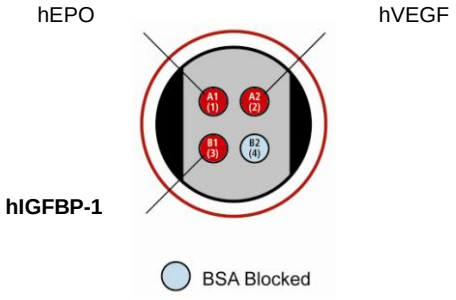
MSD® 96-Well MULTI-ARRAY®
Human IGFBP-1 Tissue Culture Assay

The following assay protocol has been optimized for analysis of human Insulin-like Growth Factor Binding Protein-1 (IGFBP-1) in tissue culture samples.

MSD Materials

	Storage
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker A Kit	RT
❑ MULTI-SPOT® 96-well 4 Spot Human Hypoxia Plate(s)	2-8 ºC
❑ SULFO-TAG™ Anti-hIGFBP-1 Antibody (100X)[1]	2-8 ºC
❑ Diluent 1	2-8 ºC
❑ Diluent 100	2-8 ºC
❑ Human IGFBP-1 Calibrator (1 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.

Notes:

Read the entire thorough instructions before beginning work.

Other Materials & Equipment (not supplied)

- Deionized water for diluting Wash Buffer and Read Buffer.
- Phosphate Buffered Saline with 0.05% Tween-20 (PBS-T) for plate washing
- Adhesive plate seals
- Microtiter plate shaker
- Automatic 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, 50, and 150µL into a 96-well microplate

Protocol at a Glance

The following protocol describes a preferred assay format. The protocol can be completed in approximately 4 hours if each reagent is prepared during the preceding incubation. This time can reduced to 2.5 hours if the blocking reagent is added the night before.

1. Block plates for 1 hour at room temperature (alternatively block plates overnight at 4 ^{o}C).
2. Wash.
3. Add Detection Antibody Reagent and Calibrator and/or sample and incubate 2 hours.
4. Wash.
5. Add Read Buffer and analyze immediately.

Preparation Instructions

Prepare Blocker A Kit:

Prepare Blocker A solution following the instructions included in 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 IGFBP-1 Calibrator stock solution and prepare the required Calibrator dilution series using the stock solution and Diluent 1.

Notes:

- A recommended Calibrator dilution procedure is listed below for up to 4 replicates of 7 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
- *Prepare 200 µL of a high Calibrator containing 100 ng/mL IGFBP-1 by combining 20 µL of 1 µg/mL IGF-BP1 Calibrator solution with 180 µL of Diluent 1.*
- *Prepare 6 additional 1:4 serial dilutions, beginning with the high combined Calibrator, by adding 50 µL of the Calibrator to 150 µL Diluent 1.*
- *This will create 7 Calibrators with 100000, 25000, 6250, 1563, 391, 98, 24 pg/mL of IGFBP-1.*
- *The recommended 8th dilution is Diluent 1 alone (e.g. zero Calibrator).*

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

Prepare Detection Antibody Reagent:

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

2. In a 15 mL tube combine:
   a. 2.97 mL Diluent 100
   b. 30 µL of 100X SULFO-TAG Anti-hIGFBP-1 Antibody (final concentration: 1X)

3. Detection Antibody Reagent is stable at room temperature for a few hours.

Prepare Diluted Read Buffer:

Diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.

1. Determine total number of wells in experiment. Each well will receive 150 µL of 1X Read Buffer T, with surfactant.

2. Dilute 4X Read Buffer T, with surfactant to 1X with deionized water.

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

Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Human Hypoxia plate.
No pre-treatment is necessary.

1. Add 150 µL/well of blocking solution A and incubate at room temperature for 1 hour or overnight at 4 ^{o}C.

2. Wash plates 3 times with Phosphate Buffered Saline + 0.05% Tween-20 (PBS-T).

3. Dispense 25 µL/well of Detection Antibody Reagent and 25 µL/well Calibrator, or sample, and incubate at room temperature with shaking for 2 hours.

4. Wash plates 3 times with PBS-T.

5. Prepare SECTOR Imager such that plate can be read immediately after Read Buffer addition.

6. Add 150 µL/well 1X Read Buffer T.

7. Analyze immediately with SECTOR Imager.