MSD® MULTI-SPOT Assay System

Human MIP-3β Kit

1-Plate Kit	K151OWD-1
5-Plate Kit	K151OWD-2
25-Plate Kit	K151OWD-4

MSD Cytokine Assays

Human MIP-3β Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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Ordering Information

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Introduction

Macrophage inflammatory protein 3 beta (MIP-3β) (CCL19) is a homeostatic C-C chemokine associated with CCR7, of which CCL21 is also a ligand, expressed and secreted in abundance by stromal cells of the lymph nodes.[1,2] Its function is central to chemotactic migration of dendritic cells to the lymph nodes, orientation within the nodes, and activation of T cells once there.[1,3]

MIP-3β's chemotactic effect on lymphocytes induces a proinflammatory response in organs and areas of non-lymphoid origin that results in de novo formation of lymphoid tissue. This suggests a role in inflammatory disorders that include rheumatoid arthritis,[4] inflammatory bowel disease,[5] and atherosclerosis,[2] evidenced by increased expression and elevated levels of MIP-3β, its receptor CCR7, and related chemokine, CCL21.[1-5] Moreover, MIP-3β is thought to play a role in HIV infection, with MIP-3β serum levels positively correlated with HIV progression.[6,7] The proposed mechanism of action creates a detrimental pathogenic feedback loop wherein HIV infection increases the presence of MIP-3β and CCL21, leading to inappropriate inflammation that further promotes HIV replication in activated T cells.[6,7] This phenomenon is observed independent of highly active anti-retroviral therapy.[7]

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-3β 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 a SECTOR® Imager 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.

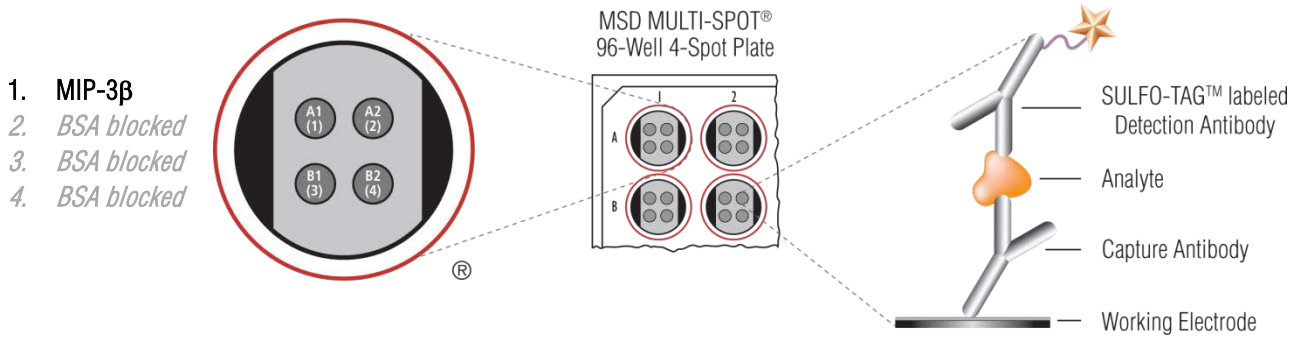


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K1510WD-1	K1510WD-2	K1510WD-4
MULTI-SPOT 96-Well 4-Spot Human MIP-3β Plate N4510WA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu MIP-3β Antibody[1] (50X) D210W-2 (75 µL), D210W-3 (375 µL)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human MIP-3β Calibrator (0.2 µg/mL) C010W-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 safety information is available in the product Material Safety Data Sheet, 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-3β 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. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator, then add to diluent at room temperature to make the standard curve solutions.

Standard	Human MIP-3β (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 2-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 2-fold in Diluent 43. Samples with extremely high levels of cytokines may require additional 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-3β 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 the SECTOR Imager. 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.

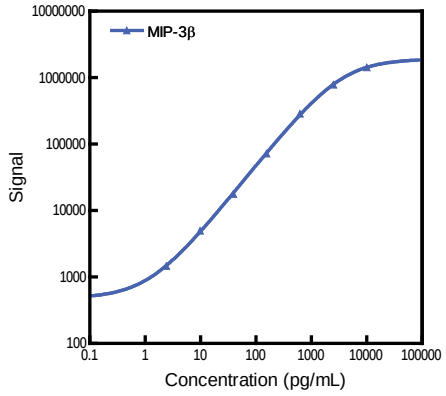
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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-3β		
Conc. (pg/mL)	Average Signal	%CV
0	347	2.4
2.4	1466	0.6
9.8	4976	1.6
39	17 747	5.5
156	71 978	2.8
625	285 532	6.6
2500	782 451	1.1
10 000	1 425 088	5.2

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-3β
Average LLOD (pg/mL)	0.090

Specificity

To assess specificity of the MIP-3β 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-4, 100 pg/mL; and MIP-5, 1200 pg/mL. Less than 0.1% non-specific binding was observed with each protein.

Assay Components

Calibrator

The assay calibrator uses recombinant human MIP-3β, (residues 22–98), expressed in *E.coli*.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MDS Detection Antibody
MIP-3β	Goat Polyclonal	Goat Polyclonal

References

1. Haessler U, et al. Dendritic cell chemotaxis in 3D under defined chemokine gradients reveals differential response to ligands CCL21 and CCL19. Proc Natl Acad Sci U S A. 2011 Apr 5;108(14):5614-9.
2. Damas JK, et al. Enhanced expression of the homeostatic chemokines CCL19 and CCL21 in clinical and experimental atherosclerosis: possible pathogenic roles in plaque destabilization. Arterioscler Thromb Vasc Biol. 2007 Mar;27(3):614-20.
3. Muthuswamy R, et al. PGE2 transiently enhances DC expression of CCR7 but inhibits the ability of DCs to produce CCL19 and attract naïve T cells. Blood. 2010 Sep 2;116(9):1454-9.
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5. Middel P, et al. Increased number of mature dendritic cells in Crohn's disease: evidence for a chemokine-mediated retention mechanism. Gut. 2006 Feb;55(2):220-7.
6. Damas JK, et al. Enhanced levels of the CCR7 ligands CCL19 and CCL21 in HIV infection: correlation with viral load, disease progression and response to highly active antiretroviral therapy. AIDS. 2009 Jan 2;23(1):135-8.
7. Damas JK, et al. Homeostatic chemokines CCL19 and CCL21 promote inflammation in human immunodeficiency virus-infected patients with ongoing viral replication. Clin Exp Immunol. 2009 Sep;157(3):400-7.

Summary Protocol

Human MIP-3β Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human MIP-3β 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 2-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 SECTOR Imager.

Plate Diagrams