MSD® MULTI-SPOT Assay System

Human ProInflammatory 9-Plex Ultra-Sensitive Kit

1-Plate Kit	K15007C-1
5-Plate Kit	K15007C-2
25-Plate Kit	K15007C-4

MSD Biomarker Assays

Human ProInflammatory 9-Plex Ultra-Sensitive Kit

IL-2, IL-8, IL-12p70, IL-1β, GM-CSF, IFN-γ, IL-6, IL-10, TNF-α

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Table of Contents

Ordering Information

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Introduction

Inflammatory processes are involved in many physiological events, including infection, the healing response, and other disease states such as autoimmunity. Cytokines and chemokines are small, soluble proteins that can help mediate both acute and chronic inflammatory responses.

Interleukin (IL)-2 is produced primarily by activated $CD4^+$ T cells. IL-2 is an important regulator of proliferation and maintenance of several T- and NK-cell subsets. The presence of IL-2 has also been demonstrated to play an important role in the long-term survival of activated helper T cells (Th) and $CD8^+$ cytotoxic T cells (Tc).

IL-8, also known as CXCL8, is a chemokine responsible for the attraction of neutrophils to vascular endothelium and extravasation into inflammed tissues. It is produced primarily by activated macrophages in response to toll-like receptor agonists and certain bacterial pathogens.

IL-12p70 is the active heterodimer of IL-12, consisting of the p40 and p35 subunits. IL-12 participates in the differentiation of naïve T cells in Th1 cells. It stimulates the secretion of IFN-γ and TNF-α and inhibits IL-4 induced proliferation of lymphocytes. IL-12 plays an important role in the mediation of the cytotoxic activity of NK cells and $CD8^+$ Tc. It is produced by dendritic cells, monocytes, macrophages, and B-cells in response to intra-cellular pathogens.

IL-1β is produced by dendritic cells, monocytes, macrophages and certain epithelial cells. IL-1β is produced in response to infection induced inflammation. It induces the production of adhesion molecules that enable the transmigration of leukocytes into inflammed tissues. IL-1β also participates in fever induction by the hypothalamus.

Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) is secreted in response to inflammation by macrophages, T cells, endothelial cells and other non-lymphoid cells. It is responsible for the stimulation of stem cells to produce neutrophils, basophils, eosinophils and monocytes.

Interferon-γ (IFN-γ), also known as type two interferon, plays a role in the recruitment of leukocytes to the site of infection. IFN-γ is produced by Th1 cells and NK cells. IFN-γ activates macrophages by increasing the expression of major histocompatibility complex (MHC) molecules and antigen processing components. It has also been show to contribute to immunoglobulin (Ig) class switching and suppress Th2 responses. IFN-γ enhances the effects of type one interferons, such as IFN-β.

IL-6 is a proinflammatory cytokine secreted by monocytes, macrophages and certain non-lymphoid cell types in response to tissue damage or infection. It plays a role in the acute phase response, the regulation of fever, and the generation of plasma B cells. IL-6 has been recently shown to act in concert with TGF-β to induce the differentiation of IL-17 producing helper T cells from naïve progenitors.

IL-10 inhibits the production of proinflammatory cytokines by T cells, and it is a potent suppressor of monocyte and macrophage functions. As such, it plays an important role in the regulation and termination of inflammatory responses. IL-10 also plays an important role in the growth and differentiation of B cells, NK cells, Th cells, and cytotoxic T cells. IL-10 is produced by macrophages and certain T cell subsets, including CD4+CD25+Foxp3+ regulatory T cells.

Tumor Necrosis Factor-α (TNF-α) plays a key role in the acute phase reaction and systemic inflammation. TNF-α is primarily produced by activated macrophages, but it is also secreted by a variety of other cell types under pathogenic conditions. Upon receptor binding, it has been shown to trigger diverse cell signaling pathways including apoptosis, proliferation, differentiation, chemoattraction, hypothalamic regulation, and cytokine production. TNF-α can also contribute to tumorigenesis and viral replication.

