pbl assay science

VeriKine™ Human IFN-α Multi-Subtype ELISA Kit

Product #41105

High Sensitivity: 12.5 – 500 pg/ml
Extended Range: 156 – 5000 pg/ml

Store **all** components at 2 - 8°C

INTRODUCTION

Interferons (IFNs) are a family of mammalian cytokines initially characterized by their ability to inhibit viral infection. They are synthesized and secreted by most cell types in response to pathogens. In addition to their antiviral properties, IFNs have also been shown to exhibit anti-proliferative, immunomodulatory, and many other activities.

In humans, IFN-α consists of a group of proteins that are greater than 85% homologous by amino acid sequence. Numerous individual human IFN-α subtypes have been identified; many display different properties. It remains unclear why there are multiple IFN-α subtypes. A variety of studies suggested they possess overlapping but also unique sets of biological activities.

The PBL Assay Science VeriKine™ Human IFN-α Multi-Subtype ELISA kit is specifically formulated to detect 14 out of 15 identified human IFN-α subtypes. They are: IFN-αA, IFN-α2, IFN-αD, IFN-αB2, IFN-αC, IFN-αG, IFN-αH, IFN-αI, IFN-αJ1, IFN-αK, IFN-α1, IFN-α4a, IFN-α4b, and IFN-αWA.

MATERIALS PROVIDED

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

ADDITIONAL MATERIALS REQUIRED *(NOT PROVIDED)*

- Microtiter plate reader capable of reading a wavelength of 450 nm
- Variable volume microtiter pipettes
- Adjustable multi-channel 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 alpha in media using a sandwich immunoassay.[1,2] The kit is based on an ELISA with anti-detection antibody conjugated to horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. The assay is based on the international reference standard for human interferon alpha (Hu-IFN-α) provided by the National Institutes of Health.[3]

Speed: Incubation time, 3 hr 15 min

Specificity: Human IFN-α. No cross reactivity with human IFN-γ, human IFN-β or human IFN-ω. No cross-reactivity with: mouse or rat IFN-α, IFN-β, or IFN-γ; bovine IFN-τ.

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

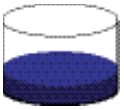
Please note that the concentrations of the Detecting Antibody and HRP differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (COA) for their preparation.

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

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

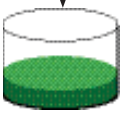
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
3 hr, 15 min



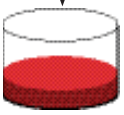
Add **100 µl** Standard, Blank or Sample

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



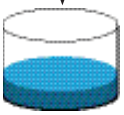
Add **100 µl** Diluted Ab Solution

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



Add **100 µl** Diluted HRP Solution

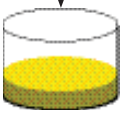
Incubate **1 hr**
Aspirate and Wash **4x**



Add **100 µl** TMB Substrate

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

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



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

PREPARATION OF REAGENTS

Wash Buffer: Dilute 50 ml of the Wash Solution Concentrate to a final volume of 1000 ml with distilled or deionized water. Mix thoroughly before use. The diluted wash buffer can be stored at 22-25°C.

Human Interferon Alpha Solution: Dilute the Human IFN Alpha Standard, provided at 10,000 pg/ml, in Dilution Buffer as indicated. 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:
Construct a High Sensitivity standard curve 12.5 – 500 pg/ml or Extended Range standard curve 156 – 5000 pg/ml.

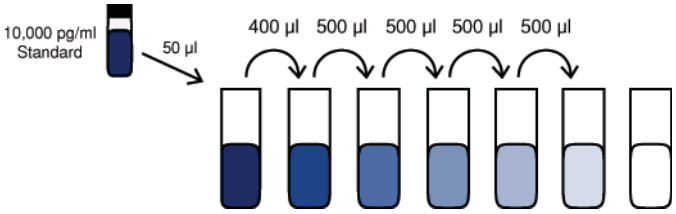
a) Label six polypropylene tubes (S1-S6).
b) Fill tubes with Dilution buffer as indicated.
c) Using polypropylene tips add the Human IFN-α Standard to S6 and mix gently. Change tips between each dilution.
d) Remove indicated amount from S6 and add to S5. Repeat to complete series to S1.
e) Refrigerate until use in step 1 of the assay procedure.

