MSD® MULTI-ARRAY Assay System

Human IL-18 Kit

1-Plate Kit	K151MCD-1
5-Plate Kit	K151MCD-2
25-Plate Kit	K151MCD-4

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MSD® Cytokine Assays

Human IL-18 Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Ordering Information

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Introduction

Interleukin 18 (IL-18) is an 18 kDa cytokine and a co-stimulatory factor that is produced in Kuppfer cells, activated macrophages, keratinocytes, and intestinal epithelial cells.[1] One of the main functions of IL-18 is to promote the production of IFN-γ from T and NK cells, particularly in the presence of IL-12p70. IL-18 also promotes the secretion of other proinflammatory cytokines like TNF-α, IL-1β, and GM-CSF that enhance the migration and activation of neutrophils during microbial infections.[2] In addition, IL-18 enhances cytotoxic activity and proliferation of CD8+ T and NK cells and has been shown to stimulate the production of IL-13 and other Th2 cytokines.[2,3] Dysregulation of IL-18 may therefore contribute to inflammatory-associated disorders, unchecked infections, autoimmune diseases such as rheumatoid arthritis, acute and chronic kidney injury, cancer, and pathogenic conditions related to metabolic syndrome.[2,3]

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 IL-18 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.

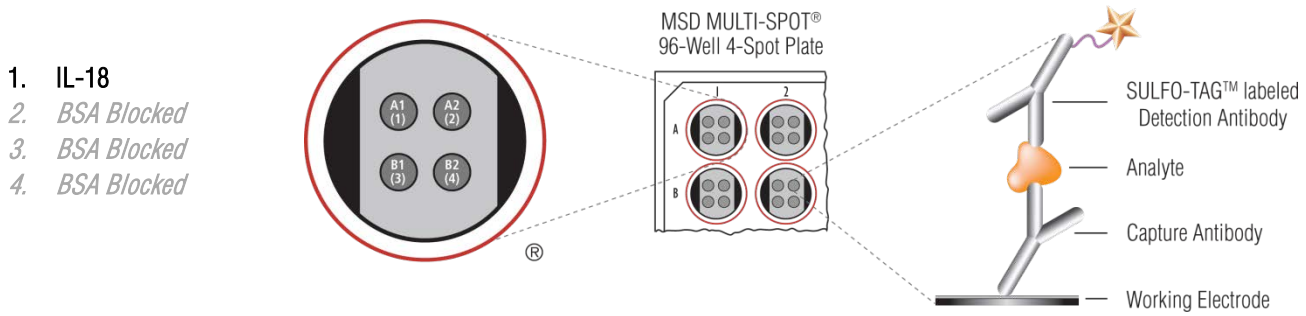


Figure 1. *Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K151MCD-1	K151MCD-2	K151MCD-4
MULTI-SPOT 96-Well 4-Spot Human IL-18 Plate N451MCA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu IL-18 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human IL-18 Calibrator (0.5 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 (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

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 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 Human IL-18 Kit at 200-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. Prepare the standard solutions at room temperature.

Standard	IL-18 Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	500 000	
STD-01	2500	200
STD-02	625	4
STD-03	156	4
STD-04	39	4
STD-05	9.8	4
STD-06	2.4	4
STD-07	0.6	4
STD-08	0	n/a

To prepare 7 standard solutions plus a zero calibrator blank for up to 3 replicates:

1) Prepare the highest standard (STD-01) by adding 10 µL of stock calibrator to 1990 µL of Diluent 10. Mix well.
2) Prepare the next standard (STD-02) by transferring 75 µL of STD-01 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 blank.

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 a 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

MSD recommends reviewing current literature and protocols for preparing other matrices such as urine,[4,5] CSF,[6] and tissue homogenates.[7]

Other Diluents

In addition to MSD Diluent 10, MSD Diluents 2, 7, and 8 are suitable for preparation of samples and calibrators. However, the Human IL-18 assay was optimized with MSD Diluent 10 and the data provided as part of this protocol was generated with this diluent. Assay performances may differ in other diluents.

Prepare Detection Antibody Solution

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

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu IL-18 Antibody
- ❑ 2940 µL 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 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:** Wash the plate 3 times with 300 µL/well of PBS-T. 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.

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 keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

   You may prepare diluted read buffer during incubation.

   Bubbles introduced when adding read buffer will interfere with plate imaging and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

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.

