MSD® MULTI-SPOT Assay System

Human MIP-5 Kit

1-Plate Kit	K151RMD-1
5-Plate Kit	K151RMD-2
25-Plate Kit	K151RMD-4

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MSD Cytokine Assays

Human MIP-5 Kit

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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Ordering Information

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Introduction

Macrophage inflammatory protein 5 (MIP-5) (CCL15/NCC3/SCYA15) is a C-C chemokine attractant for neutrophils, monocytes, and lymphocytes.[1] MIP-5 is highly expressed in the liver, small intestine, and colon;[1,2] expressed to a lesser degree in the lungs;[2,3] and secreted by T and B lymphocytes, NK cells, monocytes, and monocyte-derived dendritic cells.[2] It primarily acts through CCR1 but can also bind CCR3.[1,2]

MIP-5 is implicated in asthma[2] and sarcoidosis,[3] where increased expression may be linked to disease progression and pathogenesis.[2,3] MIP-5 also shows promise as a potential blood biomarker for Alzheimer's disease in lieu of cerebrospinal fluid biomarkers, showing decreased monocyte levels and a corresponding increased plasma concentration in diseased and early symptom patients.[4] While the exact mechanism of action is unknown, research suggests abnormal cytokine and chemokine levels lead to improper and/or impaired regulation of immune cells, resulting in neuroinflammatory processes.[4] MIP-5 is also shown to be involved in atherosclerosis, as its presence stimulates the secretion of matrix metalloprotein-9, which is associated with atherosclerotic plaque destabilization and rupture.[5]

Principle of the Assay

MSD cytokine assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human MIP-5 is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG) 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 detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

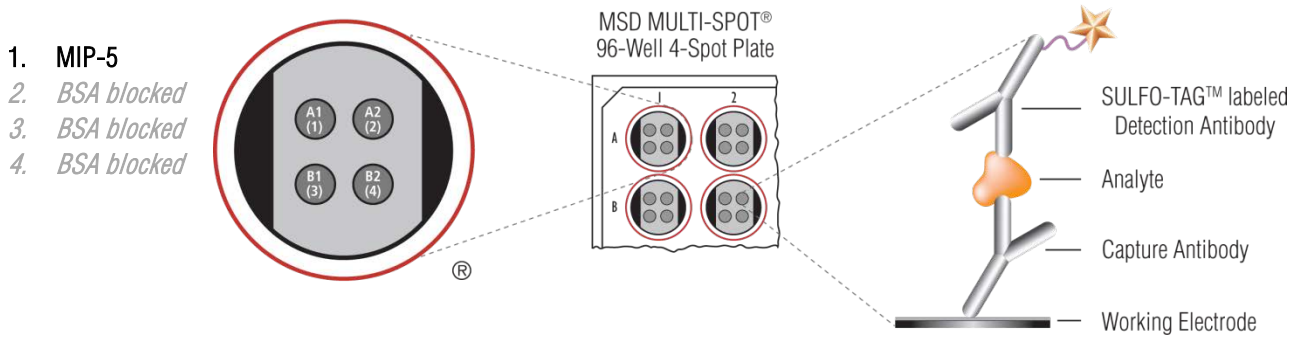


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K151RMD-1	K151RMD-2	K151RMD-4
MULTI-SPOT 96-Well 4-Spot Human MIP-5 Plate N451RMA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu MIP-5 Antibody[1] (50X) D21RM-2 (75 µL), D21RM-3 (375 µL)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human MIP-5 Calibrator (0.2 µg/mL) C01RM-2	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 43 R50AG-1 (10 mL), R50AG-2 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 3 R51BA-4 (5 mL), R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL ea)	5 bottles (25 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Material and Equipment (not supplied)

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

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines. Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service.

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

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 43 and Diluent 3 into aliquots appropriate for the size of your needs before refreezing.

Prepare Standards

MSD supplies calibrator for the Human MIP-5 Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator, then add to diluent at room temperature to make the standard curve solutions.

Standard	Human MIP-5 (pg/mL)	Dilution Factor
Stock Calibrator	200 000	
STD-01	10 000	20
STD-02	2500	4
STD-03	625	4
STD-04	156	4
STD-05	39	4
STD-06	9.8	4
STD-07	2.4	4
STD-08	0	n/a

To prepare 7 standard solutions plus a zero calibrator blank for up to 4 replicates:

1) Prepare the highest standard by adding 50 µL of stock calibrator to 950 µL of Diluent 43. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 300 µL of Diluent 43. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 43 as the blank.

Dilute Samples

Serum and Plasma

Plasma prepared in heparin tubes commonly displays additional clotting following thawing of the sample. Remove clots and all solid material by centrifugation. Avoid multiple freeze–thaw cycles for serum and plasma samples. Dilute serum and plasma samples at least 10-fold* in Diluent 43; however, you may adjust dilution factors for the sample set under investigation.

Tissue Culture

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. Dilute tissue culture supernatant samples at least 10-fold* in Diluent 43. Samples with extremely high levels of cytokines may require additional dilution.

