pbl assay science

VeriKine Human IFN Beta ELISA Kit

Catalog No. 41410

Assay Range: 50 – 4000 pg/ml

Store **all** components at 2 - 8°C

INTRODUCTION

Interferon beta (IFN-β) is synthesized and secreted by fibroblasts and many other cell types in response to pathogens. These pathogens include viruses and bacteria and signaling can occur through Toll-like receptor dependent or independent pathways. Following secretion, IFN-β binds to type I interferon receptors on proximal or distal cells activating the JAK1-STAT signaling pathway. Activation of this signal transduction pathway leads to the expression of 2'-5' oligoadenylate synthetases (2'5' OAS), protein kinase R (PKR), MxA proteins, and interferon regulatory factor 7 (IRF-7). The upregulation of IRF-7 expression can exert a positive feedback on IFN-β production, whereas the induction of 2'5' OAS activates a latent endonuclease known as RNase L. RNase L cleaves both viral and cellular single stranded mRNA, thereby limiting viral replication and dissemination.

The PBL Assay Science VeriKine Human IFN-β ELISA kit uses the sandwich immunoassay technique for the quantitative measurement of IFN-β in media. It is developed for superior performance with intra-assay and inter-assay CVs of ≤ 8%.

MATERIALS PROVIDED

- Pre-coated microtiter plate(s)
- Plate sealers
- Wash Solution Concentrate
- Human Interferon Beta Standard, 100,000 pg/ml
- Sample Diluent
- Antibody Concentrate
- HRP Conjugate Concentrate
- Concentrate Diluent
- TMB Substrate Solution
- Stop Solution

ADDITIONAL MATERIALS REQUIRED *(NOT PROVIDED)*

- Microtiter plate reader capable of reading a wavelength of 450 nm
- Variable volume microtiter pipettes
- Adjustable multichannel pipette (50-200 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)

Specifications: This kit quantitates human interferon beta in buffers or tissue culture media (TCM) using a sandwich immunoassay.[1,2] The lowest concentration of Hu-IFN-β that can be detected in a test sample is 50 pg/ml. This kit is not designed to measure Hu-IFN-β in human serum. The kit is based on an ELISA with streptavidin conjugated to horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. The assay is based on the international reference standard for human interferon beta (Hu-IFN-β) provided by the National Institutes of Health.[3]

Speed: Incubation time, 3 hr 15 min

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

Storage Conditions/Comments: For retention of full activity, all reagents should be kept at 2-8°C in the dark.

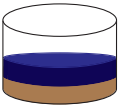
Please note that the dilutions of the Antibody Concentrate and HRP Conjugate Concentrate differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (COA) for their preparation.

CAUTION: The Wash Solution Concentrate, Sample Diluent and Concentrate Diluent contain 0.1% Kathon CG/ICP as a preservative; they should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

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

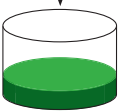
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
3 hr, 15 min



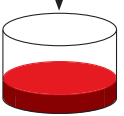
Add **50 µl** Sample Diluent
Add **50 µl** Standard, Blank or Sample

Incubate **1 hr**
Aspirate and Wash **3x**



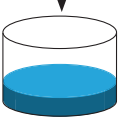
Add **100 µl** Diluted Ab Solution

Incubate **1 hr**
Aspirate and Wash **3x**



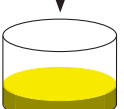
Add **100 µl** Diluted HRP Solution

Incubate **1 hr**
Aspirate and Wash **3x**



Add **100 µl** TMB Substrate

Incubate **15 min** in the dark
Do not seal or wash.



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

Note: ALL incubations are at room temperature (22-25°C)

PREPARATION OF REAGENTS

Before starting the assay, plate(s), Wash Solution Concentrate, applicable dilution matrices (e.g. tissue culture media), Sample Diluent, Concentrate Diluent, Stop Solution, and samples should be equilibrated to room temperature (RT), 22-25°C. The TMB Substrate Solution should be equilibrated to RT (22-25°C) during step 3 of the Assay Procedure. Supplied Human IFN Beta Standard, Antibody Concentrate, and HRP Conjugate Concentrate should be kept on ice (4°C).

Wash Buffer: 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 Buffer by adding 50 ml of Wash Solution Concentrate to 450 ml of distilled or deionized water. Mix thoroughly before use. The diluted Wash Buffer can be stored at RT (22-25°C).

