

# MSD MULTI-ARRAY<sup>®</sup> Assay System

## Human IL-17D Kit

1-Plate Kit

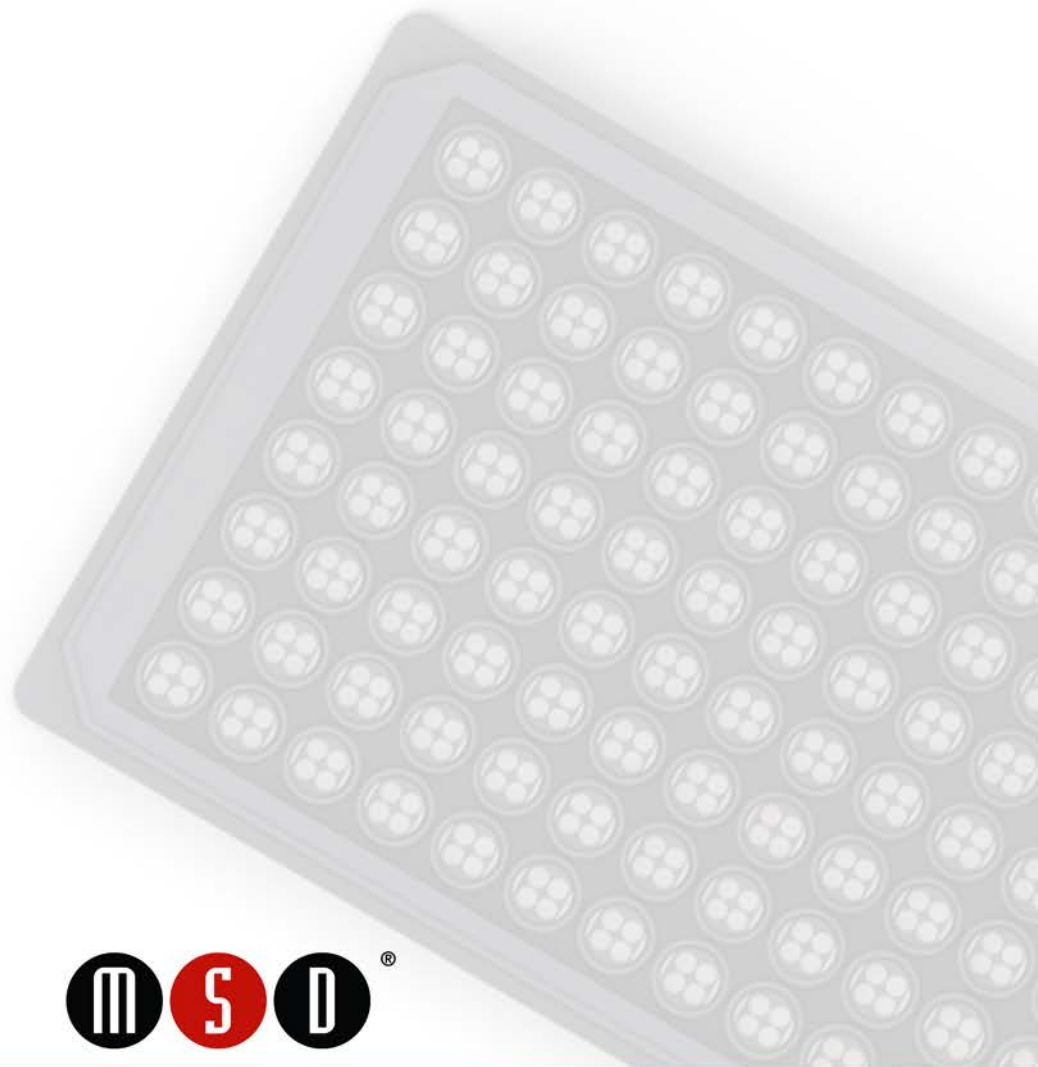
K151MMD-1

5-Plate Kit

K151MMD-2

25-Plate Kit

K151MMD-4



# MSD<sup>®</sup> Cytokine Assays

## Human IL-17D 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<sup>®</sup>**

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## Ordering Information

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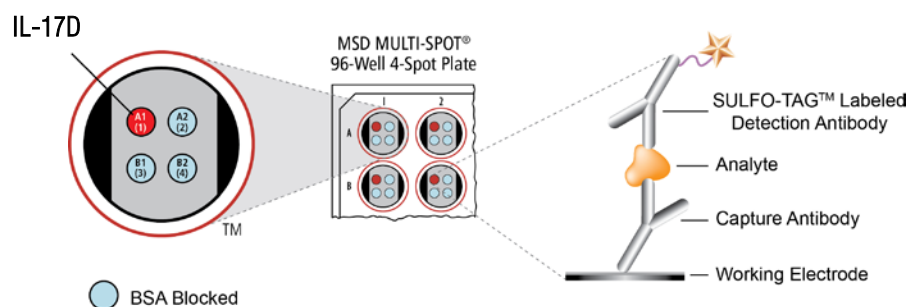
# Introduction

The **Interleukin-17 (IL-17)** cytokines are emerging as key regulators of initiation and maintenance of the proinflammatory immune response. The family consists of six related molecules: IL-17A, IL-17B, IL-17C, **IL-17D**, IL-17E, and IL-17F. These molecules have a molecular mass of 20–30 kDa and share 20–50% homology to IL-17A. In general, IL-17 induces several pro-inflammatory molecules, such as IL-6, IL-8, GM-CSF, IL-1, TNF- $\alpha$ , and MCP-1, from different cell types including fibroblasts, endothelial cells, epithelial cells, and tissue-specific macrophages. Thus, it makes sense that these cytokines are linked to inflammatory diseases including rheumatoid arthritis, asthma, lupus, allograft rejection, and tumorigenicity. However, differences in expression patterns and production levels appear to give specificity of action and role to each of the family members.<sup>1</sup>

