MSD® MULTI-SPOT Assay System

Human FasL Kit

1-Plate Kit	K151KDD-1
5-Plate Kit	K151KDD-2
25-Plate Kit	K151KDD-4

MSD Biomarker Assays

Human FasL 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®
A division of Meso Scale Diagnostics, LLC.
1601 Research Boulevard
Rockville, MD 20850-3173 USA
www.mesoscale.com

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Ordering Information

MSD Customer Service

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

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

Fas ligand (FasL or CD95L) is a 40 kDa, homotrimeric, type II membrane protein that belongs to the tumor necrosis factor family (TNF).[1] FasL is an essential effector in immune system functions; the protein induces apoptosis in Fas(CD95/APO-1)-expressing cells. In hematopoietic cells, intracellular FasL is stored in secretory lysosomes and is mobilized to the immunological synapses upon activation, a process mediated by trafficking and cytosolic reorganization adaptor proteins. FasL surface expression is regulated by post-translational ectodomain shedding by disintegrin and subsequent regulated intramembrane proteolysis, resulting in the release of soluble FasL into serum.[2,3] FasL binding to the FasL receptor results in receptor trimerization, internalization of the Fas complex, initiation of the apoptotic signaling cascade, and cell death.[4] FasL deficiences have been implicated in tumor drug resistance, oncogenesis, and autoimmune disorders.[5]

Principle of the Assay

MSD biomarker assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human FasL 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.

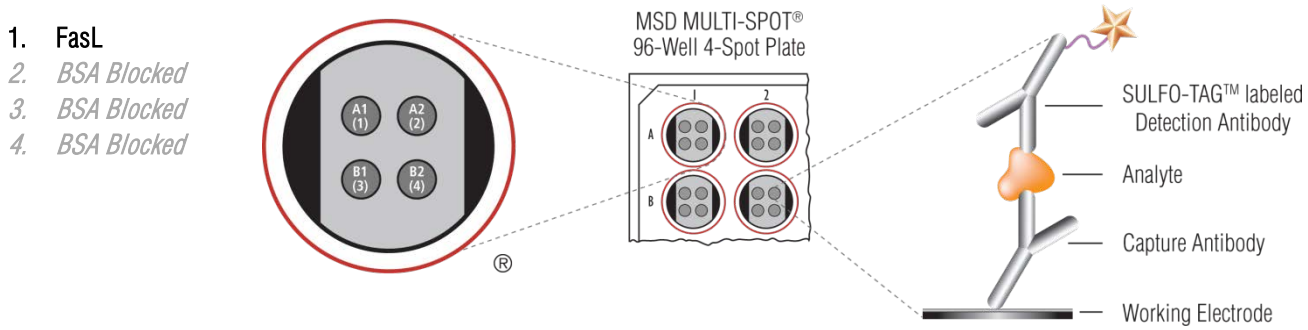


Figure 1. *Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K151KDD-1	K151KDD-2	K151KDD-4
MULTI-SPOT 96-Well, 4-Spot Human FasL Plate N451KDA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu FasL Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human FasL Calibrator (50 ng/mL)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 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.

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

[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 FasL Kit at 20-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	FasL Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	50 000	
STD-01	2500	20
STD-02	625	4
STD-03	156	4
STD-04	39	4
STD-05	9.8	4
STD-06	2.4	4
STD-07	0.61	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 25 µL of stock calibrator to 475 µL of Diluent 10 . Mix well.
2) Prepare the next standard (STD-02) by transferring 100 µL of the highest standard to 300 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.

Dilute Samples

For serum and plasma samples, MSD recommends 2-fold dilution in Diluent 10 ; however, you may adjust dilution factors for the sample set under investigation. To dilute sample 2-fold, add 60 µL of sample to 60 µL of Diluent 10 .

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 FasL 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 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.

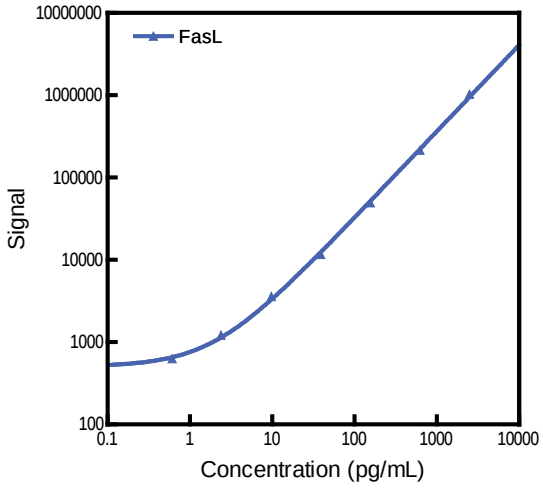
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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.



FasL		
Conc. (pg/mL)	Average Signal	%CV
0	430	6.6
0.61	628	7.0
2.4	1219	3.8
9.8	3578	1.3
39	11 588	2.6
156	49 385	0.8
625	214 525	1.4
2500	1 030 610	4.2

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).

	FasL
LLOD (pg/mL)	0.28
LLOD Range (pg/mL)	0.20–0.40

Tested Samples

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

Sample Type	Statistic	FasL
Serum	Median (pg/mL)	15.0
	Range (pg/mL)	6.4-46
	Number of Samples	20
	Samples in Quantitative Range	20

Assay Components

Calibrator

The assay calibrator uses recombinant human FasL protein, residues 134–281, expressed in Chinese Hamster Ovary cells.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
FasL	Mouse Monoclonal	Mouse Monoclonal

References

1. Ashkenazi A. Targeting death and decoy receptors of the tumor-necrosis factor super family. Nat Rev Cancer. 2002 Jun;2(6):420-430.
2. Jodo S, et al. Bioactivities of Fas Ligand-Expressing Retroviral Particles. J Immunol 2000 May 15;164(10):5062-5069.
3. Voss M, et al. Posttranslational regulation of Fas ligand function. Cell Commun Signal 2008 Dec 29;6:11.
4. Voss M, et al. Identification of SH3 domain interaction partners of human FasL (CD178) by phage display screening. BMC Immunol 2009 Oct 6;10:53.
5. Pitti, RM, et al. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Nature. 1998 Dec 17;396(6712):699-703.

Summary Protocol

Human FasL Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human FasL 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 20-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 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 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