Sample Preparation: Prepare test samples of unknown IFN concentration to be tested using Dilution Buffer as required. Measurements in duplicate are recommended. Refrigerate until use in step 1 of the assay procedure.

Antibody Solution: Dilute Antibody Concentrate with Dilution Buffer. Refer to the lot specific Certificate of Analysis (COA) for the correct amount of Antibody Solution to prepare. Refrigerate until use in step 2 of the assay procedure.

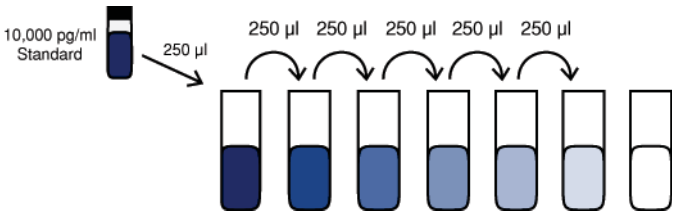
Figure 1: 6-Point Standard Curve Prepared in Dilution Buffer

High Sensitivity



Dilution Buffer Label	S6	S5	S4	S3	S2	S1	Blank
Dilution Buffer (µl)	950	600	500	500	500	500	500
IFN-α Conc. (pg/ml)	500	200	100	50	25	12.5	0

Extended Range



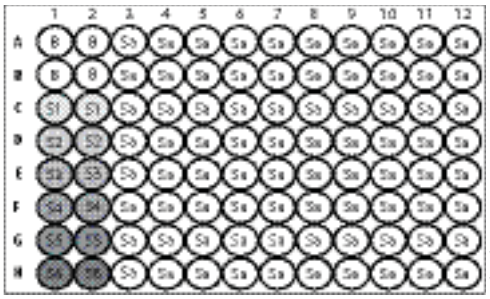
Dilution Buffer Label	S6	S5	S4	S3	S2	S1	Blank
Dilution Buffer (µl)	250	250	250	250	250	250	250
IFN-α Conc. (pg/ml)	5000	2500	1250	625	312	156	0

HRP Solution: Dilute HRP Conjugate Concentrate with Concentrate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct amount of HRP Solution to prepare. Refrigerate until use in step 3 of the assay procedure.

ASSAY PROCEDURE

All incubations should be performed in a closed chamber at 24°C or alternatively at room temperature (22-25°C) keeping the plate away from drafts and other temperature fluctuations. Use plate sealers to cover the plate as directed. During all wash steps remove contents of plate by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry. All wells should be filled with a minimum of 250 µl of diluted wash solution. Refer to preparation of reagents for diluted solutions.

Figure 2: Example of a Typical Plate Setup



B = Blank
S1-S6 = Standard Curve
Sa = Sample

1. **Standards and 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. Each standard, blank and sample should be run in duplicate. We recommend using strips 1 and 2, rows A-H for serially diluted standards and blanks. 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.

After 1 hour, empty the contents of the plate and wash the wells one time only 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 time, warm the TMB Substrate Solution to room temperature (22-25°C).

After 1 hour, empty the contents of the plate and wash the wells four 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. Blank ODs should be subtracted from the standards and sample ODs to eliminate background.

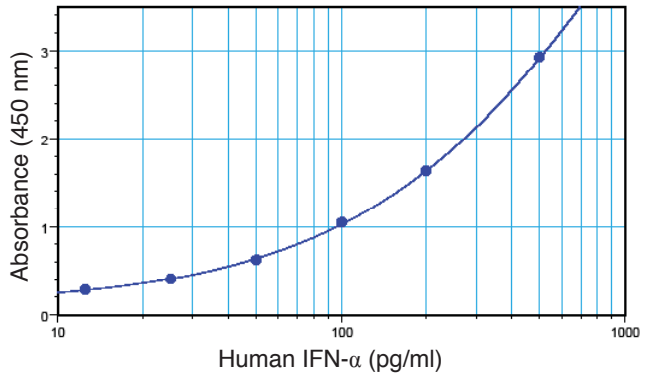
Because the interferon samples are titrated against the international standard, the values from the curves can be determined in units/ml as well as pg/ml. The conversion factor of about 3 – 5 pg/unit is applicable for human interferon alpha.[4,5] Nevertheless, this conversion factor is only an approximation.

