pbl assay science

VeriKine-HS™ Human IFN Beta Serum ELISA Kit

Catalog No. 41415

Assay Range: 2.3 - 150 pg/ml

Store **all** components at 2 - 8°C

Protocol B

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

IFN-β 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-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 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-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.

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. It appears that IFN-β 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 -β in a variety of sample matrices including serum, plasma and tissue culture media. 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.

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
- 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 µl)
- 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-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 is recombinant Human IFN Beta expressed in mammalian cells.

Speed: Incubation time, 3 hr

Specificity: Human IFN-β. No cross-reactivity detected 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 full activity, all reagents should be kept at 2-8ºC in the dark when not in use. Diluents and buffer reagents should be warmed to RT (22-25ºC) before use.

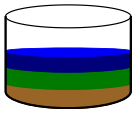
Please note that the dilutions of the Detection Antibody and HRP differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (COA) for their preparation.

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.

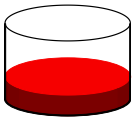
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
3 hr



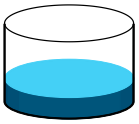
1) Add **50 µl** Sample Buffer
2) Add **50 µl** Diluted Antibody
3) Add **50 µl** Test Sample, IFN-β Standard or Blank

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



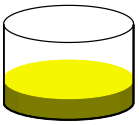
Add **100 µl** Diluted HRP Solution

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



Add **100 µl** TMB Substrate

Incubate **30 min** in the dark
Do not seal, shake or wash.



Note: ALL Incubations are at room temperature (22 to 25ºC)

Add **100 µl** Stop Solution
Read plate within 5 min (450 nm)

PREPARATION OF REAGENTS

Bring the plates, Sample Buffer, Standard Diluent, applicable dilution matrices (e.g. Serum, Plasma or Tissue Culture Media), Assay Diluent, TMB Substrate, Stop Solution and samples to room temperature (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 (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^{o}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, 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.

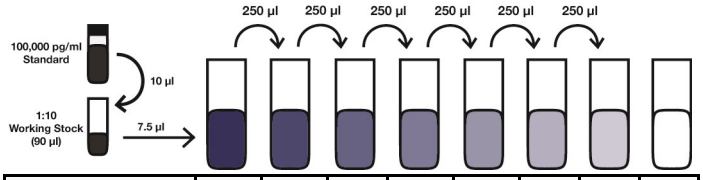
The methods associated with the collection, storage and testing of experimental samples have all been reported to affect ELISA results.[10] Although extensive testing has been carried out to minimize sample matrix effects, the user should determine whether the test sample matrix adversely affects the 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 (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: 7-Point Standard Curve Prepared in Standard Diluent



Standard Diluent Label	S7	S6	S5	S4	S3	S2	S1	Blank
Standard Diluent/Matrix Volume (µl)	492.5	250	250	250	250	250	250	250
IFN-β Conc. (pg/ml)	150.0	75.0	37.5	18.8	9.4	4.7	2.3	0

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 1).
c) Using polypropylene tips, add 10 µl of IFN Standard to 90 µl of Standard Diluent or sample matrix. Using a 100 µl or 200 µl pipette, set the volume to 80 µl and mix thoroughly by pipetting up and down 10 times.

d) Add 7.5 µl 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 250 µl, mix S7 thoroughly by pipetting up and down 10 times. Transfer 250 µl of S7 to S6 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.

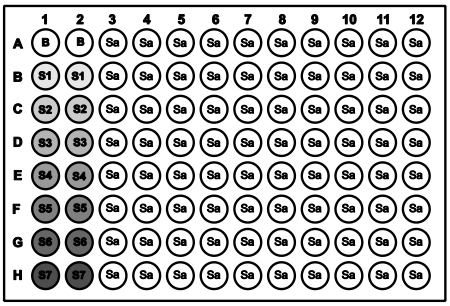
Antibody Solution: Refer to the lot specific Certificate of Analysis (COA) for the correct amount of Antibody Solution to prepare. Dilute Antibody Concentrate in recommended volume of Assay Diluent. Set aside diluted Antibody Solution at RT (22 -25ºC) until use.

HRP Solution: Refer to the lot specific Certificate of Analysis (COA) for the correct 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 (22-25ºC) 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 and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate. Wash each well with a minimum of 300 µl 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



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

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-β Standard, blanks and samples in duplicate. Remove extra microtiter 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 50 µl of Sample Buffer to each well.
Add 50 µl of diluted Antibody Solution (refer to Preparation of Reagents) to each well (Total volume = 100 µl/well).

Step B: Adding Standards, Test Samples and Blanks

Standards: Add 50 µl of Standard (refer to Preparation of Reagents) per well.
Blanks: Add 50 µl Standard Diluent or dilution matrix to wells designated for blanks.
Test samples: Add 50 µl of each sample to wells designated for samples. (Final Volume in Wells = 150 µl)

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 the working Wash Solution (refer to Preparation of Reagents).

