pbl assay science

VeriKine-HS Human IFN-α All Subtype ELISA Kit

Product #41115

Assay Range: 1.95 – 125 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 VeriKine-HS Human IFN-α All Subtype ELISA Kit is specifically formulated to detect all human subtypes.

Subtype	LLOQ	LLOD
α1 (αD)	2.31	0.76
α2a (αA)	1.36	0.42
α4a (αM1)	1.38	0.43
α5 (αG)	1.70	0.51
α6 (αK)	1.13	0.33
α7 (αJ1)	2.09	0.59
α8 (αB2)	2.81	0.82
α10 (αC)	1.77	0.65
α14 (αH)	1.76	0.51
α16 (αWA)	2.01	0.68
α17 (αI)	1.36	0.50
α21 (αF)	6.18	1.67

41115 Rev. 00

MATERIALS PROVIDED

- Pre-coated microtiter plate(s)
- Plate sealers
- Wash Solution Concentrate
- Human Interferon Alpha Standard (10,000 pg/ml)
- Sample Buffer
- Standard Diluent
- Antibody Concentrate
- HRP Conjugate Concentrate
- Antibody Diluent
- HRP 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 human sera, plasma and tissue culture media using a sandwich immunoassay.[1,2] The kit is based on an ELISA with biotinylated-detection antibody and streptavidin-conjugated 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: Typical incubation time, 22 hr 30 min

Specificity: Human IFN-α. Cross reacts with Cynomolgus/Rhesus IFN-α. No cross reactivity detected with human IFN-β, IFN-γ or IFN-ω; mouse or rat IFN-α, IFN-β or IFN-γ; or bovine IFN-τ.

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

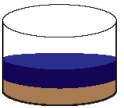
Please note that the concentrations of the Antibody Concentrate and HRP Concentrate differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (CoA) for their preparation.

CAUTION: Wash Solution Concentrate, Standard Diluent, Sample Buffer, and Antibody 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 laboratory research use only. Not for use in human diagnostic or therapeutic procedures.

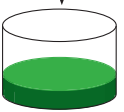
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
22 hr, 30 min



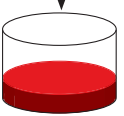
1) Add **50 µl** Sample Buffer
2) Add **50 µl** Standard, Blank or Sample

Incubate **30 sec** (shake at 550 rpm) @ RT (22-25°C)
Transfer to **4°C** and incubate **18-20 hr**
Aspirate and Wash **1x**



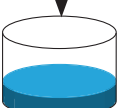
Add **100 µl** Diluted Ab Solution

Incubate **1 hr** (shake at 550 rpm) @ RT (22-25°C)
Aspirate and Wash **3x**



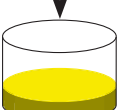
Add **100 µl** Diluted HRP Solution

Incubate **1 hr** (shake at 550 rpm) @ RT (22-25°C)
Aspirate and Wash **4x**



Add **100 µl** TMB Substrate

Incubate **30 min** in the dark @ RT (22-25°C)
Do not seal, shake or wash.



Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

PREPARATION OF REAGENTS

All components should be kept at 4°C throughout the assay, aside from the TMB Substrate and Stop Solution, which should be equilibrated to room temperature (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 1:10 working wash solution (e.g. add 50 ml of the Wash Solution Concentrate to 450 ml of distilled or deionized water). Mix thoroughly before use. The diluted Wash Buffer can be stored at RT (22-25°C) when not in use.

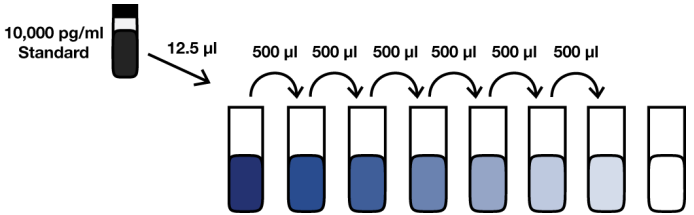
Human Interferon Alpha Solution: Dilute the Human IFN Alpha Standard, provided at 10,000 pg/ml, in the same matrix as the test samples. Examples of these matrices are endogenous IFN-α-free human serum, plasma or tissue culture medium containing 10% FBS. In the event that the sample matrix is not available, the Standard Diluent may be used to prepare the standard curve.

