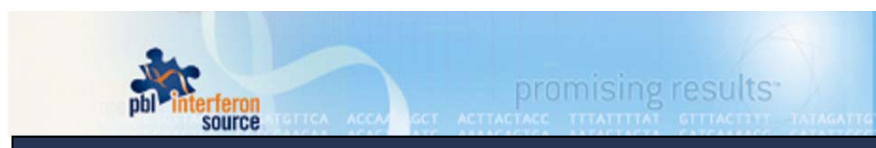

VeriKine-HS™ Human IFN-β Serum ELISA kit

Product #41415-1

Assay Range: 2.3-150 pg/mL

Store **all** components at 2-8°C

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Specifications: This kit quantitates Human Interferon-Beta (IFN-β) 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). Tetramethyl-benzidine (TMB) is the substrate. The standard provided in the kit is recombinant Human Interferon-Beta expressed in mammalian cells.

Speed: Incubation time, 3 hr

Specificity: Human IFN-β. No cross-reactivity with human IFN-α, human IFN-γ, human IFN-ω or human IL-6. No cross-reactivity with mouse IFN-α, mouse IFN-β or rat IFN-β.

Storage Conditions/Comments: For retention of activity, all reagents should be kept at 2-8ºC in the dark when not in use. Diluents and buffer reagents should be warmed to room temperature (RT) 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.

MATERIALS PROVIDED

- Pre-coated micro-titer plate
- Plate Sealers
- Wash Solution Concentrate
- Human Interferon-Beta (IFN-β) Standard, 100,000 pg/ml
- Standard Diluent
- Sample Buffer
- Antibody Concentrate
- HRP Conjugate Concentrate
- Assay Diluent
- TMB Substrate
- Stop Solution

ADDITIONAL MATERIALS REQUIRED (*NOT PROVIDED*)

- Micro-titer plate reader capable of reading an OD at a wavelength of 450nm
- Variable volume micro-titer pipettes
- Adjustable multi-channel pipette (50-300µl)
- Reagent reservoirs
- Wash bottle or plate washing system
- Distilled or deionized water
- Serological pipettes (1, 5, 10 or 25ml)
- Disposable pipette tips (polypropylene)
- Plate shaker

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 Interferon-Alpha (IFN-α) genes and at least one Interferon-Beta (IFN-β) gene in most vertebrates.[1] IFN-β is used therapeutically to treat multiple sclerosis.[2]

IFN-β expression and secretion is primarily induced by signaling from pattern recognition receptors such as the Toll-like (TLR) and RIG-I-like receptors (RLR).[3] Overall, IFN-β 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-β gene transcription.

Following expression and secretion, IFN-β binds to a transmembrane heterodimeric receptor chain consisting of IFNAR1 and 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.[4] This results in the expression of many genes including interferon regulatory factor 7 (IRF7) that up-regulates the expression of many IFN-α 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.[5]

IFN-β is biologically unique when compared to other interferons. Studies have shown that IFN-β has overlapping and distinct gene expression patterns as compared to IFN-α.[6] It appears that IFN-β binds to the Type I IFN receptor with higher affinity than the other Type I IFNs[7] and that it may regulate receptor internalization in a different manner also.[8]

The *VeriKine-HS™* Human Interferon-Beta Serum ELISA kit has been developed to measure low/basal levels of IFN-β in a variety of sample matrices including tissue culture media, serum and plasma. The basal levels of Type I IFNs, including IFN-β, are not fully understood. They are believed to be important for robust response to pathogens and may play additional roles in cellular homeostasis.[9]

PREPARATION OF REAGENTS

Bring Plates, Sample Buffer, Standard Diluent, applicable dilution matrices (e.g. Serum, Plasma, or Tissue Culture Medium), Assay Diluent, TMB Substrate, Stop Solution and samples to RT (22-25ºC) before use. Supplied Human IFN-β 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. For e.g. Add 50ml of the Wash Solution Concentrate to 450ml of distilled or deionized water, mix thoroughly. The diluted Wash Solution can be stored at (2-25°C) when not in use.

Human Interferon-Beta Solution: Using the Human IFN-β Standard, construct a standard curve (2.3-150 pg/ml), as shown in figure 1 (page 8), in the same matrix as the test samples. Examples of these matrices are IFN-β 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.[10] Although extensive testing has been carried out to minimize sample matrix affects, the user should determine whether the test sample matrix adversely affects the recovered IFN-β values.

