



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 2-8°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
Assay Buffer	SMP324-8		8 ml
Standard Diluent	SMP323-60		55 ml
Antibody Concentrate	SMP322-1		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.

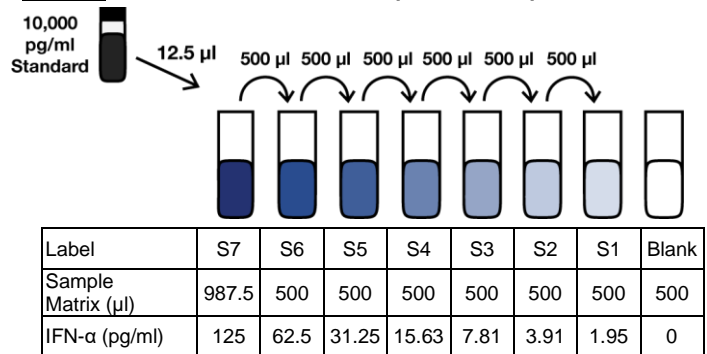
PREPARATION OF REAGENTS

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:

- 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. 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



Sample Preparation: Thaw frozen sample tubes to Room Temperature (RT) (22-25°C) in either tap water or between the fingertips. If samples require dilution, prepare using relevant matrix. Keep on ice (2-8°C) 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 (22-25°C).

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate						
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 (22-25°C).

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

CAUTION: Components should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

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ASSAY PROCEDURE

	Bring to RT (22-25°C)	Keep at 2-8°C
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 2-8°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.

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 2-8°C 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.

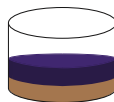
7. Using a microplate reader, determine the absorbance at 450 nm within 2 minutes after the addition of Stop Solution.

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) (22-25°C)*

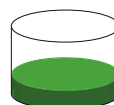
DAY 1



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 2-8°C and incubate 18-22 hr

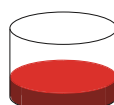
DAY 2

Aspirate and Wash 1x



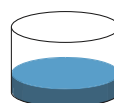
Add 100 µl diluted Antibody Solution
Incubate 1 hr (shake at 550 rpm) at RT*

Aspirate and Wash 3x

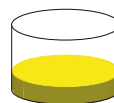


Add 100 µl diluted HRP Solution
Incubate 1 hr (shake at 550 rpm) at RT*

Aspirate and Wash 4x



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

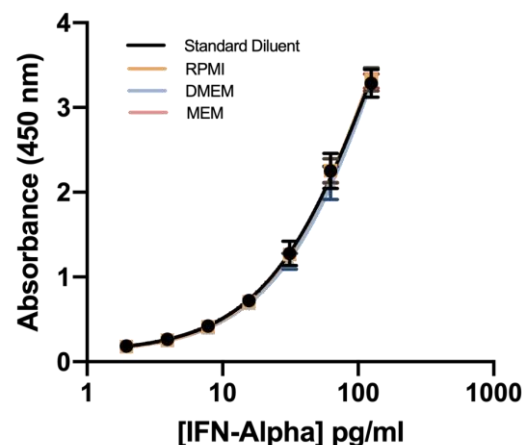


Add 100 µl Stop Solution
Read plate within 2 min (450 nm)

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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