MSD® 96-Well MULTI-ARRAY® Human IFN-β Assay

The following assay protocol has been optimized for the quantitative measurement of human interferon beta (IFN-β) in tissue and cell culture samples.

Materials Included

	Storage
❑ MSD GOLD™ 96-well Avidin SECTOR® Plates	2-8°C
❑ Anti-hIFN-β Biotinylated Capture Antibody (50X)	2-8°C
❑ SULFO-TAG™ Anti-hIFN-β Antibody (50X)[1]	2-8°C
❑ Human IFN-β Calibrator[2]	≤-70°C
❑ Diluent 1[2]	2-8°C
❑ Diluent 100	2-8°C
❑ Read Buffer T (4X)	RT

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines. Additional safety information is available in the product safety data sheet, which can be obtained from MSD Customer Service.

[1] SULFO-TAG-conjugated detection antibodies should be stored in the dark.

[2] Human IFN-β Calibrator and Diluent 1 are not included in the Base Kit.

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting concentrated buffers
- ❑ Phosphate-buffered saline (PBS) for washing plates
- ❑ 500 mL bottle
- ❑ 50 mL tubes
- ❑ 15 mL tubes
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Various microcentrifuge tubes for making serial dilutions of lysates (if desired)
- ❑ Automated 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 µL and 150 µL into a 96-well microplate

Protocol at a Glance

Notes:

Read the entire detailed instructions before beginning work.

1. Wash plate.
2. Add capture antibody, incubate 1 hour.
3. Wash plate (optional).
4. Add detection antibody and samples, incubate 2 hours, wash.
5. Add Read Buffer T and analyze plate.

The protocol can be completed in approximately 3.5 hours if each reagent is prepared during the preceding incubation.

Preparation Instructions

Prepare Capture Antibody.

1. Determine total number of wells to be used in the experiment. Each well will require 20 µL of capture antibody.
2. Prepare capture antibody by diluting Anti-hIFN-β Biotinylated Capture Antibody in Diluent 100 to a final concentration of 1X.
3. Diluted capture antibody should be kept at 2-8°C until used.

Prepare Detection Antibody. You will need 3 mL per plate.

Detection antibody solution should be stored at 2–8ºC after preparation.

In a 15 mL tube combine:

- ❑ 2.94 mL Diluent 100
- ❑ 60 µL 50X Anti-hIFN-β Antibody (Final concentration: 1X)

Diluted detection antibody should be kept at 2-8°C until used.

Prepare Read Buffer T. You will need 20 mL per plate at a final 1X concentration.

In a 50 mL tube, combine:

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

Notes:

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

Prepare Standards.

MSD supplies Human IFN-β Calibrator at a stock concentration of 2.5 µg/mL. This is 25-fold higher concentration than the recommended highest standard. MSD recommends running at least two replicates of each standard. Each well will require 50 µL of standard. Prepare the desired concentrations by serially diluting the provided Human IFN-β Calibrator stock into Diluent 1.

To prepare 8 standard solutions for up to 3 replicates:

A recommended dilution procedure is listed below for preparing 3 replicates of each standard.

- ❑ Prepare the highest standard by adding 20 µL of the supplied calibrator stock (2.5 µg/mL) to 480 µL of Diluent 1. Mix thoroughly.
- ❑ Prepare the next standard by transferring 75 µL of the highest standard to 225 µL of Diluent 1. Mix well.
- ❑ Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
- ❑ Use Diluent 1 as the 8th standard (i.e. zero calibrator).
- ❑ This will create 7 standards with 100,000; 25,000; 6,250; 1,563; 391; 98; and 24 pg/mL of IFN-β.

Dilutions should be prepared immediately before use.

A minimum of two replicates of standards and samples should be assayed.

Calibrators should be kept at 2-8^{o}C if not used immediately.

This yields the following calibrator concentrations:

Calibrator	IFN-β (pg/mL)
STD 1	100,000
STD 2	25,000
STD 3	6,250
STD 4	1,563
STD 5	391
STD 6	98
STD 7	24
STD 8	0

Spot the Difference™

Assay Protocol

Notes:

Begin with an MSD GOLD 96-well Avidin SECTOR plate.

STEP 1 Wash Plate and Add Capture Antibody Solution.

a) **Wash** plate 3 times with 300 µL/well of PBS.

b) **Add** 20 µL/well of 1X capture antibody solution.

c) **Incubate** at room temperature with shaking for 1 hour.

Shaking the plate accelerates analyte capture.

STEP 2 Wash Plate (optional). Add Detection Antibody Solution and Samples.

a) **Wash** plate 3 times with 300 µL/well of PBS. **Note:** This wash step may be omitted.

b) **Add** 20 µL/well of detection antibody solution.

c) **Add** 50 µL/well calibrator or sample.

d) **Incubate** at room temperature with shaking for 2 hours.

STEP 3 Wash Plate and Read.

Bubbles in the read buffer will interfere with reliable imaging of the plate if carried into the wells.

a) **Wash** plate 3 times with 300 µL/well of PBS.

b) **Add** 150 µL/well of 1X Read Buffer T.

Read plate immediately after adding read buffer.

c) **Analyze** plate on SECTOR Imager.