pbl assay science

VeriKine™ Mouse IFN Alpha ELISA Kit

Catalog No. 42120

Assay Range: 12.5 - 400 pg/ml

Store all components at 2 - 8°C

INTRODUCTION

Interferons (IFNs) are a group of cytokines which exhibit pleitropic activities that play major roles in both innate and adaptive immunity. Type I IFNs consist of multiple IFN-α genes and at least one IFN-β gene in most vertebrates, and a few other family members such as limitin in the mouse.[1] There are 14 IFN-α subtypes in the mouse which share at least 75% identity in protein sequence. In the human, IFN-α is used to treat viral diseases and cancer[2-4] and is studied in mouse models of these diseases.[5,6] Recent evidence has suggested that IFN-α may play a role in development of certain autoimmune diseases[7,8] and that treatment with IFN-α is useful for other autoimmune diseases.[9]

The expression of interferons is regulated by a group of interferon regulatory factor proteins (IRF). The expression of the individual IFN-α subtypes is regulated by the cellular levels of IRF3 and IRF7. Most of the IFN-α subtypes require IRF7 for expression. IRF7 is constitutively expressed in some cell types and is inducible in others. Inducible IRF7 expression is induced by IFN-β and IFN-α4 in mice and leads to expression of the other IFN-α subtypes. Murine IFN-β may be required for the production of IFN-α by fibroblasts but may not be required for IFN-α production by other cells.[10] 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.[11]

Plasmacytoid dendritic cells are the major producers of IFN-α, but a large variety of cells can also produce lower levels of these proteins.[12,13] The particular subtypes that are expressed appear to be somewhat cell and stimulus specific.[14-16]

The VeriKine^{TM} Mouse IFN-α ELISA kit has been developed to measure levels of IFN-α in tissue culture media. It is important with this kit and any ELISA kit to run the standard curve in the same matrix as your samples unless you have demonstrated that the matrix does not affect the signal.

MATERIALS PROVIDED

• Pre-coated microtiter plate(s)
• Plate Sealers
• Wash Solution Concentrate
• Mouse Interferon Alpha (IFN-α) Standard (10,000 pg/ml)
• Sample Buffer
• Concentrate Diluent
• Antibody Concentrate
• HRP Conjugate Concentrate
• Assay Diluent
• TMB Substrate Solution
• Stop Solution

ADDITIONAL MATERIALS REQUIRED (*NOT PROVIDED)*

• Microtiter plate 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 VeriKine[TM] kit quantitates interferon alpha in tissue culture media (10% FBS) using a sandwich immunoassay.[17,18] **The quantitation of mouse interferon alpha in serum and plasma samples using this product has not been fully evaluated by PBL.** The kit is based on an ELISA with anti-detection antibody conjugated to horseradish per-oxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. All reagents are supplied. The assay is based on the international reference standard for mouse interferon alpha (Mu-IFN-α) provided by the National Institutes of Health.[19] Typical standard curves for each lot are included with the procedure.

Speed: Maximum incubation time, 27 hr 15 min

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

Storage Conditions/Comments: For retention of full activity, all reagents should be kept at 2-8ºC in the dark. Deionized or distilled water should be used for preparation of all reagents. All dilutions should be made with polypropylene tubes and pipette tips. Pipette tips should be changed between each dilution tube. All measurements for standards and samples should be performed in duplicate. At least two control wells (wells with Sample Buffer only) should be used for each assay; these control values should be subtracted from all readings prior to any calculations or plots of the data.

CAUTION: Wash Solution Concentrate, Sample Buffer and HRP/Ab 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 material safety data sheet (MSDS) for the product.

