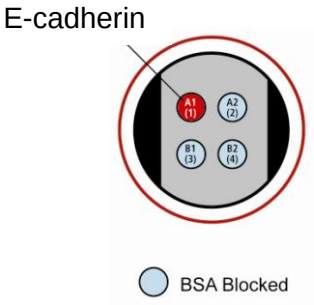
MSD® 96-Well MULTI-ARRAY® Human E-Cadherin Assay

The following assay protocol has been optimized for analysis of E-cadherin in human serum or plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker A Kit	RT
❑ MULTI-SPOT® 96-well 4 Spot Human E-cadherin Plate(s)	2-8 °C
❑ SULFO-TAG™ Anti-hE-cadherin Antibody (50X)[1]	2-8 °C
❑ Diluent 100	2-8 °C
❑ Diluent 11	≤-10 °C
❑ Diluent 7	≤-10 °C
❑ Human E-cadherin Calibrator (10 µg/mL)	≤-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.

Other Materials & Equipment (not supplied)

- Deionized water for diluting Read Buffer
- Phosphate buffered saline for plate washing
- Adhesive plate seals
- Microtiter plate shaker
- Plate washer, or other efficient multi-channel pipetting equipment for washing 96-well plates
- Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 and 150 µL into a 96-well microplate

Notes:

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The assay protocol was optimized for human serum samples. For significantly different sample matrices, it is recommended to use a Calibrator diluent that is similar to the sample matrix (e.g. lysis buffer + carrier protein).

The protocol can be completed in approximately 5.5 hours if each reagent is prepared during the preceding incubation. This time can reduced to **4.5 hours if the blocking reagent is added the night before**.

Step 1. Add Blocking solution, incubate 1-2 hours, wash.

Step 2. Add 25 µL of Diluent 7.
Add 25 µL of samples or Calibrator, incubate 2 hour, wash.

Step 3. Add 25 µL of Detection Antibody, incubate 2 hour, wash.

Step 4. Add 150 µL of Read Buffer and analyze plate.

Preparation Instructions

Prepare Blocker A solution:

Follow instructions included with the Blocker A Kit.

Prepare Calibrator dilutions:

1. Determine how many Calibrator levels and replicates will be run. Each well will require 25 µL of Calibrator. Thaw one vial of E-cadherin Calibrator stock solution and prepare the required Calibrator dilution series using the stock solution and Diluent 100.
   - A recommended Calibrator dilution procedure is listed below for up to 3 replicates of 7 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.

Notes:

- *Prepare 200 µL of a high Calibrator containing 1000 ng/mL E-cadherin by adding 20 µL of the Calibrator stock solution at 10 µg/mL to 180 µL Diluent 100.*
- *Prepare 6 additional 1:5 serial dilutions, beginning with the high Calibrator, by adding 50 µL of the Calibrator to 200 µL Diluent 100.*
- *This will create 7 Calibrators with 1000, 200, 40, 8, 1.6, 0.32 and 0.064 ng/mL of E-cadherin.*
- *The recommended 8th dilution is Diluent 100 alone (i.e. zero Calibrator).*

❖ Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately.

2. Calibrators are stable at room temperature for at least a few hours.

Prepare Samples:

Dilute serum or plasma samples 10-fold in Diluent 100.
Each well will require 25 µL of diluted sample.

Prepare Detection Antibody Reagent:

1. Each well requires 25 µL of Detection Antibody Reagent. Prepare 3 mL per plate.

2. In a 15 mL tube combine:
   a. 2.94 mL Diluent 11
   b. 60 µL of 50X SULFO-TAG Anti-hE-cadherin Antibody (final concentration: 1X)

Prepare Read Buffer Solution:

Diluted Read Buffer may be stored at room temperature for later use.

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 Human E-cadherin plate.
No pre-treatment is necessary.

1. Add 150 µL/well of Blocker A and incubate at room temperature for 1-2 hours.

2. Wash plates 3 times with phosphate buffered saline (PBS)

3. Add 25 µL/well of Diluent 7.
   Add 25 µL/well Calibrator or 10-fold diluted sample and incubate at room temperature with shaking for 2 hours.

4. Wash plates 3 times with PBS.

5. Add 25 µL/well Detection Antibody Reagent and incubate at room temperature with shaking for 2 hours.

6. Wash plates 3 times with PBS.

7. Prepare SECTOR Imager so that plate can be read immediately after Read Buffer addition.

8. Add 150 µL/well 1X Read Buffer T. Avoid bubbles.

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

9. Analyze immediately with SECTOR Imager.