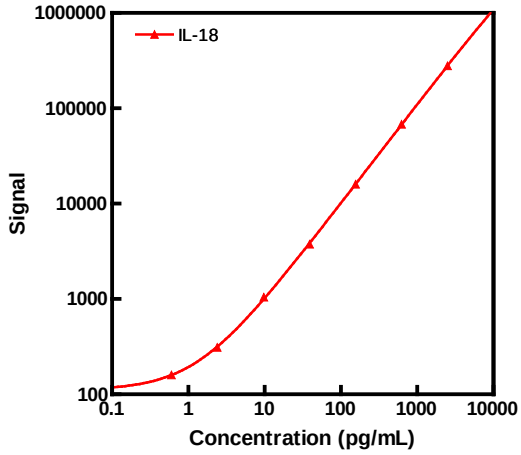
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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-18		
Conc. (pg/mL)	Average Signal	%CV
0	121	18.0
0.6	160	7.0
2.4	310	4.0
9.8	1045	7.0
39	3760	14.0
156	15 938	2.0
625	68 104	12.0
2500	278 480	4.0

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-18
Average LLOD (pg/mL)	0.71

Dilution Linearity

To assess linearity, human serum, EDTA plasma, and heparin plasma were diluted 4-fold, 8-fold, and 16-fold with Diluent 10. The measured concentrations were corrected for dilution factor to determine the actual IL-18 levels in the sample. The dilution adjusted calculated concentration of each dilution point was compared to the calculated recovery of the previous dilution. Human serum recovery ranged from 97 to 112% with an average recovery of 107%. Human EDTA plasma recovery ranged from 100 to 124% with an average recovery of 111%, and human heparin plasma recovery ranged from 102 to 155% with an average recovery of 119%.

Spike Recovery

Human serum, EDTA plasma, and heparin plasma were spiked with human IL-18 calibrator at multiple levels throughout the range of the assay. The samples were then diluted 2-fold and tested for recovery. Calibrator recovery from human serum ranged from 87 to 112% with an average recovery of 97%. Calibrator recovery from human EDTA plasma ranged from 81 to 108% with an average recovery of 93%, and calibrator recovery from human heparin plasma ranged from 60 to 107% with an average recovery of 81%.

Samples

Normal human serum (n=13), EDTA plasma (n=10), and heparin plasma (n=4) samples from commercial sources were evaluated for human IL-18. The samples were diluted 2-fold and the measured concentrations were corrected for dilution factor to determine the actual IL-18 levels in the sample. The analyte was quantifiable in all 13 serum samples with a median measurement of 136 pg/mL. The human IL-18 levels in these serum samples ranged from 71 to 207 pg/mL. Human IL-18 was quantifiable in all 10 EDTA plasma samples with median level measured at 410 pg/mL (range from 155 to 1370 pg/mL). Human IL-18 was also quantifiable in all heparin plasma samples with a median level of 139 pg/mL (range from 109 to 191 pg/mL).

Assay Components

Calibrator

The assay calibrator uses recombinant human IL-18 protein expressed in *E. coli.*

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
IL-18	Mouse Monoclonal	Rat Monoclonal

References

1. Arend WP, et al. IL-1, IL-18, and IL-33 families of cytokines. Immunol Rev. 2008 Jun;223:20-38.
2. Sahoo M, et al. Role of the inflammasome, IL-1β, and IL-18 in bacterial infections. ScientificWorldJournal. 2011;11:2037-50.
3. Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol. 2007 Jan;27(1):98-114.
4. Zhou H, et al. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney. 2006;69:1471-76.
5. Thomas CE, et al. Urine collection and processing for protein biomarker discovery and quantification. Cancer Epidemiol Biomarkers & Prevention. 2010;19:953-59.
6. Schoonenboom NS, et al. Effects of processing and storage conditions on amyloid beta (1-42) and tau concentrations in cerebrospinal fluid: implications for use in clinical practice. Clin Chem. 2005; 51:189-95.
7. Castle JD. Purification of organelles from mammalian cells. Curr Protoc Immunol. 2003; Chapter 8: Unit 8.1B

Summary Protocol

Human IL-18 Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human IL-18 assay.

Sample and Reagent Preparation

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

Prepare Blocker A solution.

Prepare 7 standard solutions using the supplied calibrator:

- Dilute the stock calibrator 200-fold in Diluent 10.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.

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

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

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

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

Add 50 µL/well of sample (standards, controls, or unknowns).

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.

Plate Diagrams