



VeriKine-HS™ Human Interferon Alpha All Subtype ELISA Kit

Certificate of Analysis & Protocol

Assay Range: 1.95 - 125 pg/ml
 Compatibility: Serum, Plasma, Tissue Culture Media
 Assay Length: 22 hr 30 min

Catalog No: 41115-1

Lot No:

Expiration:

Store all components at 2-8°C

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP217		1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60		2 x 50 ml
Human IFN-Alpha Standard, 10,000 pg/ml	SMP049-400		1 vial
Sample Buffer	SMP220-8		8 ml
Standard Diluent	SMP218-60		55 ml
Antibody Concentrate	SMP219-1		1 vial
Antibody Diluent	SMP304-15		15 ml
HRP Conjugate Concentrate	SMP056-240		1 vial
HRP Diluent	ASDHRP-15		15 ml
TMB Substrate Solution	KET-15		15 ml
Stop Solution	SCY-15		15 ml

Authorization

Released by: _____

Date: _____

INTRODUCTION

PBL's Human IFN-Alpha All Subtype ELISA quantifies all human IFN-Alpha subtypes and is suitable for use with human sera, plasma, and tissue/cell culture media samples. The standard curve is generated using a single human IFN-Alpha subtype protein standard.

Note: Use shaker at 550 rpm speed for optimal assay results.

Visit PBL's website
pblassaysci.com/documentation
 for additional information including
 technical data sheet

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

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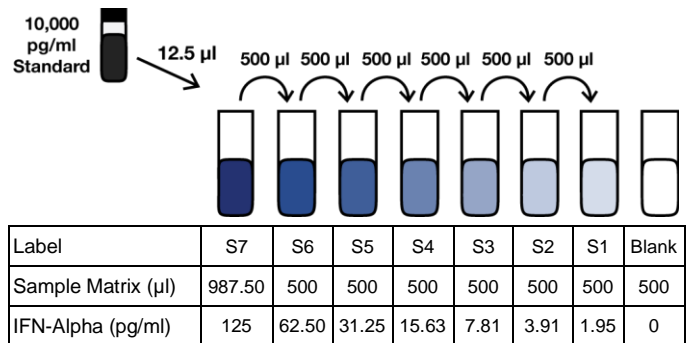
PREPARATION OF REAGENTS

Wash Buffer: Wash Solution Concentrate may contain crystals; place 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. Keep at RT (22-25°C).

Human IFN-Alpha Standard Curve Preparation:

- Label seven polypropylene tubes (S1 – S7).
- Add indicated volume of Sample Matrix or Standard Diluent to each tube as indicated in [Figure 1](#).
- Using polypropylene tips, add indicated volume of Human IFN-Alpha Standard to S7 and mix gently. *Change tips between each dilution.*
- Remove indicated amount from S7 and add to S6. Repeat to complete series to S1. Set aside on ice (2-8°C) until use in step 1.

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



Sample Preparation: Prepare test samples as required. Measurements in duplicate are recommended. Refrigerate until use in step 1.

Antibody Solution: 15 minutes prior to use in step 2, dilute Antibody Concentrate in the volume of Antibody Diluent as shown below. Keep at RT (22-25°C).

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (µl)						
Antibody Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

HRP Solution: 15 minutes prior to use in step 3, dilute HRP Conjugate Concentrate in the volume of HRP Diluent as shown below. Keep at RT (22-25°C).

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)						
HRP Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

ASSAY PROCEDURE

	Bring to RT (22-25°C)	Keep at 2-8°C
Day 1	Plate/Plate Sealers	All other components
	Standard Diluent	
	Sample Buffer	
	Matrices/Samples	
Day 2	Wash Buffer	All other components
	Antibody Solution	
	HRP Solution	
	TMB Substrate Solution	
	Stop Solution	

- **Incubations:** All incubations should be conducted in a closed chamber at RT, keeping the plate away from drafts. (**Note:** The overnight incubation is at 4°C and does not require shaking.)
- **Plate Washing:** All wells should be filled with a minimum of 300 µl of Wash Buffer. Remove plate contents by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry.

DAY 1

1. Determine the number of microplate strips required. 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 Sample Buffer** to every well.

Step B: Add **50 µl of Standard, Test Samples or Blanks** (Standard Diluent or appropriate dilution matrix) 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 18-20 hours without shaking.

DAY 2

After 18-20 hours, empty plate contents and wash wells one time.

2. Add **100 µl** of diluted **Antibody Solution** to each well. Cover with Plate Sealer and shake plate at 550 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells three times.

3. Add **100 µl** of diluted **HRP Solution** to each well. Cover with Plate Sealer and shake plate at 550 rpm at RT for 1 hour. Warm **TMB Substrate Solution** and **Stop Solution** to RT.

After 1 hour, empty the plate contents and wash wells four times.

4. 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 and **DO NOT SHAKE** during the incubation.

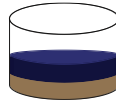
5. After 30 minutes, **DO NOT EMPTY THE WELLS AND DO NOT WASH**. Add **100 µl** of **Stop Solution** to each well.

6. Using a microplate reader, determine the absorbance at 450 nm within 2 minutes after the addition of Stop Solution.

HUMAN IFN-ALPHA ELISA (41115) ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 22 hr 30 min

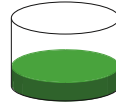
DAY 1



1. Add **50 µl** Sample Buffer
2. Add **50 µl** Standard, Sample or Blank
*Incubate 30 sec (shake at 550 rpm) at RT**
Transfer to 4°C and incubate 18-20 hr

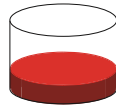
DAY 2

Aspirate and Wash 1x



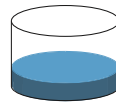
- Add **100 µl** diluted Antibody Solution
*Incubate 1 hr (shake at 550 rpm) at RT**

Aspirate and Wash 3x

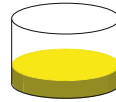


- Add **100 µl** diluted HRP Solution
*Incubate 1 hr (shake at 550 rpm) at RT**

Aspirate and Wash 4x



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



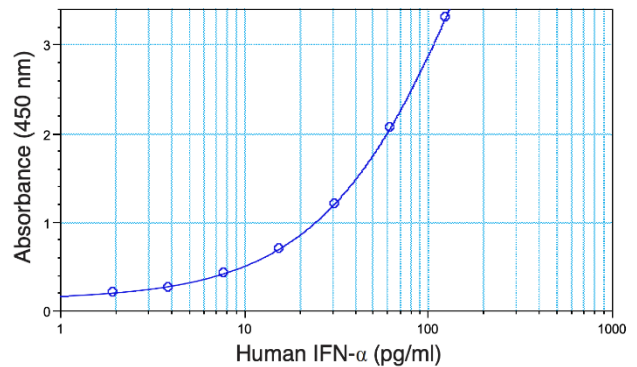
- Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

Note: All incubations are at Room Temperature (RT) (22-25°C)*

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. Use the conversion factor of 3-5 pg/unit to approximate titers in units/ml. Blank ODs may be subtracted from the standards and sample ODs to eliminate background.

Figure 2: Typical Standard Curve



Visit PBL's website
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technical data sheet