MSD® MULTI-SPOT Assay System

Human sFas Kit

1-Plate Kit	K151KBD-1
5-Plate Kit	K151KBD-2
25-Plate Kit	K151KBD-4

MSD Biomarker Assays

Human sFas 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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Table of Contents

Ordering Information

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Introduction

Soluble Fas (sFas or sCD95) is a 48 kDa, type I membrane protein that plays a critical role in apoptosis.[1] Several sFas isoforms are generated by alternative mRNA splicing; the predominant form lacks the transmembrane domain, a result of exon 6 deletion, while less active isoforms lack combinations of exons 3, 4, 6, and 7. Functionally, sFas inhibits extracellular Fas/FasL binding, impairing the homeostatic regulation of immune responses such as Fas-induced apoptosis.[2]

sFas is present in activated human lymphocytes and tumor cell lines supernatants. Elevated levels have been observed in patients suffering from systemic lupus erythematosus and hematopoietic malignancies, suggesting that sFas modulates autoimmune diseases and oncogenesis.[3-4]

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 sFas 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.

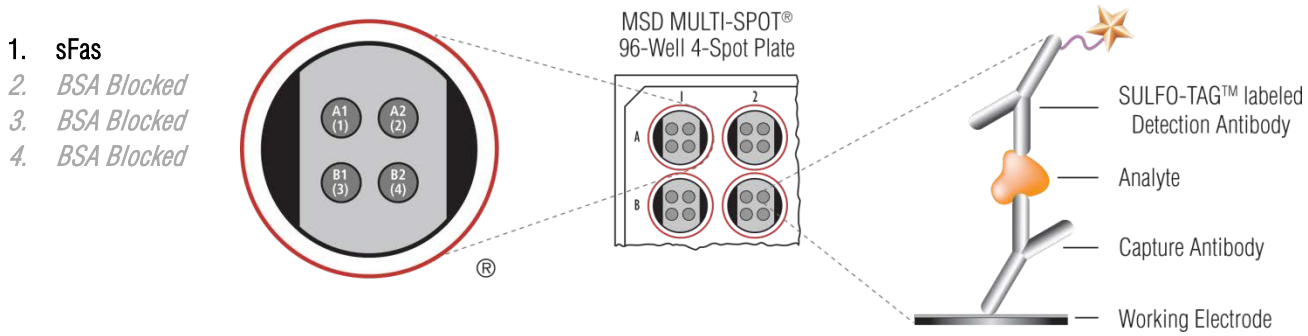


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K151KBD-1	K151KBD-2	K151KBD-4
MULTI-SPOT 96-Well, 4-Spot Human sFas Plate N451KBA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu sFas Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human sFas Calibrator (100 ng/mL)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 100 R50AA-4 (50 mL), R50AA-2 (200 mL)	2–8°C	1 bottle (50 mL)	2 bottles (200 mL ea)	10 bottles (200 mL ea)
Diluent 12 R50JA-4(10 mL), R50JA-3 (50 mL)	≤-10°C	1 bottle (10 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 12 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 sFas 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	sFas Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	100 000	
STD-01	5000	20
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 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 100. Mix well.
2) Prepare the next standard (STD-02) by transferring 100 µL of the highest standard to 300 µL of Diluent 100. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 100 as the blank.

Dilute Samples

For serum and plasma samples, MSD recommends 100-fold dilution in Diluent 100; however, you may adjust dilution factors for the sample set under investigation. To dilute sample 100-fold, add 10 µL of sample to 990 µL of Diluent 100.

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 sFas Antibody
- ❑ 2940 µL of Diluent 12

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.

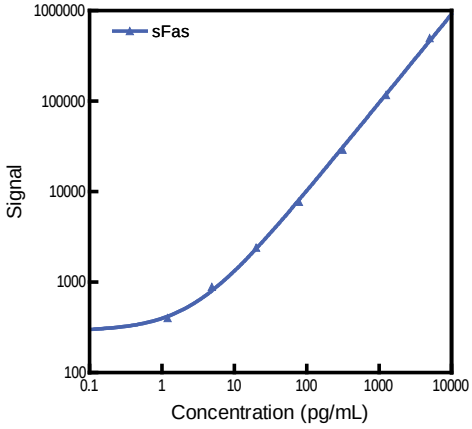
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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.



sFas		
Conc. (pg/mL)	Average Signal	%CV
0	243	6.1
1.2	401	5.1
4.9	888	4.7
20	2405	5.3
78	7719	5.3
313	29 029	8.9
1250	116 815	3.7
5000	496 984	1.7

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).

	sFas
LLOD (pg/mL)	0.44
LLOD Range (pg/mL)	0.34-0.60

Tested Samples

Normal human serum samples were diluted 200-fold and tested with the Human sFas Kit. Median and range of concentrations are displayed below. Concentrations are corrected for sample dilution.

Sample Type	Statistic	sFas
Serum	Median (pg/mL)	16 400
	Range (pg/mL)	10 800–2 800
	Number of Samples	20
	Samples in Detectable Range	20

Assay Components

Calibrator

The assay calibrator uses recombinant human sFas protein, residues 17–173, expressed in murine myeloma cell, NS0-derived.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
sFas	Goat Polyclonal	Goat Polyclonal

References

1. Papoff G, et al. An N-terminal domain shared by Fas/Apo-1 (CD95) soluble variants prevents cell death in vitro. J. Immunol. 1996;156:4622-4630.
2. Cascino I, et al. Soluble FAS/APO-1 Splicing Variants and Apoptosis. Frontiers in Bioscience. 1996 Jan 1;1:d12-18.
3. Del-Rey MJ, et al. Autoimmune lymphoproliferative syndrome (ALPS) in a patient with a new germline Fas gene mutation. Immunobiology. 2007;212(2):73-83.
4. Otsuki T, et al. Alterations of Fas and Fas-Related Molecules in Patients with Silicosis. Exp Biol Med. 2006 May;231(5):522-533.

Summary Protocol

Human sFas Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human sFas 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 100.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 100-fold in Diluent 100 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 12

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