Standard Curve Preparation:

Construct a standard curve 1.95 – 125 pg/ml.

a) Label seven polypropylene tubes (S1-S7).
b) Add indicated volumes of Sample Matrix or Standard Diluent to the labeled tubes (see Figure 1).
c) Using polypropylene tips, add the indicated volume of Human IFN-α Standard to S7 and mix gently. Change tips between each dilution.
d) Remove indicated amount from S7 and add to S6. Repeat to complete series to S1.
e) **Set aside on ice (4°C)** until use in step 1 of the Assay Procedure.

Figure 1: 7-Point Standard Curve Prepared in Sample Matrix



Sample Matrix Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Matrix Vol. (µl)	987.5	500	500	500	500	500	500	500
IFN-α Conc. (pg/ml)	125	62.5	31.25	15.625	7.813	3.906	1.953	0

Sample Preparation: Prepare test samples of unknown IFN concentration to be tested as required. Measurements in duplicate are recommended. Refrigerate until use in step 1 of the Assay Procedure.

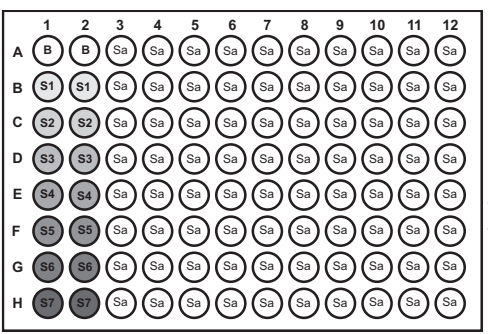
Antibody Solution: Dilute Antibody Concentrate in Antibody Diluent. Refer to the lot specific Certificate of Analysis (CoA) for the correct amount of Antibody Solution to prepare. Prepare 15 minutes prior to use in step 2 of the Assay Procedure and keep at RT (22-25°C).

HRP Solution: Dilute HRP Conjugate Concentrate in HRP Diluent. Refer to the lot specific Certificate of Analysis (CoA) for the correct amount of HRP Solution to prepare. Prepare 15 minutes prior to use in step 3 of the Assay Procedure and keep at RT (22-25°C).

ASSAY PROCEDURE – Day 1

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. The 4°C incubation does not require shaking. 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 300 µl of diluted Wash Buffer. Refer to Preparation of Reagents for diluted solutions. Any alteration of the described procedures can directly affect assay performance.

Figure 2: Example of a Typical Plate Setup



B = Blank
S1-S7 = 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. We recommend running both the IFN-α standard and samples at least 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.

Step A: Adding Sample Buffer. Add 50 µl of Sample Buffer to each well.

Step B: Adding Standard, Test Samples and Blanks.
Standard: Add 50 µl of Standard (refer to Preparation of Reagents) to each well. (Total volume = 100 µl/well).
Test Samples: Add 50 µl of each sample to each well. (Total volume = 100 µl/well).
Blanks: Add 50 µl Standard Diluent or appropriate dilution matrix in duplicate to each well. (Total volume = 100 µl/well).

Cover with Plate Sealer and incubate for 30 seconds at RT (22-25°C) with shaking at 550 rpm. Transfer the plate to 4°C and incubate for 18-20 hours without shaking.

ASSAY PROCEDURE – Day 2

After 18-20 hours, empty the contents of the plate and wash the wells one time with 300 µl of 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 at RT (22-25°C) with shaking at 550 rpm.

After 1 hour, empty the contents of the plate and wash the wells three times with 300 µl of 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 at RT (22-25°C) with shaking at 550 rpm. During this time, warm the TMB Substrate Solution and Stop Solution to RT (22-25°C).

After 1 hour, empty the contents of the plate and wash the wells four times with 300 µl of 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 30 minutes. Do not use a plate sealer during the incubation. DO NOT SHAKE.

5. **Stop Solution:** After the 30 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 2 minutes after the addition of the Stop Solution.

