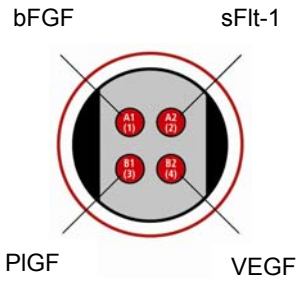
MSD® 96-Well MULTI-SPOT®
Human Growth Factor Panel I Assay

The following assay protocol has been optimized for analysis of basic fibroblast growth factor (bFGF), soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) in human serum and plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (4X)	RT
❑ MULTI-SPOT 96-well 4 Spot Human Growth Factor I Plate(s)	2-8 °C
❑ SULFO-TAG™ Anti-Human Growth Factor I Detection Antibody Blend (100X)[1]	2-8 °C
❑ Blocker C	2-8 °C
❑ Diluent 7	≤-10 °C
❑ Diluent 8	≤-10 °C
❑ Diluent 9	≤-10 °C
❑ Human Growth Factor I High Calibrator Blend (9 ng/mL each of bFGF, sFlt-1, PlGF, & VEGF)	≤-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 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

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The assay protocol was optimized for serum and plasma 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).

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**.

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

Step 2. Add 25 µL of Diluent 7.
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

Thaw Diluents:

Thaw Diluent 7, Diluent 8, and Diluent 9. Vortex briefly. If there is a precipitate, mix gently and warm to room temperature to dissolve. Diluents are stable at 4 °C for one week.

Prepare Calibrator dilutions:

1. Determine how many Calibrator levels and replicates will be tested in the experiment. Each well will require 25 µL of Calibrator. Thaw Diluent 9 and one vial of High Calibrator Blend stock solution. Vortex briefly. Prepare the required Calibrator dilution series using Diluent 9.

Notes:

2. A recommended Calibrator dilution procedure is listed below for 4 replicates of 6 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.
    i. *Prepare a seven point calibration curve using 1/4 serial dilution as follows: Begin with the Combined High Calibrator at 9 ng/mL as the top of the curve and add 50 µL of solution to 150 µL Diluent 9 to make a Calibrator solution at 2.25 ng/mL. Repeat the 1/4 serial dilution five times to make Calibrator solutions of 0.563, 0.141, 0.035, 0.009 and 0.002 ng/mL.*
    ii. *The recommended 8th dilution is Diluent 9 alone (e.g. zero Calibrator).*

3. Calibrators are stable at room temperature for a few hours. The High Calibrator Blend stock solution is stable for one day at 4 ^{o}C and for one additional freeze-thaw cycle. Diluent 9 is stable for one week at 4 ^{o}C.

Prepare the 1X Detection Antibody Solution:

a) In a 15 mL tube combine:
    - ❑ 30 µL of 100X SULFO-TAG Detection Antibody Blend
    - ❑ 2.97 mL of Diluent 8

Detection Antibody Solution is stable at RT for a few hours.

b) This will yield 3 mL of diluted Detection Antibody Solution at the working concentration with sufficient volume for one plate.

Dilute Read Buffer:

In a 50 mL tube combine (per plate):
- □ 5 mL 4X Read Buffer T
- □ 15 mL deionized water

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

Assay Protocol

Notes:

Begin with a MULTI-SPOT 96-well 4 Spot Human Growth Factor I 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.

2. Wash plates 3 times with phosphate buffered saline with 0.05% Tween-20 (PBS-T).

3. Add 25 µL/well of Diluent 7.

4. Add 25 µL/well Calibrator or sample and incubate at room temperature with shaking for 2 hours.

5. Wash plates 3 times with PBS-T.

6. Add 25 µL/well of 1X Detection Antibody Solution and incubate at room temperature with shaking for 2 hours.

7. Wash plates 3 times with PBS-T.

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

9. Add 150 µL/well 1X Read Buffer T.

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

10. Analyze immediately with SECTOR Imager.