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

ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
27 hr 15 min

1) Add **100 µl** Standard, Blank or Sample
2) Add **50 µl** Diluted Antibody Solution

Incubate **1 hr** (shake at 450 rpm)
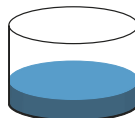Transfer plate to 4ºC and incubate **20-24 hours**

Aspirate and wash **4x**

Add **100 µl** Diluted HRP Solution

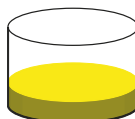Incubate **2 hr** (shake at 450 rpm)
Aspirate and wash **4x**

Add **100 µl** TMB Substrate

Incubate **15 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—DAY 1

Wash Solution: Dilute 50 ml of the Wash Solution Concentrate to a final volume of 1000 ml of distilled or deionized water and mix thoroughly. The diluted Wash Solution can be stored at RT (22-25°C) when not in use.
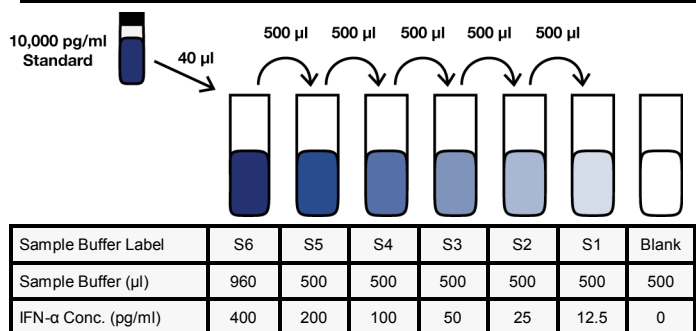
Mouse Interferon Alpha Solution: Using the Mouse IFN-α Standard, construct a standard curve (12.5-400 pg/ml), as shown in figure 1, in Sample Buffer. In certain situations, "test" samples may contain substances that can interfere with assay results. Therefore it is recommended to run the IFN standard curve diluted in your sample matrix.

Do not add the Standard and Samples to the plate until the Antibody Solution is prepared.

Standard Curve Preparation:
a) Label six polypropylene tubes (S1-S6)
b) Fill tubes with Sample Buffer or Sample Matrix as indicated
c) Using polypropylene tips, add 40 µl of the IFN Standard to S6 and mix thoroughly. Change pipette tips between each dilution.
d) Remove indicated amount from S6 and transfer to S5. Repeat to complete series to S1.
e) Set aside until use in step 1 of the assay procedure.

Figure 1: 6-Point Standard Curve Prepared in Sample Buffer

Sample Buffer Label	S6	S5	S4	S3	S2	S1	Blank
Sample Buffer (µl)	960	500	500	500	500	500	500
IFN-α Conc. (pg/ml)	400	200	100	50	25	12.5	0

Sample Preparation: Prepare test samples of unknown interferon concentration to be tested. Dilute as required in Sample Buffer. Measurements in duplicate are recommended. Set aside and proceed to the Antibody Solution preparation.

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 Concentrate Diluent. Set aside diluted Antibody Solution at RT (22-25ºC) until use.

PREPARATION OF REAGENTS—DAY 2

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—DAY 1

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-S6 = Standard Curve
Sa = Sample
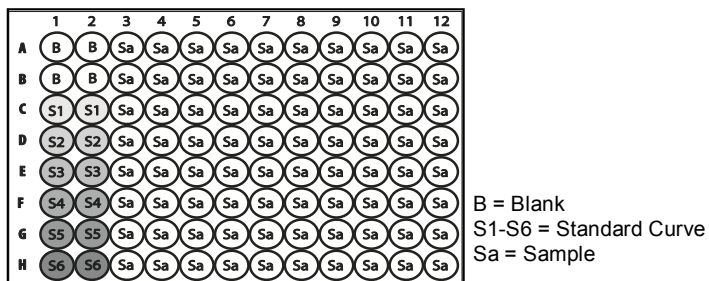
1. **Standards, Test Samples and Diluted Antibody Solution**: Determine the number of microplate strips required to test the desired number of samples plus the appropriate number of wells needed to run blanks and standards. We recommend running both the IFN-α standard, blanks and samples in duplicate. 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 Standards, Test Samples and Blanks Add 100 µl of Standard (refer to Preparation of Reagents), Test Samples or Blanks per well.

Step B: Adding diluted Antibody Solution Add 50 µl of diluted Antibody Solution (refer to Preparation of Reagents) to each well (Total volume = 150 µl/well). Change pipette tips between each addition.

Refer to the sample plate layout in Figure 2.

