



Protocol

VeriKine™ Human Interferon Alpha Multi-Subtype Serum ELISA Kit

Catalog No: 41110

Assay Range: 12.5 - 1000 pg/ml

Store all components at 2 - 8°C

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Materials Provided:

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

Additional Materials Required (NOT PROVIDED):

- Microplate reader capable of reading 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)
- Sample matrix (e.g. normal human serum, normal human plasma, tissue culture media) free of endogenous interferon alpha

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 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

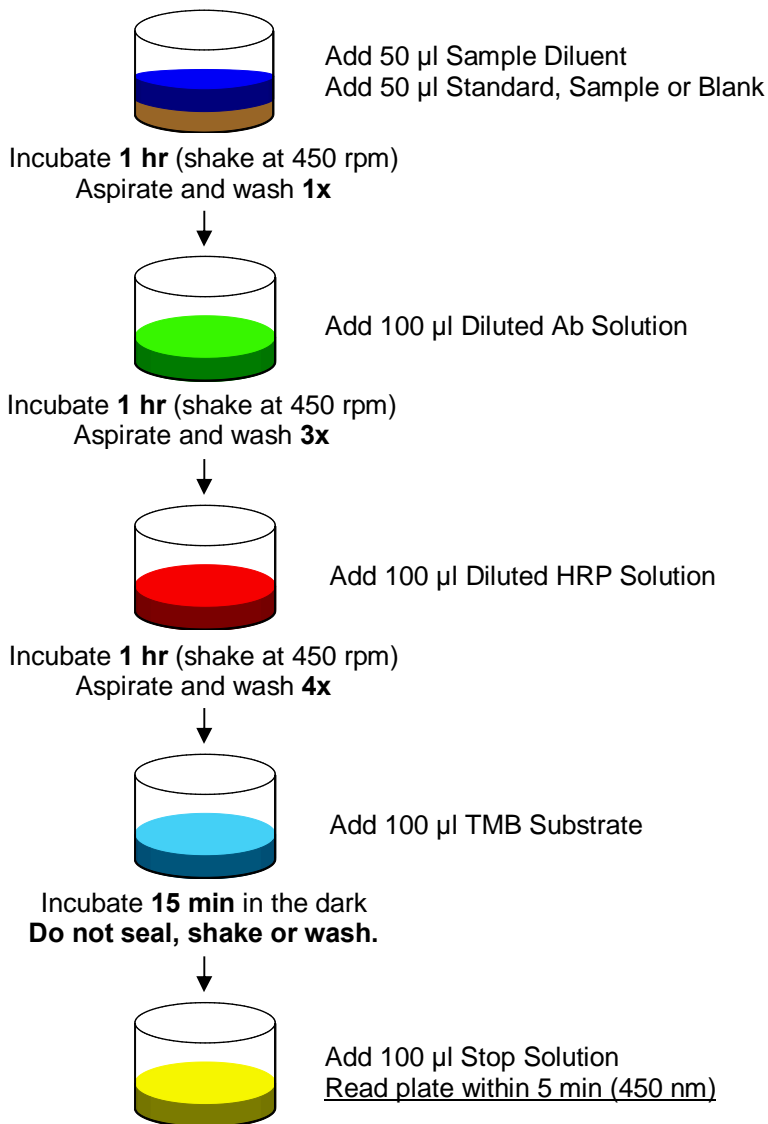
Specificity: Human IFN- α . No cross reactivity detected with human IFN- β , human IFN- γ or human IFN- δ . No cross-reactivity detected with mouse or rat IFN- α , IFN- β , or IFN- γ ; bovine IFN- α .

Storage Conditions/Comments: For retention of 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.

Assay Procedure – Quick Reference

Total Time: 3 hr 15 min



Note: All incubations are at room temperature (RT) 22-25°C.

Preparation of Reagents

Before starting the assay, the plate(s), Wash Solution Concentrate, applicable dilution matrices, Sample Diluent, Concentrate Diluent, Stop Solution, Dilution Buffer and samples should be equilibrated 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. Supplied Human IFN Alpha Standard, Antibody Concentrate and HRP Conjugate Concentrate should be kept on ice (4°C).

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 solution by adding 50 ml of Wash Solution Concentrate to 950 ml of distilled or deionized water. Mix thoroughly before use. Diluted Wash Buffer can be stored at RT (22-25°C) when not in use.

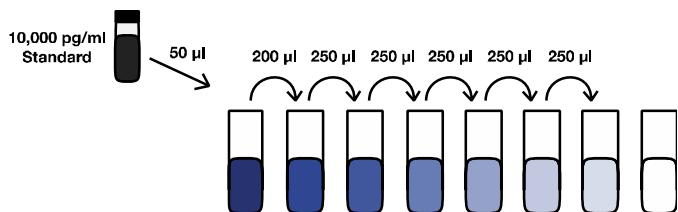
Human Interferon Alpha Solution: Dilute the Human Interferon Alpha Standard, provided at 10,000 pg/ml, in the sample matrix (normal human serum, normal human plasma, tissue culture media or buffer) as indicated below. *Due to matrix effects, it is required to prepare the standard curve in the sample matrix. A standard curve prepared in the supplied Sample Diluent cannot be used to accurately extrapolate concentrations of human interferon alpha in serum and plasma.*

Standard Curve Preparation:

Construct a standard curve 12.5-1000 pg/ml in the sample matrix.

