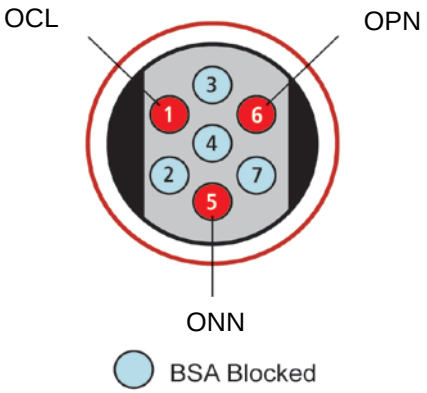
# *MSD® 96-Well MULTI-SPOT® Human Bone Panel II*

*The following assay protocol has been optimized for analysis of osteocalcin (OCL), osteonectin (ONN), and osteopontin (OPN) in human serum and plasma samples.*

## *MSD Materials*

| | Storage |
|---|---|
| ❑ MULTI-SPOT 96-well 7-Spot Human Bone Panel II Plate(s) | 2–8ºC |
| ❑ SULFO-TAG™ Anti-hOsteocalcin Antibody (50X)[1] | 2–8ºC |
| ❑ SULFO-TAG Anti-hOsteonectin Antibody (50X)[1] | 2–8ºC |
| ❑ SULFO-TAG Anti-hOsteopontin Antibody (50X)[1] | 2–8ºC |
| ❑ Human Bone Panel II Calibrator Blend<br>Osteocalcin *(OCL – 0.2 µg/mL)*<br>Osteonectin[2] *(ONN – 2 µg/mL)*<br>Osteopontin *(OPN – 0.2 µg/mL)* | ≤-70°C |
| ❑ Diluent 7 | ≤-10°C |
| ❑ Diluent 11 | ≤-10°C |
| ❑ Blocker A Kit | RT |
| ❑ MSD Read Buffer T (4X) | RT |




**The SECTOR® Imager data file will identify spots according to their well location, not by the coated capture antibody name.**

[1] SULFO-TAG-conjugated detection antibodies should be stored in the dark.

[2] 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.

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

**Note:** Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines. Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service.

***Notes:***

## *Other Materials & Equipment (not supplied)*

- ❑ Deionized water for diluting concentrated buffers
- ❑ Phosphate buffered saline (PBS) for plate washing
- ❑ 15 mL and 50 mL tubes
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Microcentrifuge tubes for making dilutions of standards
- ❑ Automated plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- ❑ Appropriate liquid handling equipment for desired throughput capable of accurately dispensing 25 µL and 150 µL into a 96-well microplate

## *Protocol at a Glance*

**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.

*Read the entire detailed instructions before beginning work.*

The full protocol that follows describes the most conservative approach to achieving highly sensitive results using MSD technology to quantify OCL, ONN, and OPN. 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.

## *Preparation Instructions*

***Notes:***

**Thaw Reagents.** Thaw MSD Human Bone Panel II Calibrator Blend, 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 Blocker A Solution.** Prepare MSD Blocker A solution following the instructions included in the MSD Blocker A Kit.

*Blocker A solution may be stored at 2–8°C for up to 5 weeks.*

**Prepare Detection Antibody Solution.** MSD provides the detection antibodies blended in a 50X stock solution. The working detection antibody solution is 1X. You will need 3 mL per plate.

*Detection antibody solution is stable on ice for a few hours.*

In a 15 mL tube, combine:

- ❑ 2.82 mL Diluent 11
- ❑ 60 µL of 50X SULFO-TAG Anti-hOsteocalcin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hOsteonectin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hOsteopontin Antibody

**Prepare Read Buffer T.** MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X. You will need 20 mL per plate at a final 1X concentration.

In a 50 mL tube, combine:

- ❑ 15 mL deionized water
- ❑ 5 mL 4X Read Buffer T

**Prepare Standards.**

**Caution:** The Human Osteonectin Calibrator is a human source material (isolated from thrombin-activated human platelets) and should be handled as a Biosafety Level 2 material.

*The calibrator dilutions should be prepared immediately before use and kept on ice until use.*

MSD supplies a multi-analyte calibrator blend that contains 0.2, 2.0, and 0.2 µg/mL of OCL, ONN, and OPN, respectively.These concentrations reflect the top of the standard curve and this calibrator blend provided should be used as the highest calibrator; no dilution is necessary.
To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates:

1) Vortex the calibrator blend provided, briefly, and use it as the highest standard (STD-1).
2) Prepare the next standard (STD-2) by transferring 15 µL of the highest standard to 135 µL of Diluent 7. Mix well by vortexing. Repeat 10-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 7 as the zero standard.

This yields the following calibrator concentrations:

| Calibrator | OCL (pg/mL) | ONN (pg/mL) | OPN (pg/mL) |
|---|---|---|---|
| **STD-1** | 200,000 | 2,000,000 | 200,000 |
| **STD-2** | 20,000 | 200,000 | 20,000 |
| **STD-3** | 2,000 | 20,000 | 2,000 |
| **STD-4** | 200 | 2,000 | 200 |
| **STD-5** | 20 | 200 | 20 |
| **STD-6** | 2.0 | 20 | 2.0 |
| **STD-7** | 0.2 | 2.0 | 0.2 |
| **STD-8** | 0 | 0 | 0 |

***Notes:***

*Remaining undiluted combined Calibrator stock solutions may be refrozen on dry ice in single use aliquots and stored at ≤-70°C.*

**Prepare Samples.** Dilute samples with Diluent 7. For serum and plasma samples, MSD recommends a 20-fold sample dilution; however, depending on the sample set under investigation, you may need to use a higher dilution factor. For example, to dilute 20-fold, add 10 µL of sample to 190 µL of Diluent 7.

## *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 Blocker A solution to each well of the Human Bone Panel II plate. Seal the plate, and incubate for 30 minutes to 1 hour at room temperature.
   Prepare samples and calibrators during this time.

2) Wash plates 3 times with 200 µL per well 1X PBS. Add 25 µL of Diluent 7 to all wells. Next, add 25 µL of calibrator dilutions to appropriate calibrator wells, and 25 µL of diluted serum/plasma samples to sample wells. Seal the plate and incubate with shaking for two hours at room temperature.

*Shaking the plate accelerates analyte capture.*

3) Wash plates 3 times with 200 µL per well 1X PBS. Add 25 µL of detection antibody solution to each well. Seal the plate and incubate with shaking for 1 hour at room temperature.

4) Wash plates 3 times with 200 µL per well 1X PBS. Add 150 µL of 1X MSD Read buffer T solution to each well and read immediately on the MSD instrument.

*Bubbles in the read buffer will interfere with reliable imaging of the plate if carried into the wells.*