

**Product #41110****Human IFN Alpha Serum Sample ELISA Kit Protocol****High Sensitivity:** 12.5 – 500 pg/ml**Extended Range:** 156 – 5000 pg/ml

Version 1.6

Specifications: This kit quantitates human interferon alpha in human sera and media using a sandwich immunoassay.^{1,2} The kit is based on an ELISA with anti-secondary 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 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.

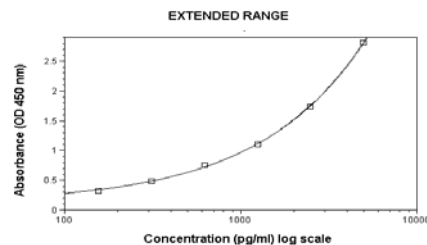
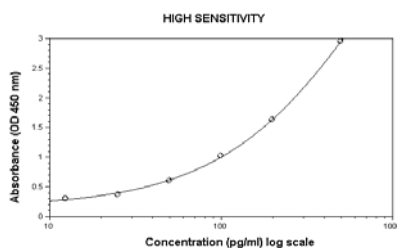
Materials Provided:

- Pre-coated micro-titer plates
- Plate Sealers
- Wash Solution Concentrate
- Human IFN Alpha Standard
- Sample Diluent
- Antibody Concentrate
- HRP Conjugate Concentrate
- HRP Conjugate Diluent
- TMB Substrate
- Stop Solution
- Antibody Diluent

Additional Materials Required (not provided):

- Micro-titer plate reader capable of reading a wavelength of 450nm
- Variable volume micro-titer pipettes
- Adjustable multi-channel pipette (50-200 μ l)
- Reagent reservoirs
- Wash bottle or plate washing system
- Distilled or deionized water
- Serological pipettes (1, 5, 10 or 25ml)
- Disposable pipette tips (polypropylene)

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.



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

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Preparation of Reagents

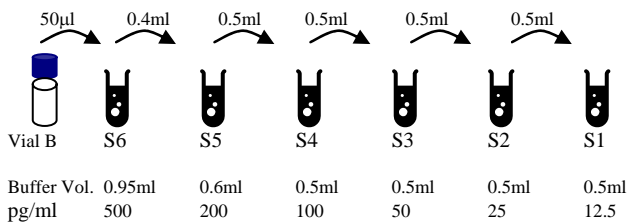
Wash Buffer: Dilute 50mL of the Wash Solution Concentrate to a final volume of 1000mL with distilled or deionized water. Mix thoroughly before use. The diluted wash buffer can be stored at (2-25°C).

Human IFN Alpha Solution: Dilute the Human Interferon Alpha Standard, provided at 10,000pg/ml, in Sample Diluent 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.

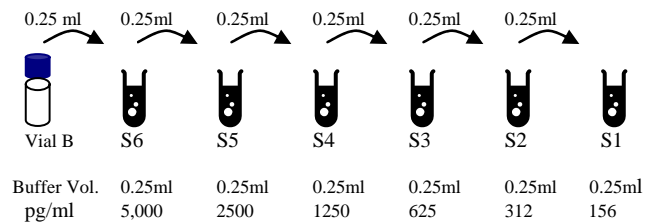
Construct a High Sensitivity standard curve 12.5 – 500 pg/ml or Extended Range standard curve 156- 5000 pg/ml.

- Label six polypropylene tubes (S1-S6).
- Fill tubes with Sample Diluent as indicated.
- Using polypropylene tips add the Human IFN Alpha 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.

High Sensitivity



Extended Range



Sample Preparation: Prepare samples of unknown interferon concentration using Sample Diluent as required. For serum samples dilution in the provided Sample Diluent is required to achieve accurate results. PBL recommends a minimum 1:2 dilution (50% serum) for optimal performance. Measurements in duplicate are recommended. Refrigerate until use in step 1 of the assay procedure.

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

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

Assay Procedure- Quick Reference

Step	Reagent	Volume/well	Incubation	Wash	Comments
1	Standard and Samples	100 µl	60 min	1X	Include blanks containing Sample Diluent only
2	Diluted Antibody Solution	100 µl	60 min	3X	
3	Diluted HRP Solution	100 µl	60 min	4X	During incubation, warm TMB to room temp.
4	TMB Substrate	100 µl	15 min	DO NOT WASH	Incubate in the dark
5	Stop Solution	100 µl	0 min	DO NOT WASH	Read plate at 450nm, within 5 minutes
6	Read Plate at 450nm				

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



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 250µl of diluted wash solution. Refer to preparation of reagents for diluted solutions.

1. **Standards and Samples:** Determine the number of micro-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 micro-titer 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 the interferon standards, blanks and samples. Cover 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.

2. **Antibody:** Add 100µl of diluted antibody solution to all wells. Cover 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 100µl of diluted HRP solution (refer to preparation of reagents) to all wells. Cover and incubate for 1 hour. During this incubation period, 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 (refer to preparation of reagents).

4. **TMB Substrate:** Add 100 µl 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 incubation of TMB, DO NOT WASH. Add 100 µl of Stop Solution to each well.

6. **Read:** Using a micro-plate 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 OD's should be subtracted from the standards and sample OD's to eliminate background. Typical standard curves for this assay are shown on the COA. These examples are for the purpose of illustration only, and should not be used to calculate unknowns.

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.

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