Human Interferon Beta Solution: Using the Human IFN Beta Standard, provided at 100,000 pg/ml, construct a standard curve (50 - 4000 pg/ml), as shown in Figure 1, in the same matrix as the test samples. In the event that the sample matrix is not available, the Sample Diluent may be used to prepare the sample curve. In certain situations, test samples may contain substances that can interfere with assay results.

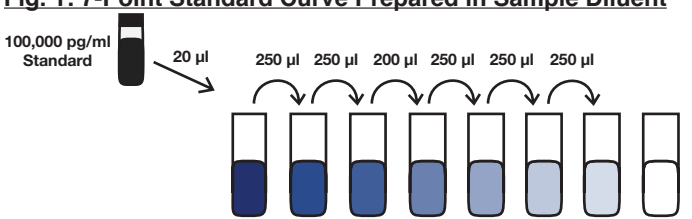
The methods associated with the collection, storage, and testing of environmental samples have all been reported to affect ELISA results. For example, we have found that protein-free media provides poor results.

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 S7 dilution (Figure 1) and 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 Sample Diluent



Sample Dilution Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Diluent Vol. (µl)	480	250	250	300	250	250	250	250
IFN-β Conc. (pg/ml)	4000	2000	1000	400	200	100	50	0

Standard Curve Preparation:

a) Label seven polypropylene tubes (S7-S1).
b) Add indicated volumes of Sample Diluent or sample matrix to the labeled tubes as indicated in Figure 1.
c) Using polypropylene tips, add 20 µl of the Human IFN-β Standard to S7 and mix thoroughly to recover all material adhered to the inside of the pipette tip.
d) Using a pipette set at 250 µl, mix S7 thoroughly by pipetting up and down 10 times.
e) Transfer indicated amount from 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.

Sample Preparation: Prepare test samples of unknown IFN concentration to be tested using Sample Diluent (or applicable matrices) as required. Measurements in duplicate are recommended. Set aside on ice (4°C) until use in step 1 of the Assay Procedure.

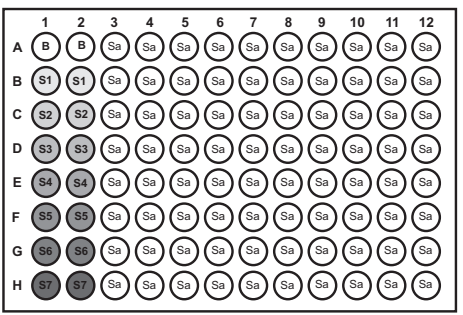
Antibody Solution: Refer to the lot specific Certificate of Analysis (COA) for the correct volume of Antibody Solution to prepare. Dilute Antibody Concentrate in recommended volume of Concentrate Diluent. Prepare 15 minutes prior to use in step 2 of the Assay Procedure and keep at RT (22-25°C).

HRP Solution: Refer to the lot specific Certificate of Analysis (COA) for the correct volume of HRP Solution to prepare. Dilute HRP Conjugate Concentrate in recommended volume of Concentrate Diluent. Prepare 15 minutes prior to use in step 3 of the Assay Procedure and keep at RT (22-25°C).

ASSAY PROCEDURE

All incubations should be performed at room temperature (RT), 22-25°C, keeping the plate away from drafts and other temperature fluctuations. 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 250 µl of diluted Wash Buffer at each wash step. Refer to Preparation of Reagents for details on dilution of concentrated solutions.

Figure 2: Example of a Typical Plate Setup



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

1. **Standards and Test Samples:** 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 the Human IFN Beta Standard, blanks, and samples in duplicate or triplicate (see Figure 2 for example plate setup). A standard curve is required for each assay. 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.

Add 50 µl Sample Diluent to the wells. Add 50 µl of the diluted Standard Curve, blanks, or test samples. Cover with plate sealer and incubate for 1 hour.

After 1 hour, empty the contents of the plate and wash the wells three times with diluted Wash Buffer (refer to Preparation of Reagents).

2. **Antibody Solution:** Add 100 µl of diluted Antibody Solution (refer to Preparation of Reagents) to each well. Cover with plate sealer and incubate for 1 hour.

After 1 hour, empty the contents of the plate and wash the wells three times with diluted Wash Buffer.

3. **HRP:** Add 100 µl of diluted HRP Solution (refer to Preparation of Reagents) to each well. Cover with plate sealer and incubate for 1 hour. During this incubation period, warm the TMB Substrate Solution to RT (22-25°C).

After 1 hour, empty the contents of the plate and wash the wells three times with diluted Wash Buffer.

