

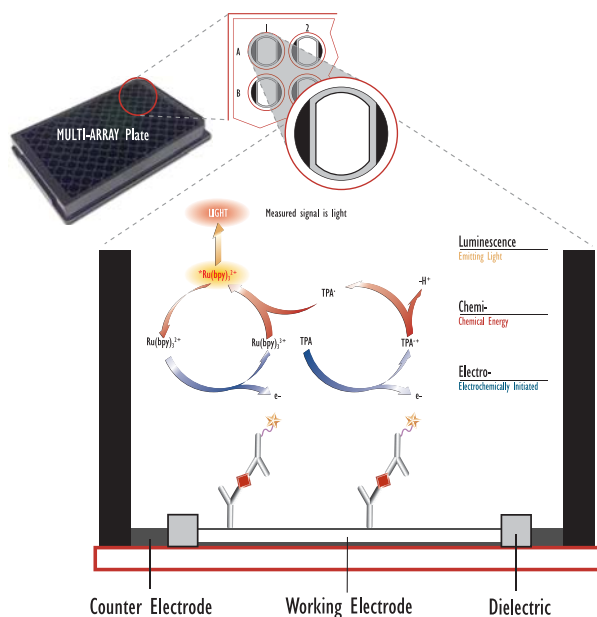


Mouse and Rat Cytokine Assay Products from Meso Scale Discovery

In this poster, we present a collection of products and applications that demonstrate the power of MSD technology as a foundation for high performance mouse and rat 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). In multiplexed cytokine assays, the preferred combination of cytokines depends on the particular application and system being studied. The Mouse/Rat TH1/TH2 9-Plex and Rat Demonstration Panel are presented here as an illustration of higher order multiplex products that are available from the MSD catalog. Sample data is given including spot layout, standard curves, and detection limits. Functional performance data is presented (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.

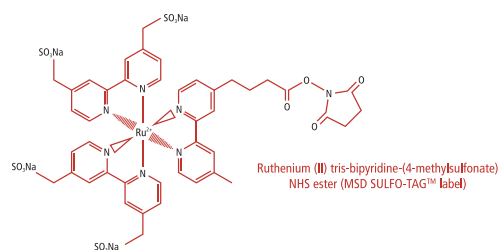
The MSD[®] Platform

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

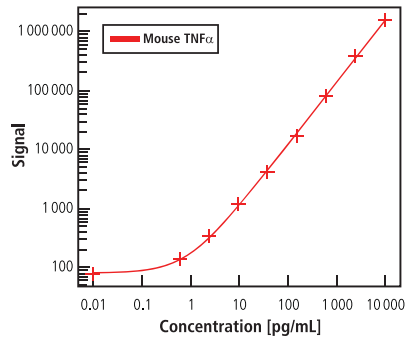
Ultra-Sensitive Kit, Serum/Plasma Protocol

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

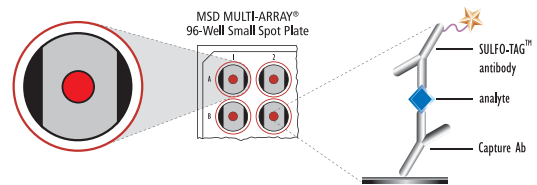
Mouse TNF- α Assay

Singleplex mouse and rat cytokine assays from MSD provide a means for rapid measurement of analyte in a simple format. In the examples below, Tissue Culture Kits and Ultra-Sensitive Kits for Mouse TNF- α and Ultra-Sensitive Kits for Rat IL-6 offer highly sensitive assays with a wide dynamic range.

