MSD MULTI-ARRAY® Assay System

Human MIF Kit

1-Plate Kit	K151LVD-1
5-Plate Kit	K151LVD-2
25-Plate Kit	K151LVD-4

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MSD® Cytokine Assays

Human MIF 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 Blvd.
Rockville, MD 20850 USA
www.mesoscale.com

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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 Migration Inhibitory Factor (MIF) is a 12 kDa, pleiotropic protein, involved in both innate and adaptive immune responses. The protein was one of the first cytokines discovered through its role in delayed type sensitivity and regulation of macrophage action against bacterial pathogens.[1] MIF is ubiquitously expressed in tissues such as the anterior pituitary, gut and kidney epithelia, skin, and in neuronal and non-neuronal cells of the brain. MIF has been shown to be a key regulator of acute and chronic inflammatory conditions including rheumatoid arthritis (RA), atherosclerosis, and systemic lupus erythematosus (SLE).[2,3]

MIF displays a unique regulatory role in its ability to sustain inflammatory responses despite the presence of endogenous or exogenous glucocorticoids.[1,3] Once released, it "overrides" or counter-regulates the immunosuppressive effects of steroids on immune cell activation and cytokine production. This property makes MIF an attractive therapeutic target for autoimmune and inflammatory diseases.[2,4]

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. The Human MIF Assay 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 intensity of emitted light to provide a quantitative measure of analytes in the sample.

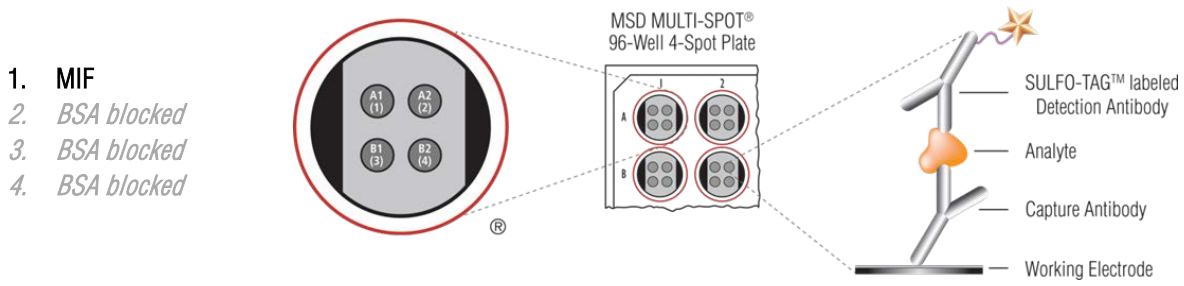


Figure 1. *Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K151LVD-1	K151LVD-2	K151LVD-4
MULTI-SPOT 96-Well 4-Spot Human MIF Plate N451LVA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu MIF Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human MIF Calibrator (1.5 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 10 R55BB-5 (10 mL), R55BB-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 11 R55BA-4 (5 mL), R55BA-3 (50 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 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 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

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.

[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 10 and Diluent 11 into aliquots appropriate to the size of your assay needs. These diluents can go through 3 freeze–thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Standards

MSD recommends an 8-point standard curve with 4-fold serial dilution steps and a zero calibrator. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	MIF Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	1 500 000	
STD-01	15 000	100
STD-02	3750	4
STD-03	938	4
STD-04	234	4
STD-05	59	4
STD-06	15	4
STD-07	3.7	4
STD-08	0	n/a

To prepare 8 standard solutions for up to 3 replicates:

1) Prepare the highest standard by adding 10 µL of the stock calibrator to 990 µL of Diluent 10. Mix well.
2) Prepare the next standard by transferring 75 µL of the highest standard to 225 µL of Diluent 10. Mix Well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 10 as the 8th standard (i.e. zero calibrator).

Notes

a. Alternatively, standards may be prepared in the sample matrix or diluent of choice. 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.
b. You may modify the standard solutions as necessary to meet specific assay requirements.

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 10.

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. Samples with extremely high levels of cytokines may require dilution. Dilute tissue culture supernatant samples at least 2-fold in Diluent 10.

Other Matrices

For information on preparing samples in other matrices, such as sputum, CSF, and tissue homogenates, contact MSD Scientific Support at 1-301-947-2025 or ScientificSupport@mesoscale.com

Prepare Detection Antibody Solution

MSD provides detection antibody in a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu MIF Antibody
- ❑ 2.94 mL of Diluent 11

Prepare Read Buffer

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

For 1 plate, combine:

- ❑ 10 mL Read Buffer T (4X)
- ❑ 10 mL 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 (see 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.