Cover with a Plate Sealer and incubate for 1 hour at RT (22-25°C) with shaking at 450 rpm.

After 1 hour, transfer the plate to 4°C and incubate 20-24 hours without shaking.

ASSAY PROCEDURE—DAY 2

After 20-24 hours, empty the contents of the plate and wash the wells <u>four times</u> with diluted 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 incubate for 2 hours at RT (22-25°C) with shaking at 450 rpm.

After 2 hours, empty the contents of the plate and wash the wells <u>four times</u> with diluted 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 15

minutes. Do not use a plate sealer during the incubation. DO NOT SHAKE.

4. **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.

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. Blank ODs should be subtracted from the standards and sample ODs to eliminate background. A typical standard curve for this assay is shown in the enclosed pages. This example is for the purpose of illustration only, and should not be used to calculate unknowns.

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.

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 10-20 pg/unit is applicable for mouse interferon alpha.[3] Nevertheless, this conversion factor is only an approximation.

Figure 3: Typical Standard Curve
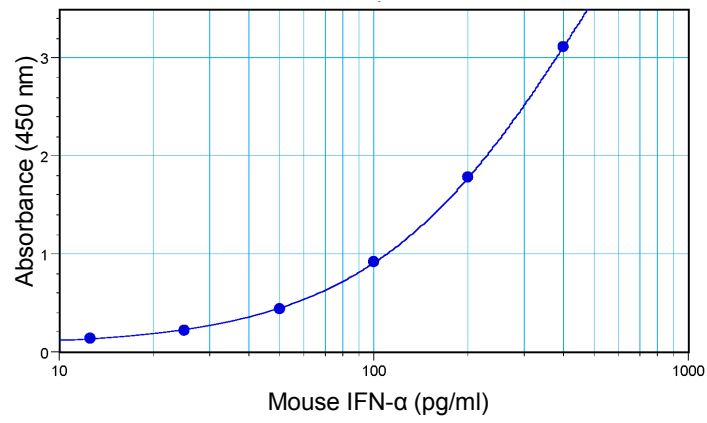
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PLATE LAYOUT
Use this plate layout as a record of standards and samples assayed.
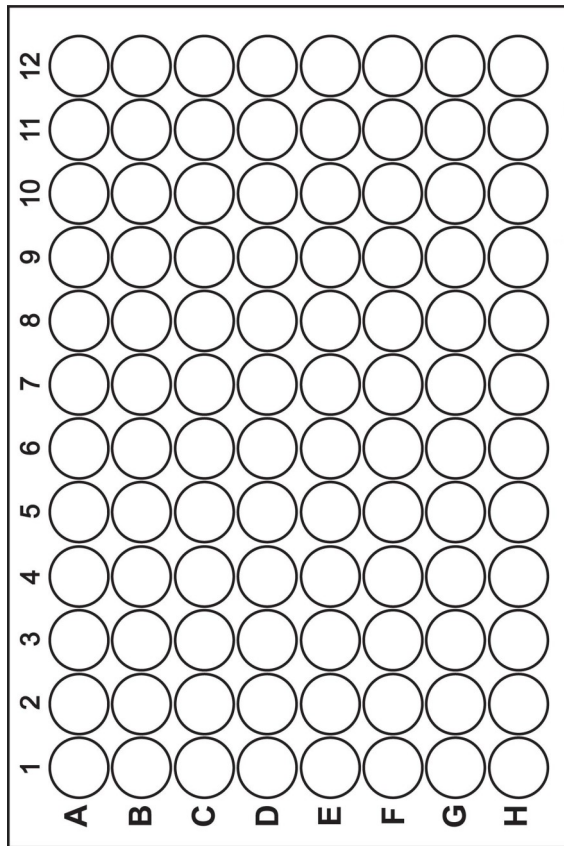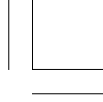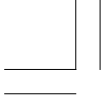
PBL Assay Science
131 Ethel Road West, Suite 6, Piscataway, NJ 08854 USA

Tel: +1 732-777-9123, Fax: +1 732-777-9141
Email: info@pblassaysci.com, Website: www.pblassaysci.com