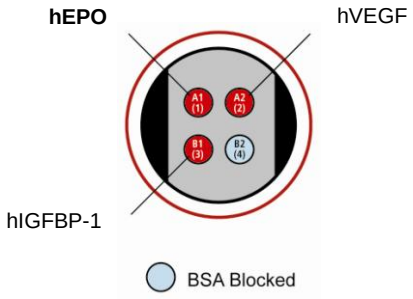
MSD® 96-Well MULTI-ARRAY®
Human EPO Tissue Culture Assay

The following assay protocol has been optimized for analysis of human Erythropoietin (EPO) 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-hEPO Antibody (100X)[1]	2-8 ºC
❑ Diluent 1	2-8 ºC
❑ Diluent 100	2-8 ºC
❑ Human EPO Calibrator (20 IU/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 150 µL 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 EPO 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 10 IU/mL of EPO by adding 100 µL of the 20 IU/mL EPO stock solution to 100 µL of Diluent 1.*
- *Prepare 6 additional 1:4 serial dilutions, beginning with the high Calibrator, by adding 50 µL of the Calibrator to 150 µL Diluent 1.*
- *This will create 7 Calibrators with 10000, 2500, 625, 156, 39, 9.8, 2.4 mIU/mL EPO.*
- *The recommended 8th dilution is Diluent 1 alone (e.g. zero Calibrator).*

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

3. The human EPO calibrator has been anchored and referenced to an international standard. The table below summarizes the reference information.

Analyte	WHO Standard Reference Number	WHO Standard Units / µg	MSD Calibrator 1µg = WHO Units	WHO Units
h EPO	88/574	127	130	IU

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-hEPO Antibody (final concentration: 1X)

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

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

Prepare Diluted Read Buffer:

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.*

Notes:

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 °C.

Plates may also be blocked overnight at 4ºC and stored for up to a week with blocker.

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.

Shaking a 96-well MSD MULTI-ARRAY® or MULTI-SPOT plate typically accelerates capture at the working electrode.

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.

Bubbles in the Read Buffer will interfere with reliable imaging of the plate.

7. Analyze immediately with SECTOR Imager.