Human TNF-β Kit

1-Plate Kit	K151LWD-1
5-Plate Kit	K151LWD-2
25-Plate Kit	K151LWD-4

MSD®
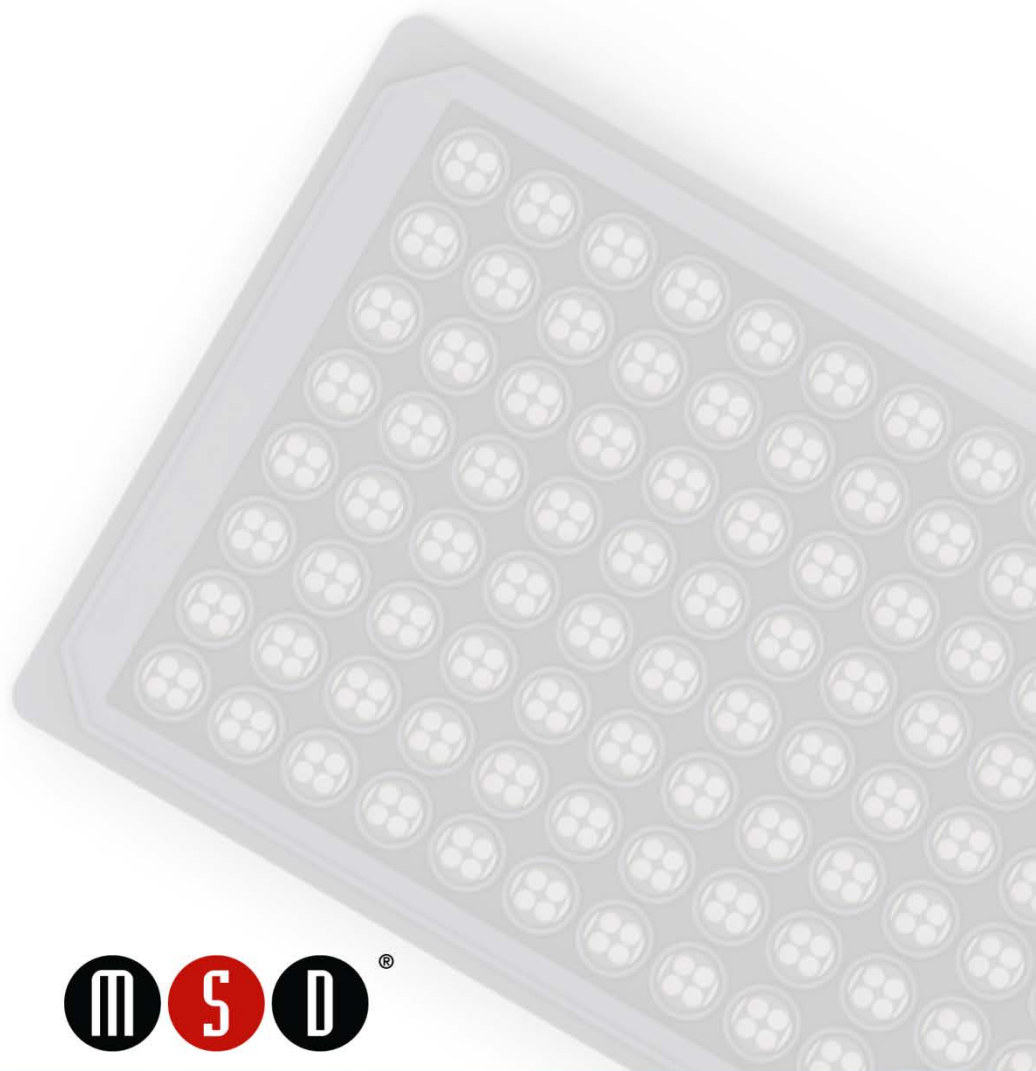
MSD Cytokine Assays

Human TNF-β 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

MSD Customer Service

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MSD Scientific Support

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Introduction

Tumor necrosis factor-beta (TNF-β), also known as Lymphotoxin-alpha, is a 25 kDa protein and member of the cytokine TNF-family, a group of cytokines known to contribute to apoptosis. TNF-β is both structurally and functionally similar to TNF-α with 35% protein sequence homology, and both bind to the same cell surface receptors (TNF-RI and TNF-RII).[1] It is produced by TH1 type T-cells after antigenic or mitogenic stimulation and is cytotoxic for a range of tumor cells. Genetic polymorphism within the regulatory regions of TNF-α and TNF-β (specifically 252 A to G in TNF-β) are linked to various cancer malignancies.[2] TNF-β is a mediator of inflammatory, immunostimulatory, and antiviral responses.[3] It may also be involved in the proper development of secondary lymphoid organs such as lymph nodes and Peyer's patches.[4] TNF-β has also been shown to contribute to the susceptibility of several autoimmune diseases, including graft-versus-host and rheumatoid arthritis[5,6], as well diabetes [7] and other disorders.