Tissue Culture Kit

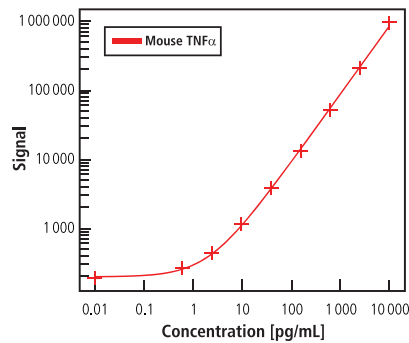


Mouse TNF- α TC		
Concentration (pg/mL)	Mean	% CV
0	77	3.2
0.61	141	3.6
2.4	337	3.1
9.8	1163	5.9
39	4129	4.9
156	16622	2.8
625	79048	5.4
2500	387944	3.6
10000	1562514	4.0



Mouse TNF- α TC	
LLOD (pg/ml)	0.2

Ultra-Sensitive Kit



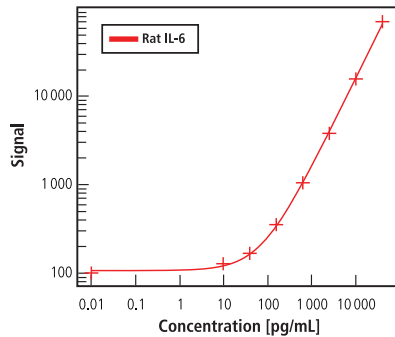
Mouse TNF- α US		
Concentration (pg/mL)	Mean	% CV
0	191	3.0
0.61	264	2.9
2.4	439	0.5
9.8	1146	2.8
39	3839	4.7
156	13016	5.9
625	51522	2.5
2500	209567	2.9
10000	958534	1.1

Mouse TNF- α US	
LLOD (pg/ml)	0.2

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.

Rat IL-6 Assay

Ultra-Sensitive Kit



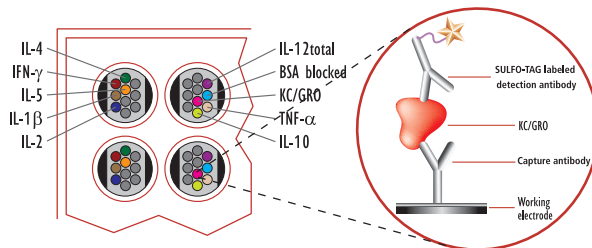
Rat IL-6 US		
Concentration (pg/mL)	Mean	% CV
0	100	4.6
10	127	4.0
39	167	5.4
156	353	2.0
625	1052	2.0
2500	3820	6.4
10000	15800	6.8
40000	70342	4.8

Rat IL-6 US	
LLOD (pg/ml)	14.6

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.

Multiplexed MSD Cytokine Assays

MSD Mouse TH1/TH2 9-Plex Cytokine Assay

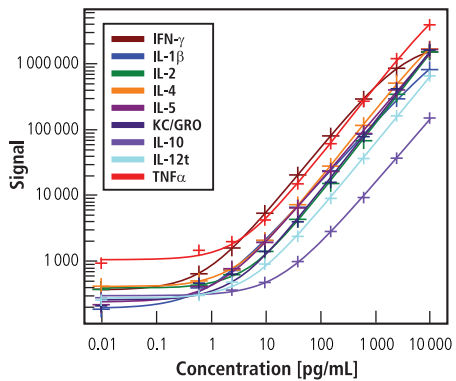


Each well in an MSD MULTI-SPOT® plate contains multiple spots, each with a capture antibody for a particular biological assay. The assays are independent of one another and each is optimized for maximum performance in detecting its particular analyte.

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).

