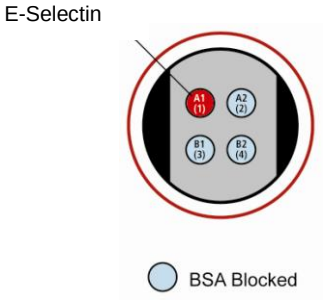
MSD® 96-Well MULTI-ARRAY® E-Selectin Assay

The following assay protocol has been optimized for analysis of E-selectin in human serum and plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker A Kit	RT
❑ MULTI-SPOT® 96-well 4 Spot Human E-Selectin Plate(s)	2-8 ºC
❑ SULFO-TAG™ Anti-hE-Selectin Antibody (50X)[1]	2-8 ºC
❑ Diluent 10	≤-10 °C
❑ Human E-Selectin Calibrator (10 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.

Notes:

Other Materials & Equipment (not supplied)

- Deionized water for diluting Wash Buffer and 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
- Liquid handling equipment for desired throughput that must accurately dispense 10, 25, 40, and 150 µL into a 96-well micro plate

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The protocol can be completed in approximately 4 hours if each reagent is prepared during the preceding incubation. This time can be reduced to **3 hours if the blocking step is performed overnight** prior to performing the assay. All reagents can be prepared hours ahead of time if desired.

Step 1. Add Blocking Solution, incubate 1 hour, wash.
(alternatively, block plates overnight at 4 ^{o}C).

Step 2. Add 40 µL of Diluent 10.
Add 10 µL of Samples or Calibrator, incubate 2 hours, wash.

Step 3. Add 25 µL of Detection Antibody, incubate 1 hour, wash.

Step 4. Add 150 µL of Read Buffer, read plate and analyze data.

Preparation Instructions

Prepare Blocker A Kit:

Follow instructions included with the Blocker A Kit.

Notes:

Prepare Calibrator dilutions:

1. Determine how many Calibrator levels and replicates will be tested. Each well will require 10 µL of Calibrator. Thaw one vial of Calibrator stock solution and prepare the required Calibrator dilution series using Diluent 10.
   a) A recommended Calibrator dilution procedure is listed below for 4 replicates of 6 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
      - *Prepare 100 µL of E-Selectin Calibrator at a concentration of 1000 ng/mL by adding 10 µL of the E-Selectin stock solution at 10 µg/mL to 90 µL of Diluent 10. Vortex briefly, and let the solution equilibrate for approximately 15 minutes.*
      - *Prepare a seven point calibration curve using 1/7 serial dilution as follows: Begin with the above diluted solution of E-selectin at 1000 ng/mL as the top of the curve and add 10 µL of solution to 60 µL Diluent 10 to make a Calibrator solution at 143 ng/mL. Repeat the 1/7 serial dilution five times to make Calibrator solutions of 20, 2.9, 0.42, 0.06, and 0.008 ng/mL.*
      - *The recommended 8th dilution is Diluent 10 alone (e.g. zero Calibrator).*
   b) Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately to produce the desired standard curve.

2. Calibrators should be kept at 4ºC (for up to 4 hours) if not used immediately. The Diluent 10 is stable for one week at 4 ^{o}C. For longer storage, aliquot and store at -20 ^{o}C. Diluent 10 may be refrozen twice.

Prepare the 1X Detection Antibody Solution

a) In a 15 mL tube combine:
   - ❑ 60 µL of 50X SULFO-TAG Anti-hE-Selectin Antibody
   - ❑ 2.94 mL of Diluent 10

Detection Antibody Solution is stable at room temperature for a few hours and should be stored in the dark when not in use.

b) This will yield 3 mL of diluted Detection Antibody Solution at the working concentration with sufficient volume for one plate.

Dilute Read Buffer:

Diluted Read Buffer may be stored at room temperature for later use.

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

Notes:

Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Human E-Selectin Plate.
No pre-treatment is necessary.

1. Add 150 µL/well of Blocker A Solution and incubate on a plate shaker at room temperature for 1 hour or without shaking, overnight at 4 °C.

2. Wash plates 3 times with 200 µL per well phosphate buffered saline with 0.05% Tween-20 (PBS-T).

3. Add 40 µL Diluent 10.

4. Add 10 µL/well Calibrator or sample and incubate at room temperature with shaking for 2 hours.

5. Wash plates 3 times with 200 µL per well PBS-T.

6. Add 25 µL/well of 1X Detection Antibody Solution and incubate at room temperature with shaking for 1 hour.

7. Wash plates 3 times with 200 µL per well PBS-T.

8. Prepare SECTOR Imager so that plate can be read immediately after Read Buffer addition.

9. Add 150 µL/well 1X Read Buffer T. Avoid bubbles. The use of an electronic multi-pipettor at moderate speed setting is recommended.

Bubbles introduced to the well during Read Buffer addition will interfere with reliable imaging of the plate.

10. Read plate immediately following Read Buffer T dispense on the SECTOR Imager.

Sample Plate Layout:

		1	2	3	4	5	6	7	8	9	10	11	12
ng/mL Calibrator (7- fold dilutions)	A	1000											
	B	143											
	C	20											
	D	2.9											
	E	0.42											
	F	0.06											
	G	0.008											
	H	0											
		Calibrator			**samples**								