

# MSD 96-Well MULTI-ARRAY Human IFN- $\beta$ Assay

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

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## Storage

### MSD Materials

<input type="checkbox"/> Read Buffer T (4X)	RT
<input type="checkbox"/> Diluent 1 <sup>1</sup>	2-8 °C
<input type="checkbox"/> Diluent 100	2-8 °C
<input type="checkbox"/> MULTI-ARRAY <sup>®</sup> 96-well Avidin Plates	2-8 °C
<input type="checkbox"/> SULFO-TAG <sup>™</sup> Anti-hIFN- $\beta$ Antibody (50X)	2-8 °C
<input type="checkbox"/> Anti-hIFN- $\beta$ Biotinylated Capture Antibody (50X)	2-8 °C
<input type="checkbox"/> Human IFN- $\beta$ Calibrator <sup>1</sup>	$\leq$ -70 °C

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### Other Materials & Equipment (not supplied)

- Deionized water for diluting Read Buffer
- Phosphate buffered saline for plate washing.
- Plate Shaker
- Adhesive plate seals
- 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 20, 50, and 150  $\mu$ L into a 96-well micro plate

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<sup>1</sup> Diluent 1 and Human IFN- $\beta$  Calibrator are not included in the Base Kit.

FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



**Notes:**

*Read the entire detailed instructions before beginning work.*

## **Protocol at a Glance**

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

1. Wash plates 3 times with PBS.
2. Add Capture Antibody and incubate 1 hour with shaking.
3. Wash plates 3 times with PBS. Note: This wash step may be omitted.
4. Add Detection Antibody and Calibrator and incubate for 2 hours with shaking.
5. Wash plates 3 times with PBS.
6. Add Read Buffer and read on SECTOR<sup>®</sup> Imager.

## **Preparation Instructions**

### **Prepare Capture Antibody:**

1. Determine total number of wells to be used in the experiment. Each well will require 20  $\mu\text{L}$  of Capture Antibody.
2. Prepare Capture Antibody by diluting Anti-hIFN- $\beta$  Biotinylated Capture Antibody in Diluent 100 to a final concentration of 1X.
3. Diluted Capture Antibody should be kept at 4  $^{\circ}\text{C}$  until used.

### **Prepare Calibrator Dilutions:**

1. Determine how many replicates of each Calibrator concentration will be used in the experiment. MSD<sup>®</sup> recommends running at least two replicates of each Calibrator concentration. Each well will require 50  $\mu\text{L}$  of Calibrator. Prepare the desired concentrations by serially diluting the provided Human IFN- $\beta$  Calibrator stock into Diluent 1. *A recommended Calibrator dilution procedure is listed below, for preparing three replicates of each Calibrator.*
  - Prepare the high Calibrator, containing 100000 pg/mL of Human IFN- $\beta$ , by adding 16  $\mu\text{L}$  of the supplied Calibrator stock (2.5  $\mu\text{g}/\text{mL}$ ) to 400  $\mu\text{L}$  of Diluent 1. Mix thoroughly.
  - Prepare 6 serial dilutions starting with the 100000 pg/mL high Calibrator and diluting by a factor of 4 (add 75  $\mu\text{L}$  high Calibrator to 225  $\mu\text{L}$  Diluent 1) to prepare the remainder of the dilution series.
  - This will create seven Calibrators with 100000, 25000, 6250, 1563, 391, 98, and 24 pg/mL of IFN- $\beta$ .
  - Reserve 200  $\mu\text{L}$  of Diluent 1 as the 8<sup>th</sup> (zero) Calibrator.
2. Calibrators should be kept at 4  $^{\circ}\text{C}$  if not used immediately.



**Prepare Detection Antibody:**

1. Prepare 3.0 mL per plate
2. In a 15 mL tube combine:
  - a. 2.94 mL Diluent 100
  - b. 60  $\mu$ L 50X Anti-hIFN- $\beta$  Antibody (Final concentration: 1X)
3. Diluted Detection Antibody should be kept at 4°C until used.

**Dilute Read Buffer:**

1. Determine total number of wells to be used in the experiment. Each well will receive 150  $\mu$ L of Read Buffer T. Prepare 20% extra over the required volume for use with a multi-channel pipettor.
2. Dilute 4X Read Buffer T to 1X with deionized water.
3. Diluted Read Buffer may be stored at room temperature for later use.

***Assay Protocol***

Begin with a MULTI-ARRAY 96-well Avidin plate.

1. Wash plates 3 times with phosphate buffered saline (PBS).
2. Add 20  $\mu$ L/well of 1X Capture Antibody and incubate at room temperature with shaking for 1 hour.
3. Wash plates 3 times with phosphate buffered saline (PBS).  
Note: This wash step may be omitted.
4. Add 20  $\mu$ L/well of 1X Detection Antibody and 50  $\mu$ L/well Calibrator or sample and incubate at room temperature with shaking for 2 hours.
5. Wash plates 3 times with PBS.
6. Add 150  $\mu$ L/well 1X Read Buffer T. *Avoid bubbles.* An electronic multi-pipettor at moderate speed setting is recommended.
7. Analyze with SECTOR instrument.

