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

The following assay protocol has been optimized for analysis of Human Vascular Endothelial Growth Factor (VEGF) in human serum and plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker C	2-8 °C
❑ MULTI-ARRAY 96-well Small Spot VEGF Plate(s)	2-8 °C
❑ SULFO-TAG™ Anti-hVEGF Antibody (100X)[1]	2-8 °C
❑ Diluent 7	≤-10 °C
❑ Diluent 8	≤-10 °C
❑ Diluent 9	≤-10 °C
❑ Human VEGF Calibrator (1µg/mL)	≤-70 °C

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting 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 and 150 µL into a 96-well microplate

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

Protocol at a Glance

The protocol can be completed in approximately 5.5 hours if each reagent is prepared during the preceding incubation. This time can reduced to **4.5 hours if the blocking reagent is added the night before**.

1. Block plates for 1-2 hours at room temperature (alternatively block plates overnight at 4 °C).
2. Wash.
3. Add Diluent 7 and Calibrator and/or sample and incubate for 2 hours with shaking.
4. Wash.
5. Add Detection Antibody Reagent and incubate for 2 hours with shaking.
6. Wash.
7. Add Read Buffer and read immediately.

Preparation Instructions

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 9.
   - 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 VEGF by combining 20 µL of VEGF stock solution at 1 µg/mL with 180 µL of Diluent 9.*
   - *Prepare 6 additional 1:4 serial dilutions, beginning with the high Calibrator, by adding 50 µL of the Calibrator to 150 µL Diluent 9.*
   - *This will create 7 Calibrators with 100000, 25000, 6250, 1563, 391, 98, and 24 pg/mL of VEGF.*
   - *The recommended 8th dilution is Diluent 9 alone (e.g. zero Calibrator).*

   ❖ 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.

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

Notes:

Read the entire detailed instructions before beginning work.

The assay protocol was optimized for human serum samples. For significantly different sample matrices, it is recommended to use a Calibrator diluent that is similar to the sample matrix (e.g. lysis buffer + carrier protein).

Notes:

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 requires 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-hVEGF Antibody (final concentration: 1X)

Dilute Read Buffer:

1. Determine total number of wells in the experiment. Each well will receive 150 μL of Read Buffer T. Prepare an extra 20%.
2. Dilute 4X Read Buffer T to 1X with deionized water.
3. Diluted Read Buffer may be stored at room temperature for later use.

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

Assay Protocol

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

1. Add 150 μL/well of Blocker C and incubate at room temperature for 1-2 hours 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. Add 25 μL/well of Diluent 7.
   Add 25 μL/well Calibrator or sample and incubate at room temperature with shaking for 2 hours.

4. Wash plates 3 times with PBS-T.

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

5. Add 25 μL/well Detection Antibody Reagent and incubate at room temperature with shaking for 2 hours.

6. Wash plates 3 times with PBS-T.

7. Prepare SECTOR® Imager so that plate can be read immediately after Read Buffer addition.

8. Add 150 μL/well 1X Read Buffer T.

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

9. Analyze immediately with SECTOR Imager.