Note: Due to the inherent nature of human IFN-β 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-β 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 S7 dilution (page 8 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.

Standard Curve Preparation:

a) Label seven polypropylene tubes (S1-S7)

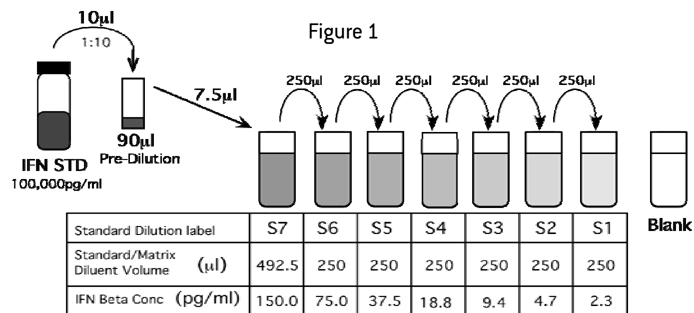
b) Add indicated volumes of Standard Diluent or sample matrix to the labeled tubes (see figure below)

c) Using polypropylene tips, add 10µl of IFN Standard to 90µl of Standard Diluent or sample matrix. Using a 100µl or 200µl pipette , set the volume to 80µl and mix thoroughly by pipeting up and down 10 times.

d) Add 7.5µl of the 1:10 prediluted standard to S7 and mix thoroughly to recover all material adhered to the inside of the pipette tip.

e) Using a pipette set at 250µl, mix S7 thoroughly by pipeting up and down 10 times. Transfer 250µl of S7 to S6 and mix thoroughly by pipeting up and down 5 times. Repeat to complete series to S1.

f) Set aside until use in step 1 of the assay procedure



Standard Dilution label	S7	S6	S5	S4	S3	S2	S1
Standard/Matrix Diluent Volume (µl)	492.5	250	250	250	250	250	250
IFN Beta Conc (pg/ml)	150.0	75.0	37.5	18.8	9.4	4.7	2.3

Figure 1

Antibody Solution: Refer to the lot specific Certificate of Analysis (COA) for the correct amounts of Antibody Solution to prepare. Dilute Antibody Concentrate in recommended volume of Assay Diluent. Set aside diluted Antibody Solution at RT (22-25ºC) until use.

HRP Solution: Refer to the lot specific Certificate of Analysis (COA) for the correct amounts 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 (22-25ºC) until use.

ASSAY PROCEDURE

All incubations should be performed in a closed chamber at RT (22-25ºC) 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 over a sink and blotting the plate on lint-free absorbent paper; tap the plate. Wash each well with a minimum of 300µl of diluted Wash Solution for each wash step. See Preparation of Reagents for details on dilution of concentrated solutions.

1. **Standards, Test Samples and diluted Antibody Solution**:
   Determine the number of micro-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-β standard, blanks and samples in duplicate. Remove extra micro-titer strips from the frame, seal in the foil bag provided and store at 2-8ºC. Unused strips can be used in later assays.

   Step A: Adding Sample Buffer and diluted Antibody Solution
   Add 50µl of Sample Buffer to each well
   Add 50µl of diluted Antibody Solution (refer Preparation of Reagents) to each well (Total volume = 100µl /well)

   Step B: Adding Standards, Test Samples and Blanks
   Standards: Add 50µl of Standard (refer to Preparation of Reagents) per well.

Blanks: Add 50µl Standard Diluent or dilution matrix to wells designated for blanks.
Test samples: Add 50µl of each sample to wells designated for samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
B	S1	S1	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
C	S2	S2	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
D	S3	S3	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
E	S4	S4	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
F	S5	S5	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
G	S6	S6	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
H	S7	S7	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa

B = Blank
S1-S7 = Standard Curve
Sa = Samples

Cover with Plate Sealer and shake plate at RT (22-25ºC) for 2 hours

After 2 hours, empty the contents of the plate and wash the wells 3 times with the working Wash Solution (refer to Preparation of Reagents)

2. **HRP:** Add 100µl of diluted HRP Solution (refer to Preparation of Reagents) to each well. Cover with Plate Sealer and shake plate at RT (22-25ºC) for 30 minutes.

   After 30 minutes, empty the contents of the plate and wash the wells 4 times with working Wash Solution.

3. **TMB Substrate:** Add 100µl of the TMB Substrate Solution to each well. Incubate, in the dark, at RT (22-25ºC) for 30 minutes. Do not use a plate sealer during the incubation. DO NOT SHAKE.

