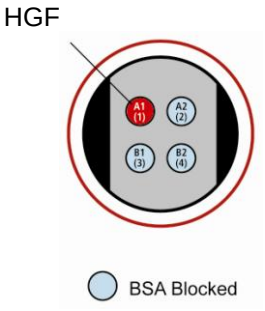
MSD® 96-Well MULTI-ARRAY®
Human Hepatocyte Growth Factor (HGF) Assay

The following assay protocol has been optimized for analysis of HGF in human serum and plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (with surfactant) (4X)	RT
❑ Blocker A Kit	RT
❑ MULTI-SPOT® 96-well 4 Spot Human HGF Plate	2-8 °C
❑ SULFO-TAG™ Anti-hHGF Antibody (100X)[1]	2-8 °C
❑ Diluent 8	≤-10 °C
❑ Diluent 13	≤-10 °C
❑ Human HGF Calibrator (1 µg/mL)	≤-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 (PBS) 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 protocol can be completed in approximately 5.5 hours if each reagent is prepared during the preceding incubation. This time can be 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 13 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 Blocker A:
Prepare Blocker A solution from Blocker A kit according to kit instructions.

Solutions containing Blocker A should be kept at 4ºC and discarded after 14 days.

Dilute Read Buffer:
Dilute 4X Read Buffer T to 1 X with deionized water.
Approximately 20 mL of 1X Read Buffer is required per plate.

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

Thaw Diluents
Thaw diluents. Vortex briefly. If there is a precipitate, mix gently and warm to room temperature to dissolve.

Prepare Calibrator dilutions:

1. Determine how many Calibrator levels and replicates will be tested. Each well will require 25 µL of Calibrator. Thaw one vial of Human HGF Calibrator stock solution. Vortex briefly. Prepare the required concentrations by serially diluting Calibrator stock into Diluent 13.

Notes:

2. *A recommended Calibrator dilution procedure for 3 replicates is to prepare an initial high Calibrator at 100000 pg/ml by adding 10 µL of Human HGF Calibrator at 1 µg/mL to 90 µL Diluent 13, and then prepare six additional 1:10 serial dilutions. Use Diluent 13 alone as the zero Calibrator concentration. The resulting Calibrator levels will be 100000, 10000, 1000, 100, 10, 1, 0.1, and 0 pg/mL HGF. Once the concentration range of samples is known, a smaller calibration range can be used.*

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-hHGF Antibody (final concentration: 1X)

Assay Protocol

Begin with a MULTI-SPOT 96-well 4 Spot Human HGF plate.
No pre-treatment is necessary.

1. Add 150 µL/well of Blocker A 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 (PBS).
3. Add 25 µL/well of Diluent 13.
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.
6. Add 25 µL/well Detection Antibody Reagent and incubate at room temperature with shaking for 2 hours.
7. Wash plates 3 times with PBS.
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. Read plate on SECTOR Imager immediately after Read Buffer addition. Discard plate after reading.