- a) Label polypropylene tubes as S7-S1.
- b) Fill tubes with Sample Matrix/Sample Diluent as indicated.
- c) Using polypropylene tips add the Human IFN Alpha 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) Refrigerate 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)	450	300	250	250	250	250	250	250
IFN- Conc. (pg/ml)	1000	400	200	100	50	25	12.5	0

Sample Preparation: For samples in which the expected concentration of interferon alpha is greater than 1000 pg/ml, we recommend pre-diluting the samples in the sample matrix to concentrations within the range of the curve.

Antibody Solution: Dilute Antibody Concentrate with Dilution Buffer. Refer to the lot specific Certificate of Analysis (COA) for the correct amount of Antibody Concentrate 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 with Concentrate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct amount of HRP Conjugate Concentrate 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 in a closed chamber 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. **For consistent results, we recommend that incubations in steps 1, 2, and 3 be performed with shaking at 450 rpm.** 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 μ l of diluted Wash Buffer. Refer to Preparation of Reagents for diluted 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	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
B	S1	S1	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
C	S2	S2	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
D	S3	S3	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
E	S4	S4	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
F	S5	S5	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
G	S6	S6	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa
H	S7	S7	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa	Sa

B = Blank
 S1 . S7 = Standard Curve
 Sa = Sample

1. **Standards and Samples:** Determine the number of microtiter plate 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 50 μ l of Sample Diluent to each well. To this, add 50 μ l of the standards, blanks and test samples per well. Cover with plate sealer and shake plate (450 rpm) at RT (22-25°C) for 1 hour.

After 1 hour, empty the contents of the plate and wash the wells one time only with diluted Wash Buffer.

2. **Antibody:** Add 100 μ l of diluted antibody solution (refer to Preparation of Reagents) to each well. Cover with plate sealer and shake plate (450 rpm) at RT (22-25°C) 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 shake plate (450 rpm) at RT (22-25°C) 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, for 15 minutes. Do not use a plate sealer during the incubation.

5. **Stop Solution:** After the 15 minute TMB incubation, 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

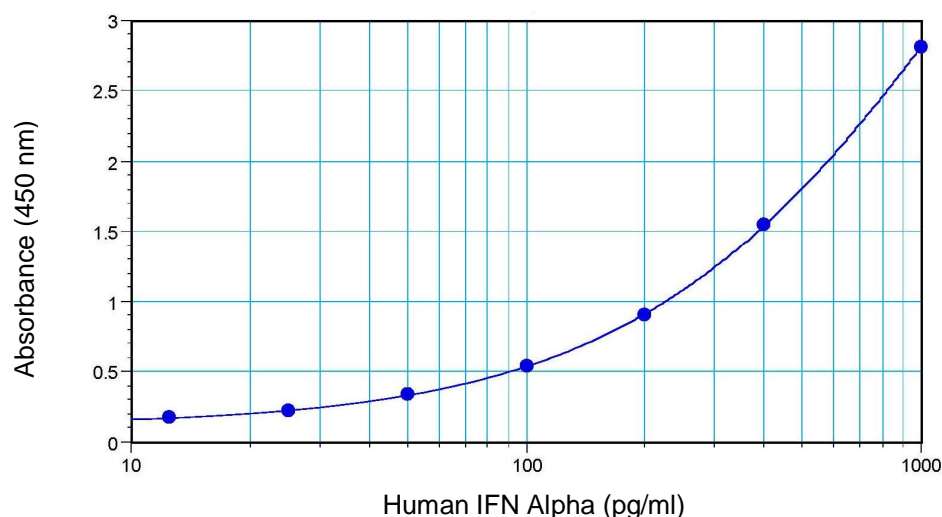
By plotting the optical densities (OD) using a 4-parameter fit for the standard curve 1000-0 pg/ml*, the interferon titer in the samples can be determined. Blank ODs should be subtracted from the standards and sample ODs to eliminate background. A typical standard curve for this assay is shown below. This example is for the purpose of illustration only, and should not be used to calculate unknowns.

****We have observed variable performance for the 12.5 pg/ml point in the standard curve prepared in normal human plasma. We thus strongly recommend that the 12.5 pg/ml point be excluded when plotting ODs for standard curve in normal human plasma.***

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 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.

Figure 3: Typical Standard Curve



Results of a typical standard curve using a 4-parameter fit is 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.



References

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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- 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.