

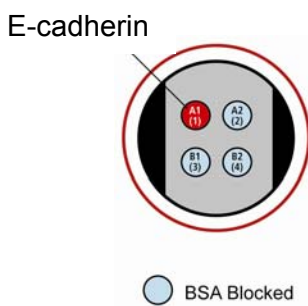
MSD[®] 96-Well MULTI-ARRAY[®] Human E-Cadherin Assay

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

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 E-cadherin Plate(s) | 2-8 °C |
| <input type="checkbox"/> SULFO-TAG [™] Anti-hE-cadherin Antibody (50X) ¹ | 2-8 °C |
| <input type="checkbox"/> Diluent 100 | 2-8 °C |
| <input type="checkbox"/> Diluent 11 | ≤-10 °C |
| <input type="checkbox"/> Diluent 7 | ≤-10 °C |
| <input type="checkbox"/> 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.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.



Notes:

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

Read the entire detailed instructions before beginning work.

Protocol at a Glance

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

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).

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 $\mu\text{g}/\text{mL}$ 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:

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

1. Add 150 μ L/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 μ L/well of Diluent 7.
Add 25 μ L/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 μ L/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 μ L/well 1X Read Buffer T. Avoid bubbles.
9. Analyze immediately with SECTOR Imager.

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

