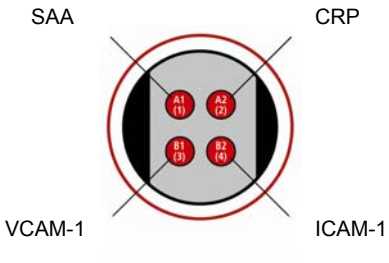
MSD® 96-Well MULTI-SPOT® Vascular Injury Panel II Assay

The following assay protocol has been optimized for analysis of serum amyloid A (SAA), C-reactive protein (CRP), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) 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 Vascular II Plate(s)	2-8 ºC
❑ SULFO-TAG™ Anti-Human Vascular Injury II Detection Antibody Blend (50X)[1]	2-8 ºC
❑ Diluent 15	≤-10 °C
❑ Human Vascular Injury II Calibrator Blend *Calibrators for SAA, CRP[2], VCAM-1, and ICAM-1 are blended at 1 µg/mL each*	≤-70 °C



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

[1] SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.
[2] The CRP Calibrator in this kit is derived from human source material which has been tested and found to be negative for HBsAg, HIV-1 and HIV-2 antibodies, and HCV. This material should be handled and disposed of in accordance with local, state and federal guidelines.

Notes:

Other Materials & Equipment (not supplied)

- Deionized water for diluting Read Buffer
- Phosphate buffered saline (PBS) for dilution of Blocker A for sample dilution
- 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 5, 10, 25, 40, 150, and 200 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 reagent is added the night before**. 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 15.
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.
This will yield a 5% (w/v) Blocker A Solution.

Notes:

Diluent 15 is stable for one week at 4 °C. For longer storage, aliquot and store at ≤-10°C. Diluent 15 may be refrozen twice.

Prepare Calibrator Dilutions:

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 II Calibrator Blend, and prepare the required Calibrator dilution series using Diluent 15.

1) 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 II Calibrator Blend as the top of the curve (1000 ng/mL) and add 10 µL of solution to 60 µL Diluent 15 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 15 alone (e.g. zero Calibrator).*

2) Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately to produce the desired standard curve.

Prepare 1% Blocker A Solution for Sample Diluent:

1. Determine the amount of 1% Blocker A Solution needed for the experiment. Each sample requires approximately 300 µL 1% Blocker A Solution when diluting sample according to the recommendation below.

2. Dilute 5% Blocker A Solution to 1% with PBS.

Sample preparation:

Dilute samples 1/200 in 1% Blocker A Solution. For example a recommended two-step dilution is as follows:

- Prepare an initial 10-fold dilution by adding 10 µL of sample to 90 µL of Blocker A solution and mix thoroughly.
- Prepare the 200X diluted sample by starting with the 10X diluted sample and diluting by a factor of 20; add 10 µL of the 10X diluted sample to 190 µL of Blocker A solution.

Notes:

Prepare the 1X Detection Antibody Solution

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

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 room temperature for a few hours and should be stored in the dark when not in use.

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 stored at room temperature for later use.

Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Vascular II Plate.
No pre-treatment is necessary.

1. Add 150 µL/well of 5% Blocker A Solution and incubate at room temperature for 1 hour with shaking (or overnight at 4 ^{o}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/well of Diluent 15.
4. Add 10 µL/well Calibrator or diluted 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.
10. **Analyze immediately with SECTOR Imager.** This is important because the VCAM-1 assay signal decreases about 20% within the first 5 minutes of Read Buffer T incubation.

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**								