

MSD[®] 96-Well MULTI-ARRAY[®] Thrombomodulin Assay

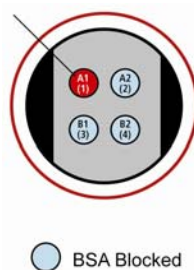
The following assay protocol has been optimized for analysis of thrombomodulin in human serum and plasma samples.

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 Human Thrombomodulin Plate(s)	2-8 °C
<input type="checkbox"/> SULFO-TAG [™] Anti-hThrombomodulin Antibody (50X) ¹	2-8 °C
<input type="checkbox"/> Diluent 10	≤-10 °C
<input type="checkbox"/> Human Thrombomodulin Calibrator (10 µg/mL)	≤-70 °C

Thrombomodulin



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 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 3 replicates of 6 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
 - Prepare 100 μL of Thrombomodulin Calibrator at a concentration of 1000 ng/mL by adding 10 μL of the Thrombomodulin stock solution at 10 $\mu\text{g/mL}$ 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 Thrombomodulin 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 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 μL of 50X SULFO-TAG Anti-hThrombomodulin 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 Thrombomodulin 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 $^{\circ}$ 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											
		Calibrator			samples								

