Meso Scale Discovery®

MULTI-ARRAY® Assay System

Mouse Cytokine Assay
Ultra-Sensitive Kit

Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Di

MSD MULTI-ARRAY Assay

Ultra-Sensitive Kit

Mouse Cytokine Assay

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.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

Table of Contents

table of contents

Ordering Information

Ordering information

MSD Customer Service

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

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

Introduction

introduction

Cytokines are a category of signaling molecules that interact with cells in order to regulate the body's response to both normal and pathological conditions.[1] They are soluble proteins, peptides or glycoproteins that mediate both acute and chronic inflammatory responses. Most cytokines have both anti-inflammatory and pro-inflammatory functions.[2] Cytokines are also named according to the type of cell that produces it or its function. Lymphokines are produced from lymphocytes, monokines are generated from monocytes, chemokines have chemotactic activities whereas interleukins are made by one leukocyte and act on other leukocytes.[1,3,4] Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action).[5,6] Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines.

Cytokines typically bind to their specific receptors and regulate cell activation, hematopoiesis, apoptosis, cell migration, and cell proliferation.[7-9] Thus, they are involved in almost all aspects of innate and adaptive immune response. Structurally, cytokines have been classified into different types:

1. Four α-helix bundle family – This has three sub-families, the IL-2 sub-family, the interferon sub-family and the IL-10 sub-family
2. The IL-1 family which includes IL-1 and IL-18
3. The IL-17 family, which has not yet been completely characterized

Functionally, cytokines have been classified into two categories: type 1 cytokines which enhance cytokine responses and type 2 cytokines, which affect antibody responses.[10]

The cytokine receptors have been divided into several families based on their structure and functions.[11,12] Hematopoietin family receptors are dimers or trimers with conserved cysteines in their extracellular domains. Additionally, they also contain a conserved Trp-Ser-X-Trp-Ser sequence. Interferon family receptors have the conserved cysteine residues but not the Trp-Ser-X-Trp-Ser sequence. This family includes the receptors for IFNα, IFNβ, and IFNγ. Tumor Necrosis Factor family receptors have four extracellular domains. The receptors for soluble TNFα and TNFβ as well as membrane-bound CD40 and Fas belong to this family. Chemokine family receptors have seven transmembrane helices and interact with G protein.[13] Examples in this family are the receptors for IL-8, MIP-1 and RANTES.

Principle of the Assay

principle of the assay

MSD® Cytokine Assays measure one to ten cytokines in a 96-well MULTI-ARRAY or MULTI-SPOT® plate. The assays employ a sandwich immunoassay format where capture antibodies are coated in a single spot, or in a patterned array, on the bottom of the wells of a MULTI-ARRAY or MULTI-SPOT (Figure 1) plate. Cytokine assays are available from MSD in 1-spot MULTI-ARRAY and 4-, 7-, and 10-spot MULTI-SPOT 96-well plate formats. The user adds the sample and a solution containing the labeled detection antibody— anti-cytokine antibody labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. The cytokine in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound cytokine 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 provide a quantitative measure of cytokine present in the sample.

This product insert outlines the Mouse Ultra-Sensitive Cytokine Assay protocol that can be used to measure analytes in different matrices such as cell culture media, serum, plasma and other biological fluids. MSD cytokine assays may be provided on SMALL-SPOT or MULTI-SPOT plates. The different plate geometries are shown below.

Small Spot **4-Spot** **7-Spot** **10-Spot**

Figure 1. *The above diagram shows the different spot orientations of MSD Cytokine Assays. 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.*

III Reagents Supplied

reagents supplied

Product Description	Storage
MULTI-ARRAY® or MULTI-SPOT® 96-well Mouse Cytokine Plate(s)	2–8°C
SULFO-TAG™ Detection Antibody[1] (50X)	2–8°C
Cytokine Calibrators (1 µg/mL)	≤-70°C
Diluent 4 R52BB-4 (8 mL) R52BB-3 (40 mL)	≤-10°C
Diluent 5 R52BA-4 (5 mL) R52BA-5 (25 mL)	≤-10°C
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT

IV Required Materials and Equipment - not supplied

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

V Safety

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] Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

VI Reagent Preparation

reagent preparation

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

Important: Upon first thaw, separate Diluent 4 and Diluent 5 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

Dilute Calibrators in Diluent 4. MSD recommends the preparation of an 8-point standard curve consisting of at least 2 replicates for each point. Each well requires 25 µL of Calibrator. For the assay, MSD recommends 4-fold serial dilution steps and Diluent 4 alone for the 8th point.

