and reduces the chance that sample dilution will be required in cases where the analyte level is high relative to the calibration curve. Multiplexing also provides numerous advantages in efficiency (limited sample volumes, reduced testing time required to generate data for multiple assays), experimental control (allows for on-board internal controls), and consistency (fewer manipulations to achieve same dataset as multiple singleplex assays).

Human TH1/TH2 10-Plex Assay

Ultra-Sensitive Kit



	IFNγ	IL-1β	IL-2	IL-4	IL-5
LLOD (pg/mL)	0.4	0.2	0.7	0.3	0.1

	IL-8	IL-10	IL-12p70	IL-13	TNF-α
LLOD (pg/mL)	0.1	0.4	2.3	1.8	0.5

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator. The indicated values represent the average LLOD over several kit lots.

Endogenous Levels

		IFNγ (pg/mL)	IL-1β (pg/mL)	IL-2 (pg/mL)	IL-4 (pg/mL)	IL-5 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	IL-12p70 (pg/mL)	IL-13 (pg/mL)	TNF-α (pg/mL)
Serum	Min	n/d	n/d	n/d	n/d	0.3	1.6	n/d	n/d	n/d	2.0
	Max	1.0	1.8	25.8	0.7	4.5	10.2	20.2	33.7	48.4	4.8
	Median	0.6	0.3	n/d	n/d	0.4	7.1	0.9	7.2	n/d	3.6
EDTA Plasma	Min	n/d	0.3	n/d	n/d	0.3	4.7	n/d	n/d	n/d	3.8
	Max	1.0	3.2	25.3	n/d	4.4	45.6	28.6	31.5	48.1	7.3
	Median	0.8	0.5	0.7	n/d	0.4	6.3	0.5	3.6	n/d	5.2
Heparin Plasma	Min	0.4	n/d	n/d	n/d	0.2	1.7	n/d	n/d	n/d	3.8
	Max	1.2	2.0	29.9	n/d	4.5	14.6	28.7	33.1	43.7	8.5
	Median	0.9	0.9	1.0	n/d	0.5	5.2	0.5	11.9	n/d	6.9

Endogenous levels were measured using 8 sera, EDTA plasma, and heparin plasma from normal human samples. If measured values are below the LLOD, they are indicated as n/d (not detectable).

Precision: Multi-Day Study

	Control	Runs	Average %CV: Average Conc. (pg/mL)	Average %CV: Intra-Plate	Average %CV: Inter-Plate
IFNγ	High	13	1910	4.7	6.5
	Medium	13	176	5.3	6.6
	Low	13	18.4	4.9	10
IL-1β	High	13	2076	4.3	4.7
	Midium	13	201	6.3	11
	Low	13	19.2	5.7	13
IL-2	High	13	2017	4.8	7.1
	Midium	13	199	6.4	8.5
	Low	13	18.4	4.3	9.7
IL- 4	High	13	1990	6.6	4.8
	Midium	13	204	7.5	7.5
	Low	13	18.8	7.9	10
IL-5	High	13	2118	4.5	7.0
	Midium	13	197	4.7	7.9
	Low	13	19.2	4.1	8.6
IL-8	High	13	2039	4.9	4.7
	Midium	13	203	5.4	8.9
	Low	13	19	4.7	12
IL-10	High	13	2009	5.4	8.1
	Midium	13	194	5.4	13
	Low	13	18.7	5.3	14
IL-12p70	High	13	1916	4.9	6.9
	Midium	13	213	6.5	6.3
	Low	13	19.1	5.3	8.8
IL-13	High	13	2081	5.8	6.2
	Midium	13	167	6.4	11
	Low	13	16.7	4.6	12
TNF-α	High	13	1887	5.5	7.7
	Midium	13	146	4.9	12
	Low	13	12.7	5.8	15

Precision data is obtained when control samples containing high, mid, and low levels of each analyte were measured in triplicate on multiple days using multiple plate lots. Each triplicate measurement is defined as a run.

