



pbl assay science

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# VeriKine-HS™ Human IFN Beta Serum ELISA Kit

Catalog No. 41415

Assay Range: 1.2 - 150 pg/ml

Store **all** components at 2 - 8°C

## Protocol A

**(Recommended)**

**Enhanced protocol for improved  
performance in Serum evaluation**

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## INTRODUCTION

Interferons (IFNs) are a group of cytokines which exhibit pleiotropic activities that play major roles in both innate and adaptive immunity. Type I IFNs consist of multiple IFN Alpha ( $\alpha$ ) genes and at least one IFN Beta ( $\beta$ ) gene in most vertebrates. IFN- $\beta$  is used therapeutically to treat multiple sclerosis.

IFN- $\beta$  expression and secretion is primarily induced by signaling from pattern recognition receptors such as Toll-like (TLR) and RIG-I-like receptors (RLR). Overall, IFN- $\beta$  is part of the first wave of cytokine response in cells. Pathogen infection can result in the activation of interferon regulatory factor 3 (IRF3) that functions in *trans* to activate IFN- $\beta$  gene transcription.

Following expression and secretion, IFN- $\beta$  binds to a trans-membrane heterodimeric receptor chain consisting of IFNAR1 & IFNAR2 on infected (autocrine) or neighboring cell (paracrine) surfaces. Receptor binding promotes a signal transduction cascade consisting of components of the JAK-STAT signaling pathway. This results in the expression of many genes including interferon regulatory factor 7 (IRF7) that upregulates the expression of many IFN- $\alpha$  subtype proteins. The IRF3/IRF7 signaling cascade is important for the initial and progressive responses to pathogens wherein hundreds of genes are regulated in a coordinated, temporal manner.

IFN- $\beta$  is biologically unique when compared to other interferons. Studies have shown that IFN- $\beta$  has overlapping and distinct gene expression patterns as compared to IFN- $\alpha$ . It appears that IFN- $\beta$  binds to the Type I IFN receptor with higher affinity than other Type I IFNs and that it may also regulate receptor internalization in a different manner.

This kit has been developed to measure low/basal levels of IFN- $\beta$  in a variety of sample matrices including serum, plasma and tissue culture media. The basal levels of Type I IFNs, including IFN- $\beta$ , are not fully understood. They are believed to be important for robust response to pathogens and may play additional roles in cellular homeostasis.

#### **MATERIALS PROVIDED**

- Pre-coated microtiter plate
- Plate Sealers
- Wash Solution Concentrate
- Human Interferon Beta Standard, 100,000 pg/ml
- Standard Diluent
- Sample Buffer
- Antibody Concentrate
- HRP Conjugate Concentrate
- Diluent Additive III
- Assay Diluent
- TMB Substrate
- Stop Solution

#### **ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)**

- Microplate reader capable of reading an OD at a wavelength of 450 nm
- Variable volume microtiter pipettes
- Adjustable multichannel pipette (50-300  $\mu$ l)
- Reagent reservoirs
- Wash bottle or plate washing system
- Distilled or deionized water
- Serological pipettes (1, 5, 10 or 25 ml)
- Disposable pipette tips (polypropylene)
- Plate shaker

*Specifications:* This kit quantitates Human IFN- $\beta$  in sera, plasma and tissue culture media by sandwich enzyme linked immunosorbent assay (ELISA). Interferon binds to plates coated with antibody and detection is accomplished using a biotinylated secondary antibody followed by streptavidin conjugated to horseradish peroxidase (HRP). Tetramethylbenzidine (TMB) is the substrate. The standard provided is recombinant Human IFN Beta expressed in mammalian cells.

*Speed:* Incubation time, 3 hr 30 min

*Specificity:* Human IFN- $\beta$ . No cross-reactivity detected with human IFN- $\alpha$ , human IFN- $\gamma$ , human IFN- $\omega$  or human IL-6. No cross-reactivity with mouse IFN- $\alpha$ , mouse IFN- $\beta$  or rat IFN- $\beta$ .

*Storage Conditions/Comments:* For retention of full activity, all reagents should be kept at 2-8°C in the dark when not in use. Diluents and buffer reagents should be warmed to RT (22-25°C) before use.

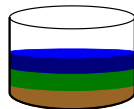
Please note that the dilutions of the Detection Antibody and HRP differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (COA) for their preparation.

