pbl assay science

VeriKine-HS Human IFN-α All Subtype TCM ELISA Kit

Product #41135

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 refers to a family of proteins comprised of 12 highly homologous protein subtypes (greater than 85% by amino acid sequence), encoded by 13 genes, that exhibit pleiotropic biologic activities. Measuring the levels of the 12 different human IFN-α subtypes is essential for understanding their triggers and subsequent effects on the immune system. The ability to measure all the subtypes may provide an indication of the total IFN-α level.

PBL's VeriKine-HS Human IFN-α All Subtype TCM ELISA Kit is specifically formulated to detect all human IFN-Alpha subtypes.

Subtype	LLOQ*	LLOD*
α1 (αD)	1.17	0.36
α2a (αA)	1.22	0.37
α4a (αM1)	1.76	0.53
α5 (αG)	1.14	0.36
α6 (αK)	0.73	0.25
α7 (αJ1)	1.11	0.35
α8 (αB2)	1.51	0.46
α10 (αC)	1.89	0.61
α14 (αH)	2.92	0.97
α16 (αWA)	2.29	0.77
α17 (αI)	1.97	0.62
α21 (αF)	2.88	0.93

*The LLODs and LLOQs were determined by adding three or ten standard deviations, respectively, to the mean O.D. value of forty zero standard replicates and interpolating the corresponding concentrations.

MATERIALS PROVIDED

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

ADDITIONAL MATERIALS REQUIRED *(NOT PROVIDED)*

- Microtiter plate reader capable of reading a wavelength of 450 nm
- Plate shaker (recommended shaking capability of 550 rpm)
- Variable volume microtiter pipettes
- Adjustable multi-channel 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)

Specifications: This kit quantitates human interferon alpha in tissue culture media (TCM) 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]

Assay Length: 20 hr 30 min - 24 hr 30 min

Specificity: Human IFN-α. Cross reacts with Cynomolgus/Rhesus IFN-α (13%). No cross reactivity detected with human IFN-β, IFN-γ or IFN-ω; mouse or rat IFN-α, IFN-β or IFN-γ; pig IFN-α; or bovine IFN-τ.

Precision: Human IFN-α was spiked into tissue culture media at three known concentrations.

To assess precision *within* an assay, or **Intra-Assay CV**, 20 replicates of each concentration were tested on a single plate.

	Intra-Assay Precision		
TC Media Spike Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/ml)	2.39	12.90	87.16
Standard Deviation	0.13	0.46	4.58
CV (%)	5.4	3.6	5.3

To assess precision *between* assays, or **Inter-Assay CV**, 3 independent assays testing each spike concentration were run by the same operator. The results in the following table represent averaged data from 3 operators.

	Inter-Assay Precision		
TC Media Spike Sample	**1**	**2**	**3**
n	9	9	9
Mean (pg/ml)	3.29	15.13	93.69
Standard Deviation	0.16	0.83	6.07
CV (%)	4.9	5.5	6.5

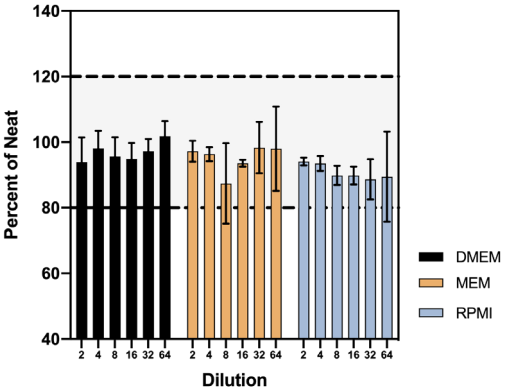
To assess **Intermediate Precision**, 25 assays were run by ten operators using 3 lots of components.

	Intermediate Precision		
TC Media Spike Sample	**1**	**2**	**3**
n	25	25	25
Mean (pg/ml)	3.29	15.30	94.00
Standard Deviation	0.44	1.75	7.37
CV (%)	13.4	11.4	7.8

Spike Recovery: Human IFN-α was spiked into tissue culture media at three known concentrations in 25 independent assays.

	Spike Recovery		
TC Media Spike Sample	**1**	**2**	**3**
n	25	25	25
Average Recovery (%)	109	102	104
Range	86-143	81-122	90-120

Linearity: Human IFN-α was spiked into three tissue culture media (RPMI, DMEM and MEM) at 80 pg/ml and then serially diluted with Standard Diluent to assess assay linearity.



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

Please note that the concentration of HRP Concentrate may 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 preparation.