Spike Recovery

	IFNγ				IL-1β				IL-2				IL-4				IL-5			
	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery
Spiked Serum	1	1	21		2	2	99		3	3	17		0	0	16		1	1	7.6	
	23	22	5.0	95	24	20	5.9	86	28	23	6.9	82	21	21	4.2	99	22	23	2.3	104
	226	215	6.0	95	221	198	3.8	90	253	212	6.0	84	222	225	2.8	101	211	224	3.6	106
	2237	2221	3.4	99	2272	2209	4.1	97	1914	1904	6.6	99	2289	2376	4.0	104	2363	2795	3.4	118
Spiked Heparin Plasma	1	1	19		0	0	19		2	2	15		0	0	23		1	1	9.1	
	23	22	3.3	96	22	21	2.3	97	27	22	7.2	84	21	22	4.4	106	23	23	5.6	102
	225	215	2.9	95	219	207	3.1	95	252	205	8.0	81	222	242	2.7	109	212	222	4.4	105
	2236	2196	3.5	98	2270	2262	4.1	100	1913	1797	5.7	94	2289	2405	6.4	105	2363	2337	33	99
Spiked EDTA Plasma	1	1	49		0	0	27		1	1	27		0	0	42		1	1	11	
	23	20	2.9	86	22	22	2.8	100	26	27	6.3	105	21	22	2.9	106	22	23	2.2	104
	225	194	2.0	86	219	220	2.0	101	251	247	9.0	98	222	226	3.3	102	211	223	0.9	105
	2236	2001	2.5	89	2270	2436	2.7	107	1912	2163	10	113	2289	2311	4.0	101	2363	2747	1.3	116

	IL-8				IL-10				IL-12p70				IL-13				TNF-α			
	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery
Spiked Serum	18	18	3.6		2	2	9.3		6	6	15		5	5	17		4	4	8.7	
	40	34	2.3	84	26	24	4.2	90	28	27	4.0	94	27	30	3.0	113	26	25	3.1	100
	237	209	4.4	88	231	221	3.4	96	237	244	10	103	211	267	9.5	127	224	232	4.9	103
	2164	2092	4.3	97	1989	1518	12	76	2016	2173	3.6	108	2416	2934	4.8	121	2183	2105	7.6	96
Spiked Heparin Plasma	6	6	4.2		12	12	9.2		76	76	13		29	29	7.6		5	5	3.9	
	28	26	3.8	94	36	29	6.1	81	98	75	13	77	51	44	4.3	87	26	23	3.0	86
	225	218	3.6	97	240	236	4.0	98	307	296	6.0	96	236	266	3.0	113	225	190	2.9	84
	2152	2101	5.1	98	1999	1921	7.7	96	2086	2182	4.4	105	2440	2794	5.7	115	2184	1745	4.8	80
Spiked EDTA Plasma	3	3	6.6		2	2	16		3	3	33		4	4	39		5	5	9.1	
	25	21	26	85	26	24	4.0	95	25	28	3.4	114	26	31	3.4	120	27	25	3.3	94
	222	208	3.5	94	230	219	2.6	95	234	246	6.8	105	211	276	4.6	131	226	213	6.6	94
	2149	2094	3.3	97	1988	2015	2.7	101	2013	2244	3.4	111	2415	3094	6.2	128	2184	2048	4.2	94

Spike and Recovery data is obtained when pooled samples of human serum, heparin plasma, and EDTA plasma were spiked with calibrators at multiple levels throughout the range of the assay. Each spike was done in 3 replicates. An average of pooled samples is shown for each case.

% Recovery = measured / expected * 100

Dilutional Linearity

		IFNγ			IL-1β			IL-2			IL-4			IL-5		
	Fold Dilution	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery
Spiked Serum	Neat	724	2.9		681	5.4		745	6.0		645	5.6		737	4.2	
	2	772	3.2	107	708	6.7	104	633	11	85	730	3.2	113	748	3.6	102
	4	759	4.7	98	741	6.9	105	705	14	111	780	2.0	107	745	2.5	100
	8	764	3.1	101	697	9.0	94	681	17	97	753	5.7	97	694	5.9	93
Spiked Plasma	Neat	755	4.0		703	8.8		740	15		691	8.3		793	3.0	
	2	816	2.8	108	769	5.3	109	763	11	103	784	8.0	113	776	6.8	98
	4	755	4.0	93	694	4.7	90	705	13	92	782	9.1	100	701	4.7	90
	8	726	4.8	96	648	4.8	93	627	9.2	89	799	5.0	102	671	1.2	96