Mouse TH1/TH2 9-Plex Assay

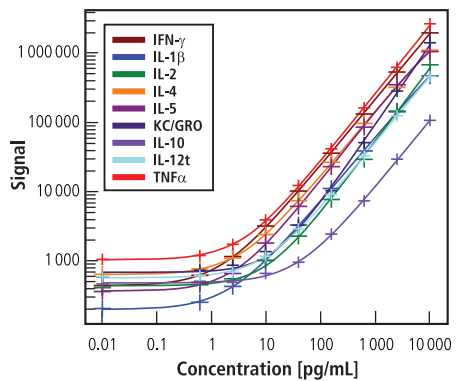
Tissue Culture Kit



	IFN γ	IL-1 β	IL-2	IL-4	IL-5
LLOD (pg/mL)	0.2	1.7	1.6	0.8	0.5

	KC/GRO	IL-10	IL-12total	TNF- α
LLOD (pg/mL)	1.2	9.9	3.6	1.2

Ultra-Sensitive Kit



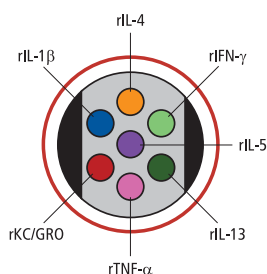
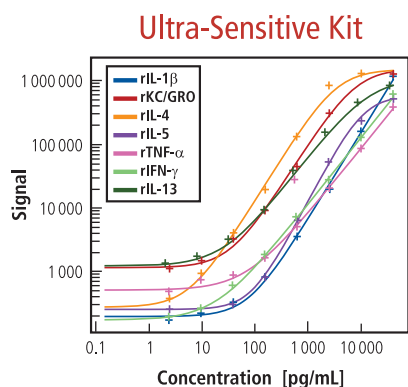
	IFN γ	IL-1 β	IL-2	IL-4	IL-5
LLOD (pg/mL)	0.4	1.5	2.6	0.8	0.7

	KC/GRO	IL-10	IL-12total	TNF- α
LLOD (pg/mL)	2.7	8.4	2.9	1.1

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.

Rat Demonstration Panel

MSD's Ultrasensitive Rat 7-Plex Cytokine Panel measures seven rat cytokines simultaneously from a single sample (serum or plasma). Typical standard curves for this panel are shown in the figure below. The lower limit of detection (LLOD) for each analyte was determined by calculating 2.5 standard deviations above the average background (no analyte). LLOD's range from 2 pg/mL to 41 pg/mL (see table). The linear dynamic range is very large: these assays remain linear beyond 20,000 pg/mL. The typical %CV for the standard curves and samples were less than 10%.



	rIL-1 β	rKC/GRO	rIL-4	rIL-5	rTNF- α	rIFN- γ	rIL-13
LLOD (pg/mL)	25	9	2	41	10	5	6

	Limits of Detection Across Lots (pg/mL)						
	rIL-1 β	rKC/GRO	rIL-4	rIL-5	rTNF- α	rIFN- γ	rIL-13
Median	20	3.0	1.1	32	11	6.5	4.4
Max	50	9.0	1.9	128	59	20	6.0
Min	11	1.3	0.3	20	4.0	5.2	3.2
N of Lots	10	6	7	5	9	7	5

Precision: Multi-Day Study

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.

	Control	Runs	Average Conc. (pg/mL)	Average %CV	
				Intra-Plate	Inter-Plate
rIL-1 β	High	13	7665	4.2	5.4
	Medium	12	1494	4.5	8.7
	Low	22	327	5.3	8.9
rKC/GRO	High	13	8471	6.0	7.9
	Medium	12	3285	2.7	8.4
	Low	22	640	2.2	9.2
rIL-4	High	13	7942	2.5	9.3
	Medium	12	2689	5.4	7.8
	Low	22	458	2.6	6.1
rIL-5	High	13	7202	4.3	5.4
	Medium	12	1936	2.3	9.1
	Low	22	546	5.6	9.5
rTNF- α	High	13	10000	3.7	9.9
	Medium	12	1471	3.7	6.0
	Low	22	342	3.3	8.0
rIFN- γ	High	13	5257	5.9	7.1
	Medium	12	3082	3.5	7.7
	Low	22	810	3.3	9.6
rIL-13	High	13	6682	4.9	7.1
	Medium	12	1206	2.6	8.6
	Low	22	112	3.0	10.5