2. **HRP:** Add 100 µl 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 µl 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 minute incubation of TMB, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add 100 µl 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.

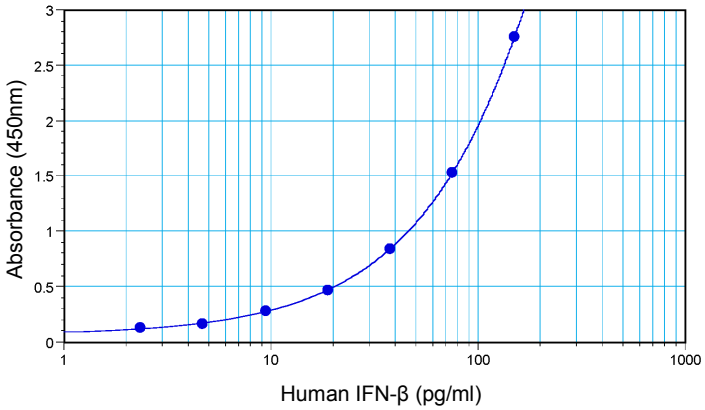
CALCULATION OF RESULTS

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



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)

REFERENCES

1. Krause CD, Pestka S. Evolution of the Class 2 cytokines and receptors, and discovery of new friends and relatives. *Pharmacol Ther*. 2005 Jun;106(3):299-346.

2. Weinstock-Guttman B, Ramanathan M, Zivadinov R. Interferon-beta treatment for relapsing multiple sclerosis. *Expert Opin Biol Ther*. 2008 Sep;8(9):1435-47.

3. Lee MS, Kim YJ. Pattern-recognition receptor signaling initiated from extracellular, membrane, and cytoplasmic space. *Mol Cells*. 2007 Feb 28;23(1):1-10.

4. Heim MH. The Jak-STAT pathway: cytokine signaling from the receptor to the nucleus. *J Recept Signal Transduct Res*. 1999 Jan-Jul;19(1-4):75-120.

5. Taniguchi T, Takaoka A. The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr Opin Immunol*. 2002 Feb;14(1):111-6.

6. Lewerenz M, Mogensen KE, Uzé G. Shared receptor components but distinct complexes for alpha and beta interferons. *J Mol Biol*. 1998 Sep 25;282(3):585-99.

7 . Jaks E, Gavutis M, Uzé G, Martal J, Piehler J. Differential receptor subunit affinities of type I interferons govern differential signal activation. *J Mol Biol*. 2007 Feb 16;366 (2):525- 39.

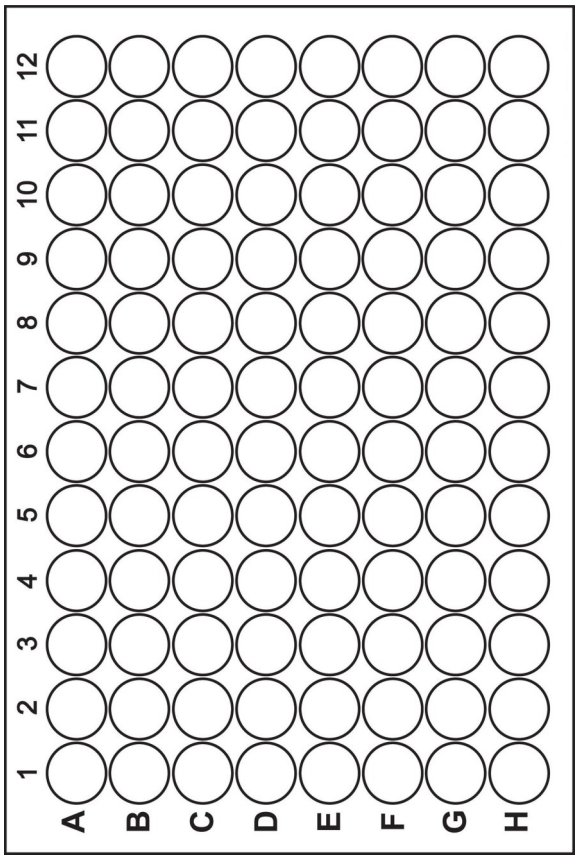
8. Marijanovic Z, Ragimbeau J, van der Heyden J, Uzé G, Pellegrini S. Comparable potency of IFNalpha2 and IFN beta on immediate JAK/STAT activation but differential down-regulation of IFNAR2. *Biochem J.* 2007 Oct 1;407(1):141-51.

9. Taniguchi T, Takaoka A. A weak signal for strong responses: interferon-alpha/beta revisited. *Nat Rev Mol Cell Biol*. 2001 May;2(5):378-86.

10. Aziz N, Nishanian P, Mitsuyasu R, Detels R, Fahey JL. Variables That Affect Assays for Plasma Cytokines and Soluble Activation Markers. *Clin Diagn Lab Immunol*. 1999 Jan: (6)1:89-95.

PLATE LAYOUT

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



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