

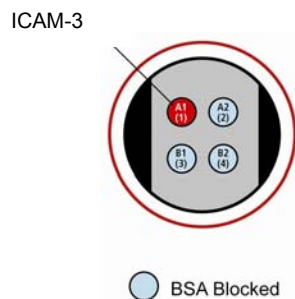
# MSD<sup>®</sup> 96-Well MULTI-ARRAY<sup>®</sup> ICAM-3 Assay

The following assay protocol has been optimized for analysis of intercellular adhesion molecule-3 (ICAM-3) 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 <sup>®</sup> 96-well 4 Spot Human ICAM-3 Plate(s)	2-8 °C
<input type="checkbox"/> SULFO-TAG <sup>™</sup> Anti-hICAM-3 Antibody (50X) <sup>1</sup>	2-8 °C
<input type="checkbox"/> Diluent 10	≤-10 °C
<input type="checkbox"/> Human ICAM-3 Calibrator (10 µg/mL)	≤-70 °C



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

<sup>1</sup> 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  $\mu\text{L}$  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  $\mu\text{L}$  of Diluent 10.  
Add 10  $\mu\text{L}$  of Samples or Calibrator, incubate 2 hours, wash.
- Step 3.** Add 25  $\mu\text{L}$  of Detection Antibody, incubate 1 hour, wash.
- Step 4.** Add 150  $\mu\text{L}$  of Read Buffer, read plate and analyze data.

## *Preparation Instructions*

### **Prepare Blocker A Kit:**

Follow instructions included with the Blocker A Kit.



**Prepare Calibrator dilutions:**

1. Determine how many Calibrator levels and replicates will be tested. Each well will require 10  $\mu\text{L}$  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 3 replicates of 6 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
    - Prepare 100  $\mu\text{L}$  of ICAM-3 Calibrator at a concentration of 1000 ng/mL by adding 10  $\mu\text{L}$  of the ICAM-3 stock solution at 10  $\mu\text{g/mL}$  to 90  $\mu\text{L}$  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 ICAM-3 at 1000 ng/mL as the top of the curve and add 10  $\mu\text{L}$  of solution to 60  $\mu\text{L}$  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.
    - The recommended 8<sup>th</sup> 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.

**Prepare the 1X Detection Antibody Solution**

- a) In a 15 mL tube combine:
  - 60  $\mu\text{L}$  of 50X SULFO-TAG Anti-hICAM-3 Antibody
  - 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.

*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.*



**Notes:**

## Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Human ICAM-3 Plate.  
No pre-treatment is necessary.

1. Add 150  $\mu$ L/well of Blocker A Solution and incubate on a plate shaker at room temperature for 1 hour or without shaking, overnight at 4  $^{\circ}$ C.
2. Wash plates 3 times with 200  $\mu$ L per well phosphate buffered saline with 0.05% Tween-20 (PBS-T).
3. Add 40  $\mu$ L Diluent 10.
4. Add 10  $\mu$ L/well Calibrator or sample and incubate at room temperature with shaking for 2 hours.
5. Wash plates 3 times with 200  $\mu$ L per well PBS-T.
6. Add 25  $\mu$ L/well of 1X Detection Antibody Solution and incubate at room temperature with shaking for 1 hour.
7. Wash plates 3 times with 200  $\mu$ L per well PBS-T.
8. Prepare SECTOR Imager so that plate can be read immediately after Read Buffer addition.
9. Add 150  $\mu$ 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											
		Calibrator			samples								

Meso Scale Discovery, Meso Scale Diagnostics, www.mesoscale.com, MSD, MSD (design), Discovery Workbench, Quickplex, Multi-Array, Multi-Spot, Sulfo-Tag and Sector are trademarks of Meso Scale Diagnostics, LLC.  
© 2009 Meso Scale Discovery a division of Meso Scale Diagnostics, LLC. All rights reserved.

