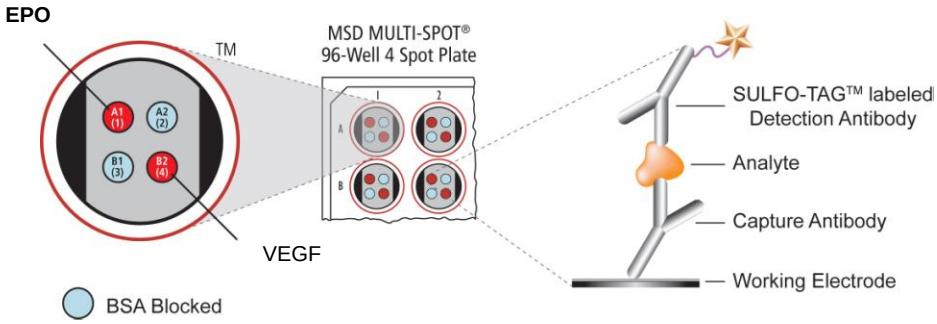
MSD® MULTI-ARRAY® Mouse/Rat EPO Tissue Culture Assay

The following assay protocol has been optimized for analysis of Mouse/Rat 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 Mouse/Rat Hypoxia Plate(s)	2-8 ºC
❑ SULFO-TAG™ Anti-m/r EPO Antibody (100X stock)[1]	2-8 ºC
❑ Diluent 1	2-8 ºC
❑ Diluent 8	≤-10 °C
❑ Mouse EPO Calibrator (0.1 µg/mL)	≤-70 °C
❑ Rat EPO Calibrator (0.1 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:

Other Materials & Equipment (not supplied)

- Deionized water for diluting Wash Buffer and Read Buffer
- Phosphate Buffered Saline + 0.05% Tween-20 (PBS-T) for plate washing
- Adhesive plate seals
- Microtiter plate shaker
- Plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- Liquid handling equipment for desired throughput that must accurately dispense 25, 50, and 150 µL into a 96-well micro plate

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The following protocol describes a preferred assay format. 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.

Step 1. Add Blocking Solution, incubate 1-2 hours, wash. (alternatively, block plates overnight at 4 ^{o}C).

Step 2. Add 25 µL of Samples or Calibrator, incubate 2 hours, wash.

Step 3. Add 25 µL of Detection Antibody, incubate 2 hours, wash.

Step 4. Add 150 µL of Read Buffer, read plate and analyze data.

Preparation Instructions

Prepare Blocker A Kit:

Prepare Blocker A solution following the instructions included in the Blocker A kit.

Notes:

Prepare Calibrator dilutions:

Depending on the desired application, the following procedure can be applied to Mouse or Rat Calibrators.

1. Determine the number of Calibrator concentrations and replicates that will be tested. 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 Calibrator stock solution and Diluent 1. A sample plate layout is shown in Figure 1.
   a) A recommended Calibrator dilution procedure is listed below for 3 replicates of 7 Calibrator concentrations, plus 1 zero-Calibrator point.
      - *Prepare 200 µL of a 10 ng/mL EPO Calibrator by adding 20 µL of the 0.1 µg/mL EPO Calibrator stock solution to 180µL of Diluent 1.*
      - *Prepare a seven point calibration curve using 1/4 serial dilution as follows: Begin with the above diluted solution of EPO at 10 ng/mL as the top of the curve and add 50 µL of solution to 150 µL Diluent 1 to make a Calibrator solution at 2500 pg/mL. Repeat the 1/4 serial dilution five times to create 7 Calibrators containing 10000, 2500, 625, 156, 39, 9.8, & 2.4 pg/mL of EPO.*
      - *The recommended 8th Calibrator is Diluent 1 (e.g. zero Calibrator).*

   b) Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately.
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 8
   b) 30 µL of 100X SULFO-TAG Anti-m/r EPO Antibody (final concentration: 1X)

Detection Antibody Reagent is stable at room temperature for a few hours and should be stored in the dark when not in use.

Prepare Diluted Read Buffer:

1. Approximately 20 mL per plate is required.
2. Dilute 4X Read Buffer T (with surfactant) to 1X with deionized water.

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

Notes:

Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Mouse/Rat Hypoxia Plate. No pre-treatment is necessary.

1. Add 150 µL/well of blocking solution and incubate at room temperature for 1 hour or overnight at 4 °C.
2. Wash plates 3X with PBS-T.
3. Dispense 25 µL/well of Calibrator or sample, and incubate at room temperature with shaking for 2 hours.
4. Wash plates 3X with PBS-T.
5. Dispense 25 µL/well of Detection Antibody Reagent and incubate at room temperature with shaking for 2 hours.
6. Wash plates 3X with PBS-T.
7. Prepare SECTOR Imager such that plate can be read immediately after Read Buffer addition.
8. Add 150 µL/well 1X Read Buffer T.
9. Analyze immediately with SECTOR Imager. This is important because the EPO assay signal decreases about 20% over 5 minutes.

Bubbles introduced to the well during Read Buffer addition will interfere with reliable imaging of the plate.

		1	2	3	4	5	6	7	8	9	10	11	12
Calibration curve dilution series	A	Cal7											
	B	Cal6											
	C	Cal5											
	D	Cal4											
	E	Cal3											
	F	Cal2											
	G	Cal1											
	H	0											
		Rat or Mouse calibrators			**samples**								

Figure 1. Sample plate layout that can be used for this assay.