*MSD® 96-Well MULTI-ARRAY® Human **TGF-β1 Assay***

The following assay protocol has been optimized for analysis of transforming growth factor β1 (TGF-β1) in human serum samples.

MSD Materials

	storage
❑ MSD Read Buffer T(With Surfactant), 4X	RT
❑ MSD Blocker A Kit	RT
❑ MULTI-ARRAY 96-well Small Spot TGF-β1 plate(s)	2-8ºC
❑ MSD SULFO-TAG™ Anti-hTGF-β1 Antibody (50X)[1]	2-8ºC
❑ Diluent 100	2-8ºC
❑ Diluent 7	≤-10 °C
❑ Diluent 9	≤-10 °C
❑ Human TGF-β1 Calibrator (1 μg/mL)	≤-70 °C

Other Materials & Equipment (not supplied)

- ❑ Various microcentrifuge tubes for making serial dilutions of test solutions
- ❑ Phosphate buffered saline + 0.05% Tween-20 (PBS-T)
- ❑ Ultrapure water
- ❑ Plate washer or other efficient multi-channel pipetting equipment for washing 96-well plate.
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Appropriate liquid handling equipment for desired throughput.
- ❑ Acid solution (1N HCl)
- ❑ Neutralization solution (1.2N NaOH/0.5M HEPES)

[1] Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

Protocol at a Glance

The protocol can be completed in approximately 4.5 hours if each reagent is prepared during the preceding incubation.

Step 1. Add Blocking solution, incubate 1 hour, wash.

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 1 hour, wash.

Step 4. Add 150 µL of Read Buffer and analyze plate.

Preparation Instructions

Prepare Blocker A solution:

Follow instructions included with the Blocker A Kit.

Prepare Read Buffer Solution:

In a 50 mL tube combine (per plate):

- ☐ 5 mL 4X Read Buffer T
- ☐ 15 mL deionized water

Thaw Diluents

Thaw Diluent 7, and Diluent 9. Vortex briefly once thawed. If there is a precipitate, mix gently and warm to room temperature to dissolve. Keep all materials on ice until use. The remaining amounts of the diluents after use can be aliquotted and refrozen as needed.

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 a vial of TGF-β1 Calibrator on ice. Vortex briefly. The highest point on the calibration curve is recommended to be 0.1 µg/mL. Prepare this solution by diluting 15 µL of the Human TGF-β1 Calibrator with 135 µL of Diluent 9. Then, prepare a calibration curve by serially diluting the 0.1 µg/mL Calibrator solution 1:10 into Diluent 9. This can be achieved by taking 15 µL of 0.1 µg/mL Calibrator solution into 135 µL of Diluent 9. Repeat the 1:10 dilution 5 more times.
2) The recommended 8th dilution is Diluent 9 alone (e.g. zero Calibrator)
3) The Calibrator dilutions should be prepared immediately before use and kept on ice until use.

Prepare Samples

1) Each well will require 25 µL of diluted sample that has been activated by acid-treatment and subsequent neutralization. Sera and suitably collected platelet-poor EDTA plasma may be used.
2) To 40 µL serum/plasma, add 20 µL of 1N HCl, mix well and incubate 10 minutes at room temperature.
3) Neutralize the acidified sample by adding 20 µL of 1.2N NaOH/0.5M HEPES; mix well.

Notes:

Read the entire detailed instructions before beginning work.

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

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

Notes:

4) Dilute the activated sample 4 fold with Diluent 9. Prepare this solution by diluting 25 µL of activated sample with 75µL of Diluent 9. The samples should be prepared immediately before use and kept on ice until use.

Prepare Detection Antibody Solution

1) Determine the total number of wells in the experiment. Each well will require 25 µL of Detection Antibody Solution.
2) In a 15 mL tube combine:
   a. 60 µL 50X SULFO-TAG Anti-hTGF-β1 Antibody
   b. 2.94 mL Diluent 100
3) Detection Antibody Solution is stable on ice for a few hours.

Assay Protocol

Begin with a MULTI-ARRAY 96-well Small Spot TGF-β1 plate.
No pre-treatment is necessary.

1. Add 150 µL of MSD Blocker A per well of the TGF-β1 plate, cover and incubate for 30 min to 1 hour at room temperature.
2. During blocking prepare Calibrator dilutions, serially diluting using 10-fold dilutions into Diluent 9. Mix gently between dilution steps and keep solutions on ice.
3. Wash three times with 200 µL of phosphate buffered saline + 0.05% Tween-20 (PBS-T).
4. Add 25 µL of Diluent 7 to all wells. Next, 25 µL of Calibrator dilutions can be added to appropriate calibration wells, and 25 µL of treated and diluted serum/plasma samples to sample wells.
5. Cover plate and incubate with shaking for two hours at room temperature.
6. During the incubation, prepare Detection Antibody Solution as indicated above, and keep on ice until use.
7. Wash three times with 200 µL of PBS-T
8. Add 25 µL of the Detection Antibody Solution to each well. Cover and incubate with shaking for 1 hour at room temperature.
9. Wash three times with 200 µL PBS-T
10. Add 150 µL of the 1X MSD Read buffer T solution to each well and read immediately on the MSD SECTOR® Imager.

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