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

The following assay protocol has been optimized for analysis of human Vascular Endothelial Growth Factor (VEGF) in tissue culture samples.

MSD Materials

	Storage
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker A Kit	RT
❑ MULTI-ARRAY 96-well Small Spot VEGF Plate(s)	2-8 ºC
❑ SULFO-TAG™ Anti-hVEGF Antibody (100X)[1]	2-8 ºC
❑ Diluent 1	2-8 ºC
❑ Diluent 100	2-8 ºC
❑ Human VEGF Calibrator (1µg/mL)	≤-70 °C

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
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25, 50, and 150 µL into a 96-well micro plate

[1] SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

Protocol at a Glance

Notes:

Read the entire detailed instructions before beginning work.

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 VEGF Calibrator stock solution and prepare the required Calibrator dilution series using the stock solution and Diluent 1.
   - 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 400 µL of a high Calibrator containing 25 ng/mL VEGF by combining 10 µL of VEGF stock solution at 1 µg/mL with 390 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 25000, 6250, 1563, 391, 98, 24, 6 pg/mL of VEGF.*
   - *The recommended 8th dilution is Diluent 1 alone (e.g. zero Calibrator).*

Notes:

- ❖ Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately. NOTE: At very high VEGF levels (greater than 25000 pg/mL), the calibration curve may hook. It is recommended that samples with VEGF levels in this range be diluted so that they are measured in the linear portion of the calibration curve.

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

3. The human VEGF calibrator has been anchored and referenced to international standards. The table below summarizes the reference information.

Analyte	WHO Standard Reference Number	WHO Standard Units / μg	MSD Calibrator 1μg = WHO Units	WHO Units
h VEGF	01/424	n/a	0.5	μg
h VEGF	02/286	1,000	360	U

** MSD VEGF Calibrator previously used in Human VEGF Kits and Human Hypoxia Kits was anchored to WHO Standard Reference 01/424 with 1 μg of MSD Calibrator = 1 μg of WHO Standard

Prepare Detection Antibody Reagent:

1. Each well will require 25 μL of Detection Antibody Reagent. Prepare 4 mL per plate.

2. In a 15 mL tube combine:
   a. 3.99 mL Diluent 100
   b. 10 μL of 100X SULFO-TAG Anti-hVEGF Antibody (final concentration: 0.25X)

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

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.

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

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

Notes:

Assay Protocol

Begin with a MULTI-ARRAY 96-well Small Spot VEGF Plate.
No pre-treatment is necessary.

1. Add 150µL/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.