4. **Stop Solution:** After the 30 minutes incubation of TMB, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add 100µl of Stop Solution.

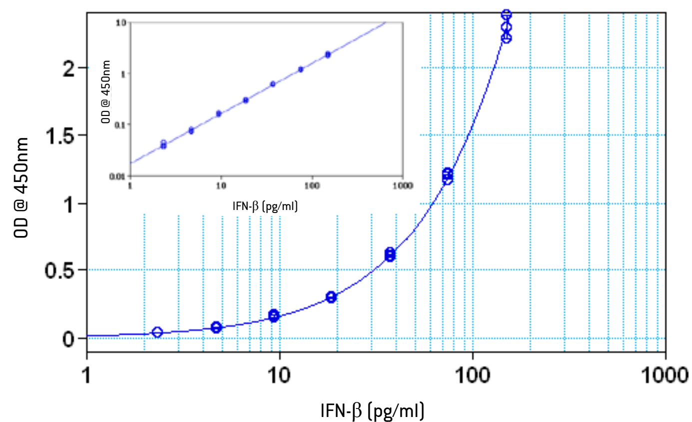
5. **Read:** Using a micro-plate reader, determine the absorbance at 450nm within 5 minutes after the addition of the Stop Solution.

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.

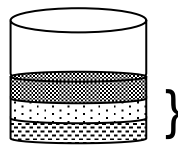
A shift in optical densities is typical between users and kit lots. The back fit concentration extrapolated from the standard curve is a more accurate determination of the sample titer and performance of the kit. Variations from the typical curve provided can be a result of operator technique, altered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

Results of a typical standard curves 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.



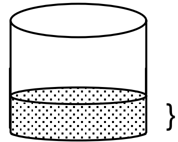
ASSAY PROCEDURE—QUICK REFERENCE

Total time: 3 hours



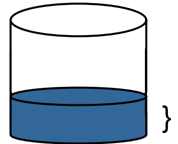
1) Add 50µl Sample Buffer
2) Add 50µl Diluted Antibody
3) Add 50µl Test Sample or IFN-β standard

Incubate 2 hours with shaking (450 rpm)
Aspirate and wash 3 x



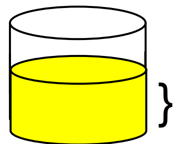
Add 100µl Diluted HRP Solution

Incubate 30 minutes with shaking (450 rpm)
Aspirate and wash 4 x



Add 100µl TMB Substrate

Incubate 30 minutes in the dark
Do not shake. Do not wash



Add 100µl Stop Solution

Read plate within 5 minutes at 450nm

PERFORMANCE CHARACTERIZATION STUDIES

1. Normal Human Serum Screen:

Twenty five lots of normal Human Serum were screened. Only two lots had detectable signal corresponding to 2-6 pg/ml on a standard curve.

2. Spike Recovery in Normal Human Serum:

High medium and low spikes were prepared in 14 normal low background lots of Human Sera and in Standard Diluent. The concentrations of the spikes were calculated from a Standard Curve prepared in Standard Diluent.

Spikes in Normal Human Serum:

	Concentration (pg/ml)	% Recovery Range	Average % Recovery
Low Spike	5	77-102%	91%
Med Spike	25	81-110%	100%
High Spike	100	84-107%	95%

Spikes in Standard Diluent:

	Concentration (pg/ml)	% Recovery range	Average % Recovery
Low Spike	5	80-123%	101%
Med Spike	25	81-126%	104%
High Spike	100	78-117%	98%

3. Intra-Assay and Inter-Assay %CV:

	Standard Diluent	Human Serum	Tissue Culture Media (10% FBS)
Intra-Assay CV	3.9% (n=8 assays)	3.6% (n=26 assays)	4% (n=18 assays)
Inter-Assay CV	6.8% (n=8 assays)	7.9% (n=26 assays)	7.4% (n=18 assays)

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NOTES

NOTES

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HERE'S HOW:

PBL is in the process of compiling an extensive list of references on the use of Interferon and Interferon-related products. This reference list, including selected abstractions, will be made available to the scientific community through the PBL web site: www.interferonsource.com. If you have a published scientific paper, scientific poster or other scientific publication which mentioned the use of a PBL InterferonSource product, we will send you a free Interferon T-shirt in return for your citation.

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