MSD® MULTI-SPOT Assay System

Mouse MIP-3α Kit

1-Plate Kit	K152MSD-1
5-Plate Kit	K152MSD-2
25-Plate Kit	K152MSD-4

MSD Cytokine Assays

Mouse MIP-3α 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®
A division of Meso Scale Diagnostics, LLC.
1601 Research Boulevard
Rockville, MD 20850-3173 USA
www.mesoscale.com

MESO SCALE DISCOVERY, MESO SCALE DIAGNOSTICS, MSD, MSD GOLD, DISCOVERY WORKBENCH, MULTI-ARRAY, MULTI-SPOT, QUICKPLEX, SECTOR, SECTOR PR, SECTOR HTS, SULFO-TAG, U-PLEX, S-PLEX, V PLEX, STREPTAVIDIN GOLD, MESO, www.mesoscale.com, SMALL SPOT (design), 96 WELL 1, 4, 7, & 10-SPOT (designs), 384 WELL 1 & 4-SPOT (designs), MSD (design), U-PLEX (design), S-PLEX (design), V-PLEX (design), and SPOT THE DIFFERENCE are trademarks and/or service marks of Meso Scale Diagnostics, LLC. © 2012, 2015 Meso Scale Diagnostics, LLC. All rights reserved.

Table of Contents

Introduction 4
Principle of the Assay 4
Reagents Supplied 5
Required Material and Equipment (not supplied) 5
Safety 5
Reagent Preparation 6
Protocol 8
Curve Fitting 8
Typical Data 9
Sensitivity 9
Specificity 9
Assay Components 10
References 10
Summary Protocol 11
Plate Diagrams 13

Ordering Information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

Macrophage inflammatory protein 3 alpha (MIP-3α) (LARC/CCL20) is a C-C chemokine with inflammatory and homeostatic functions.[1,2] Initially identified in the liver, it is expressed in lymphatic tissue, lung tissue, macrophages, dendritic cells, B- and T-lymphocytes, and eosinophilic granulocytes as well as in normal colon, pancreas, prostate, uterine cervix, and skin.[2] To date, MIP-3α is the only known ligand for the CCR6 receptor; it is also chemotactic for CCR6$^+$ cells such as Th17.[1-3]

MIP-3α is implicated in a broad spectrum of disorders, including colorectal cancer and tumor metastasis,[1] rheumatoid arthritis,[3] psoriasis,[4] obesity,[5] and wound healing.[6] Disrupting the MIP-3α and CCR6 interaction may ultimately prove to be a viable therapeutic strategy,[4,7] as CCR6 deletion resulted in reduced atherosclerotic lesion area and reduced macrophage presence at atherosclerotic plaque sites in models of atherogenesis.[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. Mouse MIP-3α 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.

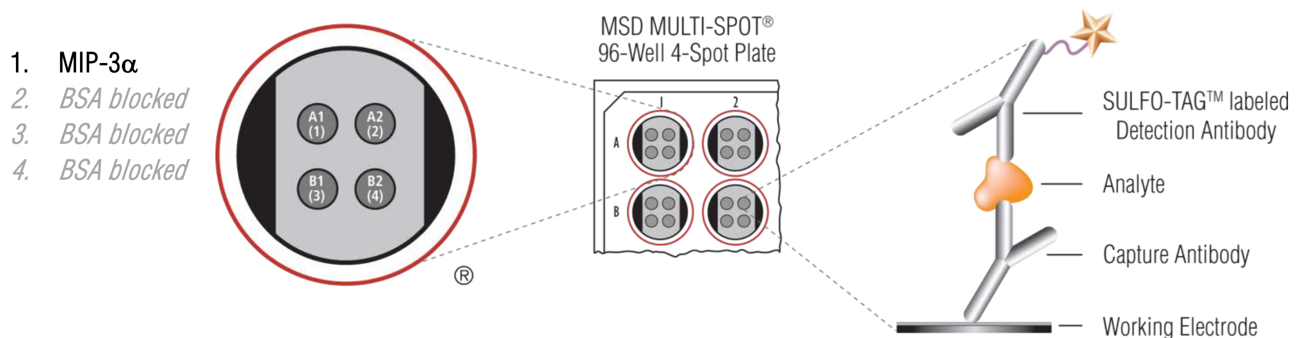


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K152MSD-1	K152MSD-2	K152MSD-4
MULTI-SPOT 96-Well 4-Spot Mouse MIP-3α Plate N452MSA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-ms MIP-3α Antibody[1] (50X) D22MS-2 (75 µL), D22MS-3 (375 µL)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Mouse MIP-3α Calibrator (0.05 µg/mL) C02MS-2	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 41 R50AH-1 (10 mL), R50AH-2 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 45 R50AI-1 (5 mL), R50AI-2 (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 41 and Diluent 45 into aliquots appropriate for the size of your needs before refreezing.