CALCULATION OF RESULTS

By plotting the optical densities (OD) using a 4-parameter logistic 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 standard and sample ODs to eliminate background. To approximate determined titers in units/ml, use the conversion factor of 3 – 5 pg/unit.

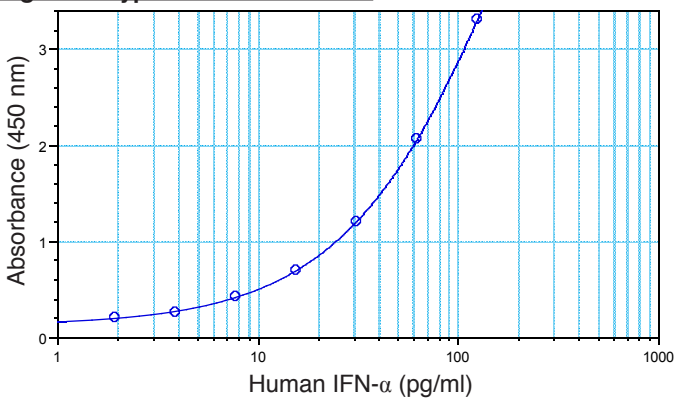
Because the interferon samples are titrated against the international standard, the values from the curves can be determined in units/ml as well as pg/ml. The conversion factor of about 3 – 5 pg/unit is applicable for human interferon alpha.[4,5] Nevertheless, this conversion factor is only an approximation.

A shift in optical densities is typical between users and kit lots. The back fit concentration interpolated 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 logistic 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



SPIKE RECOVERY, ACCURACY, PRECISION, SUBTYPE SENSITIVITY DATA

Heterophilic antibody interference

Human heterophilic antibodies in serum/plasma samples can bind to the antibodies used in an immunoassay to yield false positives or inaccurate measurements. The quantitation of IFN-alpha by this kit is not affected by heterophilic antibodies that may be found in serum/plasma from donors with autoimmune diseases such as Rheumatoid Arthritis, Lupus, etc. For more information, visit: pblassaysci.com/content/hs-human-ifn-alpha-all-subtype-elisa.

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.

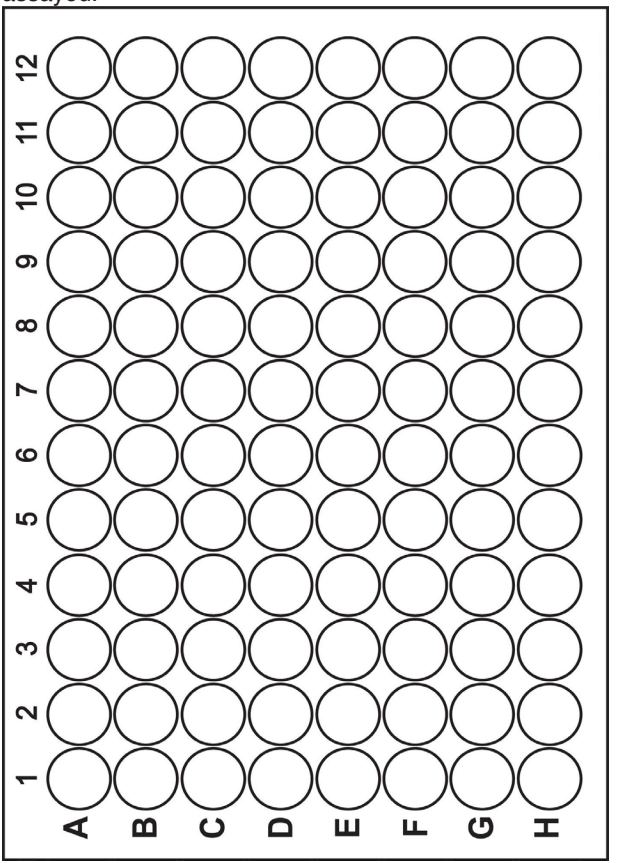
3. Human IFN-α international reference standard provided by the NIH, reference no. Gxa01-901-535. Pestka, S. (1986) "Interferon Standards and General Abbreviations," in *Methods in Enzymology*, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 14-23.

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.



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