Assay Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Sample or Calibrator:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 50 µL of calibrator or diluted sample 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.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of 1X detection antibody solution into each well 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.

   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.

4. **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 of the MSD plate. 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.

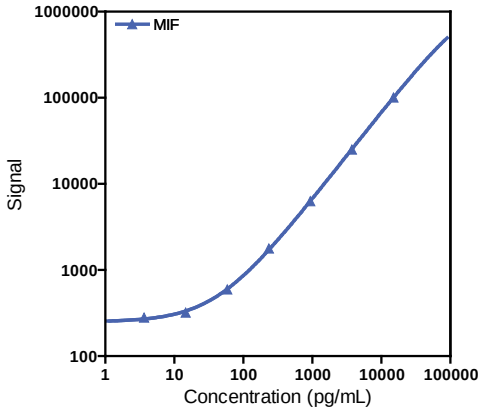
   Due to the varying nature of each research application, you should assess stability before allowing plates to sit with read buffer for extended periods.

Analysis of Results

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate a 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) which allows accurate quantification without the need for dilution in many cases. 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 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.



	MIF	
Conc. (pg/mL)	Average Signal	%CV
0	218	2.4
3.7	281	19.4
15	320	1.6
59	595	1.3
234	1779	1.2
938	6294	6.0
3750	24 997	5.8
15 000	100 775	4.8

Sensitivity

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

	MIF
LLOD (pg/mL)	2.4

Assay Components

Calibrator

The assay calibrator uses recombinant human MIF protein expressed in *E. coli.*

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
MIF	Mouse Monoclonal	Goat Polyclonal

References

1. Calandra T & Roger T. Macrophage migration inhibitory factor: a regulator if innate immunity. Nature reviews Immunology. 2003 Oct;3:791-800.
2. Santos LL, Morand EF. Macrophage migration inhibitory factor: a key cytokine in RA, SLE and atherosclerosis. Clin Chim Acta. 2009 Jan;399(1-2): 1-7.
3. Flaster H, Bernhagen J, Calandra T, Bucala R. The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. Mol Endocrinol. 2007 Jun;21(6):1267-80.
4. Kleemann R, Bucala R. Macrophage migration inhibitory factor: critical role in obesity, insulin resistance, and associated comorbidities. Mediators Inflamm. 2010 2010:610479.

Summary Protocol

MSD 96-well Human MIF Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human MIF assay.

Sample and Reagent Preparation

Bring all reagents to room temperature, and thaw the calibrator on ice.

Prepare Blocker A solution.

Prepare 8 standard solutions using the supplied calibrator as described in the "Prepare Standards" section.

Dilute samples 2-fold in Diluent 10 before adding to the plate.

Prepare detection antibody solution by diluting 50X detection antibody 50-fold in Diluent 11.

Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2: Wash and Add Sample or Calibrator

Wash plate 3 times with 300 µL/well of PBS-T.

Add 50 µL/well of calibrator or diluted 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 300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