Standard	Concentration (pg/mL)	Dilution Factor
100X Stock	1000000	
STD-01	10000	100
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 this 8-point standard curve for up to 4 replicates:

1) Prepare the highest Calibrator point (STD-01) by transferring 10 µL of the Mouse Cytokine stock Calibrator to 990 µL Diluent 4.
2) Prepare the next Calibrator by transferring 50 µL of the STD-01 to 150 µL Diluent 4. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Diluent 4 (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. Serum and plasma samples may be run neat in the MSD Mouse Cytokine Assay.

Tissue Culture

Tissue culture supernatant samples may not require dilution prior to being used in the MSD Mouse Cytokine 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 is provided at 50X stock concentration. 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 into 2.94 mL of Diluent 5.

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 specific analyte. 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.

VII Assay Protocol

assay protocol

1. **Addition of Diluent 4:** Dispense 25 µL of Diluent 4 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.
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.

Notes

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

Lower sample volumes such as 10 µL are feasible, but it may reduce assay sensitivity. If lower sample volume is utilized, then the volume of the Calibrators / Standard Curve should be adjusted in parallel.

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

VIII Analysis of Results

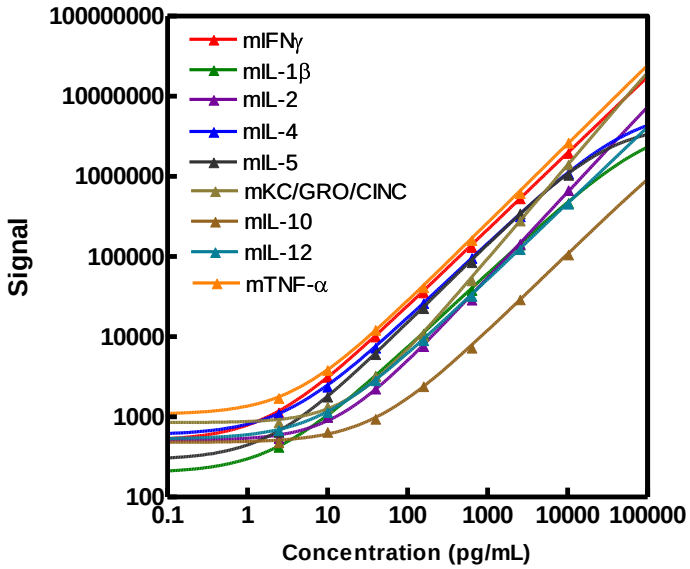
analysis of results

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.

IX Typical Standard Curve

typical standard curve

The following standard curves demonstrate the wide dynamic range (3-4 logs) of the Mouse TH1/TH2 9-Plex Ultra-Sensitive Kit. 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.



X Sensitivity

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 value below represents the average LLOD over multiple kit lots.

Analyte	LLOD (pg/mL)
Mouse IFN-γ	0.47
Mouse IL-1β	2.1
Mouse IL-2	3.0
Mouse IL-4	0.87
Mouse IL-5	0.70
Mouse KC/GRO/CINC	2.9
Mouse IL-10	11
Mouse IL-12 Total	5.3
Mouse TNF-α	1.0