Cross-Reactivity of Analytes

	rIL-1 β	rKC/GRO	rIL-4	rIL-5	rTNF- α	rIFN- γ	rIL-13
rIL-1 β spot	100.00%	0.12%	0.12%	0.09%	0.04%	0.08%	0.02%
rKC/GRO spot	0.01%	100.00%	-0.12%	-0.16%	-0.45%	-0.09%	-0.16%
rIL-4 spot	0.20%	0.08%	100.00%	0.15%	0.08%	0.19%	0.04%
rIL-5 spot	0.08%	0.11%	0.08%	100.00%	0.06%	0.12%	0.04%
rTNF- α spot	0.42%	0.17%	0.24%	0.37%	100.00%	0.11%	0.35%
rIFN- γ spot	0.08%	0.06%	0.10%	0.15%	0.24%	100.00%	0.06%
rIL-13 spot	-0.50%	-0.02%	-0.51%	-0.39%	-0.75%	0.03%	100.00%

Cross-reactivity of the assays within the same well was measured using a single analyte at 10,000 or 40,000 pg/mL. We chose these high values to yield a high specific signal and to provide a stringent test for cross reactivity. The signals measured for the other analytes were used to calculate cross-reactivity. The % cross-reactivity was less than 0.5% for all analytes and typically undetectable at levels below 0.2%.

Custom Cytokines

To complement the Mouse and Rat 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 mouse (or rat) cytokine assays can be multiplexed with other secreted proteins and biomarkers. Combinations that have been done previously are cytokine assays combined with metabolic assays (metabolikine) as well as 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 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 mouse and rat 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

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; and use of cytokines to compare sample handling and analysis across organizations and studies. 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-1 β , 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-1 β , IL-6, TNF- α
Human Pro-Inflammatory 4-Plex II	IL-1 β , IL-6, IL-8, TNF- α
Human Pro-Inflammatory 7-Plex	IFN- γ , IL-1 β , IL-6, IL-8, IL-10, IL-12p70, TNF- α
Human Pro-Inflammatory 9-Plex	GM-CSF, IFN- γ , IL-1 β , 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-1 β , TARC
Panel	Analytes
Human Chemokine 9-Plex	Eotaxin, Eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1 β , TARC
Human Demonstration 4-Plex	IL-1 β , IL-2, IL-6, TNF- α
Human Demonstration 7-Plex	GM-CSF, IL-1 β , IL-2, IL-5, IL-6, IL-8, TNF- α
Human Demonstration 10-Plex	GM-CSF, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF- α
Mouse TH1/TH2 9-Plex	IFN- γ , IL-1 β , IL-2, IL-4, IL-5, KC/GRO/CINC, IL-10, IL-12 total, TNF- α
Mouse Pro-Inflammatory 7-Plex	IFN- γ , IL-1 β , IL-6, IL-10, IL-12p70, KC/GRO/CINC, TNF- α
Rat Demonstration 7-Plex	IFN- γ , IL-1 β , IL-4, IL-5, IL-13, KC/GRO/CINC, TNF- α

Individual Cytokine Assays						
Human		Mouse		Rat		
Eotaxin	IL-4	IL-12p70	MCP-4	MMP-10	GM-CSF	IL-10*
Eotaxin-3	IL-5	IL-13	MDC	RANTES	IFN γ	IL-13
G-CSF	IL-6	IL-17	MIP-1 α	TARC	IL-1 α	KC/GRO/CINC (CXCL1)
GM-CSF	IL-6R	IL-18*	MIP-1 β	TIMP-1	IL-1 β	MCP-1
IFN β	IL-7*	IP-10	MIP-3 α	TNF- α	IL-2*	MIP-3 α
IFN γ	IL-8	I-TAC	MMP-1	TNF-RI	IL-4	TNF- α
IL-1 α *	IL-10	MIG*	MMP-2	TNF-RII	IL-5	
IL-1 β	IL-12	M-CSF	MMP-3		IL-6	
IL-2	IL-12p40	MCP-1	MMP-9			
						* 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 (most detection limits ~0.2-10 pg/mL)
- Ultra-Sensitive Kits are recommended for complex matrices (serum / plasma)
- Wide dynamic range assays enable measurement of low and high level cytokines in the same sample without dilution
- 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 mouse and rat samples
- Simple and rapid workflows / protocols enable more efficient use of time
- Comparable assay performance across the technology platform