* You may use PBS for your initial dilution step (i.e., 1:5) and then use Diluent 43 for the final 1:2 dilution.

Prepare Detection Antibody Solution

MSD provides each detection antibody in a 50X stock solution. The working detection antibody solution is 1X. Avoid exposing 1X detection antibody solution to light to prevent elevated background signals.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu MIP-5 Antibody
- ❑ 2940 µL of Diluent 3

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Notes

1. Add Sample or Calibrator: Add 50 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare detection antibody solution during incubation.

   Shaking the plate typically accelerates capture at the working electrode.

2. Wash and Add Detection Antibody Solution: Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare diluted read buffer during incubation.

   You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

3. Wash and Read: Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. No incubation in read buffer is required before reading the plate.

   Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

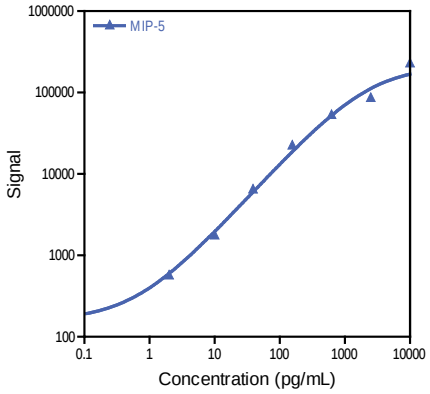
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the software uses 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 Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards.



MIP-5		
Conc. (pg/mL)	Average Signal	%CV
0	162	11.7
2.4	580	1.9
9.8	1796	6.3
39	6598	0.4
156	22 832	0.6
625	54 166	0.1
2500	87 797	6.6
10 000	233 040	3.6

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank).

	MIP-5
Average LLOD (pg/mL)	0.20

Dilutional Linearity

Freshly collected human blood was stimulated with LPS and co-stimulated with peptidoglycan and zymosan for two different lengths of time. The citrate plasma was then collected, and 4 plasma samples were used to assess the linearity of the Human MIP-5 assay. The 4 samples were diluted 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution was calculated by dividing the calculated concentration (dilution adjusted) by the expected concentration, i.e., the dilution-adjusted concentration of the previous dilution. The average percent recovery shown below is based on samples within the quantitative range of the assay.

% Recovery=measured/expected∗100

		MIP-5	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range
Citrate Plasma (N=4)	4	95	78–100
	8	92	80–139
	16	130	137–145

Spike Recovery

Normal human plasma samples were spiked with human MIP-5 calibrator at multiple levels throughout the range of the assay. The samples were then diluted 2-fold and tested for recovery. The average percent recovery shown below is based on samples within the quantitative range of the assay. % Recovery=measured/expected∗100

	MIP-5		
Sample Type	Spike Conc. (pg/mL)	Average %Recovery	% Recovery Range
Plasma (N=3)	990–1280	100	99–101
	460–490	88	83–92
	160–140	100	93–108

Specificity

To assess the specificity of the Human MIP-5 assay, the kit was tested with the following recombinant human proteins: fractalkine, 35 000 pg/mL; I-TAC, 1500 pg/mL; MCP-2, 250 pg/mL; MIP-3β, 275 pg/mL; and MIP-4, 100 pg/mL. Less than 0.1% non-specific binding was observed with each protein.

Assay Components

Calibrator

The assay calibrator uses recombinant human MIP-5, (residues 22–113), expressed in *E. coli*.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MDS Detection Antibody
MIP-5	Mouse Monoclonal	Goat Polyclonal

References

1. Richter R, et al. Quantum proteolytic activation of chemokine CCL15 by neutrophil granulocytes modulates mononuclear cell adhesiveness. J Immunol. 2005 Aug 1;175(3):1599-608.
2. Joubert P, et al. Expression and regulation of CCL15 by human airway smooth muscle cells. Clin Exp Allergy. 2011 Jan;42(1):85-94.
3. Kwon, SH, et al. Chemokine Lkn-1/CCL15 enhances matrix metalloproteinase-9 release from human macrophages and macrophage-derived foam cells. Nutr Res Pract. 2008;2(2);134-7.
4. Arakelyan A, et al. Protein levels of CC chemokine ligand (CCL)15, CCL16 and macrophage stimulating protein in patients with sarcoidosis. Clin Exp Immunol. 2009 Mar;155(3):457-65.
5. Hochstrasser T, et al. Two blood monocytic biomarkers (CCL15 and p21) combined with the mini-mental state examination discriminate Alzheimer's disease patients from healthy subjects. Dement Geriatr Cogn Dis Extra. 2011 Jan;1(1):297-309.

Summary Protocol

Human MIP-5 Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human MIP-5 assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare standard solutions using the supplied calibrator:
  - Dilute the stock calibrator 20-fold in Diluent 43.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples 10-fold in Diluent 43 before adding to the plate.
- ❑ Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 3.
- ❑ Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Sample

- ❑ Add 50 µL/well of sample (standards, controls, or unknowns).
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 2: Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3: Wash and Read Plate

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 150 µL/well of 2X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.

Plate Diagrams