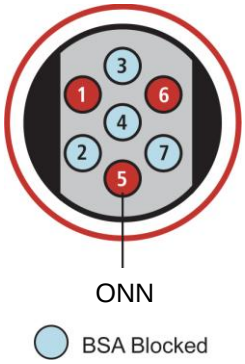
MSD® 96-Well MULTI-ARRAY® Human Osteonectin Assay

The following assay protocol has been optimized for analysis of osteonectin (ONN) in human serum and plasma samples.

MSD Materials

	Storage
❑ MSD Read Buffer T (With Surfactant), 4X	RT
❑ MSD Blocker A Kit	RT
❑ MULTI-SPOT® 96-well 7 Spot Human Bone Panel II Plate(s)	2-8ºC
❑ MSD SULFO-TAG^{M} Anti-hOsteonectin Antibody (50X)[1]	2-8ºC
❑ Diluent 7	≤-10 °C
❑ Diluent 11	≤-10 °C
❑ Human Bone Panel II Calibrator Blend Osteocalcin *(OCL – 0.2 µg/mL)* Osteonectin[2] *(ONN – 2 µg/mL)* Osteopontin *(OPN – 0.2 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.

[2] The Osteonectin in the Calibrator Blend is derived from human source material which has been tested and found to be negative or non reactive for HBsAg, anti-HB core, Syphilis, anti-HVC, anti- HIV-1 antigens, anti-HIV1/2 and anti-HTLV1/2. This material should be handled and disposed of in accordance with local, state, and federal guidelines.

Notes:

Other Materials & Equipment (not supplied)

- Various microcentrifuge tubes for making serial dilutions of test solutions
- Phosphate buffered saline (PBS) for plate washing
- Ultrapure water
- Automated plate washer
- Adhesive plate seals
- Microtiter plate shaker
- Appropriate liquid handling equipment for desired throughput.

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The protocol can be completed in approximately 4.5 hours if each reagent is prepared during the preceding incubation. This time can be reduced to **3.5 hours if the blocking step is performed overnight** prior to performing the assay.

Step 1. Add Blocking Solution, incubate 1 hour, wash.

Step 2. Add 25 µL of Diluent 7.
Add 25 µL of Calibrator or diluted samples (diluted 20-fold), 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

Preparation of MSD Blocker A solution:

Prepare MSD Blocker A solution following the instructions included in the MSD Blocker A Kit. MSD Blocker A may be stored at 4°C for up to 2 weeks.

Preparation of Read Buffer Solution:

Dilute 4X MSD Read Buffer T stock solution (with surfactant) 4-fold to 1X with deionized water. Diluted Read Buffer may be stored at room temperature for later use.

Thaw Diluents:

Thaw Diluent 7, and Diluent 11. Vortex briefly once thawed. If there is a precipitate, mix gently and warm to room temperature to dissolve. Keep all materials on ice until use. The remaining amounts of the Diluents after use can be aliquoted and refrozen as needed.

Prepare Calibrator Dilutions:

Caution: The Osteonectin (ONN) in the Human Bone Panel II Calibrator Blend is a human source material (isolated from thrombin-activated human platelets) and should be handled as a Biosafety Level 2 material.

1) Determine how many Calibrator levels and replicates will be tested in the experiment; triplicate wells of each Calibrator level are typically recommended. Each well will require 25 µL of Calibrator. Thaw a vial of Human Bone Panel II Calibrator Blend on ice. The undiluted Calibrator is at a concentration of 2 µg/mL. Vortex briefly. Prepare the required subsequent concentrations by serially diluting the Calibrator 1:10 into Diluent 7. For example, add 15 µL of the Calibrator to 135 µL of Diluent 7 and repeat this dilution five more times. Use Diluent 7 alone as a zero Calibrator condition. Remaining undiluted Calibrator stock solutions may be refrozen on dry ice in single use aliquots and stored at ≤-70 °C.

2) The Calibrator dilutions should be prepared immediately before use and kept on ice until use.

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-hOsteonectin Antibody (final concentration: 1X)

3) Detection Antibody Reagent is stable on ice for a few hours.

Assay Protocol

Begin with a MULTI-SPOT 96-well 7 Spot Human Bone Panel II Plate.
No pre-treatment is necessary.

1) Add 150 µL of MSD Blocker A per well of the Human Bone Panel II plates, cover and incubate for 30 min to 1 hour at room temperature.

2) During blocking prepare Calibrator dilutions, serially diluting using 10-fold dilutions into Diluent 7 and mixing gently between dilution steps. Calibrators can be kept on ice.

3) Also prepare samples during the blocking step. Serum and plasma samples should be diluted 20-fold in Diluent 7. Store on ice.

4) Wash plates 3 times with 200 µL per well 1X PBS.

5) Add 25 µL of Diluent 7 to all wells. Next, 25 µL of Calibrator dilutions can be added to appropriate calibration wells, and 25 µL of diluted serum/plasma samples to sample wells.

6) Cover plate and incubate with shaking for two hours at room temperature.

7) During the incubation, prepare Detection Antibody mixture in Diluent 11 (3 mL per plate) to obtain the final concentrations indicated above, and keep on ice until use.

8) Wash plates 3 times with 200 µL per well 1X PBS.

9) Add 25 µL of the Detection Antibody Reagent to each well. Cover and incubate with shaking for 1 hour at room temperature.

10) Wash plates 3 times with 200 µL per well 1X PBS.

11) Prepare SECTOR Imager to read plate.

12) Add 150 µL of the 1X MSD Read buffer T solution to each well and read immediately on the MSD SECTOR Imager.