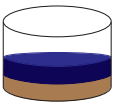
CAUTION: Wash Solution Concentrate, Standard Diluent, Assay Buffer, and Antibody Diluent contain 0.1% Kathon CG/ICP as a preservative; they should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

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

ASSAY PROCEDURE - QUICK REFERENCE

Total Time: 20 hr 30 min - 24 hr 30 min

DAY 1



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

Incubate **30 sec** *(shake at 550 rpm) at RT**
Transfer to **4°C** *and incubate* **18-22 hr**

DAY 2

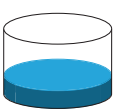
Aspirate and Wash **1x**

Add **100 µl** diluted Antibody Solution
Incubate **1 hr** *(shake at 550 rpm) at RT**

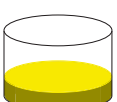
Aspirate and Wash **3x**

Add **100 µl** diluted HRP Solution
Incubate **1 hr** *(shake at 550 rpm) at RT**

Aspirate and Wash **4x**



Add **100 µl** TMB Substrate
Incubate **30 min** *in the dark at RT**
Do not seal, shake or wash.



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

*RT: Room Temperature (22-25°C)

PREPARATION OF REAGENTS

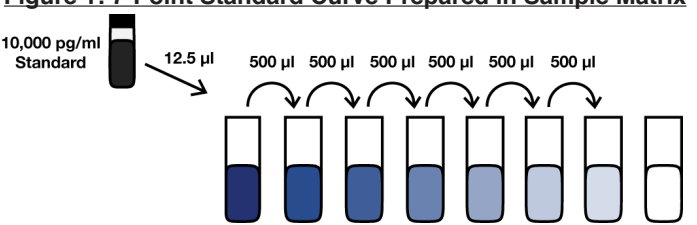
Wash Buffer: Wash Solution Concentrate may contain crystals; place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution (e.g. add 50 ml Wash Solution Concentrate to 450 ml distilled or deionized water). Mix thoroughly before use. (**Note:** Prepare fresh Wash Buffer for each assay run.)

Human Interferon Alpha Solution: Dilute the Human IFN Alpha Standard, provided at 10,000 pg/ml, in the same matrix as test samples. If sample matrix is not available, Standard Diluent may be used.

Standard Curve Preparation (1.95 – 125 pg/ml):

a. Label seven polypropylene tubes (S1 – S7).
b. Add indicated volume of Sample Matrix or Standard Diluent
c. Using polypropylene tips, add indicated volume of Human IFN Alpha Standard to S7 and mix gently. Remove indicated amount from S7 and add to S6. Repeat to complete series to S1. *Change tips between each dilution.*

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



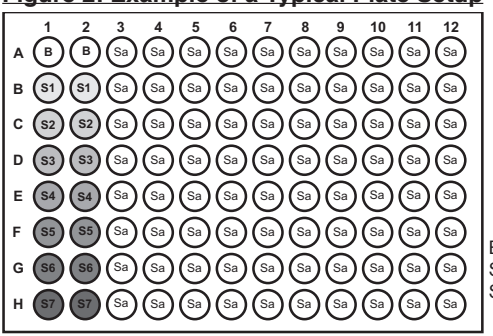
Sample Matrix Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Matrix Vol. (µl)	987.5	500	500	500	500	500	500	500
IFN-α Conc. (pg/ml)	125	62.5	31.25	15.63	7.81	3.91	1.95	0

Sample Preparation: Prepare samples of unknown IFN concentration to be tested as required. Measurements at least in duplicate are recommended.

Antibody Solution: 30 minutes prior to use in step 2 of the Assay Procedure, dilute Antibody Concentrate in Antibody Diluent. Refer to the lot specific Certificate of Analysis (CoA) for the amount to prepare. Keep at RT (22-25°C).

HRP Solution: 30 minutes prior to use in step 2 of the Assay Procedure, dilute HRP Conjugate Concentrate in HRP Diluent. Refer to the lot specific Certificate of Analysis (CoA) for the amount to prepare. Keep at RT (22-25°C).

Figure 2: Example of a Typical Plate Setup



B: Blank
S1-S7: Standard Curve
Sa: Sample

ASSAY PROCEDURE – Day 1

Notes:

- *Any modification of the described procedures can directly affect assay performance.*
- *On Day 1, bring the plate, Standard Diluent, Assay Buffer and Matrices/Samples to RT* (22-25°C) *prior to Step 1. All other components, including Standard, should be kept at* 4°C.
- **Incubations:** Use Plate Sealers to cover the plate when directed. (***Note:*** *The overnight incubation is at 4°C and does not require shaking.)*

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 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.