**CAUTION:** Wash Solution Concentrate, Standard Diluent and Sample Buffer contain 0.1% Kathon CG/ICP as a preservative. Sample buffer also contains <0.003% Sodium Azide. These components should be handled with appropriate safety precautions and discarded properly. For further information, consult the material safety data sheet (MSDS).

For laboratory research use only. Not for use in human diagnostic or therapeutic procedures.

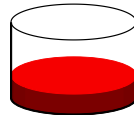
## ASSAY PROCEDURE - QUICK REFERENCE

**Total Time:**  
3 hr 30 min



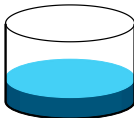
- 1) Add **50  $\mu$ l** Sample Buffer
- 2) Add **50  $\mu$ l** Diluted Antibody
- 3) Add **50  $\mu$ l** Test Sample, IFN- $\beta$  Standard or Blank

Incubate **2 hrs** (shake at 450 rpm)  
Aspirate and wash **3x**



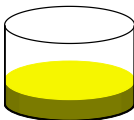
Add **100  $\mu$ l** Diluted  
HRP Solution

Incubate **30 min** (shake at 450 rpm)  
Aspirate and wash **4x**



Add **100  $\mu$ l** TMB  
Substrate

Incubate **60 min** in the dark  
**Do not seal, shake or wash.**



Add **100  $\mu$ l** Stop Solution  
Read plate within 5 min  
(450 nm)

**Note:** ALL  
Incubations are at  
room temperature  
(22 to 25°C)

## PREPARATION OF REAGENTS

Bring the plates, Sample Buffer, Standard Diluent, applicable dilution matrices (e.g. Serum, Plasma or Tissue Culture Media), Assay Diluent, Diluent Additive III, TMB Substrate, Stop Solution and samples to room temperature (RT), 22-25°C, before use. Supplied Human IFN- $\beta$  Standard, Antibody Concentrate and HRP Conjugate Concentrate should be kept on ice.

**Wash Solution:** The Wash Solution Concentrate may contain crystals. Place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution (e.g. Add 50 ml of Wash Solution Concentrate to 450 ml of distilled or deionized water and mix thoroughly). Diluted Wash Solution can be stored at RT (22-25°C) when not in use.

**Human Interferon Beta Solution:** Using the Human IFN- $\beta$  Standard, construct a standard curve (1.2-150 pg/ml), as shown in Figure 1, in the same matrix as the test samples. Examples of these matrices are IFN- $\beta$  free human serum, plasma, or tissue culture medium containing 10% FBS. In the event that the sample matrix is not available, the Standard Diluent may be used to prepare the standard curve.

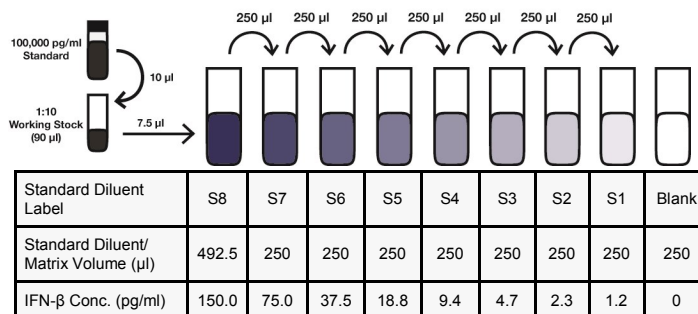
The methods associated with the collection, storage and testing of experimental samples have all been reported to affect ELISA results.<sup>10</sup> Although extensive testing has been carried out to minimize sample matrix effects, the user should determine whether the test sample matrix adversely affects the recovered IFN- $\beta$  values.

**Note:** Due to the inherent nature of human IFN- $\beta$  protein to adhere to plastic surfaces, proper pipetting technique is required to accurately prepare a standard curve and quantitate samples.

**Pipetting tips:**

- Aspirating Standard and samples  
To avoid sticking of the protein to outside walls of the pipette tip, ensure it is not immersed in the human IFN- $\beta$  Standard vial when aspirating.
- Dispensing and diluting Standard and samples  
Proper mixing technique entails pipetting up and down gently 10 times for the predilution and S8 dilution (Figure 1); 5 times for subsequent serial dilutions. Thorough, but gentle, pipetting is required to recover all material attached to the inside of the tip. Avoid excessive force or foaming to prevent denaturing of human interferon beta.

