VeriKine-HS™ Pig Interferon Alpha ELISA Kit
Certificate of Analysis & Protocol

Assay Range: 2.34 - 150 pg/ml
Compatibility: Serum, Plasma, Cell Culture Supernatant
Assay Length: 3 hr 15 min

Catalog No: 47100-1
Lot No:
Expiration:
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP256		1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60		50 ml
Pig IFN-Alpha Standard, 10,000 pg/ml	SMP257-1		1 vial
Dilution Buffer	SMP021-15		15 ml
Assay Buffer	SMP258-15		15ml
Antibody Concentrate	SMP259-1		1 vial
Antibody Diluent	SMP260-15		15 ml
HRP Conjugate Concentrate	SMP056-240		1 vial
Concentrate Diluent	SMP024-15		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.

Note: PBL recommends using a 10 µl multi-channel pipette for standard and sample preparation in order to maximize assay accuracy.

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

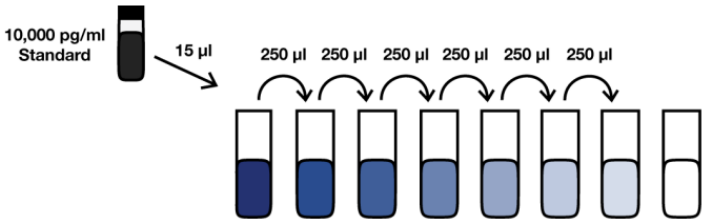
PREPARATION OF REAGENTS

Wash Buffer: Wash Solution Concentrate may contain crystals; place in a warm water bath and gently mix until completely dissolved. Prepare a 1:20 working wash solution (e.g. Add 50 ml Wash Solution Concentrate to 950 ml distilled or deionized water). Mix thoroughly before use.

Pig IFN Alpha Standard Curve Preparation:

a. Label seven polypropylene tubes (S1 – S7).
b. Add indicated volume of Sample Matrix or Dilution Buffer to each tube as indicated in Figure 1.
c. Using polypropylene tips, add 15 µl of Pig IFN-Alpha Standard to S7 and mix thoroughly to recover all material adhered to the inside of the pipette tip. **DO NOT change tips between each dilution.**
d. Remove indicated amount from S7 and add to S6. Repeat to complete series to S1.
e. Set aside on ice (2-8ºC) until step 1.

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



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Matrix (µl)	985	250	250	250	250	250	250	250
IFN-Alpha (pg/ml)	150.00	75.00	37.50	18.75	9.37	4.68	2.34	0

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

Antibody Solution: Prior to use in step 2, dilute Antibody Concentrate in the volume of Antibody Diluent as shown below. Keep on ice (2-8ºC) until use.

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: Prior to use in step 3, dilute HRP Conjugate Concentrate in the volume of Concentrate Diluent as shown below. Keep on ice (2-8ºC) until use.

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
Wash Solution Concentrate	All other components
Stop Solution	
TMB Substrate Solution (During Step 5)	

- **Incubations:** All incubations should be conducted in a closed chamber at RT, keeping the plate away from drafts.
- **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.

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 **90 µl** of **Assay Buffer** to every well.
Step B: Add **10 µl** of **Standard**, **Test Sample** or **Blank** (Dilution Buffer or appropriate dilution matrix) to each designated well.

Note: PBL recommends using a 10 µl multi-channel pipette for standard and sample preparation in order to maximize assay accuracy.

Cover with Plate Sealer and shake at 650 rpm at RT for 1 hour.

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

3. Add **100 µl** of diluted **Antibody Solution** to each well. Cover with Plate Sealer and shake at 650 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 at 650 rpm at RT for 1 hour.

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

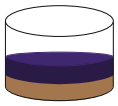
5. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 15 minutes. Do not use a Plate Sealer and DO NOT SHAKE during the incubation.

6. After 15 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.

PIG IFN-ALPHA ELISA (47100)
ASSAY PROCEDURE – QUICK REFERENCE

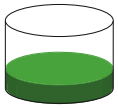
Total Time: 3 hr 15 min
Note: All incubations are at Room Temperature (RT) (22-25ºC)*



1. Add **90 µl** Assay Buffer
2. Add **10 µl** Standard, Sample or Blank

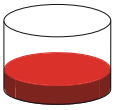
Incubate **1 hr** *(shake at 650 rpm) at RT**

Aspirate and Wash **3x**



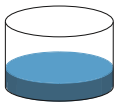
Add **100 µl** diluted Antibody Solution
Incubate **1 hr** *(shake at 650 rpm) at RT**

Aspirate and Wash **3x**

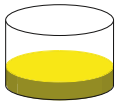


Add **100 µl** diluted HRP Solution
Incubate **1 hr** *(shake at 650 rpm) at RT**

Aspirate and Wash **3x**



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

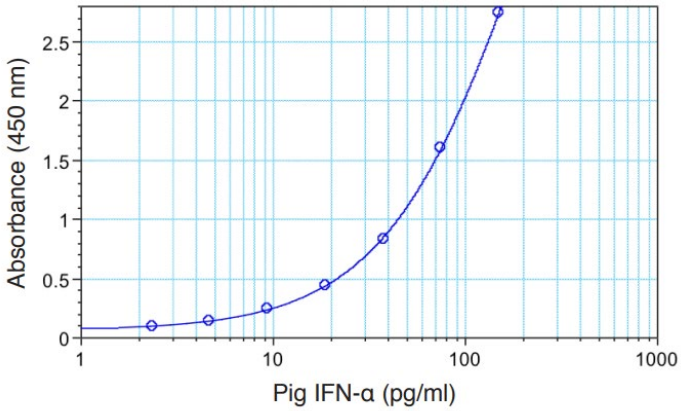


Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

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. Blank ODs may be subtracted from the standards and sample ODs to eliminate background.

Figure 2: Typical Standard Curve in Dilution Buffer



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