

# MSD<sup>®</sup> 96-Well MULTI-ARRAY<sup>®</sup> Human P-Cadherin Assay

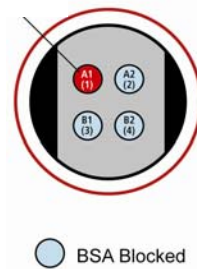
The following assay protocol has been optimized for analysis of P-cadherin in human serum or plasma samples.

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 <sup>®</sup> 96-well 4 Spot Human P-cadherin Plate(s)	2-8 °C
<input type="checkbox"/> SULFO-TAG <sup>™</sup> Anti-hP-cadherin Antibody (50X) <sup>1</sup>	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 P-cadherin Calibrator (10 µg/mL)	≤-70 °C

P-cadherin



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 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  $\mu\text{L}$  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 hour, wash.

**Step 2.** Add 25  $\mu\text{L}$  of Diluent 7.  
Add 25  $\mu\text{L}$  of samples or Calibrator, incubate 2 hour, wash.

**Step 3.** Add 25  $\mu\text{L}$  of Detection Antibody, incubate 2 hour, wash.

**Step 4.** Add 150  $\mu\text{L}$  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  $\mu\text{L}$  of Calibrator. Thaw one vial of P-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  $\mu\text{L}$  of a high Calibrator containing 1000 ng/mL P-cadherin by adding 20  $\mu\text{L}$  of the Calibrator stock solution at 10  $\mu\text{g}/\text{mL}$  to 180  $\mu\text{L}$  Diluent 100.
  - Prepare 6 additional 1:5 serial dilutions, beginning with the high Calibrator, by adding 50  $\mu\text{L}$  of the Calibrator to 200  $\mu\text{L}$  Diluent 100.
  - This will create 7 Calibrators with 1000, 200, 40, 8, 1.6, 0.32 and 0.064 ng/mL of P-cadherin.
  - The recommended 8<sup>th</sup> 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  $\mu\text{L}$  of diluted sample.**

***Prepare Detection Antibody Reagent:***

1. Each well requires 25  $\mu\text{L}$  of Detection Antibody Reagent.  
Prepare 3 mL per plate.
2. In a 15 mL tube combine:
  - a. 2.94 mL Diluent 11
  - b. 60  $\mu\text{L}$  of 50X SULFO-TAG Anti-hP-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 P-cadherin plate.  
No pre-treatment is necessary.

1. Add 150  $\mu$ 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  $\mu$ L/well of Diluent 7.  
Add 25  $\mu$ 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  $\mu$ 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  $\mu$ 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.*