**Fig. 1: 8-Point Standard Curve Prepared in Standard Diluent**



**Standard Curve Preparation:**

- Label eight polypropylene tubes (S1-S8).
- Add indicated volumes of Standard Diluent or sample matrix to the labeled tubes (see Figure 1).
- Using polypropylene tips, add 10  $\mu$ l of IFN Standard to 90  $\mu$ l of Standard Diluent or sample matrix. Using a 100  $\mu$ l or 200  $\mu$ l pipette, set the volume to 80  $\mu$ l and mix thoroughly by pipetting up and down 10 times.

- d) Add 7.5  $\mu$ l of the 1:10 prediluted standard to S8 and mix thoroughly to recover all material adhered to the inside of the pipette tip.
- e) Using a pipette set at 250  $\mu$ l, mix S8 thoroughly by pipetting up and down 10 times. Transfer 250  $\mu$ l of S8 to S7 and mix thoroughly by pipetting up and down 5 times. Repeat to complete series to S1.
- f) Set aside until use in step 1 of the assay procedure.

**Antibody Solution:** Refer to the lot specific Certificate of Analysis (COA) for the correct amount of Antibody Solution to prepare. Dilute Antibody Concentrate in recommended volume **of a mixture of Assay Diluent and Diluent Additive III**. Set aside diluted Antibody Solution at RT (22-25°C) until use.

**HRP Solution:** Refer to the lot specific Certificate of Analysis (COA) for the correct amount of HRP Solution to prepare. Prepare within 15 minutes prior to use. Dilute HRP Conjugate in recommended volume of Assay Diluent. Set aside HRP Solution at RT (22-25°C) until use.

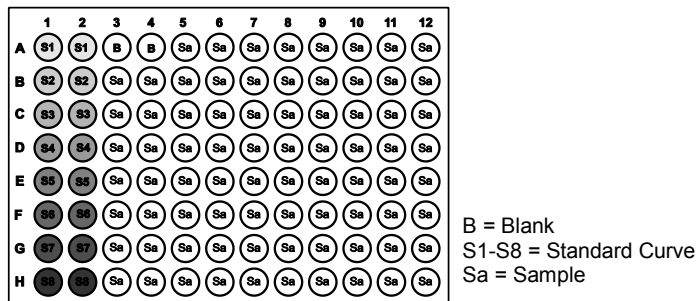
## **ASSAY PROCEDURE**

All incubations should be performed in a closed chamber at RT (22-25°C) keeping the plate away from drafts and other temperature fluctuations. Set plate shaker speed to 450 rpm where indicated. Use plate sealers to cover the plates as directed. During all wash steps, remove contents of plate by inverting and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate. If an automated plate washer is used for washing, it is recommended that the plate be soaked in Wash Solution for 30 seconds between washes.



Wash each well with a minimum of 300  $\mu$ l of diluted Wash Solution for each wash step. See Preparation of Reagents for details on dilution of concentrated solutions.

**Figure 2: Example of a Typical Plate Setup**



**1. Standards, Test Samples and diluted Antibody Solution:**  
 Determine the number of microtiter plate strips required to test the desired number of samples plus the appropriate number of wells needed to run blanks and standards. We recommend running both the IFN- $\beta$  Standard, blanks and samples in duplicate. Remove extra microtiter strips from the frame, seal in the foil bag provided and store at 2-8°C. Unused strips can be used in later assays.

**Step A: Adding Sample Buffer and diluted Antibody Solution**

Add 50  $\mu$ l of Sample Buffer to each well.  
 Add 50  $\mu$ l of diluted Antibody Solution (refer to Preparation of Reagents) to each well (Step A volume = 100  $\mu$ l/well).

**Step B: Adding Standards, Test Samples and Blanks**

Standards: Add 50  $\mu$ l of Standard (refer to Preparation of Reagents) per well.

Blanks: Add 50  $\mu$ l Standard Diluent or dilution matrix to wells designated for blanks.

Test samples: Add 50  $\mu$ l of each sample to wells designated for samples.

**(Final Volume in Wells = 150  $\mu$ l)**

Cover with plate sealer and shake plate at 450 rpm at RT (22-25°C) for 2 hours.

