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: 7603
Expiration: March 31, 2024
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP217	K7159	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60	K7157	2 x 50 ml
Human IFN-Alpha Standard, 10,000 pg/ml	SMP049-400	K7162	1 vial
Sample Buffer	SMP220-8	K7163	8 ml
Standard Diluent	SMP218-60	K7164	55 ml
Antibody Concentrate	SMP219-1	K7165	1 vial
Antibody Diluent	SMP304-15	K7166	15 ml
HRP Conjugate Concentrate	SMP056-240	K7167	1 vial
HRP Diluent	ASDHRP-15	177473	15 ml
TMB Substrate Solution	KET-15	220103D03	15 ml
Stop Solution	SCY-15	67088	15 ml

Authorization

Released by: _____

Date: March 24, 2023

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).

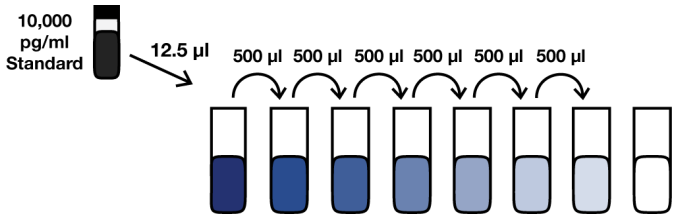
PREPARATION OF REAGENTS

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 (22-25ºC).

Human IFN-Alpha Standard Curve Preparation:

a. Label seven polypropylene tubes (S1 – S7).
b. Add indicated volume of Sample Matrix or Standard Diluent to each tube as indicated in Figure 1.
c. Using polypropylene tips, add indicated volume of 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. Set aside on ice (2-8ºC) until use in step 1.

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



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Matrix (µl)	987.50	500	500	500	500	500	500	500
IFN-Alpha (pg/ml)	125	62.50	31.25	15.63	7.81	3.91	1.95	0

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 (22-25ºC).

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (µl)	20	40	60	80	100	120
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 (22-25ºC).

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)	10	20	30	40	50	60
HRP Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

ASSAY PROCEDURE

	Bring to RT (22-25ºC)	Keep at 2-8ºC
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 4ºC 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 2-8ºC. 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 4ºC 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.

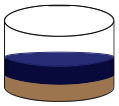
5. After 30 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

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

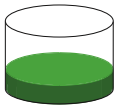


1. Add **50 µl** Sample Buffer
2. Add **50 µl** Standard, Sample or Blank

Incubate **30 sec** *(shake at 550 rpm) at RT* Transfer to* **4ºC** *and incubate* **18-20 hr**

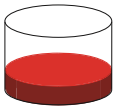
DAY 2

Aspirate and Wash **1x**



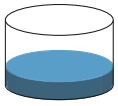
Add **100 µl** diluted Antibody Solution
Incubate **1 hr** *(shake at 550 rpm) at RT**

Aspirate and Wash **3x**

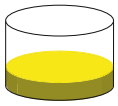


Add **100 µl** diluted HRP Solution
Incubate **1 hr** *(shake at 550 rpm) at RT**

Aspirate and Wash **4x**



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



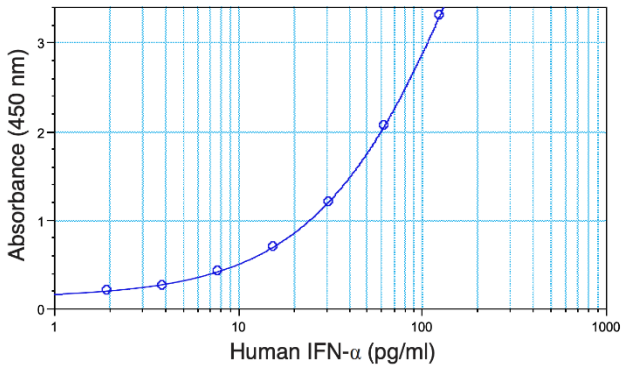
Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

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

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



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