XI References

references

1. Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol. 2003 Feb;111:S460-75.
2. Elenkov IJ, Iezzoni DG, Daly A, Harris AG, Chrousos GP. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation. 2005;12(5):255-69.
3. Dinarello, C. A. The interleukin-1 family: 10 years of discovery. FASEB J. 1994; 8: 1314–1325.
4. Nelson DS, Geczy CL. Lymphokines, monokines, and other cytokines. Aust N Z J Med. 1985;15(2):285-90.
5. Hsu SM, Hsu PL. Autocrine and paracrine functions of cytokines in malignant lymphomas. Biomed Pharmacother. 1994; 48(10):433-44.
6. Rothwell NJ. The endocrine significance of cytokines. J Endocrinol. 1991; 128(2):171-3.
7. Kong J, Grando SA, Li YC. Regulation of IL-1 family cytokines IL-1alpha, IL-1 receptor antagonist, and IL-18 by 1,25-dihydroxyvitamin D3 in primary keratinocytes. J Immunol. 2006; 76(6):3780-7.
8. Malek TR, Yu A, Zhu L, Matsutani T, Adeegbe D, Bayer AL. IL-2 family of cytokines in T regulatory cell development and homeostasis. J Clin Immunol. 2008; 28(6):635-9.
9. Kawaguchi M, Adachi M, Oda N, Kokubu F, Huang SK. IL-17 cytokine family. J Allergy Clin Immunol. 2004; 114(6):1265-73.
10. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev. 1996; 9(4):532-62.
11. Ihle JN. Cytokine receptor signaling. Nature. 1995; 377(6550):591-4.
12. Leonard WJ, Lin JX. Cytokine receptor signaling pathways. J Allergy Clin Immunol. 2000; 105(5):877-88.
13. Rossi, D., Zlotnik, A. The biology of chemokines and their receptors. Annu Rev Immunol. 2000; 18:217-42.

MSD 96-well MULTI-ARRAY and MULTI-SPOT Mouse Cytokine Assays: Ultra-Sensitive Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Mouse Cytokine Assay.

Step 1: Sample and Reagent Preparation

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

Samples may not require dilution prior to use in this assay.

Prepare Calibrator solutions and standard curve *(if applicable).*

Use the 1 µg/mL Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 4.

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

Prepare Detection Antibody Solution by diluting Detection Antibody to 1X in a final volume of 3.0 mL Diluent 5 per plate.

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

SERUM OR PLASMA SAMPLES

Step 2: Add Diluent 4

Dispense 25 µL/well Diluent 4.

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

Step 3: 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 4: Wash and Add Detection Antibody Solution

Wash plate 3 times with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

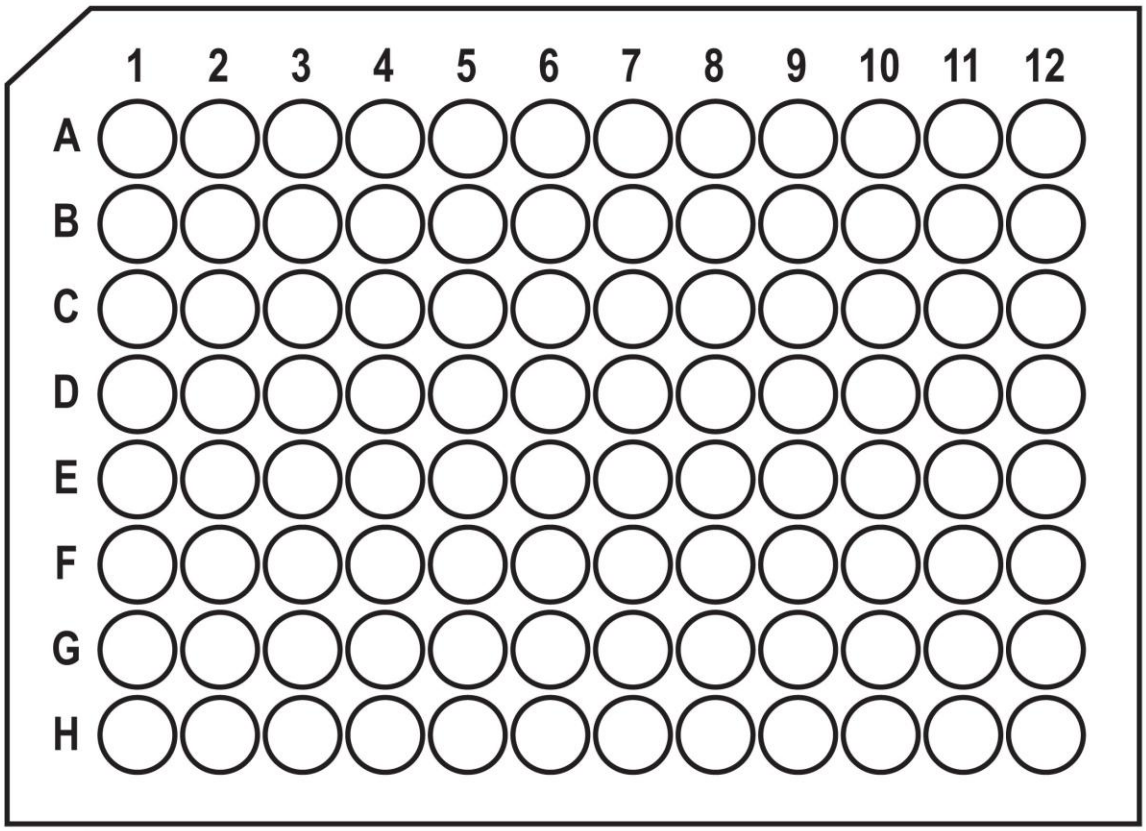
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