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 TNF-β 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.

1. **TNF-β**
2. *BSA Blocked*
3. *BSA Blocked*
4. *BSA Blocked*

MSD MULTI-SPOT®
96-Well 4-Spot Plate

SULFO-TAG™ labeled
Detection Antibody

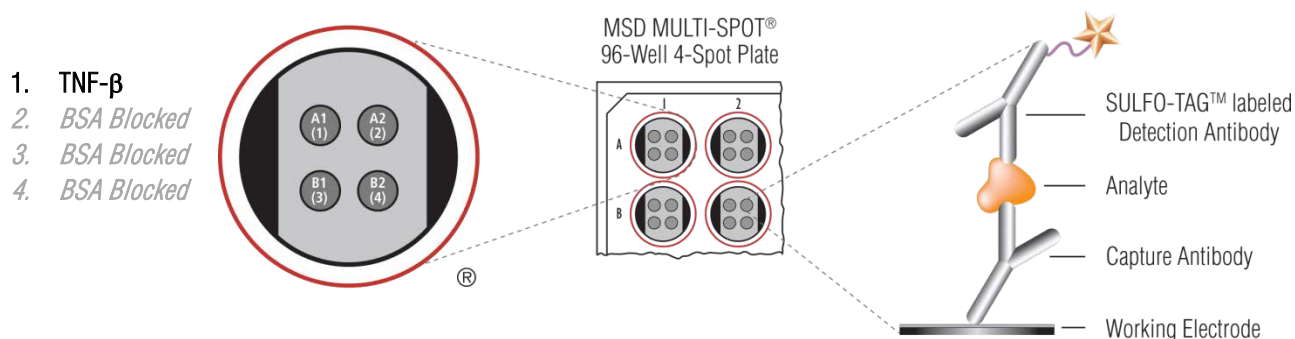Analyte

Capture Antibody

Working Electrode

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		
		K151LWD-1	K151LWD-2	K151LWD-4
MULTI-SPOT 96-Well, 4-Spot Human TNF-β Plate N451LWA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu TNF-β Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human TNF-β 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 (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.

Additional safety information is available in the product Material Safety Data Sheet, which can be obtained from MSD Customer Service.

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[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 2 and Diluent 3 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 TNF-β 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 2. Mix well.

2) Prepare the next standard by transferring 75 µL of the highest standard to 225 µL of Diluent 2. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.

3) Use Diluent 2 as the blank.

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. MSD recommends 2-fold dilution in Diluent 2; however, you may adjust dilution factors for the sample set under investigation. To dilute samples 2-fold, add 60 µL of sample to 60 µL of Diluent 2.

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 2.

Other Matrices

MSD recommends reviewing current literature and protocols for preparing other matrices such as urine,[8,9] CSF,[10] and tissue homogenates.[11]

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 TNF-β Antibody
- ❏ 2940 µL 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 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

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 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 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 the standards.
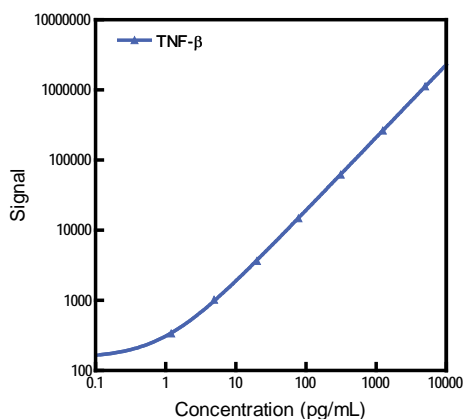
TNF-β		
Conc. (pg/mL)	Average Signal	%CV
0	121	6.7
1.2	339	6.0
4.9	1021	4.7
20	3642	6.6
78	14 855	6.1
313	62 095	5.1
1250	263 753	5.6
5000	1 127 795	6.0

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).

	TNF-β
Average LLOD (pg/mL)	0.23

Specificity

The Human TNF-β assay recognizes recombinant and native human TNF-β. This assay shows no significant non-specific binding with following recombinant human analytes: GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6,. IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, TNF-α, or VEGF.

This assay recognizes native cynomolgus monkey TNF-β.

Tested Samples

Normal human serum samples were diluted 2-fold and tested with the Human TNF-β Kit. Median and range of concentrations for the sample set are displayed below. Concentrations are corrected for sample dilution.

Sample Type	Statistic	TNF-β
Serum	Median (pg/mL)	74
	Range (pg/mL)	34–96
	Number of Samples	8
	Samples in Quantitative Range	8

Assay Components

Calibrator

The assay calibrator uses recombinant TNF-β protein, residues 35–205, expressed in *E. coli.*

Antibodies

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
TNF-β	Mouse Monoclonal	Mouse Monoclonal

References

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Spot the Difference®

Human TNF-β Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing
the Human TNF-β 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 2.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.

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

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 2.

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 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