

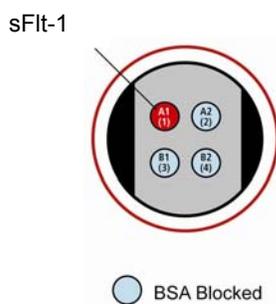
MSD[®] 96-Well MULTI-ARRAY[®] Human sFlt-1 Assay

The following assay protocol has been optimized for analysis of soluble fms-like tyrosine kinase (sFlt-1) in human serum and plasma samples.

Storage

MSD Materials

<input type="checkbox"/> Read Buffer T (4X)	RT
<input type="checkbox"/> Blocker C	2-8 °C
<input type="checkbox"/> MULTI-SPOT [®] 96-well 4 Spot Human sFlt-1 Plate	2-8 °C
<input type="checkbox"/> SULFO-TAG [™] Anti-hsFlt-1 Antibody (100X) ¹	2-8 °C
<input type="checkbox"/> Diluent 7	≤-10 °C
<input type="checkbox"/> Diluent 8	≤-10 °C
<input type="checkbox"/> Diluent 9	≤-10 °C
<input type="checkbox"/> Human sFlt-1 Calibrator (1 µg/mL)	≤-70 °C



The SECTOR[®] Imager data file will identify spots according to their well location, not by the coated capture antibody name.

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.



Notes:

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Read Buffer
- ❑ Phosphate Buffered Saline + 0.05% Tween-20(PBS-T) for plate washing
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 and 150 μL into a 96-well microplate

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The protocol can be completed in approximately 5.5 hours if each reagent is prepared during the preceding incubation. This time can be reduced to **4.5 hours if the blocking reagent is added the night before.**

The assay protocol was optimized for serum and plasma samples. For significantly different sample matrices, it is recommended to use a calibrator diluent that is similar to the sample matrix (e.g. lysis buffer + carrier protein).

- Step 1.** Add Blocking Solution, incubate 1-2 hour, wash. (alternatively, block plates overnight at 4 °C).
- Step 2.** Add 25 μL of Diluent 7.
Add 25 μL of Samples or Calibrator, incubate 2 hours, wash.
- Step 3.** Add 25 μL of Detection Antibody, incubate 2 hours, wash.
- Step 4.** Add 150 μL of Read Buffer, read plate and analyze data.

Preparation Instructions

Thaw Diluents

Thaw Diluent 7, Diluent 8, and Diluent 9. Vortex briefly. If there is a precipitate, mix gently and warm to room temperature to dissolve. Diluents are stable at 4 °C for one week.

Prepare Calibrator dilutions:

1. Determine how many Calibrator levels and replicates will be tested in the experiment. Each well will require 25 μL of Calibrator. Thaw Diluent 9 and one vial of Human sFlt-1 Calibrator (1 $\mu\text{g}/\text{mL}$). Vortex briefly. Prepare the required Calibrator dilution series using Diluent 9.



Notes:

2. A recommended Calibrator dilution procedure is listed below for 4 replicates of 7 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
 - i. Prepare 1000 μL of sFlt-1 Calibrator at a concentration of 10000 pg/mL by adding 10 μL of the sFlt-1 Calibrator at 1 $\mu\text{g}/\text{mL}$ to 990 μL Diluent 9.
 - ii. Prepare 6 additional 1:4 serial dilutions, beginning with the sFlt-1 Calibrator at 10000 pg/mL, by adding 50 μL of the Calibrator to 150 μL Diluent 9.
 - iii. This will create 7 Calibrators with 10000, 2500, 625, 156, 39, 9.8 and 2.4 pg/mL. The recommended 8th dilution is Diluent 9 alone (e.g. zero Calibrator).
3. Calibrators are stable at room temperature for a few hours. The Calibrator stock solution is stable for one day at 4 °C and for one additional freeze-thaw cycle. Diluent 9 is stable for one week at 4 °C.

Prepare the 1X Detection Antibody Solution

- a) In a 15 mL tube combine:
 - 30 μL of 100X SULFO-TAG Anti-hsFlt-1 Antibody
 - 2.97 mL of Diluent 8
- b) This will yield 3 mL of diluted Detection Antibody Solution at the working concentration with sufficient volume for one plate.

Detection Antibody Solution is stable at RT for a few hours.

Dilute Read Buffer:

- In a 50 mL tube combine (per plate):
- 5 mL 4X Read Buffer T
 - 15 mL deionized water

Diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.



Notes:

Assay Protocol

Begin with a MULTI-ARRAY 96-well 4 Spot Human sFlt-1 plate.
No pre-treatment is necessary.

1. Add 150 μ L/well of Blocker C and incubate at room temperature for 1-2 hours or overnight at 4 $^{\circ}$ C.
2. Wash plates 3 times with phosphate buffered saline + 0.05%Tween-20 (PBS-T).
3. Add 25 μ L/well of Diluent 7.
4. Add 25 μ L/well calibrator or sample and incubate at room temperature with shaking for 2 hours.
5. Wash plates 3 times with PBS-T.
6. Add 25 μ L/well of 1X Detection Antibody Solution and incubate at room temperature with shaking for 2 hours.
7. Wash plates 3 times with PBS-T.
8. Prepare SECTOR Imager so that plate can be read immediately after Read Buffer addition.
9. Add 150 μ L/well 1X Read Buffer T.
10. Analyze immediately with SECTOR Imager.

Plates may also be blocked overnight at 4 $^{\circ}$ C and stored for up to a week with Blocker C.

Avoid bubbles while adding the Read Buffer; it will interfere with accurate reading of the plate.