Principle of the Assay

MSD assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or “spot”) per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. The Human ProInflammatory 9-Plex Assay detects IL-2, IL-8, IL-12p70, IL-1β, GM-CSF, IFN-γ, IL-6, IL-10, and TNF-α in a sandwich immunoassay format (Figure 1). MSD provides a plate that has been pre-coated with capture antibody on spatially distinct spots – antibodies for IL-2, IL-8, IL-12p70, IL-1β, GM-CSF, IFN-γ, IL-6, IL-10, and TNF-α. The user adds the sample and a solution containing the labeled detection antibodies— anti-IL-2, anti-IL-8, anti-IL-12p70, anti-IL-1β, anti-GM-CSF, anti-IFN-γ, anti-IL-6, anti-IL-10, and anti-TNF-α labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the labeled detection antibodies by bound analytes completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR® instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of IL-2, IL-8, IL-12p70, IL-1β, GM-CSF, IFN-γ, IL-6, IL-10, and TNF-α present in the sample.

1 = IL-2
2 = IL-8
3 = IL-12p70
4 = IL-1β
5 = GM-CSF
6 = *BSA Blocked*
7 = IFN-γ
8 = IL-6
9 = IL-10
10 = TNF-α

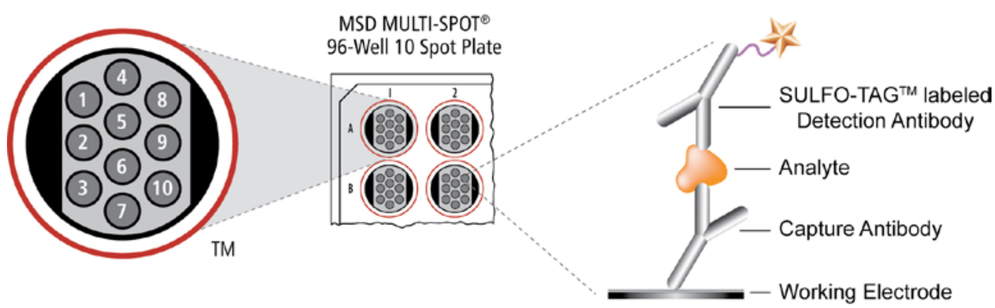


Figure 1. *Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

Reagents Supplied

Product Description	Storage	Quantity per Kit K15007C-1	K15007C-2	K15007C-4
MULTI-SPOT® 96-well 10-Spot Human ProInflammatory 9-Plex Plate N05007A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Detection Antibody Blend[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human ProInflammatory 9-Plex Calibrator Blend (Ultra-Sensitive) (1 µg/mL of each)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 2 R51BB-4 (8 mL) R51BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Diluent 3 R51BA-4 (5 mL) R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	2 bottles (200 mL ea)

Required Materials and Equipment - not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

Safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

> **Important:** Upon first thaw, separate Diluent 2 and Diluent 3 into aliquots appropriate to the size of your assay needs. These diluents can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Calibrator and Control Solutions

Calibrator for the Human ProInflammatory 9-Plex Assay is supplied at 400-fold higher concentration than the recommended highest Calibrator. Prepare a diluted stock Calibrator by diluting the stock Calibrator 100-fold in Diluent 2. MSD recommends the preparation of an 8-point standard curve consisting of at least 2 replicates of each point. Each well requires 25 µL of Calibrator. For the assay, MSD recommends 4-fold serial dilution steps and Diluent 2 alone for the 8th point:

Standard	Human ProInflammatory 9-Plex Calibrator Blend (Ultra-Sensitive) (pg/mL)	Dilution Factor
Stock Cal.	1000000	
Dil. Stock Cal.	10000	100
STD-01	2500	4
STD-02	625	4
STD-03	156	4
STD-04	39	4
STD-05	9.8	4
STD-06	2.4	4
STD-07	0.61	4
STD-08	0	n/a

