MSD® 384-Well MULTI-ARRAY® Human Vascular Endothelial Growth Factor (VEGF) Assay

The following assay protocol has been optimized for analysis of VEGF in Tissue Culture samples.

MSD Materials

	Storage
❑ Read Buffer T (4X), with surfactant	RT
❑ Blocker A Kit	RT
❑ MULTI-ARRAY 384-well Human VEGF Plate(s)	2-8 ºC
❑ SULFO-TAG™ Anti-hVEGF Antibody (100 X)[1]	2-8 ºC
❑ Diluent 1	2-8 ºC
❑ Diluent 100	2-8 ºC
❑ Human VEGF Calibrator (1µg/mL)	≤-70 ºC

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Wash Buffer and Read Buffer
- ❑ Phosphate Buffered Saline (PBS) for plate washing
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Plate washer or other efficient multi-channel pipetting equipment for washing 384-well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 10, and 35 µL into a 384-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

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 be reduced to 2.5 hours if the blocking reagent is added the night before. All reagents can be prepared hours ahead of time if desired.

1. Block plates for 1-2 hours 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.

Notes:

Read the entire detailed instructions before beginning work.

Preparation Instructions

Prepare Blocker A Kit:

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

Prepare Calibrator dilutions:

Determine how many Calibrator levels and replicates will be tested. Each well will require 10 µL of Calibrator. Thaw one vial of VEGF Calibrator stock solution and prepare the required Calibrator dilution series using the stock solutions and Diluent 1.

a) A recommended Calibrator dilution procedure is listed below for multiple replicates of 7 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.

- *Prepare 100 µL of VEGF Calibrator at a concentration of 100 ng/mL by adding 10 µL of the VEGF stock solution at 1 µg/mL to 90 µL of Diluent 1. Vortex briefly, and let the solution equilibrate for approximately 15 minutes.*
- *Prepare 6 additional 1:10 serial dilutions, beginning with the VEGF Calibrator at 100 ng/mL, by adding 10 µL of the high Calibrator to 90 µL Diluent 1.*
- *This will create 7 Calibrators with 100000, 10000, 1000, 100, 10, 1, and 0.1 pg/mL of VEGF. The recommended 8th dilution is Diluent 1 alone (e.g. zero Calibrator).*

b) Once the expected range of sample concentrations is known, the Calibrator concentrations can be adjusted appropriately.

Calibrators are stable at room temperature for a few hours.

Notes:

Prepare Detection Antibody Reagent:

1. Each well requires 10 µL of Detection Antibody Reagent. Prepare 8 mL per plate.
2. In a 15 mL tube combine:
   a. 7.92 mL Diluent 100.
   b. 80 µl of 100X SULFO-TAG Anti-hVEGF Antibody (final concentration: 1X)
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 35 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.

Assay Protocol

Begin with a MULTI-ARRAY 384-well Human VEGF Plate.
No pre-treatment is necessary.

1. Add 35µL/well of blocking solution and incubate at room temperature for 1 hour or overnight at 4 ^{o}C.

2. Wash plates 3 times with Phosphate Buffered Saline (PBS).

3. Dispense 10 µL/well of Detection Antibody Reagent and 10 µL/well Calibrator, or sample, and incubate at room temperature with shaking for 2 hours.

4. Wash plates 3 times with PBS.

5. Prepare SECTOR® Imager such that plate can be read immediately after Read Buffer addition.

6. Add 35 µL/well 1X Read Buffer T.

7. Analyze immediately with SECTOR Imager.

Avoid bubbles while adding the Read Buffer; it will interfere with accurate reading of the plate.