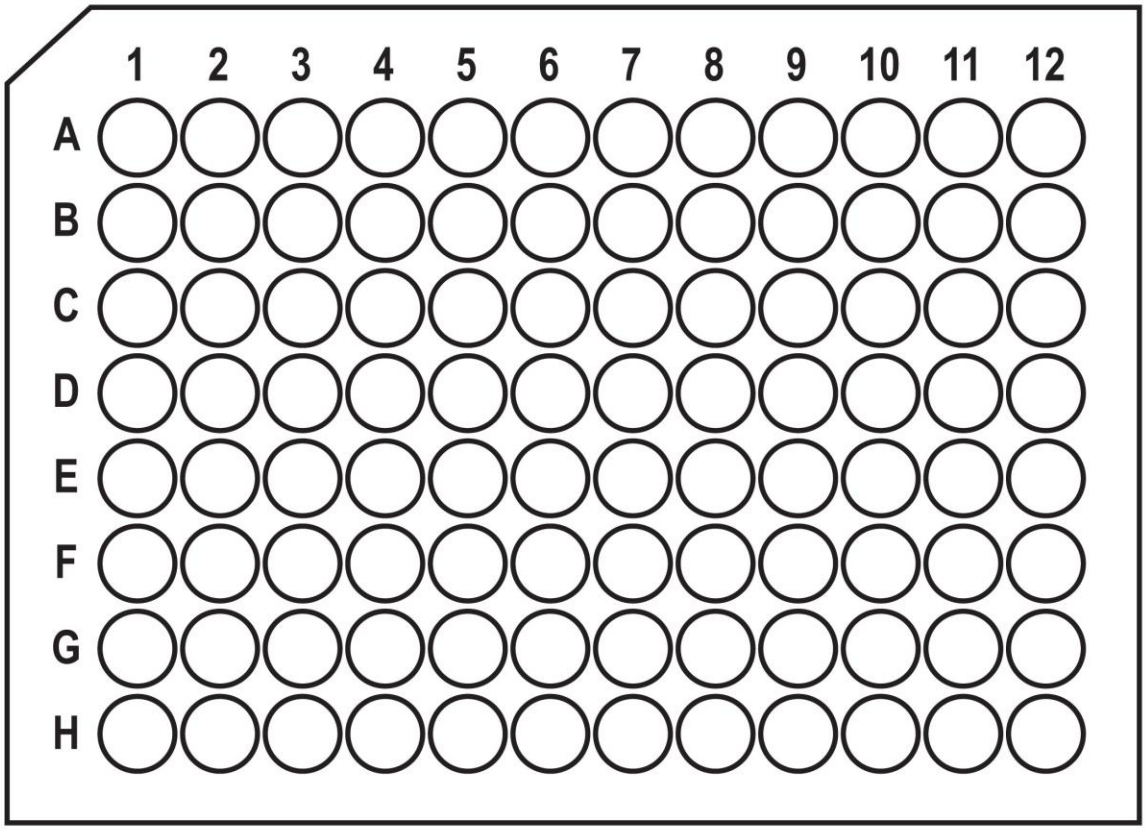
Step 5: Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 µL/well 2X Read Buffer T.

Analyze plate on SECTOR Imager instrument.


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H