To prepare this 8-point standard curve for up to 4 replicates:

1) Prepare the diluted stock Calibrator by transferring 10 µL of the Human ProInflammatory 9-Plex Calibrator Blend (Ultra-Sensitive) to 990 µL Diluent 2.
2) Prepare the highest Calibrator point (STD-01) by transferring 50 µL of the Human ProInflammatory diluted stock Calibrator to 150 µL Diluent 2. Repeat 4-fold serial dilutions 6 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Diluent 2 (i.e. zero Calibrator).

Notes:

a. Alternatively, Calibrators can be prepared in the sample matrix or diluent of choice to verify acceptable performance in these matrices. In general, the presence of some protein (for example, 1% BSA) in the sample matrix is helpful for preventing loss of analyte by adsorption onto the sides of tubes, pipette tips, and other surfaces. If your sample matrix is serum-free tissue culture media, then the addition of 10% FBS or 1% BSA is recommended.

b. The standard curve can be modified as necessary to meet specific assay requirements.

Dilution of Samples

Serum and Plasma

All solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following the thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze/thaw cycles for serum and plasma samples. Normal serum or plasma samples may not require a dilution prior to being used in the MSD Human ProInflammatory 9-Plex Assay. Serum or plasma with high levels of these analytes may require a dilution.

Tissue Culture

Tissue culture supernatant samples may not require dilution prior to being used in the MSD Human ProInflammatory 9-Plex Assay. If using serum-free medium, the presence of carrier protein (e.g., 1% BSA) in the solution is helpful to prevent loss of analyte to the labware. Samples from experimental conditions with extremely high levels of cytokines may require a dilution.

Other Matrices

Information on preparing samples in other matrices, including sputum, CSF, and tissue homogenates can be obtained by contacting MSD Scientific Support at 1-301-947-2025 or ScientificSupport@mesoscale.com.

Prepare Detection Antibody Solution

The Detection Antibody Blend is provided at 50X stock solution. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 60 µL aliquot of the stock Detection Antibody Blend into 2.94 mL of Diluent 3.

Prepare Read Buffer

The Read Buffer should be diluted 2-fold in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of 4X Read Buffer T to 10 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

Assay Protocol

Notes

1. **Addition of Diluent 2:** Dispense 25 µL of Diluent 2 into each well. Seal the plate with an adhesive plate seal and incubate for 30 min with vigorous shaking (300–1000 rpm) at room temperature.
2. **Addition of the Sample or Calibrator:** Dispense 25 µL of sample or Calibrator into separate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   Shaking a 96-well MSD plate typically accelerates capture at the working electrode.

3. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3 times with PBS-T. Dispense 25 µL of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
4. **Wash and Read:** Wash the plate 3 times with PBS-T. Add 150 µL of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

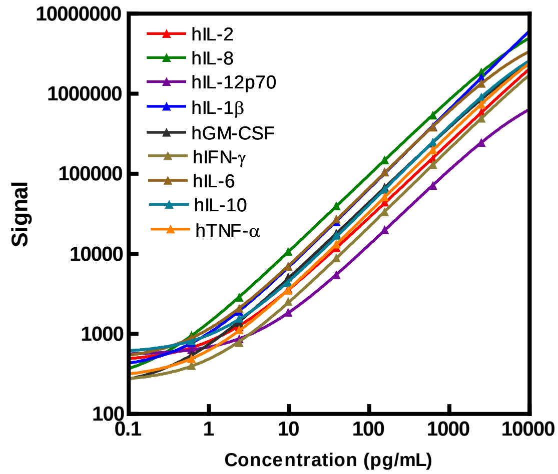
   Bubbles in the fluid will interfere with reliable reading of plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

Analysis of Results

The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Standard Curve

The following standard curves are an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