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.

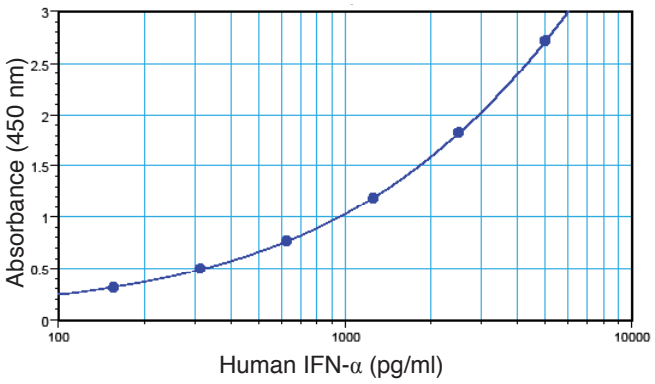
Results of a typical standard curves using a 4-parameter fit are provided for demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.

Figure 3: Typical Standard Curves

High Sensitivity



Extended Range



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 IFNa" in *Methods in Enzymology*, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 582-587.

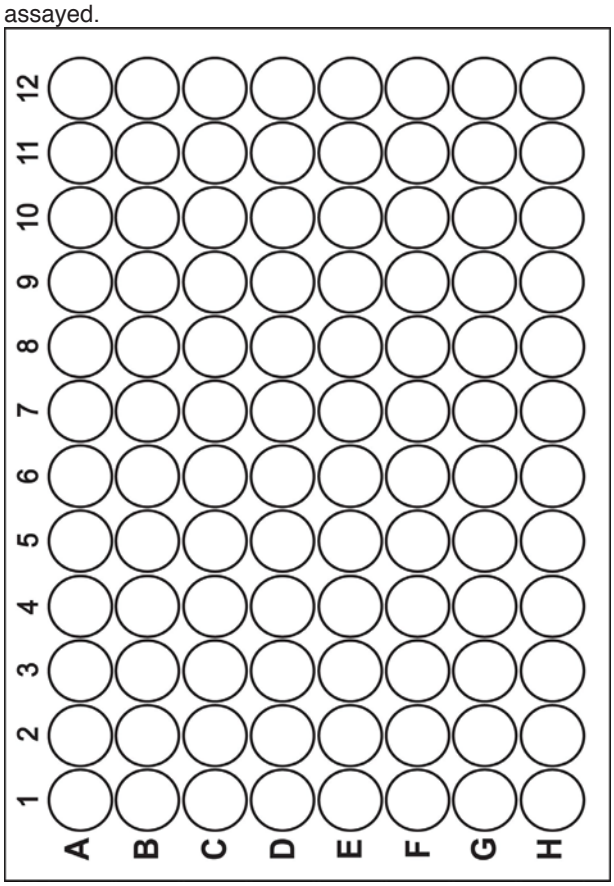
3. Human IFN-α international reference standard provided by the NIH, reference no. Gxa01-901-535. Pestka, S. (1986) "Interferon Standards and General Abbreviations," in *Methods in Enzymology*, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 14-23.

4. Rubinstein, M., Levy, W.P., Moschera, J.A., Lai, C.-Y., Hershberg, R.D., Bartlett, R.T., and Pestka, S. (1981) "Human Leukocyte Interferon: Isolation and Characterization of Several Molecular Forms," *Arch. Biochem. Biophys.* 210, 307-318.

5. Hobbs, D.S. and Pestka, S. (1982) "Purification and Characterization of Interferons from a Continuous Myeloblastic Cell Line," *J. Biol. Chem.* 257, 4071-4076.

PLATE LAYOUT

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



NOTES

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