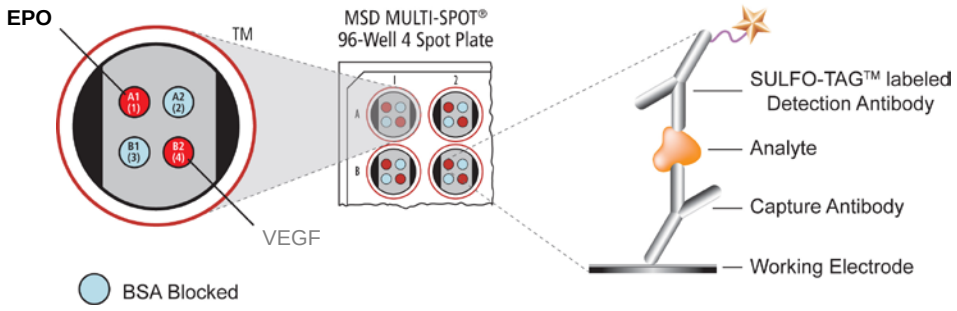
MSD® MULTI-ARRAY® Mouse/Rat EPO Assay

The following assay protocol has been optimized for analysis of Mouse/Rat EPO in Serum and Plasma samples.

MSD Materials Included

	Storage
❑ MULTI-SPOT® 96-well 4 Spot Mouse/Rat Hypoxia Plate(s)	2-8ºC
❑ SULFO-TAG™ Anti-m/r EPO Antibody (100X stock)[1]	2-8ºC
❑ Diluent 6	≤-10°C
❑ Diluent 8	≤-10°C
❑ Diluent 16	≤-10°C
❑ Mouse EPO Calibrator (0.1 µg/mL)	≤-70°C
❑ Rat EPO Calibrator (0.1 µg/mL)	≤-70°C
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker A Kit	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.

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

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 microplate

Protocol at a Glance

Read the entire detailed instructions before beginning work.

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 2-8°C).

Step 2. Add 25 µL of Diluent 16.
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 the EPO Calibrator stock solution and prepare the required Calibrator dilution series using the Calibrator stock solution and Diluent 6. A sample plate layout is shown in Figure 1.
2. 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 Calibrator containing 10 ng/mL EPO by adding 20 µL of the Calibrator stock solution containing 0.1 µg/mL of EPO to 180 µL of Diluent 6.*
    - *Prepare 200 µL of a 2,500 pg/mL Calibrator by adding 50 µL of the Calibrator at 10 ng/ml to 150 µL of Diluent 6 (1:4 dilution).*
    - *Prepare 5 additional 1:4 serial dilutions, by adding 50 µL to 150 µL of Diluent 6.*
    - *This will create 7 Calibrators containing 10,000; 2,500; 625; 156; 39; 9.8; & 2.4 pg/mL of EPO.*
    - *The recommended 8th Calibrator is Diluent 6 (e.g. zero Calibrator).*

    b) Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately.
3. 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. 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 kept in a tightly sealed container at room temperature for later use.

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 2-8°C.
2. Wash plates 3X with PBS-T.
3. Dispense 25 µL/well of Diluent 16 into each well.
4. Dispense 25 µL/well of Calibrator or sample and incubate at room temperature with shaking for 2 hours.
5. Wash plates 3X with PBS-T.
6. Dispense 25 µL/well of Detection Antibody Reagent and incubate at room temperature with shaking for 2 hours.
7. Wash plates 3X with PBS-T.
8. Prepare SECTOR Imager such that plates can be read immediately after Read Buffer addition.
9. Add 150 µL/well 1X Read Buffer T.
10. Analyze immediately on an MSD instrument. This is important because the EPO assay signal decreases about 15% over 5 minutes.

Notes:

Plates may also be blocked overnight at 2-8°C and stored for up to a week with blocker.

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

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

Changes in EPO assay signal with time in Read Buffer can also be minimized by waiting 10 minutes between Read Buffer T addition and analysis of the plate with the SECTOR Imager.

		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.