IL-2		
Conc. (pg/mL)	Average Signal	%CV
0	521	9.0
0.61	686	7.6
2.4	1227	6.2
9.8	3620	3.7
39	11914	1.6
156	44192	4.9
625	154127	6.6
2500	584570	6.2

IL-8		
Conc. (pg/mL)	Average Signal	%CV
0	231	10.8
0.61	959	1.7
2.4	2858	2.2
9.8	10699	2.8
39	39629	7.3
156	149544	4.6
625	538930	3.9
2500	1863369	5.8

IL-12p70		
Conc. (pg/mL)	Average Signal	%CV
0	582	11.3
0.61	638	4.7
2.4	864	7.8
9.8	1849	8.6
39	5467	7.9
156	20051	5.3
625	71626	9.6
2500	245830	3.6

IL-1β		
Conc. (pg/mL)	Average Signal	%CV
0	337	11.8
0.61	774	7.4
2.4	1903	6.4
9.8	6949	6.4
39	25109	3.4
156	105408	5.0
625	388319	7.0
2500	1589542	5.4

GM-CSF		
Conc. (pg/mL)	Average Signal	%CV
0	291	4.9
0.61	551	7.6
2.4	1377	7.9
9.8	5117	2.7
39	18099	6.9
156	68108	1.2
625	240731	1.1
2500	855327	0.8

IFN-γ		
Conc. (pg/mL)	Average Signal	%CV
0	288	8.4
0.61	402	6.4
2.4	777	3.2
9.8	2541	3.2
39	8862	2.8
156	33332	5.2
625	130217	3.1
2500	493194	3.9

IL-6		
Conc. (pg/mL)	Average Signal	%CV
0	463	10.0
0.61	895	5.5
2.4	2084	4.5
9.8	6989	3.6
39	27204	3.9
156	104638	3.0
625	382822	4.4
2500	1345104	4.0

IL-10		
Conc. (pg/mL)	Average Signal	%CV
0	529	7.8
0.61	816	6.6
2.4	1559	2.4
9.8	4347	7.4
39	17097	5.2
156	63335	1.7
625	248804	2.6
2500	908386	6.3

TNF-α		
Conc. (pg/mL)	Average Signal	%CV
0	268	9.0
0.61	489	5.1
2.4	1128	4.7
9.8	3500	3.8
39	13232	1.8
156	51168	3.2
625	199049	5.6
2500	739380	5.5

Sensitivity

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

	IL-2	IL-8	IL-12p70	IL-1β	GM-CSF	IFN-γ	IL-6	IL-10	TNF-α
LLOD (pg/mL)	0.35	0.090	1.4	0.36	0.20	0.53	0.27	0.21	0.50

Spike Recovery

Serum and plasma samples were spiked with Calibrator at multiple values throughout the range of the assay. Each spike was done in ≥ 3 replicates.
% Recovery = measured / expected x 100

IL-2	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	2.7	11.8	
	9.8	12	9.5	95
	39	45	9.0	109
	156	163	5.6	103
	625	669	3.4	107
EDTA Plasma	0	0.49	6.0	
	9.8	9.6	5.5	96
	39	36	10.4	93
	156	146	6.5	94
	625	637	4.1	102
Heparin Plasma	0	3.2	3.4	
	9.8	13	9.5	100
	39	45	5.2	107
	156	163	3.7	103
	625	653	1.9	104

IL-8	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	41	1.0	
	9.8	48	6.0	93
	39	70	2.9	88
	156	184	3.8	93
	625	668	3.4	100
EDTA Plasma	0	2.0	7.4	
	9.8	8.6	4.8	72
	39	29	1.7	71
	156	107	2.4	68
	625	464	2.8	74
Heparin Plasma	0	3.8	1.7	
	9.8	14	4.9	104
	39	44	4.0	103
	156	161	2.6	101
	625	647	1.1	103

