pbl assay science

VeriKine-HS™ Mouse IFN Beta Serum ELISA Kit

Catalog No. 42410

Assay Range: 0.94 – 60 pg/ml

Store **all** components at 2 - 8°C

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.[1,2]

IFN-β plays a pivotal role in the protective response to many infections and diseases.[3] However, when produced unchecked it can also contribute to the generation of clinically relevant side effects and pathological processes.[4-6] Additionally, IFN-β is a common therapeutic treatment for multiple sclerosis and some cancers with the research into these diseases often conducted in mice.[7]

The VeriKine-HS™ Mouse Interferon Beta Serum ELISA kit has been developed to quantify levels of IFN-β in tissue culture media in a sandwich immunoassay format with recombinant mouse IFN-β expressed in mammalian cells as the standard in the kit.

MATERIALS PROVIDED

- Pre-coated microtiter plate(s)
- Plate sealers
- Wash Solution Concentrate
- Mouse Interferon Beta Standard, 10,000 pg/ml
- Sample Diluent
- Serum Buffer
- Antibody Concentrate
- Antibody Diluent
- HRP Conjugate Concentrate
- HRP Diluent
- TMB Substrate
- Stop Solution

42410 Rev. 01

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)
- Timer
- Graduated Cylinder

Specifications: This VeriKine-HS™ kit quantitates Mouse Interferon Beta (IFN-β) in sera, plasma and tissue culture media by sandwich enyzme linked immunosorbent assay (ELISA). Interferon binds to plates coated with antibody and detection is accomplished using a detection antibody followed by streptavidin conjugated to horseradish peroxidase (HRP). This ELISA kit utilizes Tetramethyl-benzidine (TMB) as the substrate. The standard provided in the kit is recombinant Mouse Interferon Beta expressed in mammalian cells.

Speed: Incubation time, 1 hr 50 min

Specificity: No cross reactivity was observed when using this kit to detect the following, each at up to 5 ng/ml
Mouse: IFN-α A, IFN-α 4. IFN-α 11. IFN-α 13, IFN-γ, IL-6, Limitin, TNF-α; **Human:** IFN-α A, IFN-β, IFN-γ, IFN-ω; **Rat:** IFN-α, IFN-β, IFN-γ; **Swine:** IFN-α, IFN-β

Twenty-five different lots of pooled serum from various mouse strains (CD-1, C57, BALB/c) were screened for endogenous Mouse IFN-β. Three samples showed detectable levels at 0.304, 0.379 and 0.353 pg/ml. Out of 20 screened lots of plasma, one sample had detectable IFN-β of 0.308 pg/ml.

Precision & Recovery: Mouse IFN-β was spiked into a single lot of screened CD-1 mouse serum at three different concentrations and analyzed:
Intra-Assay CV - 24 replicates of each concentration on a single plate
Inter-Assay CV - 9 independent assays run by same operator
Average Recovery - 27 independent assays

Concentration (pg/ml)	2.5	10	50
Intra-Assay CV	5.7%	5.5%	5.5%
Inter-Assay CV	8.3%	6.3%	4.8%
Average Recovery	98%	92%	94%

Storage Conditions/Comments: For retention of full activity, all reagents should be kept at 2-8°C when not in use.
Please note that the dilutions of the Antibody Concentrate 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.

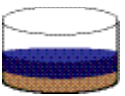
CAUTION: Wash Solution Concentrate, Sample Diluent, Antibody Diluent and Serum Buffer contain 0.1% Kathon CG/ICP as a preservative. These components should be handled with appropriate safety precautions and discarded properly. For further information, consult the material safety data sheet (MSDS).
For laboratory research use only. Not for use in diagnostic or therapeutic procedures.

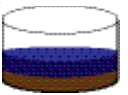
ASSAY PROCEDURE - QUICK REFERENCE

Total Time: 1 hr, 50 min

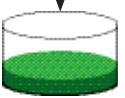
For serum & plasma samples:
1) Add **50 µl** Serum Buffer
2) Add **50 µl** Standard, Sample or Blank

For tissue culture samples:
1) Add **50 µl** Sample Diluent
2) Add **50 µl** Standard, Sample or Blank



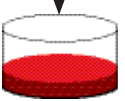


Incubate **1 hr** (shake at 650 rpm)
Aspirate and Wash **4x**



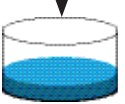
Add **50 µl** Diluted Ab Solution

Incubate **30 min** (shake at 650 rpm)
Aspirate and Wash **4x**



Add **50 µl** Diluted HRP Solution

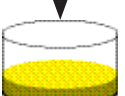
Incubate **10 min** (shake at 650 rpm)
Aspirate and Wash **4x**



Add **100 µl** TMB Substrate

Incubate **10 min** in the dark
Do not seal, shake or wash.

