pbl assay science

VeriKine™ Mouse IFN Beta ELISA Kit

Catalog No. 42400

Assay Range: 15.6 – 1000 pg/ml

Store **all** components at 2 - 8°C

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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 Interferon Alpha (IFN-α) genes and at least one Interferon Beta (IFN-β) gene in most vertebrates.

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

The VeriKine™ Mouse Interferon Beta 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, 500,000 pg/ml
- Dilution Buffer
- Antibody Concentrate
- HRP Conjugate Concentrate
- Concentrate 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)
- Timer
- Graduated Cylinder

Specifications: This VeriKine™ kit quantitates Mouse Interferon Beta (IFN-β) in sera and tissue culture media by sandwich enyzme linked immunosorbent assay (ELISA). Interferons bind to plates coated with antibody and detection is accomplished using a secondary 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, 3 hr 15 min

Specificity: Mouse IFN-β. No cross-reactivity detected with human IFN-α, IFN-γ, IFN-κ, IFN-β; rat IFN-α, IFN-β, IFN-γ; mouse IFN-α, IFN-γ; feline IFN-α; or pig IFN-α.

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

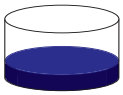
Please note that the dilutions of the Detection Antibody and HRP differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (COA) for their preparation.

CAUTION: Wash Solution Concentrate, Dilution Buffer, and Concentrate Diluent 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 safety data sheet (SDS).

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

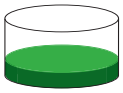
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
3 hr, 15 min



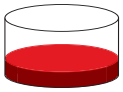
Add **100 µl** Standard, Blank, or Sample

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



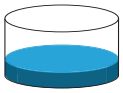
Add **100 µl** Diluted Ab Solution

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



Add **100 µl** Diluted HRP Solution

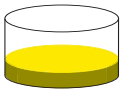
Incubate **1 hr**
Aspirate and wash **3x**



Add **100 µl** TMB Substrate

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

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), Dilution Buffer, applicable dilution matrices (e.g. Tissue culture media), Concentrate Diluent, TMB Substrate, Stop Solution, and samples should be equilibrated to Room Temperature (RT), 22-25ºC. Supplied Mouse IFN-β Standard, Antibody Concentrate, and HRP Conjugate Concentrate should be kept on ice (4ºC).

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.

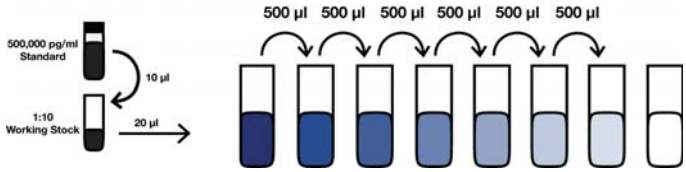
Mouse Interferon Beta Solution: Using the Mouse IFN-β Standard, construct a standard curve (15.6-1000 pg/ml), as shown in figure 1, in dilution buffer. In certain situations, "test" samples may contain substances that can interfere with assay results. Therefore it is recommended to run the IFN standard curve diluted in your sample matrix.

Standard Curve Preparation:

a) Prepare a 1:10 **working stock** of the mouse IFN-β Standard by pipetting 10 µl of IFN Standard into 90 µl of Dilution Buffer or sample matrix. Mix thoroughly by pipetting up and down 10 times.
b) Label seven polypropylene tubes (S1-S7).
c) Fill tubes with Dilution Buffer as indicated in Figure 1.
d) Add 20 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 until use in step 1 of the Assay Procedure.

Figure 1: 7-Point Standard Curve Prepared in Dilution Buffer



Sample Dilution Label	S7	S6	S5	S4	S3	S2	S1	Blank
Dilution Buffer/Matrix Vol. (µl)	980	500	500	500	500	500	500	500
IFN-β Conc. (pg/ml)	1000	500	250	125	62.5	31.3	15.6	0

Sample Preparation: Prepare test samples of unknown interferon concentration to be tested using Dilution Buffer or sample matrix as required. Set aside until use in step 1 of the Assay Procedure.