IL-12p70	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	<LLOD	12.4	
	9.8	10	4.8	94
	39	36	7.0	90
	156	152	9.1	97
	625	589	7.3	94
EDTA Plasma	0	5.3	4.3	
	9.8	12	10.8	79
	39	34	10.0	76
	156	116	5.0	72
	625	454	9.5	72
Heparin Plasma	0	1.5	8.0	
	9.8	11	5.4	89
	39	34	5.0	83
	156	148	5.0	93
	625	596	2.4	95

IL-1β	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	<LLOD	14.7	
	10	9.1	6.1	91
	39	34	8.2	88
	156	127	5.5	81
	625	526	2.9	84
EDTA Plasma	0	<LLOD	6.2	
	9.8	8.8	5.3	88
	39	33	17.6	84
	156	132	9.3	84
	625	546	1.3	87
Heparin Plasma	0	<LLOD	19.6	
	9.8	9.2	2.3	92
	39	34	10.6	87
	156	124	4.8	80
	625	475	3.0	76

GM-CSF	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	0.42	11.0	
	9.8	8.8	1.5	88
	39	38	4.4	96
	156	167	4.9	107
	625	547	2.0	88
EDTA Plasma	0	2.3	7.3	
	9.8	9.3	6.1	77
	39	32	2.9	78
	156	118	2.4	74
	625	508	4.7	81
Heparin Plasma	0	<LLOD	4.8	
	9.8	9.6	3.5	96
	39	37	5.3	95
	156	156	1.5	100
	625	596	4.0	95

IFN-γ	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	0.96	18.4	
	9.8	9.1	8.8	83
	39	35	3.8	87
	156	136	5.5	87
	625	544	4.8	87
EDTA Plasma	0	<LLOD	17.0	
	9.8	7.3	10.4	73
	39	33	7.3	84
	156	124	5.9	79
	625	548	6.2	88
Heparin Plasma	0	<LLOD	9.0	
	9.8	9.5	7.0	95
	39	40	0.4	104
	156	149	1.8	95
	625	621	4.6	99

IL-6	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	2.2	6.7	
	9.8	11	6.8	95
	39	37	0.8	89
	156	137	4.3	87
	625	747	5.2	95
EDTA Plasma	0	1.6	6.8	
	9.8	11	4.7	88
	39	41	15.0	99
	156	138	5.5	87
	625	595	4.2	95
Heparin Plasma	0	2.7	5.8	
	9.8	11	15.1	88
	39	37	3.3	88
	156	139	3.7	87
	625	581	4.3	93

IL-10	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	0.47	15.2	
	9.8	8.8	4.3	88
	39	33	3.0	86
	156	134	2.2	86
	625	539	13.5	86
EDTA Plasma	0	3.0	4.3	
	9.8	9.6	1.9	74
	39	30	3.2	70
	156	111	4.3	70
	625	464	1.8	74
Heparin Plasma	0	0.59	10.3	
	9.8	9.7	3.0	88
	39	34	4.3	85
	156	146	3.9	93
	625	565	4.3	90

TNF-α	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	1.0	4.1	
	9.8	10	2.7	93
	39	38	8.8	94
	156	154	7.5	98
	625	583	1.1	93
EDTA Plasma	0	1.5	3.2	
	9.8	10	7.7	86
	39	38	2.4	93
	156	148	2.4	93
	625	585	0.5	93
Heparin Plasma	0	1.4	2.8	
	9.8	10	1.5	93
	39	36	6.3	89
	156	145	1.9	93
	625	554	0.4	88

Linearity

Three pools each of human serum and heparin plasma were evaluated; a representative pool of each is shown below. The pooled samples were spiked with Calibrator and then diluted with Diluent 2. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).
% Recovery = (measured x dilution factor) / expected x 100