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



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

PREPARATION OF REAGENTS

Before starting the assay, the plate(s), Wash Solution Concentrate, Serum Buffer, TMB Substrate and Stop Solution should be equilibrated to room temperature (RT), 22-25ºC. Supplied Mouse IFN-β Standard, Antibody Concentrate, HRP Conjugate Concentrate, Sample Diluent, Antibody Diluent and HRP Diluent should be kept on ice (4ºC) throughout the assay.

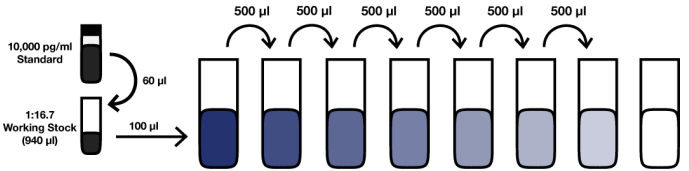
Wash Solution: The Wash Solution Concentrate may contain crystals. Place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution (e.g. Add 50 ml of Wash Solution Concentrate to 450 ml of distilled or deionized water and mix thoroughly). Diluted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

Mouse Interferon Beta Solution: Using the Mouse IFN-β Standard, construct a standard curve (0.94-60 pg/ml), as shown in figure 1, in Sample Diluent.

Standard Curve Preparation:

a) Prepare a 1:16.7 *working stock* of mouse IFN-β Standard by pipetting 60 µl of IFN Standard into 940 µl of Sample Diluent. Mix thoroughly by gently pipetting up and down 10 times.
b) Label seven polypropylene tubes (S1-S7).
c) Fill tubes with Sample Diluent as indicated in Figure 1.
d) Add 100 µl of the working stock of Mouse IFN-β Standard to S7 and mix thoroughly to recover all material adhered to the inside of the pipette tip.
e) Using a pipette set at 500 µl, mix S7 thoroughly by pipetting up and down. Transfer 500 µl of S7 to S6 and mix thoroughly by pipetting up and down. Repeat to complete series to S1.
f) **Set aside on ice (4ºC)** until use in step 1 of the Assay Procedure.

Fig. 1: 7-Point Standard Curve Prepared in Sample Diluent



Sample Dilution Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Diluent (µl)	900	500	500	500	500	500	500	500
IFN-β Conc. (pg/ml)	60	30	15	7.5	3.75	1.87	0.94	0

Test Sample Preparation: If test samples are not to be immediately assayed, it is recommended that, upon collection, they be stored at < -70ºC (avoiding multiple freeze-thaw cycles); particularly those samples in serum and plasma, which are prone to rapid degradation. If necessary, dilute test samples in Sample Diluent. Keep on ice (4ºC) until step 1 of the Assay Procedure.

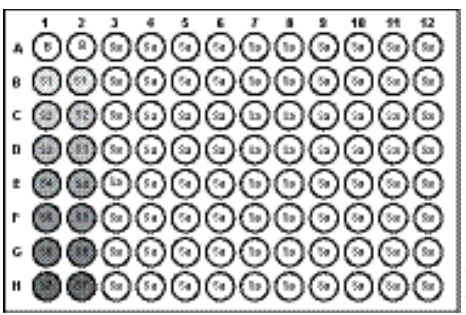
Antibody Solution: Dilute Antibody Concentrate in the volume of Antibody Diluent recommended in the lot specific Certificate of Analysis (COA). Prepare within 15 minutes prior to use and keep on ice (4ºC) until step 2 of the Assay Procedure.

HRP Solution: Dilute HRP Concentrate in the volume of HRP Diluent recommended in the lot specific Certificate of Analysis (COA). Prepare within 15 minutes prior to use and keep on ice (4ºC) until step 3 of the Assay Procedure.

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 Solution at each wash step. Refer to Preparation of Reagents for dilution of concentrated solutions.

Figure 2: Example of a Typical Plate Setup



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

1. **Standards and Test Samples:** Determine the number of microplate 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 IFN-β 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 microplate strips from the frame, seal in the foil bag provided and store at 2-8°C. Unused strips can be used in later assays.