In contrast to other IL-17 members, IL-17D is preferentially expressed in skeletal muscle, brain, adipose tissue, heart, lung, and pancreas.<sup>2</sup> IL-17D likely plays a role in local immune responses at the site of structural damage, such as that observed in trauma, myocardial infarction, or stroke. It is also possible that IL-17D is important to growth or repair of those tissues after damage. IL-17D may amplify the local primary immune response to rapidly produce a localized infiltration of T-cells to clear damaged cells, while suppressing myeloid progenitor cell production and promoting repair.<sup>3</sup>

## 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 IL-17D 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		
		K151MMD-1	K151MMD-2	K151MMD-4
MULTI-SPOT 96-Well 4-Spot Human IL-17D Plate N451MMA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu IL-17D Antibody <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human IL-17D Calibrator (0.5 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 2 R51BB-4 (8 mL), R51BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Diluent 3 R51BA-4 (5 mL), R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 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.

<sup>1</sup> 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 2 and Diluent 3 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	IL-17D Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	500 000	
STD-01	5000	100
STD-02	1250	4
STD-03	313	4
STD-04	78	4
STD-05	20	4
STD-06	4.9	4
STD-07	1.2	4
STD-08	0	n/a

To prepare 8 standard solutions for up to 3 replicates:

- 1) Prepare the highest standard by adding 10  $\mu$ L of the stock calibrator to 990  $\mu$ L of Diluent 2. Mix well.
- 2) Prepare the next standard by transferring 75  $\mu$ L of the highest standard to 225  $\mu$ L of Diluent 2. Mix Well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
- 3) Use Diluent 2 as the 8<sup>th</sup> 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 2.

### 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 2.

### 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](mailto: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  $\mu$ L of 50X SULFO-TAG Anti-hu IL-17D Antibody
- 2.94 mL of Diluent 3

## 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

1. **Add Blocker A Solution:** Add 150  $\mu\text{L}$  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.
2. **Wash and Add Sample or Calibrator:** Wash the plate 3 times with 300  $\mu\text{L}$ /well of PBS-T. Add 50  $\mu\text{L}$  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  $\mu\text{L}$ /well of PBS-T. Add 25  $\mu\text{L}$  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.
4. **Wash and Read:** Wash the plate 3 times with 300  $\mu\text{L}$ /well of PBS-T. Add 150  $\mu\text{L}$  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.

## Notes

*Shaking the plate typically accelerates capture at the working electrode.*

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

*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.*

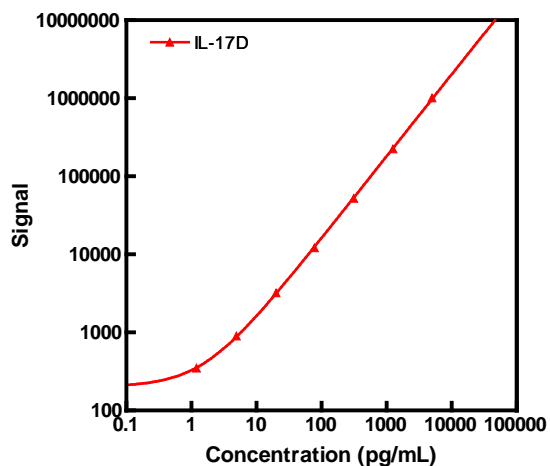
## Analysis of Results

MSD DISCOVERY WORKBENCH<sup>®</sup> 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.



IL-17D		
Conc. (pg/mL)	Average Signal	%CV
0	160	3.1
1.2	350	14.4
4.9	899	0.2
20	3223	1.3
78	12 133	3.0
313	52 089	1.6
1250	225 065	1.3
5000	1 007 511	1.3

# Sensitivity

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

IL-17D	
LLOD (pg/mL)	0.097

# Assay Components

## Calibrator

The assay calibrator uses recombinant human IL-17D protein expressed in *E. coli*.

## Antibodies

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
IL-17D	Mouse Monoclonal	Goat Polyclonal

## References

1. Pappu R, Ramirez-Carrozzi V, Sambandam A. The interleukin-17 cytokine family: critical players in host defence and inflammatory diseases. *Immunology*. 2011 Sep;134(1):8-16.
2. Li H, Chen J, Huang A, Stinson J, Heldens S, Foster J, Dowd P, Gurney AL, Wood WI. Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. *Proc Natl Acad Sci U S A*. 2000 Jan 18;97(2):773-8.
3. Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. *J Immunol*. 2002 Jul 15;169(2):642-6.

Summary Protocol  
**MSD 96-well Human IL-17D Kit**

*MSD provides this summary protocol for your convenience.  
Please read the entire detailed protocol prior to performing the  
Human IL-17D 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 2 before adding to the plate.  
Prepare detection antibody solution by diluting 50X detection antibody 50-fold in Diluent 3.  
Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

### **Step 1: Add Blocker A Solution**

Add 150  $\mu$ L/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  $\mu$ L/well of PBS-T.  
Add 50  $\mu$ L/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  $\mu$ L/well of PBS-T.  
Add 25  $\mu$ L/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  $\mu$ L/well of PBS-T.  
Add 150  $\mu$ L/well of 2X Read Buffer T.  
Analyze plate on SECTOR Imager.



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