		IL-2			IL-8		
Sample	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	1	685	1.6		675	1.8	
	2	699	3.3	102	672	2.8	100
	4	698	3.3	100	685	4.9	102
	8	669	6.0	96	631	12.2	92
Heparin Plasma	1	660	2.2		625	5.8	
	2	597	3.9	90	649	10.1	104
	4	615	11.9	103	712	1.8	110
	8	596	7.1	97	651	4.5	92

		IL-12p70			IL-1β		
Sample	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	1	650	31.4		633	2.9	
	2	628	7.3	97	656	3.4	104
	4	672	13.2	107	684	1.2	104
	8	659	6.4	98	645	3.7	94
Heparin Plasma	1	613	18.1		583	1.9	
	2	673	15.5	110	601	5.9	103
	4	668	15.0	99	584	1.3	97
	8	768	11.2	115	623	6.2	107

		GM-CSF			IFN-γ		
Sample	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	1	675	1.3		533	10.3	
	2	647	3.5	96	654	5.0	123
	4	679	3.8	105	683	5.0	104
	8	669	5.2	99	684	7.5	100
Heparin Plasma	1	621	3.4		536	2.9	
	2	647	9.7	104	599	10.0	112
	4	701	1.6	108	562	18.7	94
	8	690	1.2	98	596	9.8	106

Sample	Fold Dilution	IL-6 Conc. (pg/mL)	IL-6 Conc. % CV	IL-6 % Recovery	IL-10 Conc. (pg/mL)	IL-10 Conc. % CV	IL-10 % Recovery
Serum	1	471	4.7		577	4.0	
	2	555	9.4	118	603	5.0	104
	4	602	8.1	108	669	4.7	111
	8	589	4.7	98	630	3.4	94
Heparin Plasma	1	585	5.0		642	7.7	
	2	591	8.0	101	658	11.1	103
	4	621	8.1	105	662	2.2	101
	8	651	7.1	105	687	0.7	104

Sample	Fold Dilution	TNF-α Conc. (pg/mL)	TNF-α Conc. % CV	TNF-α % Recovery
Serum	1	645	6.9	
	2	678	5.2	105
	4	660	5.2	97
	8	633	7.8	96
Heparin Plasma	1	602	4.6	
	2	646	5.4	107
	4	613	2.3	95
	8	625	1.4	102

Samples

Eight normal human samples were measured for each of the following sample types: serum, EDTA plasma, and heparin plasma.

		IL-2 (pg/mL)	IL-8 (pg/mL)	IL-12p70 (pg/mL)	IL-1β (pg/mL)	GM-CSF (pg/mL)	IFN-γ (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)
Serum	Min	<LLOD	2.2	<LLOD	<LLOD	<LLOD	<LLOD	1.0	<LLOD	2.8
	Max	15	12	34	0.53	1.5	1.6	4.6	5.0	6.1
	Median	<LLOD	7.4	2.2	<LLOD	<LLOD	0.79	1.8	1.0	4.2
EDTA Plasma	Min	<LLOD	5.3	<LLOD	<LLOD	<LLOD	<LLOD	1.0	0.3	4.4
	Max	11	46	29	1.1	2.7	1.8	3.3	5.2	7.9
	Median	<LLOD	6.9	3.0	0.53	<LLOD	0.92	1.8	1.0	5.8
Heparin Plasma	Min	<LLOD	2.4	<LLOD	<LLOD	<LLOD	<LLOD	1.1	<LLOD	6.0
	Max	10	15	28	2.1	2.8	1.7	3.1	6.3	9.7
	Median	<LLOD	5.8	2.2	0.63	0.22	0.70	1.8	0.94	7.6

Assay Components

The human IL-2, IL-8, IL-12p70, IL-1β, GM-CSF, IFN-γ, IL-6, IL-10, and TNF-α capture and detection antibodies used in this assay are listed below.

