

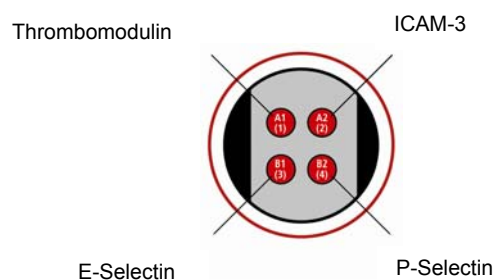
MSD[®] 96-Well MULTI-SPOT[®] Vascular Injury Panel I Assay

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.

Storage

MSD Materials

- | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| <input type="checkbox"/> Read Buffer T (4X), with surfactant | RT |
| <input type="checkbox"/> Blocker A Kit | RT |
| <input type="checkbox"/> MULTI-SPOT 96-well 4 Spot Vascular I Plate(s) | 2-8 °C |
| <input type="checkbox"/> SULFO-TAG [™] Anti-Human Vascular Injury I Detection Antibody Blend (50X) ¹ | 2-8 °C |
| <input type="checkbox"/> Diluent 10 | ≤-10 °C |
| <input type="checkbox"/> Human Vascular Injury I Calibrator Blend
<i>Calibrators for Thrombomodulin, ICAM-3, E-Selectin, and P-Selectin are blended at 1 µg/mL each</i> | ≤-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 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 °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 μ L 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 using 1/7 serial dilution as follows: Begin with the Vascular Injury Panel I Calibrator Blend as the top of the curve (1000 ng/mL) and add 10 μ L of solution to 60 μ L 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.
 - 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 4 °C (for up to 4 hours) if not used immediately. The Diluent 10 is stable for one week at 4 °C. For longer storage, aliquot and store at -20 °C. Diluent 10 may be refrozen twice.

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

Prepare the 1X Detection Antibody Solution

- a) In a 15 mL tube combine:
 - 60 μ L 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:

- 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 Vascular I Plate.
No pre-treatment is necessary.

1. Add 150 μ L/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 μ L per well phosphate buffered saline with 0.05% Tween-20 (PBS-T).
3. Add 40 μ L Diluent 10.
4. Add 10 μ L/well Calibrator or sample and incubate at room temperature with shaking for 2 hours.
5. Wash plates 3 times with 200 μ L per well PBS-T.
6. Add 25 μ L/well of 1X Detection Antibody Solution and incubate at room temperature with shaking for 1 hour.
7. Wash plates 3 times with 200 μ L per well 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. Avoid bubbles. The use of an electronic multi-pipettor at moderate speed setting is recommended.
10. Read plate immediately following Read Buffer T dispense on the SECTOR Imager.

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

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										
		blended calibrators			samples								

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