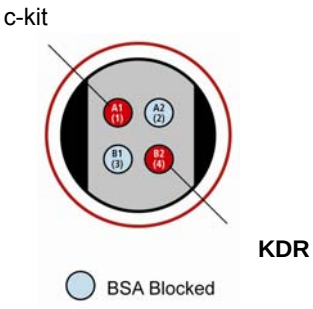
MSD® 96-Well MULTI-ARRAY® Human KDR Assay

The following assay protocol has been optimized for analysis of kinase domain insert (KDR) 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-hKDR Antibody (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 150µL 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**. All reagents can be prepared hours ahead of time if desired.

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

Step 2. Add 50 µL of Calibrator or diluted Samples (diluted 50X in Diluent 10), 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:

1. Prepare Blocker A Solution using the instructions provided with the Blocker A kit.
2. Thaw Diluent 10. Vortex briefly. Diluent is stable at 4°C for one week.

Notes:

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 a vial of Diluent 10 and one vial of High Calibrator. Vortex briefly. Prepare the required Calibrator dilution series using Diluent 10.
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, by adding 100 µL of High Calibrator to 200 µL of Diluent 10. Prepare 6 serial dilutions. The first Calibrator will be High Calibrator 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 KDR.*
   - ***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.***
3. Calibrators are stable at room temperature for a few hours. The High Calibrator 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 Detection Antibody Reagent:

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

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 11
   b. 30 µL of 100X SULFO-TAG Anti-hKDR Antibody (final concentration: 1X)

Dilute Read Buffer:

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

In a 50 mL tube combine (per plate):

1. 5 mL 4X Read Buffer T
2. 15 mL deionized water

Assay Protocol

Notes:

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 ^{o}C.

2. Wash plates 3 times with Phosphate Buffered Saline + 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 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.

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

9. Analyze immediately with SECTOR Imager.