	Source species	
Analyte	**MSD Capture Antibody**	**MSD Detection Antibody**
hIL-2	Mouse monoclonal	Mouse monoclonal
hIL-8	Mouse monoclonal	Goat polyclonal
hIL-12p70	Mouse monoclonal	Mouse monoclonal
hIL-1β	Mouse monoclonal	Goat polyclonal
hGM-CSF	Mouse monoclonal	Rat monoclonal
hIFN-γ	Mouse monoclonal	Mouse monoclonal
hIL-6	Mouse monoclonal	Goat polyclonal
hIL-10	Rat monoclonal	Rat monoclonal
hTNF-α	Mouse monoclonal	Goat polyclonal

Summary Protocol

MSD 96-well MULTI-SPOT Human ProInflammatory 9-Plex Ultra-Sensitive Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the
MSD Human ProInflammatory 9-Plex Assay.

Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

If necessary, samples should be diluted in Diluent 2.

Prepare Calibrator solutions and standard curve.

Use the Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 2.

> ***Note:*** *The standard curve can be modified as necessary to meet specific assay requirements.*

Prepare Detection Antibody Solution by diluting Detection Antibody Blend to 1X in a final volume of 3.0 mL Diluent 3 per plate.

Prepare 20 mL of 2X Read Buffer T by diluting 4X Read Buffer T with deionized water.

SERUM OR PLASMA SAMPLES

Step 1: Add Diluent 2

Dispense 25 µL/well Diluent 2.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 30 minutes.

Step 2: Add Sample or Calibrator

Dispense 25 µL/well Calibrator or sample.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

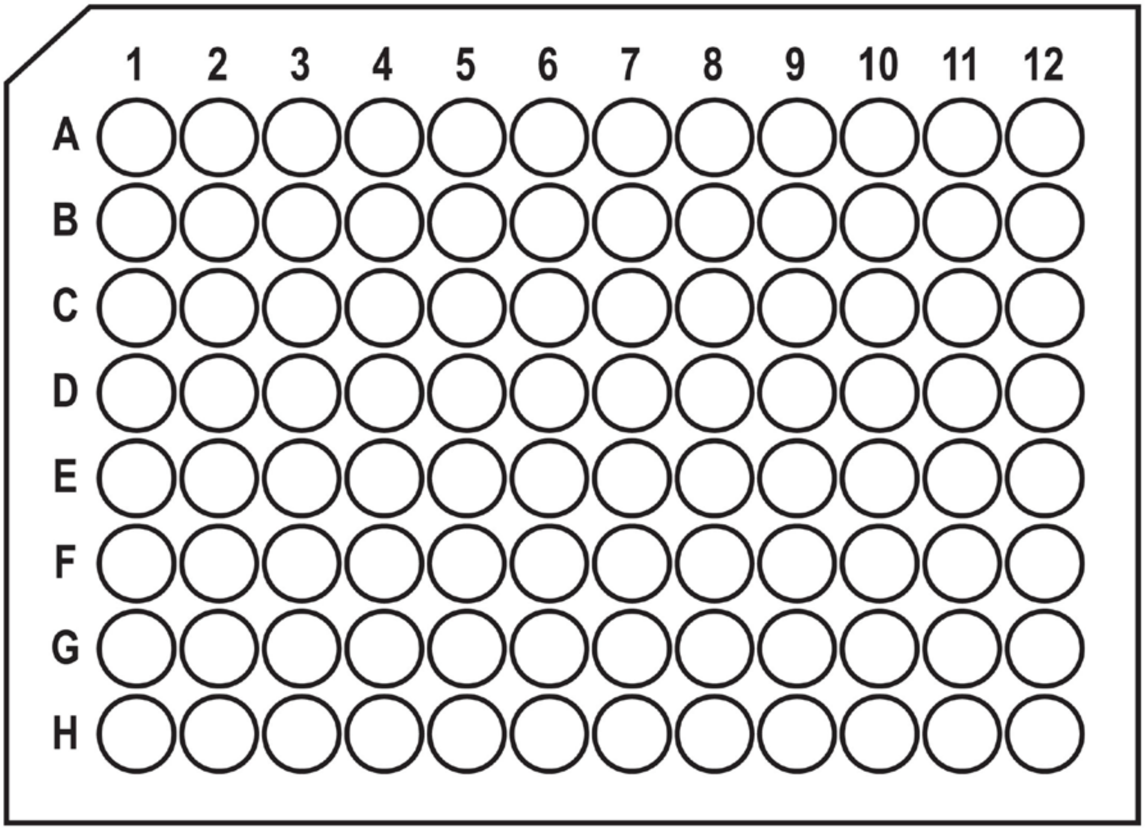
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 4: Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 µL/well 2X Read Buffer T.