		IL-8			IL-10			IL-12p70			IL-13			TNF-α		
	Fold Dilution	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery	Adjusted Conc. (pg/mL)	% CV	% Recovery
Spiked Serum	Neat	740	7.4		718	3.1		684	7.7		715	14		681	3.7	
	2	800	5.0	108	785	2.6	109	805	7.6	118	707	8.5	99	745	0.8	109
	4	776	2.8	97	803	3.1	102	766	5.3	95	671	3.9	95	696	4.4	93
	8	728	9.7	94	784	6.4	98	789	8.6	103	679	8.2	101	732	2.9	105
Spiked Plasma	Neat	699	4.7		724	3.8		842	8.9		872	12		419	4.3	
	2	785	2.5	112	768	5.0	106	898	9.6	107	763	10	88	477	2.9	114
	4	732	2.6	93	739	2.7	96	807	12	90	644	9.7	84	485	0.6	102
	8	710	2.4	97	689	2.9	93	799	1.7	99	586	1.5	91	427	11	88

To establish dilutional linearity, three pools each of human serum and human heparin plasma were evaluated; a representative pool of each is shown for each case. The pooled samples were spiked at mid level with calibrator and then diluted with Human Serum Cytokine Assay Diluent. Percent recovery is calculated as the measured concentration divided by the concentration measured for the previous dilution (expected).

% Recovery = (measured * dilution factor) / expected * 100

Cross-Reactivity

	IFNγ	IL-1β	IL-2	IL-4	IL-5	IL-8	IL-10	IL-12p70	IL-13	TNF-α
hIFNγ spot	100.00%	0.15%	0.00%	-0.02%	0.01%	-0.01%	0.01%	0.00%	0.00%	0.04%
hIL-1β spot	0.04%	100.00%	0.05%	0.09%	0.04%	0.04%	0.02%	0.05%	-0.02%	-0.11%
hIL-2 spot	0.05%	0.06%	100.00%	-0.01%	0.00%	0.12%	-0.04%	-0.03%	0.00%	0.04%
hIL-4 spot	0.14%	0.13%	0.01%	100.00%	0.10%	0.01%	-0.02%	-0.01%	-0.09%	0.07%
hIL-5 spot	0.00%	0.05%	0.00%	0.03%	100.00%	0.06%	-0.01%	-0.01%	0.00%	0.01%
hIL-8 spot	0.08%	0.05%	0.02%	0.02%	0.03%	100.00%	0.02%	0.00%	0.03%	-0.09%
hIL-10 spot	0.06%	0.04%	0.01%	0.05%	-0.01%	0.15%	100.00%	0.00%	0.00%	-0.02%
hIL-12p70 spot	0.22%	0.18%	0.07%	0.14%	0.01%	-0.07%	0.06%	100.00%	-0.10%	-0.11%
hIL-13 spot	0.08%	0.05%	0.02%	0.06%	-0.02%	0.03%	0.01%	-0.01%	100.0%	-0.06%
hTNFα spot	0.28%	0.19%	0.03%	0.05%	0.02%	0.01%	0.01%	-0.01%	0.03%	100.00%

Cross-reactivity between cytokine assays within one well is minimal. Cross-reactivity was measured using single calibrators at 2500-10000pg/mL. The high levels were selected to yield high specific signal so that the test would be sensitive to even modest levels of cross-reactivity. Cross-reactivity was calculated by determining the amount of signal on non-specific spots and comparing to signal on the specific spot. Typical levels of cross-reactivity are less than 0.1%

Custom Cytokines

To complement the Human Cytokine Kits offered by MSD, custom cytokine panels are available to support individual customer applications. The majority of cytokine assays can be multiplexed together without any problem. The MSD system was designed to have similar performance independent of the number of spots or assays within a well. Thus, a cytokine assay run in a well with only one spot typically performs the same as an assay run in a well with 10-spots. Signals are generally comparable, detection limits are within a factor of 3, and the dynamic range is similar. In addition, many of the human cytokine assays can be multiplexed with other secreted proteins and biomarkers. Combinations that have been done previously are cytokine assays combined with metabolic assays (metabolkine) as well was cytokines combined with vascular and/or growth factor markers.

There are several considerations one must take before multiplexing cytokines together. It is important to estimate the expected levels of the analytes and the assay sensitivities and dynamic ranges in preparation for multiplexing. In addition, certain antibodies may cross-react with other analytes non-specifically. In QC of MSD custom cytokine multiplexes, each kit is tested for specificity between analytes by running a single analyte calibrator at a mid level. An additional factor is appropriate selection of blockers and diluents. Since most human cytokine assays offered by MSD utilize a common protocol, this is typically not an issue. However, there are some cases where alternate diluents and/or blocker are used to achieve optimal performance. To address these issues and others related to custom human cytokines, the MSD Scientific Services department works with customers to assess needs and identify a suitable solution.