4. **TMB Substrate:** Add 100 µl of the TMB Substrate Solution to each well. Incubate, in the dark, at RT (22-25°C), for 15 minutes. Do not use a plate sealer during the incubation.

5. **Stop Solution:** After the 15 minute incubation of TMB, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add 100 µl of Stop Solution to each well.

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

CALCULATION OF RESULTS

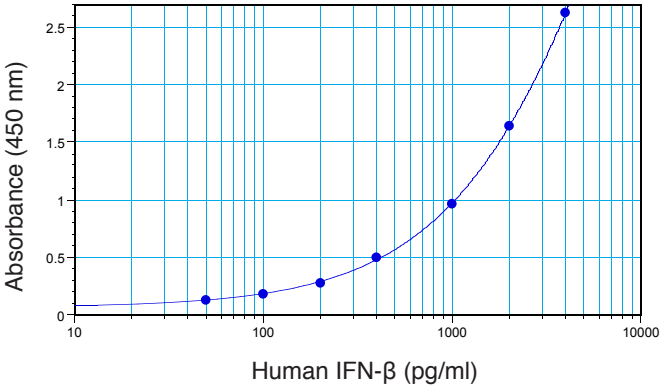
By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Based on user preference, blank ODs may be subtracted from the standards and sample ODs to eliminate background.

Because the interferon samples are titrated against the international standard, the values from the curves can be determined in units/ml as well as pg/ml. The conversion factor of about 3 – 10 pg/unit of Hu-IFN-β, mammalian, is applicable for human interferon beta.[4] Nevertheless, this conversion factor is only an approximation.

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 in Figure 3 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



REFERENCES

1. Staehelin, T., Stähli, C., Hobbs, D.S., and Pestka, S. (1981) "A Rapid Quantitative Assay of High Sensitivity for Human Leukocyte Interferon with Monoclonal Antibodies," in *Methods in Enzymology*, Vol. 79 (S. Pestka, ed.), Academic Press, New York, 589-595.

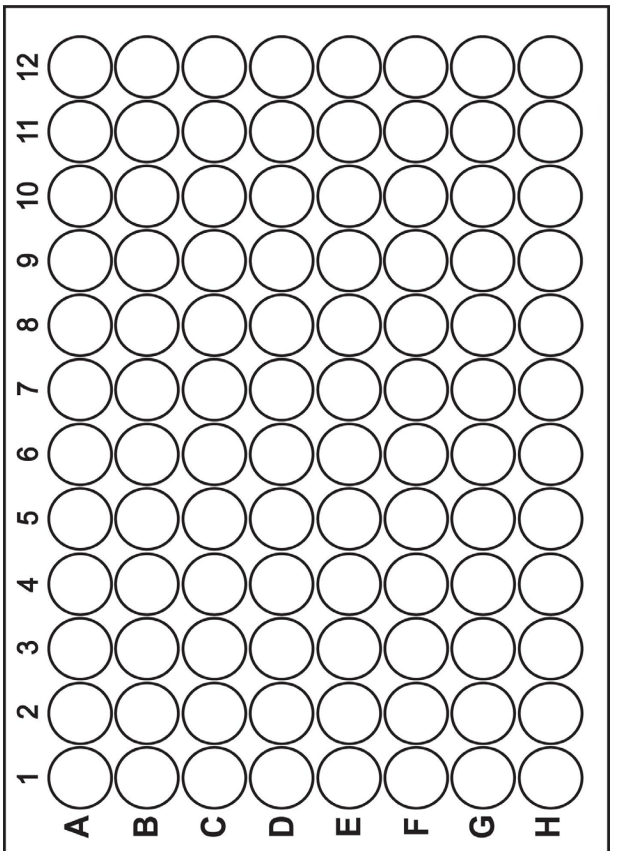
2. Kelder, B., Rashidbaigi, A., and Pestka, S. (1986) "A Sandwich Radioimmunoassay for Human IFNb," in *Methods in Enzymology*, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 582-587.

3. Human IFN-β international reference standard provided by the NIH, reference no. Gb23-902-531. Pestka, S. (1986) "Interferon Standards and General Abbreviations," in *Methods in Enzymology*, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 14-23.

4. Moschera, J., Woehle, D., Tsai, K., Chen, C. and Tarnowski, J. (1981) "Purification of Recombinant Human Fibroblast Interferon Produced in *Escherichia coli*," in *Methods of Enzymology*, Vol.119 (S. Pestka ed.), Academic Press, New York, 177 – 183.

PLATE LAYOUT

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



NOTES

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