Analyze plate on SECTOR Imager instrument.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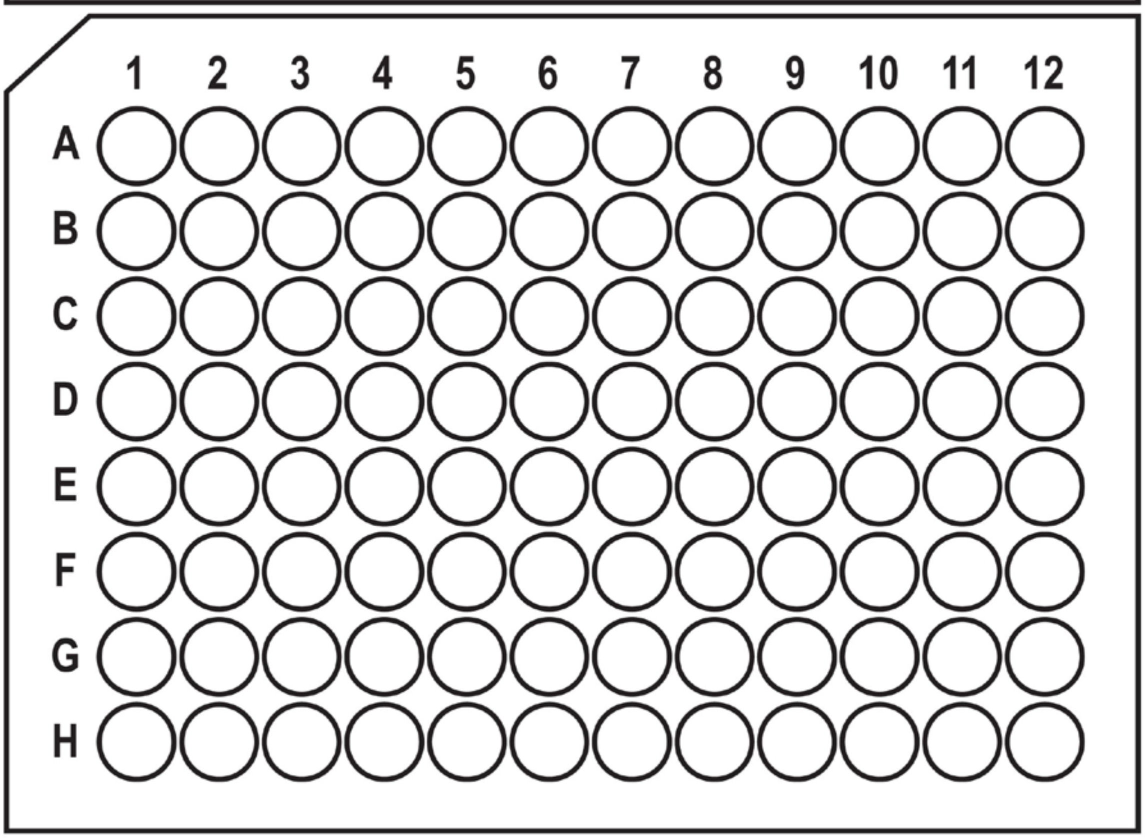
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