



pbl assay science

VeriKine™ Human IFN Alpha ELISA Kit

Catalog No. 41100

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

Store all components at 2 - 8°C

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INTRODUCTION

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

When IFN interacts with its cognate receptor, a signal is rapidly transmitted within the cell, often producing an antiviral state. The primary signal transduction cascade promoted by type I IFNs is the JAK1-STAT pathway.

Activation of this signal transduction pathway leads to increased gene expression including (2'-5') oligoadenylate synthetases, Mx proteins, and protein kinase R (PKR) that protect the cell from viral infection.

PBL Assay Science's VeriKine™ Human IFN- α ELISA kit uses the sandwich immunoassay technique for the quantitative measurement of IFN- α in media. It is developed for superior performance with intra-assay and inter-assay CVs of $\leq 8\%$.

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)

- Microplate reader capable of reading an OD at a wavelength of 450 nm
- Variable volume microtiter pipettes
- Adjustable multichannel pipette (50-200 μ l)
- 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 VeriKine™ 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 detected with human IFN- γ , human IFN- β or human IFN- ω . No cross-reactivity detected 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.

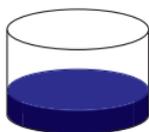
Please note that the concentrations of the Antibody Concentrate and HRP differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (COA) for their preparation.

CAUTION: Wash Solution Concentrate, 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.

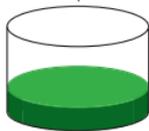
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
3 hr, 15 min



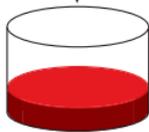
Incubate 1 hr
Aspirate and Wash 1x

Add 100 μ l Standard,
Blank or Sample



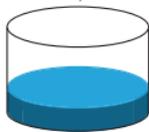
Incubate 1 hr
Aspirate and Wash 3x

Add 100 μ l Diluted
Ab Solution



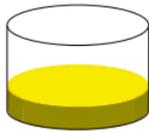
Incubate 1 hr
Aspirate and Wash 4x

Add 100 μ l Diluted
HRP Solution



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

Add 100 μ l TMB
Substrate



Add 100 μ l Stop Solution
Read plate within 5 min
(450 nm)

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

PREPARATION OF REAGENTS

All components should be kept on ice (4°C) throughout the assay, except for the Wash Solution Concentrate and Stop Solution, which should be brought to room temperature (RT), 22-25°C. The TMB Substrate should be equilibrated to RT (22-25°C) during step 3 of the Assay Procedure.

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 1:20 working Wash Buffer by adding 50 ml of Wash Solution Concentrate to 950 ml of distilled or deionized water. Mix thoroughly before use. The diluted Wash Buffer can be stored at RT (22-25°C) until use.

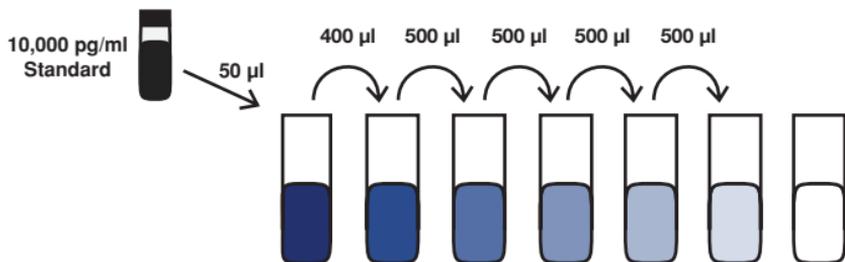
Human Interferon Alpha Solution: Dilute the Human IFN Alpha Standard, provided at 10,000 pg/ml, in Dilution Buffer as indicated. In certain situations “test” samples may contain substances that can interfere with assay results. Therefore, it is recommended to run the IFN standard curve diluted in your 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.

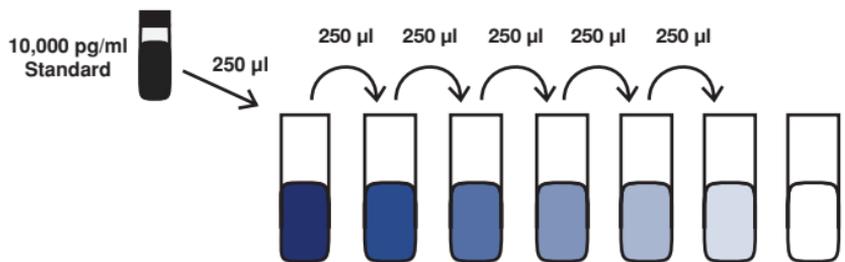
- Label six polypropylene tubes (S1-S6).
- Fill tubes with Dilution Buffer as indicated in Figure 1.
- Using polypropylene tips add the Human IFN- α Standard to S6 and mix gently. Change tips between each dilution.
- Remove indicated amount from S6 and add to S5. Repeat to complete series to S1.
- Refrigerate until use in step 1 of the Assay Procedure.