Total well volume = 100 µl (Step A + Step B)

Step A: Add 50 µl of **Assay Buffer** to every well

Step B: Add **Standard/Blanks** and **Test Samples.** (see Fig. 2)
Standard/Blanks: Add 50 µl of Standard (refer to Preparation of Reagents) or Blanks (Standard Diluent or appropriate dilution matrix) to each designated well.
Test Samples: Add 50 µl of each sample to each designated well.

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

ASSAY PROCEDURE – Day 2

Notes:

- *On Day 2, bring Wash Buffer, Antibody Diluent, HRP Diluent, TMB and Stop Solution to RT* (22-25°C)*. All other components should be kept at* 4°C.
- **Incubations:** Use Plate Sealers to cover the plate when directed. All incubations should be conducted in a closed chamber at 24°C or at RT, keeping the plate away from drafts and other temperature fluctuations.
- **Plate Washing:** All wells should be filled with a minimum of 300 µl of Wash Buffer (refer to Preparation of Reagents). Remove plate contents by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry.

After 18-22 hours, empty plate contents and wash wells one time with 300 µl of Wash Buffer, as described under *Plate Washing*.

2. **Antibody Solution:** Add 100 µl of diluted Antibody Solution (refer to Preparation of Reagents) to each well. Cover with Plate Sealer and shake at 550 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells three times with 300 µl Wash Buffer, as described under *Plate Washing*.

3. **HRP:** Add 100 µl of diluted HRP Solution (refer to Preparation of Reagents) to each well. Cover with Plate Sealer and shake at 550 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells four times with 300 µl of Wash Buffer, as described under *Plate Washing*.

4. **TMB Substrate:** Add 100 µl of TMB Substrate Solution to each well. Incubate **in the dark** at RT 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

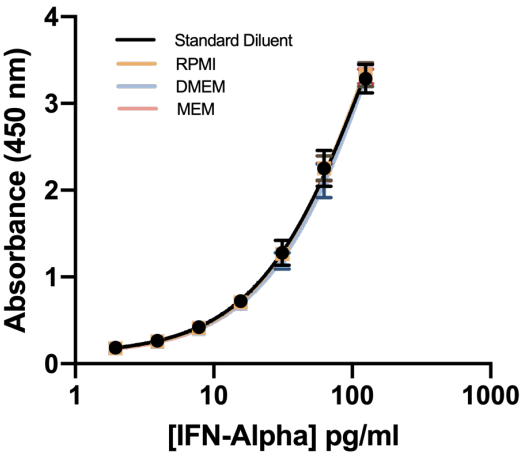
By plotting the optical densities (OD) using a 4-parameter logistic fit for the standard curve, the interferon titer in the samples can be determined. A 4-parameter logistic plot with $1/y^2$ weighted analysis is recommended for obtaining optimal fit of standard curve OD values. Based on user preference, 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 Curves in Various Matrices



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.

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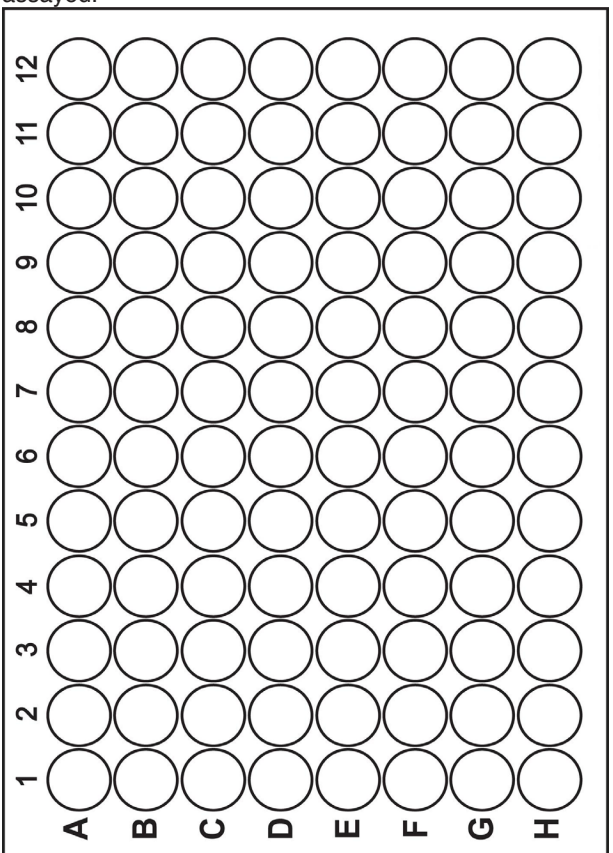
3. Human IFN-α international reference standard provided by the NIH, reference no. Gxa01-901-535. Pestka, S. (1986) "Interferon Standards and General Abbreviations," in *Methods in Enzymology*, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 14-23.

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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