After 2 hours, empty the contents of the plate and wash the wells three times with working Wash Solution (refer to Preparation of Reagents).

2. **HRP:** Add 100  $\mu$ l of diluted HRP Solution (refer to Preparation of Reagents) to each well. Cover with Plate Sealer and shake plate at 450 rpm at RT (22-25°C) for 30 minutes.

After 30 minutes, empty the contents of the plate and wash the wells four times with working Wash Solution.

3. **TMB Substrate:** Add 100  $\mu$ l of the TMB Substrate Solution to each well. Incubate, in the dark, at RT (22-25°C), for 60 minutes. Do not use a plate sealer during the incubation. DO NOT SHAKE.

4. **Stop Solution:** After the 60 minute incubation of TMB, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add 100  $\mu$ l of Stop Solution to each well.

5. **Read:** Using a microplate reader, determine the absorbance at 450 nm within 5 minutes after the addition of the Stop Solution.

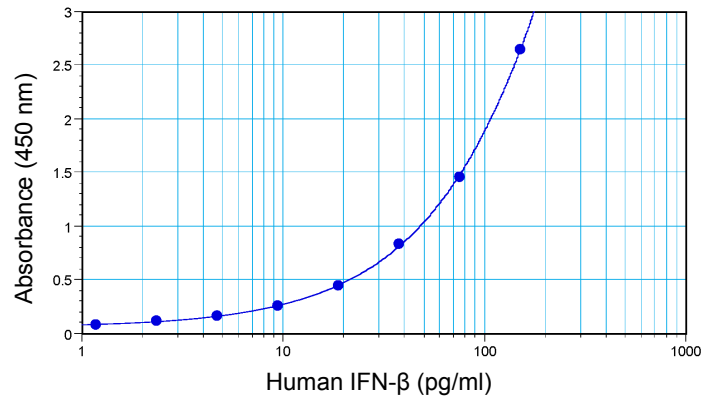
## **CALCULATION OF RESULTS**

By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Based on user preference, blank ODs may be subtracted from the standards and sample ODs to eliminate background. Use the conversion factor of 3 pg/unit to approximate titers in units/ml.

A shift in optical densities is typical between users and kit lots. The back fit concentration extrapolated from the standard curve is a more accurate determination of the sample titer and performance of the kit. Variations from the typical curve provided can be a result of operator technique, altered incubation time, fluctuations in temperature and kit age.

Results of a typical standard curve using a 4-parameter fit are provided for demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.

**Figure 3: Typical Standard Curve**



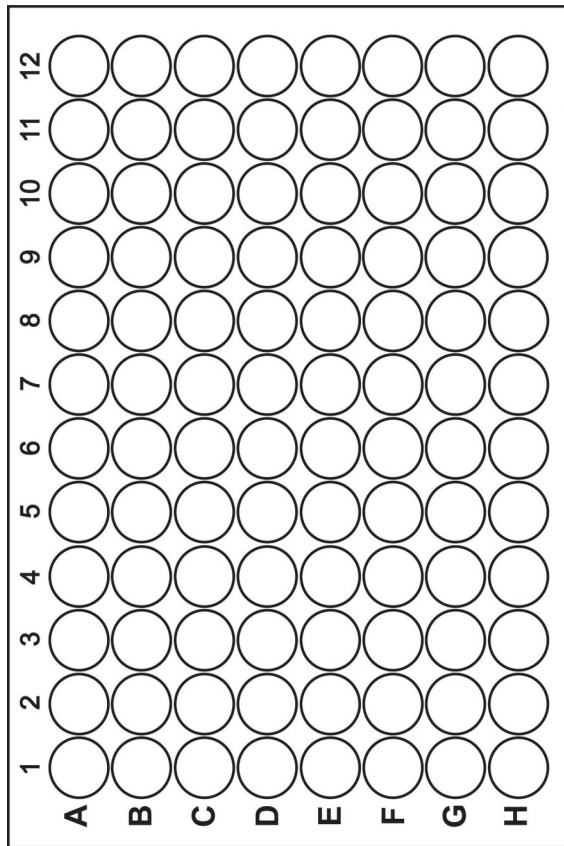
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**PLATE LAYOUT**

Use this plate layout as a record of standards and samples assayed.



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