1a. **For testing serum or plasma samples:**
- Add 50 µl of Serum Buffer to each well
- Overlay 50 µl of diluted Standard, Test Sample or Blank

1b. **For testing tissue culture samples:**
- Add 50 µl of Sample Diluent to each well
- Overlay 50 µl of diluted Standard, Test Sample or Blank

Cover with plate sealer and shake plate at 650 rpm at RT (22-25°C) for 1 hour.

After 1 hour, empty the contents of the plate and wash the wells four times with at least 250 µl of working Wash Solution (refer to Preparation of Reagents).

2. **Antibody Solution:** Add 50 µl of diluted Antibody Solution (refer to Preparation of Reagents) to each well. Cover with plate sealer and shake plate at 650 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 at least 250 µl of working Wash Solution.

3. **HRP:** Add 50 µl of diluted HRP Solution (refer to Preparation of Reagents) to each well. Cover with plate sealer and shake plate at 650 rpm at RT (22-25°C) for 10 minutes.

After 10 minutes, empty the contents of the plate and wash the wells four times with at least 250 µl of working Wash Solution.

4. **TMB Substrate:** Add 100 µl of the TMB Substrate Solution to each well. Incubate, in the dark, at RT (22-25°C), for 10 minutes. Do not use a plate sealer and do not shake during the incubation.

5. **Stop Solution:** After the 10 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

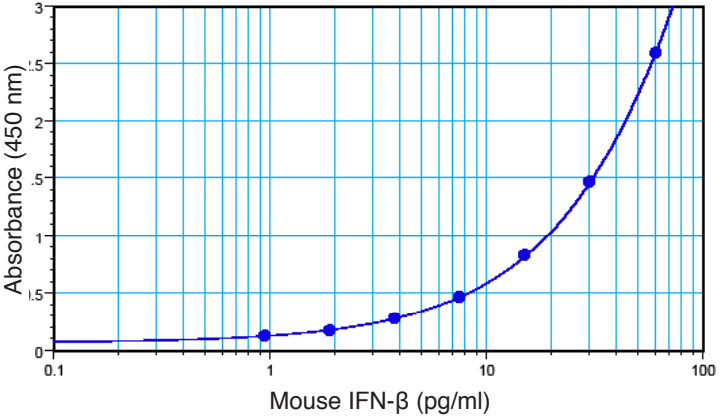
By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Based on user preference, blank ODs may be subtracted from the standards and sample ODs to eliminate background.

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.

Figure 3: Typical Standard Curve



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6. Hill, N. and Sarvetnick, N. (2002) "Cytokines: promoters and dampeners of autoimmunity." *Curr Opin Immunol.* 14(6):791-7.

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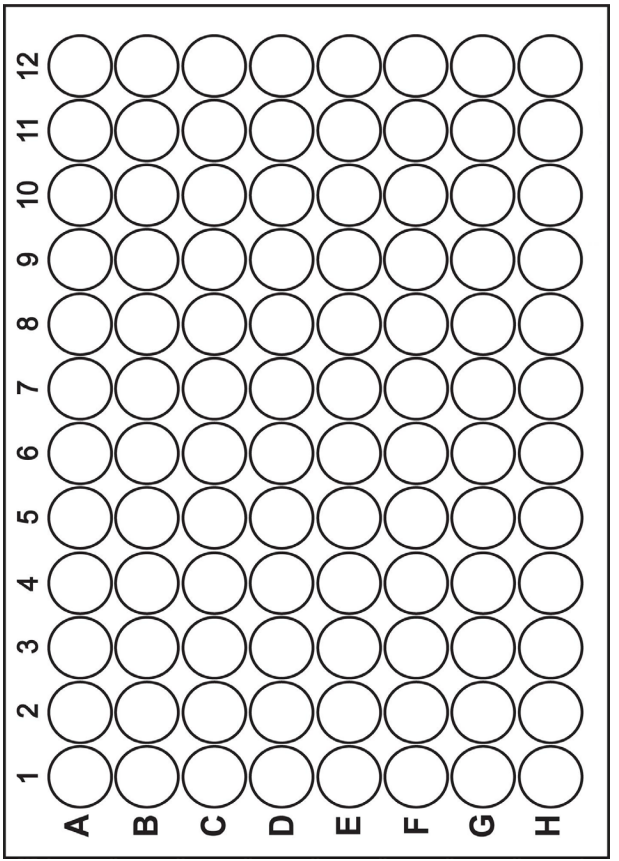
PLATE LAYOUT

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



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