VeriKine™ Human Interferon Alpha Multi-Subtype Serum ELISA Kit
Certificate of Analysis & Protocol

Assay Range: 12.5 - 1000 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 3 hr 15 min

Catalog No: 41110-1
Lot No:
Expiration:
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP047		1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60		50 ml
Human IFN-Alpha Standard, 10,000 pg/ml	SMP049-1		1 vial
Sample Diluent	SMP233-30		30 ml
Antibody Concentrate	SMP048-1		1 vial
HRP Conjugate Concentrate	SMP050-240		1 vial
Concentrate Diluent	SMP024-15		15 ml
TMB Substrate Solution	KET-15		15 ml
Stop Solution	SCY-15		15 ml
Dilution Buffer	SMP231-30		30 ml

Product Performance Specifications

Intra-Assay CV	≤ 8%
Inter-Assay CV	≤ 8%

Authorization

Released by: _____

Date:

Visit the product page on PBL's website (https://pblassaysci.com/documentation) to view our technical supplement and additional product information

CAUTION: Wash Solution Concentrate, Dilution Buffer and Concentrate 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).

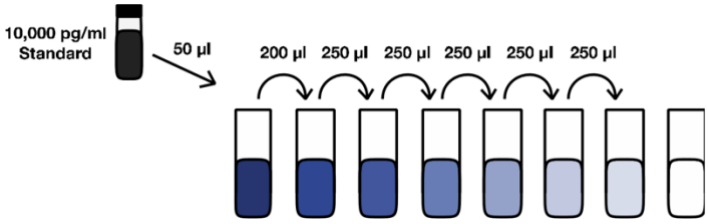
PREPARATION OF REAGENTS

Wash Buffer: Dilute 50 ml of Wash Solution Concentrate to a final volume of 1000 ml with distilled or deionized water. Mix thoroughly before use. Keep at RT (22-25ºC).
Note: Prepare fresh Wash Buffer for each assay run.

Human IFN-Alpha Standard Curve Preparation:
Dilute Human IFN-Alpha Standard in Dilution Buffer as indicated. In certain situations, "test" samples may contain substances that can interfere with assay results. It is recommended to run the IFN standard curve diluted in your sample matrix.

a. Label six polypropylene tubes (S1 – S7).
b. Add volume of Sample Matrix/Sample Diluent to each tube as indicated in Figure 1.
c. Using polypropylene tips, add indicated amount 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.
e. Keep on ice (2-8ºC) until use in Step 1 of the assay procedure.

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



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Matrix (µl)	450	300	250	250	250	250	250	250
IFN-Alpha (pg/ml)	1000	400	200	100	50	25	12.50	0

Sample Preparation: Prepare test samples of unknown IFN concentration to be tested using Dilution Buffer as required. Keep on ice (2-8ºC) until use. Measurements in duplicate are recommended.

Antibody Solution: Dilute Antibody Concentrate in volume of Dilution Buffer shown below. Prepare 15 minutes prior to use in step 2 of assay procedure.

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

HRP Solution: Dilute HRP Conjugate Concentrate in volume of Concentrate Diluent shown below. Prepare 15 minutes prior to use in step 3 of assay procedure.

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)						
Concentrate 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
Plate Sealers	All Other Components
Wash Solution Concentrate	
Sample Diluent	
Concentrate Diluent	
Stop Solution	
Dilution Buffer	
TMB Substrate Solution (During Step 3)	
Samples/Matrices	

- **Incubations:** Use plate sealers to cover the plate when directed. All incubations should be conducted in a closed chamber at 22-25ºC or at RT, keeping the plate away from drafts.
- **Plate Washing**: All wells should be filled with a minimum of 250 µl of Wash Buffer. Remove plate contents by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry.

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 Standard Diluent to each designated well.
Step B: Add **50 µl** of **Standard**, **Sample** or **Blank** (Dilution Buffer or appropriate dilution matrix) to each designated well.

Cover with Plate Sealer and shake at 450 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells <u>one time</u>.

2. Add **100 µl** of diluted **Antibody Solution** to each well.

Cover with Plate Sealer and shake at 450 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells <u>three times</u>.

3. Add **100 µl** of **HRP Solution** to each well.

Cover with Plate Sealer and shake at 450 rpm at RT for 1 hour. During this time, warm the **TMB Substrate Solution** to RT.

After 1 hour, empty plate contents and wash wells <u>four times</u>.

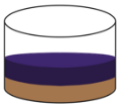
4. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 15 minutes. Do not use a Plate Sealer during the incubation.

5. After 15 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 5 minutes after the addition of Stop Solution.

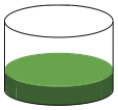
HUMAN IFN-ALPHA MULTI-SUBTYPE ELISA (41110)
ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 3 hr 15 min
Note: Unless otherwise specified, all incubations are at Room Temperature (RT) (22-25ºC)*



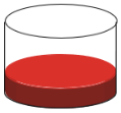
Add **50 µl** Sample Diluent
Add **50 µl** Standard, Sample, or Blank
Incubate **1 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **1x**



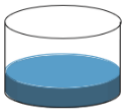
Add **100 µl** diluted Antibody Solution
Incubate **1 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **3x**

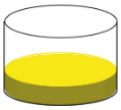


Add **100 µl** diluted HRP Solution
Incubate **1 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **4x**



Add **100 µl** TMB Substrate
Incubate **15 min** *<u>in the dark</u> at RT**
<u>Do not seal, shake, or wash.</u>



Add **100 µl** Stop Solution
<u>Read plate within 5 min (450 nm)</u>

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 ODs may be subtracted from the standards and sample ODs to eliminate background. An approximate conversation factor of about 3-5 pg/ml is applicable. A standard curve must be run for each set of samples assayed.

<u>Figure 2: Typical Standard Curve</u>