MSD® MULTI-SPOT Assay System

Mouse IL-23 Kit

1-Plate Kit	K152LKD-1
5-Plate Kit	K152LKD-2
25-Plate Kit	K152LKD-4

MSD Cytokine Assays

Mouse IL-23 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

Interleukin-23 (IL-23) is a heterodimeric cytokine that is structurally and functionally related to IL-12. Both share the 40 kDa (p40) subunit of IL-12, and this forms a disulfide link with a 19 kDa (p19) subunit to make the active IL-23 molecule.[1] IL-23 is produced by activated macrophages and dendritic cells in response to certain bacterial and viral pathogens and may play a key role in sustaining inflammatory responses that link innate and adaptive immunity.[2] The receptor of IL-23 is formed by the beta 1 subunit of IL-12 (IL-12RB1) and an IL-23 specific subunit, IL-23R. Similar to IL-12 action, IL-23 mediates the early inflammatory response to infection by activating STAT4 and increases interferon-γ (IFN-γ) production in limited amounts.[1-3]

The main role of IL-23 involves the differentiation of TH17 cells, a novel subset of $CD4^+$ memory T cells, and their stimulation to produce the cytokine, IL-17. IL-17 enhances T cell priming and stimulates the production of additional proinflammatory cytokines such as IL-1, IL-6, TNF-α, and chemokines, thus sustaining the ongoing inflammatory response.[4] Elevated levels of IL-23 and IL-17 have been consistently observed in autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, and diabetes. Chronic over-expression of IL-23 may also play a role in angiogenesis and increased tumor growth and is often lethal in several mouse models of chronic inflammatory disease.[2-4] Taken together, these actions suggest that IL-23 is a key player in sustaining cellular immunity by promoting the survival and effector cytokine production of TH1 memory cells.

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 IL-23 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.

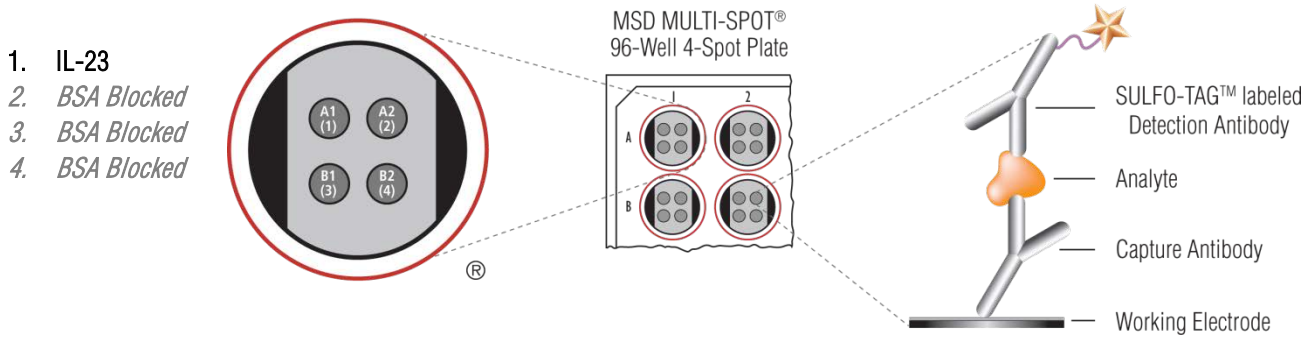


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K152LKD-1	K152LKD-2	K152LKD-4
MULTI-SPOT 96-Well 4-Spot Mouse IL-23 Plate N452LKA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-ms IL-23 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Mouse IL-23 Calibrator (100X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 4 R52BB-4(8 mL), R52BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Diluent 5 R52BA-4(5 mL), R52BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL ea)	5 bottles (25 mL ea)
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer) R93AA-2 (250 mL)	RT	1 kit (250 mL)	1 kit (250 mL)	5 kits (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 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

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

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.

Reagent Preparation

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

> Important: Upon first thaw, separate Diluent 4 and Diluent 5 into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Standards

MSD supplies calibrator for the Mouse IL-23 Kit at 100-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 and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

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

1) Prepare the highest standard by adding 10 µL of stock calibrator to 990 µL of Diluent 4. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 300 µL of Diluent 4. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 4 as the blank.

Dilute Samples

For mouse serum and plasma samples, MSD recommends 2-fold dilution in Diluent 4; however, you may adjust dilution factors for the sample set under investigation. To dilute sample 2-fold, add 100 µL of sample to 100 µL of Diluent 4.

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-ms IL-23 Antibody
- ❑ 2940 µL of Diluent 5

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 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 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.

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. 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.

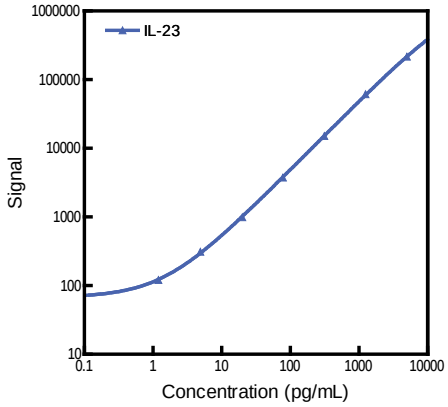
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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.



IL-23		
Conc. (pg/mL)	Average Signal	%CV
0	64	12.2
1.2	121	0.0
4.9	312	7.0
20	991	1.3
78	3739	2.5
313	15 078	2.7
1250	61 161	1.5
5 000	215 576	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).

	IL-23
Average LLOD (pg/mL)	0.78

Assay Components

Calibrator

The assay calibrator uses mouse IL-23 heterodimer composed of IL-23p40 (residues 23–335) and IL-23p19 (residues 20–196) expressed in *Spodoptera frugiperda* (Sf 21).

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MDS Detection Antibody
IL-23	Goat Polyclonal	Rat Monoclonal

References

1. Langrish CL, et al. IL-12 and IL-23: master regulators of innate and adaptive immunity. Immunol Rev. 2004 Dec;202:96-105.
2. Kunz M, Ibrahim SM. Cytokines and cytokine profiles in human autoimmune diseases and animal models of autoimmunity. Mediators Inflamm. 2009:979258.
3. D'Elios MM, et al. Targeting IL-23 in human diseases. Expert Opin Ther Targets. 2010 Jul;14(7):759-74.
4. Langrish CL, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005 Jan 17;201(2):233-40.

Summary Protocol

MSD 96-well MULTI-SPOT Mouse IL-23

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Mouse IL-23 assay.

Sample and Reagent Preparation

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

Prepare Blocker A solution.

Prepare standard solutions using the supplied calibrator:

- Dilute the stock calibrator 100-fold in Diluent 4.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 2-fold in Diluent 4 before adding to the plate.

Prepare detection antibody solution by diluting the stock detection antibody 50-fold in Diluent 5.

Prepare 2X Read Buffer T by diluting stock 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 50X 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.

Plate Diagrams