Figure 1a: 6-Point Standard Curve Prepared in Dilution Buffer (High Sensitivity)



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

Figure 1b: 6-Point Standard Curve Prepared in Dilution Buffer (Extended Range)



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

Sample Preparation: Prepare test samples of unknown interferon 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 in Dilution Buffer. Refer to the lot specific Certificate of Analysis (COA) for the correct volumes to use. Prepare 15 minutes prior to use in step 2 of the Assay Procedure and keep at RT (22-25°C).

HRP Solution: Dilute HRP Conjugate Concentrate in Concentrate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct volumes to use. 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 plate 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. All wells should be filled with a minimum of 250 µl of diluted Wash Buffer at each wash step. Refer to Preparation of Reagents for dilution of concentrated solutions.

Figure 2: Example of a Typical Plate Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	Sa									
B	B	B	Sa									
C	S1	S1	Sa									
D	S2	S2	Sa									
E	S3	S3	Sa									
F	S4	S4	Sa									
G	S5	S5	Sa									
H	S6	S6	Sa									

B = Blanks
S1-S6 = Std Curve
Sa = Samples

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. 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 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.

Add 100 μ l of the interferon standard, blank or sample 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 one time only with diluted Wash Buffer (refer to Preparation of Reagents).

2. **Antibody Solution:** Add 100 μ l 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 μ l 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 four times with diluted Wash Buffer.

4. **TMB Substrate:** Add 100 μ l 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 μ l 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 3a: Typical Standard Curve (High Sensitivity)

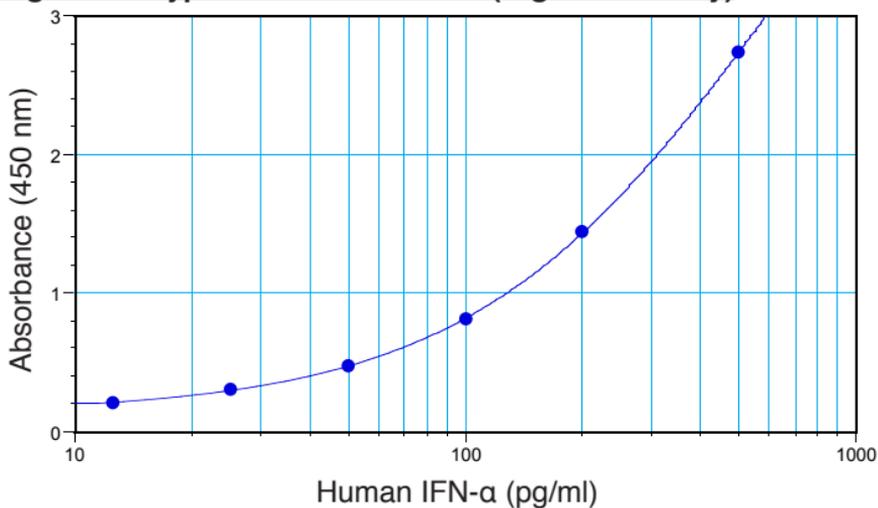
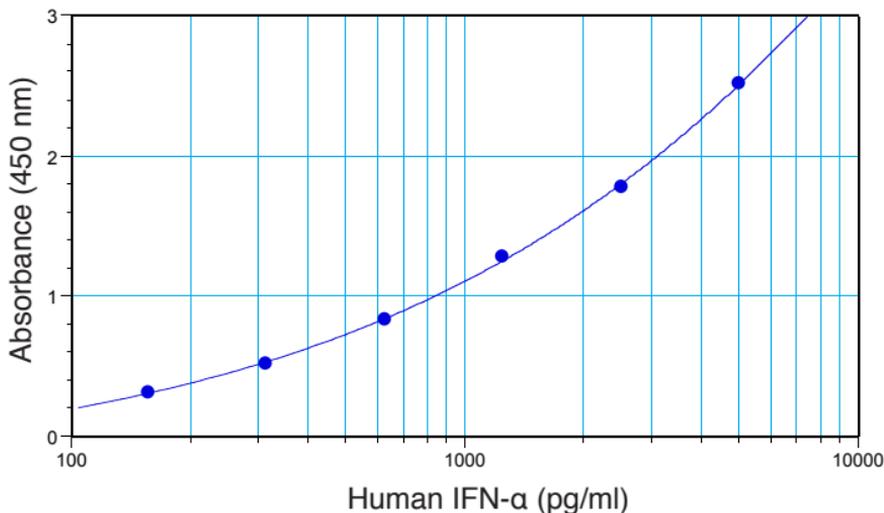


Figure 3b: Typical Standard Curve (Extended Range)



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 IFN α " 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, (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.

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

NOTES

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