VeriKine-HS™ Human Interferon Alpha All Subtype TCM ELISA Kit
Certificate of Analysis & Protocol

Assay Range: 1.95 - 125 pg/ml
Compatibility: Tissue Culture Media (TCM)
Assay Length: 20 hr 30 min – 24 hr 30 min

Catalog No: 41135-1
Lot No:
Expiration:
Store all components at 2-8ºC

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
Assay Buffer	SMP324-8		8 ml
Standard Diluent	SMP323-60		55 ml
Antibody Concentrate	SMP219-1		1 vial
Antibody Diluent	SMP315-15		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:

Visit the product page on PBL's website (https://pblassaysci.com) to view the full protocol, including performance characterization and kit specifications.

CAUTION: Components 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. (**Note:** Prepare fresh Wash Buffer for each assay run.)

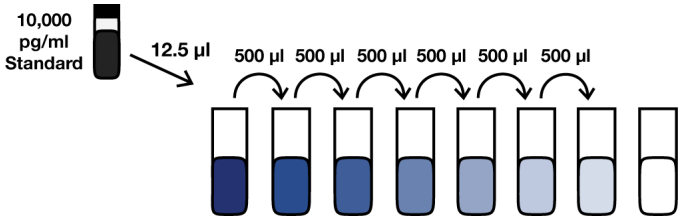
Human IFN Alpha Standard Curve Preparation:

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. 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



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

Sample Preparation: Thaw frozen sample tubes to Room Temperature (RT) (22-25ºC) in either tap water or between the fingertips. If samples require dilution, prepare using relevant matrix. Keep on ice (2-8ºC) until step 1. Measurements in duplicate are recommended.

Antibody Solution: 30 minutes prior to use in step 3, 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)						
Antibody Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

HRP Solution: 30 minutes prior to use in step 4, 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)						
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	
	Assay Buffer	
	Matrices/Samples	
Day 2	Wash Buffer	Human IFN Alpha Standard
	Antibody Diluent	Antibody Concentrate
	HRP Diluent	HRP Conjugate Concentrate
	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 2-8ºC 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.

2. Total well volume = 100 µl (Step A + Step B)
Step A: Add **50 µl** of **Assay 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 2-8ºC and incubate for 18-22 hours without shaking.

DAY 2

After 18-22 hours, empty plate contents and wash wells one time.

3. 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.

4. 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.

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

5. 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.

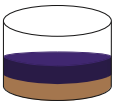
6. After 30 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

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

HUMAN IFN ALPHA TCM ELISA (41135)
ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 20 hr 30 min – 24 hr 30 min
Note: All incubations are at Room Temperature (RT) (22-25ºC)*

DAY 1

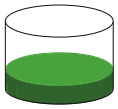


1. Add **50 µl** Assay Buffer
2. Add **50 µl** Standard, Sample or Blank

Incubate **30 sec** *(shake at 550 rpm) at RT**
Transfer to **2-8ºC** *and incubate* **18-22 hr**

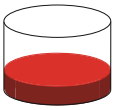
DAY 2

Aspirate and Wash **1x**



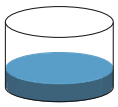
Add **100 µl** diluted Antibody Solution
Incubate **1 hr** *(shake at 550 rpm) at RT**

Aspirate and Wash **3x**

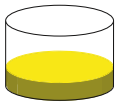


Add **100 µl** diluted HRP Solution
Incubate **1 hr** *(shake at 550 rpm) at RT**

Aspirate and Wash **4x**



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

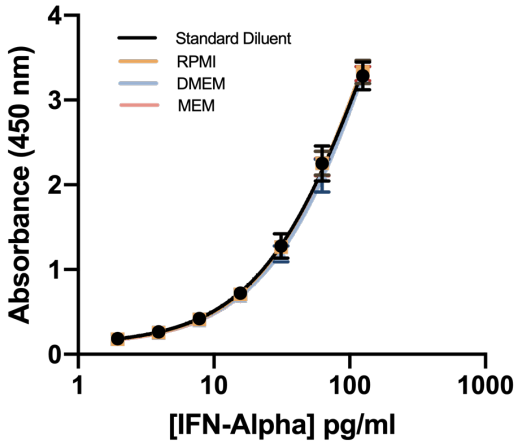


Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

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. A 4-parameter logistic plot with $1/y^2$ weighted analysis is recommended for obtaining optimal fit of standard curve OD values. Blank ODs may be subtracted from the standards and sample ODs to eliminate background.

Figure 2: Typical Standard Curves in Various Matrices



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