Prepare Standards

MSD supplies calibrator for the Mouse MIP-3α 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	Mouse MIP-3α(pg/mL)	Dilution Factor
Stock Calibrator	50 000	
STD-01	2500	20
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 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 41. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 300 µL of Diluent 41 Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 41 as the blank.

Sample Collection and Handling

If available, published guidelines for mouse sample collection, storage, and handling should be used.[8,9] Note, however, that sample stability under such guidelines has not been evaluated.

When preparing serum, allow samples to clot for two hours at room temperature. Centrifuge both serum and plasma for 20 minutes at 2000 x g prior to use. After centrifugation, test samples immediately or freeze in alliquots and store at ≤-20ºC. Avoid freezing and thawing samples more than once. Centrifuge thawed samples at 2000 x g for 3 minutes to remove particulates prior to use.

Dilute Samples

For mouse serum and plasma samples, MSD recommends 2-fold dilution in Diluent 41; however, you may need to adjust the dilution factor for the sample set under investigation. Sample volume may be conserved by using a higher 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-ms MIP-3α Antibody
- ❑ 2940 µL of Diluent 45

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.

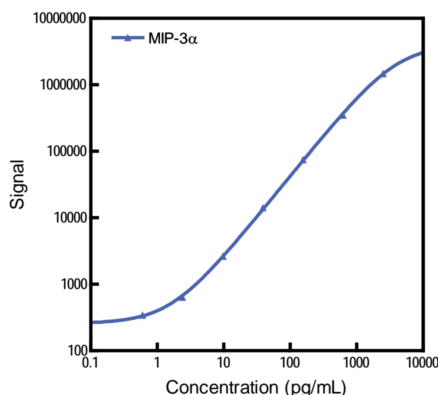
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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-3α		
Conc. (pg/mL)	Average Signal	%CV
0	225	6.9
0.61	343	6.7
2.4	639	2.6
9.8	2634	3.5
39	14 134	2.1
156	74 901	1.3
625	348 338	3.4
2500	1 470 863	2.2

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-3α
Average LLOD (pg/mL)	0.33

Specificity

To assess specificity of the MIP-3α assay, the kit was tested with the following recombinant mouse proteins: IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, KC, IL-10, IL-12, TNFα, IL-23, GM-CSF, IL-13, VEGF, RANTES, TNF-RI, TNF-RII, IL-12/IL-23 p40, and MCP-1 at 1250 pg/mL. Less than 0.1% non-specific binding was observed with each protein.

Assay Components

Calibrator

The assay calibrator uses recombinant mouse MIP-3α, (residues 27–96), expressed in *E.coli*.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MDS Detection Antibody
MIP-3α	Rat Monoclonal	Rat Monoclonal

References

1. Schutyser E, et al. Regulated production and molecular diversity of human liver and activation-regulated chemokine/macrophage inflammatory protein-3 alpha from normal and transformed cells. J Immunol. 2000 Oct 15;165(8):4470-7.
2. Ghadjar P, et al. The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. Int J Cancer. 2009 Aug 15;125(4):741-5.
3. Hirota K, et al. Preferential recruitment of CCR-6 expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. J Exp Med. 2007 Nov 26;204(12):2803-12.
4. Harper EG, et al. Th17 cytokines stimulate CCL20 expression in keratinocytes *in vitro* and *in vivo*: implications for psoriasis pathogenesis. J Invest Dermatol. 2009 Sep;129(9):2175-83.
5. Duffant C, et al. Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. Arterioscler, Thromb, Vasc Biol. 2009 Oct;29(10):1608-14.
6. Kennedy-Crispin M, et al. Human keratinocytes' response to injury upregulates CCL20 and other genes linking anniate and adaptive immunity. J Invest Dermatol. 2012 Jan;132(1):105-13.
7. Wan W, et al. Genetic deletion of chemokine receptor Ccr6 decreases atherogenesis in ApoE-deficient mice. Circ Res. 2011 Aug 5;109(4):374-81.
8. Hem A, et al. Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, ferret and mink. Lab Anim. 1998 Oct;32(4):364-8.
9. Removal of blood from laboratory animals and birds: First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement. Lab Animal 1993 Jan;27(1):1-22.

Summary Protocol

Mouse MIP-3α Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Mouse MIP-3α 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 41.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 2-fold in Diluent 41 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 45.

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