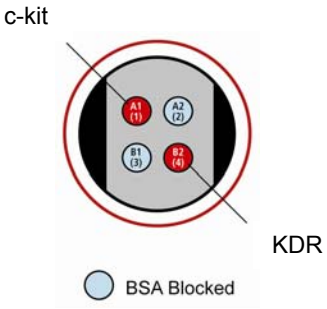
MSD® 96-Well MULTI-SPOT®
Human Growth Factor panel II Assay

The following assay protocol has been optimized for analysis of kinase domain insert (KDR) and the kit ligand receptor (c-Kit) in human serum and plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (4X) with surfactant	RT
❑ Blocker A Kit	RT
❑ MULTI-SPOT 96-well 4 Spot Human Growth Factor II Plate(s)	2-8 °C
❑ SULFO-TAG™ Anti-Human Growth Factor II Detection Antibody Blend (100X)[1]	2-8 °C
❑ Diluent 10	≤-10 °C
❑ Diluent 11	≤-10 °C
❑ Human Growth Factor II High Calibrator Blend (150 ng/mL c-Kit, 15 ng/mL KDR)	≤-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 washing equipment 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 micro plate

Read the entire detailed instructions before beginning work.

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**.

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

Step 2. Add 50 µL of Calibrator or Samples (diluted 50X), 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:

Prepare Blocker A Solution using the instructions provided with the Blocker A kit.

Thaw Diluent:

Thaw Diluent 10. Vortex briefly. Diluent is stable at 4^{o}C for one week.

Prepare Calibrator and Sample dilutions:

1. Determine how many Calibrator levels and replicates will be tested in the experiment. Each well will require 50 µL of Calibrator or 50 µL of diluted sample per well. Thaw Diluent 10 and one vial of High Calibrator Blend. Vortex briefly. Prepare the required Calibrator dilution series using Diluent 10.

Notes:

2. A recommended Calibrator dilution procedure is listed below for 3 replicates of 6 Calibrator concentrations spanning a wide range, plus 1 zero-Calibrator point.

   - *Prepare 1:3 serial dilutions beginning with the High Calibrator Blend, by adding 100 µL of High Calibrator Blend to 200 µL of Diluent 10. Prepare 6 serial dilutions. The first Calibrator will be High Calibrator Blend stock and the 8th Calibrator should be Diluent 10 alone.*

   - *This will create seven Calibrators with 15 ng/mL, 5 ng/mL, 1.67 ng/mL, 0.556 ng/mL, 0.185 ng/mL, 0.062 ng/mL, 0.021 ng/mL, and 0 ng/mL.* ***The c-Kit concentrations will be 10X higher****.*

   - ***Since the sample will be diluted 1:50, the concentrations of the Calibrators need to be multiplied by 50 if samples are read directly from the calibration curve. Thus, the dilution-corrected High KDR Calibrator is 750 ng/mL, and the dilution-corrected High c-Kit Calibrator is 7500 ng/mL.***

3. Calibrators are stable at room temperature for a few hours. The High Calibrator Blend stock solution is stable for one day at 4 °C or one additional freeze-thaw. Diluent 10 is stable for one week at 4 °C.

4. Dilute samples 1:50 in Diluent 10. Each well will require 50 µL of diluted sample.

Prepare the 1X Detection Antibody Solution:

1. In a 15 mL tube combine:
   - ❑ 30 µL of 100X SULFO-TAG Detection Antibody Blend
   - ❑ 2.97 mL of Diluent 11

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

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

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.

Notes:

Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Human Growth Factor II Plate. No pre-treatment is necessary.

1. Add 150 µL/well of Blocker A Solution and incubate at room temperature for 1-2 hours or overnight at 4 °C.

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

3. Add 50 µL/well of Calibrator or diluted sample and incubate at room temperature with shaking for 2 hours.

4. Wash plates 3 times with PBS-T.

5. Add 25µL/well of 1X Detection Antibody Solution 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.

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

9. Analyze immediately with SECTOR Imager.