MSD also has the capability to build plates and detection antibodies for assays where customers may choose to do optimization. These assays are offered through prototype services and are included in the assay list below.

Applications

Human Cytokine Assay Products from MSD have been used for a wide variety of applications in the pharmaceutical, biotechnology, and academic communities. These include: use of cytokines as biomarkers to monitor biochemical state and activity associated with disease progression and recovery; studies of inflammatory response in response to therapeutic treatments; use of cytokines to compare sample handing and analysis across organizations and studies; and characterization of transcriptional machinery regulation via downstream cytokine expression. For specific examples of these applications and more, please visit our website: http://www.mesoscale.com/CatalogSystemWeb/WebRoot/literature/publications.aspx

Current Available Cytokine Assays and Kits

Cytokine Multiplex Panels

Panel	Analytes
Human TH1/TH2 7-Plex	IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13
Human TH1/TH2 10-Plex	IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, TNF-α
Human Pro-Inflammatory 4-Plex I	IFN-γ, IL-1β, IL-6, TNF-α
Human Pro-Inflammatory 4-Plex II	IL-1β, IL-6, IL-8, TNF-α
Human Pro-Inflammatory 7-Plex	IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α
Human Pro-Inflammatory 9-Plex	GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF-α
Human MMP 2-Plex	MMP-2, MMP-10
Human MMP 3-Plex	MMP-1, MMP-3, MMP-9
Human Chemokine 7-Plex	Eotaxin, IL-8, IP-10, MCP-1, MCP-4, MIP-1β, TARC
Human Chemokine 9-Plex	Eotaxin, Eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1β, TARC
Human Demonstration 4-Plex	IL-1β, IL-2, IL-6, TNF-α
Human Demonstration 7-Plex	GM-CSF, IL-1β, IL-2, IL-5, IL-6, IL-8, TNF-α
Human Demonstration 10-Plex	GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α
Mouse TH1/TH2 9-Plex	IFN-γ, IL-1β, IL-2, IL-4, IL-5, KC/GRO/CINC, IL-10, IL-12 total, TNF-α
Mouse Pro-Inflammatory 7-Plex	IFN-γ, IL-1β, IL-6, IL-10, IL-12p70, KC/GRO/CINC, TNF-α
Rat Demonstration 7-Plex	IFN-γ, IL-1β, IL-4, IL-5, IL-13, KC/GRO/CINC, TNF-α

Individual Cytokine Assays

Human				
Eotaxin	IL-4	IL-12p70	MCP-4	MMP-10
Eotaxin-3	IL-5	IL-13	MDC	RANTES
G-CSF	IL-6	IL-17	MIP-1α	TARC
GM-CSF	IL-6R	IL-18*	MIP-1β	TIMP-1
IFNβ	IL-7*	IP-10	MIP-3α	TNF-α
IFNγ	IL-8	I-TAC	MMP-1	TNF-RI
IL-1α*	IL-10	MIG*	MMP-2	TNF-RII
IL-1β	IL-12	M-CSF	MMP-3	
IL-2	IL-12p40	MCP-1	MMP-9	

Mouse		
GM-CSF	IL-13*	TNF-α
IFNγ	IL-12p40	TNF-RI
IL-1β	IL-12p70	TNF-RII
IL-2	KC/GRO/CINC (CXCL1)	
IL-4	MIP-1α*	
IL-5	MIP-1β*	
IL-6	MCP-1	
IL-10	RANTES	
IL-12		

Rat	
GM-CSF	IL-10*
IFNγ	IL-13
IL-1α	KC/GRO/CINC (CXCL1)
IL-1β	MCP-1
IL-2*	MIP-3α
IL-4	TNF-α
IL-5	
IL-6	

* available as prototype

Conclusions

- Diverse product offering including singleplex, multiplex, and custom cytokine assays
- Our cytokine assays can be easily multiplexed with other assays for vascular, growth factor, and metabolic markers
- Highly sensitive cytokine assays (detection limits ~0.2-3 pg/mL)
- Ultra-Sensitive Kits are recommended for complex matrices (serum / plasma) and achieving greater sensitivity in cell culture applications
- Wide dynamic range assays 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 performance studies show robust assays suitable for demanding 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

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