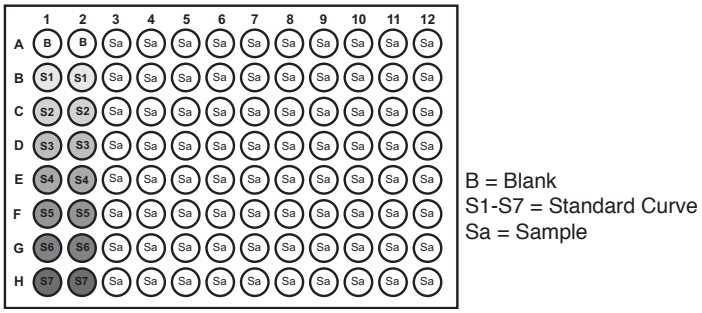
Antibody Solution: Refer to the lot specific Certificate of Analysis (COA) for the correct amount of Antibody Solution to prepare. Prepare within 15 minutes prior to use. Dilute Antibody Concentrate in recommended volume of Concentrate Diluent. Set aside diluted Antibody Solution at RT (22-25°C) until 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 Concentrate 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). Optionally, the incubations can be carried out at room temperature 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 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 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 IFN-β Standard, blanks, and samples in duplicate. Remove extra microtiter strips from the frame, seal in the foil bag provided, and store at 2-8°C. Unused strips can be used in later assays.

Add 100 µl per well of interferon standard, blank, or sample. Cover with plate sealer and incubate for 1 hour at RT (22-25°C).

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

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 at RT (22-25°C).

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

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 at RT (22-25°C).

After 1 hour, empty the contents of the plate and wash the wells three times with 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 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

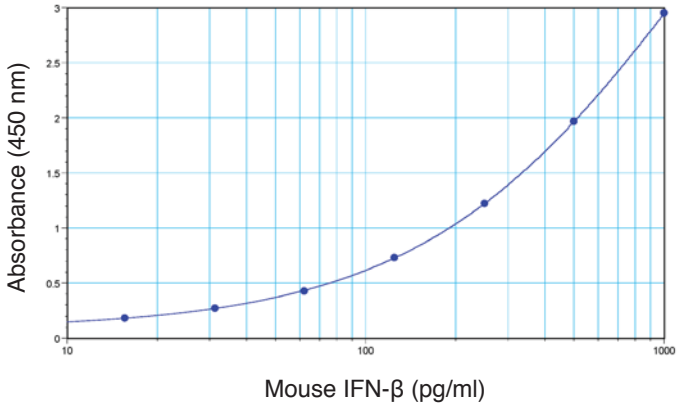
By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Based on user preference, blank ODs may be subtracted from the standards and sample ODs to eliminate background.

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



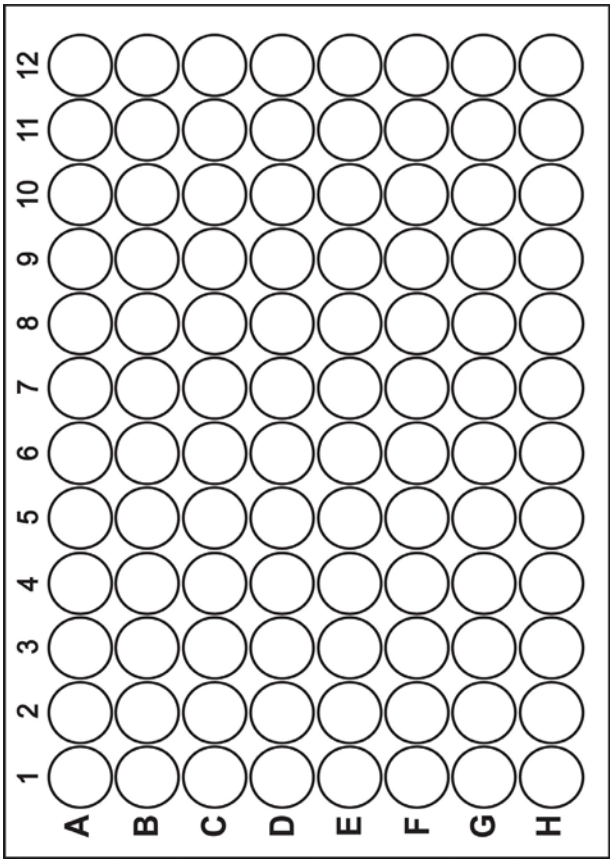
REFERENCES

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

2. Balachandran, S., Thomas, E., and Barber, G. (2004) “A FADD-dependent innate immune mechanism in mammalian cells,” in *Nature*, Vol. 432, 401-405.

PLATE LAYOUT

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



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