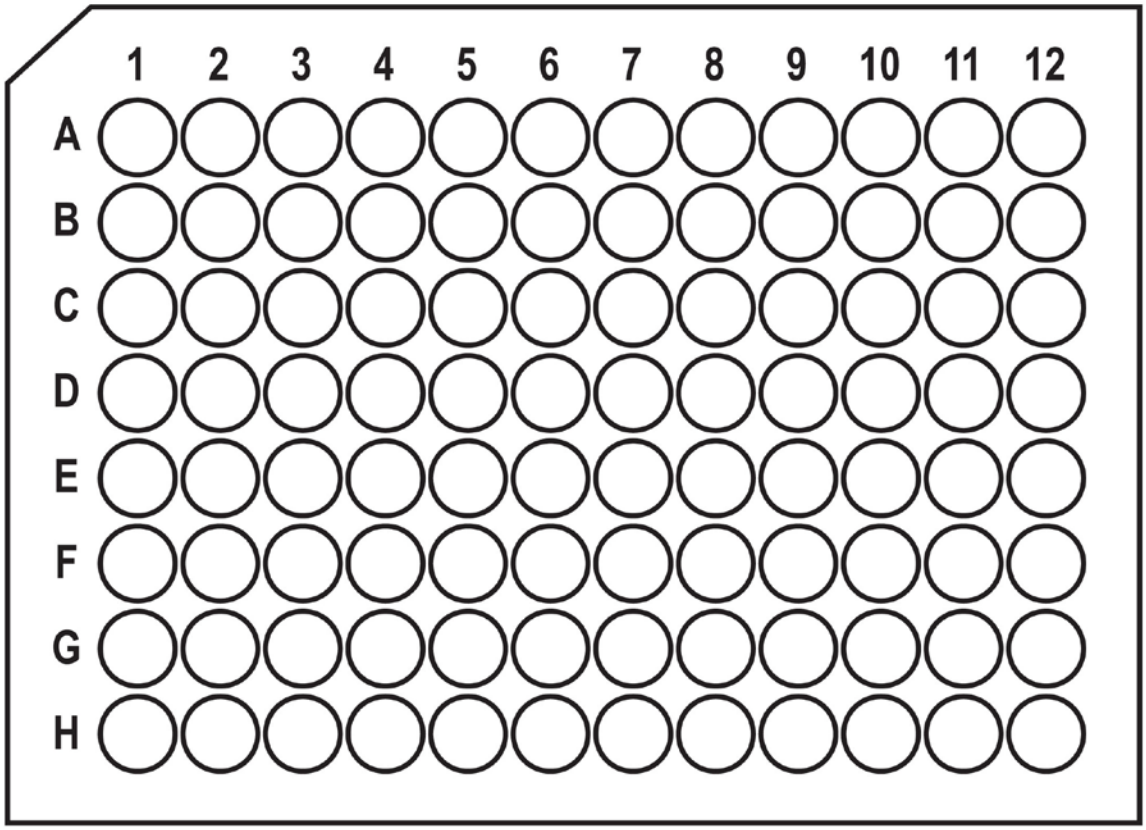
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4: 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.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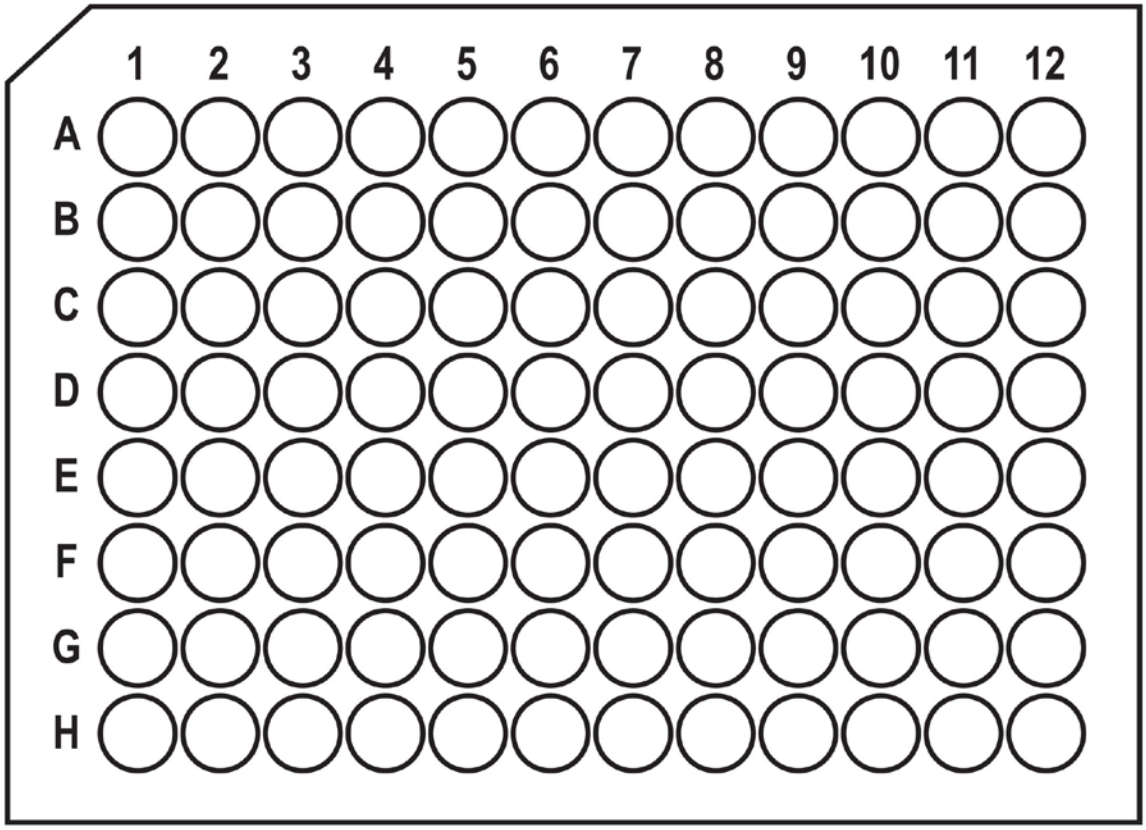
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