MSD® 96-Well MULTI-SPOT Vascular Injury Panel I Assay

The following assay protocol has been optimized for analysis of thrombomodulin, intercellular adhesion molecule-3 (ICAM-3), E-selectin, and P-selectin in human serum and plasma samples.

MSD Materials

	Storage
❑ MULTI-SPOT® 96-well 4 Spot Vascular I Plate(s)	2-8 ºC
❑ SULFO-TAG™ Anti-Human Vascular Injury I Detection Antibody Blend (50X)[1]	2-8 ºC
❑ Human Vascular Injury I Calibrator Blend *Calibrators for Thrombomodulin, ICAM-3, E-Selectin, and P-Selectin are blended at 1 µg/mL each*	≤-70 °C
❑ Diluent 10	≤-10 °C
❑ Blocker A Kit	RT
❑ Read Buffer T (4X)	RT

1. Thrombomodulin
2. ICAM-3
3. E-Selectin
4. P-Selectin

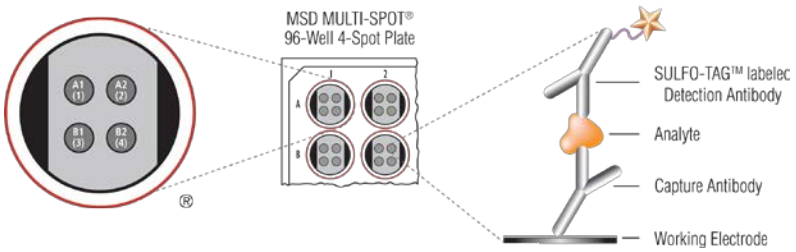

Figure1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

[1] SULFO-TAG labeled detection antibodies should be stored in the dark.

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 safety data sheet, which can be obtained from MSD Customer Service.

Notes:

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting concentrated buffers
- ❑ 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

1. Add blocking solution; incubate 1 hour; wash.
   (Alternatively, block plates overnight at 2-8 °C).
2. Add Diluent 10.
   Add samples or calibrator; incubate 2 hours; wash.
3. Add detection antibody; incubate 1 hour; wash.
4. Add Read Buffer T and analyze plate.

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.

Preparation Instructions

Prepare Blocker A Kit:

Follow instructions included with the Blocker A Kit.

Prepare Calibrator dilutions:

Notes:

1. Determine how many calibrator levels and replicates will be tested. Each well will require 10 µL of calibrator. Thaw one vial of Human Vascular Injury I calibrator Blend, and prepare the required calibrator dilution series using Diluent 10.
   a) A recommended calibrator dilution procedure is listed below for 3 replicates of combined Calibrator concentrations spanning a wide range, plus 1 zero-calibrator point (sample plate layout shown below).
      - *Prepare a seven point calibration curve as follows: Begin with the Vascular Injury Panel I Calibrator Blend as the top of the curve (1000 ng/mL) and add 20 µL of solution to 60 µL Diluent 10 to make a calibrator solution at 250 ng/mL. Repeat the 4-fold serial dilution five times to make calibrator solutions of 63, 16, 3.9, 0.98, and 0.24 ng/mL.*
      - *This will create 7 calibrators for all analytes. 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 2-8 ^{o}C (for up to 4 hours) if not used immediately. The Diluent 10 is stable for one week at 2-8 ^{o}C. For longer storage, aliquot and store at -20 ^{o}C. Diluent 10 may be refrozen twice.

Prepare the 1X Detection Antibody Solution

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

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

b) This will yield 3 mL of diluted detection antibody solution at the working concentration with sufficient volume for one plate.

Diluted read buffer may be stored at room temperature for later use.

Dilute Read Buffer T:

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

Assay Protocol

Notes:

Begin with a MULTI-SPOT 96-well 4 Spot Vascular I 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 2-8 °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 MSD instrument 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 an MSD instrument.