pbl assay science

VeriKine-HS Pig IFN-α ELISA Kit

Product #47100

Assay Range: 2.34 – 150 pg/ml

Store **all** components at 2 - 8°C

INTRODUCTION

Interferons (IFNs) are a group of cytokines which exhibit pleiotropic activities that play major roles in both innate and adaptive immunity. Type I IFNs consist of at least one IFN-β gene and protein as well as multiple IFN-α genes and proteins in most vertebrate species.[1] IFN-α expression and secretion is primarily induced by signaling events processed through pattern recognition receptors such as the Toll-like and RIG-I like receptors (TLR and RLR, respectively). While IFN-α can be produced by most cell types, strong evidence suggests that plasmacytoid dendritic cells are a major source of IFN-α *in vivo*[2]. Following expression and secretion, IFN-α binds to a hetero-dimeric receptor chain consisting of IFNAR1 and IFNAR2 subunits on proximal and distal cell surfaces. Receptor binding promotes a signal transduction cascade consisting of components of the JAK-STAT signaling pathway. Hundreds of genes are regulated subsequent to binding of the IFNAR receptor subunits to IFN-α, thus leading to the antiviral, anti-proliferative, and immunomodulatory activities of the cytokine.

The domestic pig (*Sus scrofa* or *Sus domestica*) is an important livestock animal. As such, diseases that affect livestock are frequently studied in this animal.[3] Furthermore, it is also a significant model of human infectious disease.[4,5] One of the hallmarks of viral and other infectious diseases is the production of interferon. A number of studies have already highlighted the importance of the IFN system in pigs.[6,7,8]

The Verikine-HS Pig IFN-α ELISA kit will enable determination of IFN-α levels in serum, plasma, and tissue culture media. As such, it should prove an important tool in virology, immunomodulation, and immunotoxicology studies conducted in pigs.

47100 Rev. 00

MATERIALS PROVIDED

- Pre-coated microtiter plate
- Plate sealers
- Wash Solution Concentrate
- Pig Interferon Alpha Standard (10,000 pg/ml)
- Dilution Buffer
- Assay Buffer
- Antibody Concentrate
- Antibody Diluent
- HRP Conjugate Concentrate
- Concentrate Diluent
- TMB Substrate Solution
- Stop Solution

ADDITIONAL MATERIALS REQUIRED *(NOT PROVIDED)*

- Microtiter plate reader capable of reading an OD at a wavelength of 450 nm
- Variable volume microtiter pipettes
- Adjustable multi-channel pipette (10 - 200 µl)*
- Reagent reservoirs
- Wash bottle or plate washing system
- Distilled or deionized water
- Serological pipettes (1, 5, 10 or 25 ml)
- Disposable pipette tips (polypropylene)
- Timer
- Graduated cylinder

***PBL recommends using a 10 µl multi-channel pipette for standard and sample preparation in order to maximize assay accuracy. (See page 10)**

Specifications: This kit quantitates pig interferon alpha in human sera, plasma, and tissue culture media using a sandwich immunoassay. The kit is based on an ELISA with biotinylated-detection antibody and streptavidin-conjugated horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate.

Speed: Typical incubation time, 3 hr 15 min

Specificity: Pig IFN-α. No cross reactivity was observed when using this kit to detect the following, each at up to 20 ng/ml.
Pig: IFN-β, IFN-γ; **Human:** IFN-α, IFN-β, IFN-γ, IFN-ω;
Mouse: IFN-α, IFN-β, IFN-γ; **Rat:** IFN-α

Precision & Recovery: Pig IFN-α was spiked into a single lot of normal pig serum at three different concentrations and analyzed:
Intra-Assay CV - 16 replicates of each concentration on a plate
Inter-Assay CV - 5 independent assays run by same operator
Average Recovery - 15 independent assays

Concentration (pg/ml)	4	20	100
Intra-Assay CV	5.7%	4.7%	3.3%
Inter-Assay CV	3.8%	2.5%	2.2%
Average Recovery	93%	109%	118%

Storage Conditions/Comments: For retention of full activity, all reagents should be kept at 2-8°C in the dark.

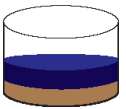
Please note that the concentrations of Antibody Concentrate and HRP Conjugate Concentrate differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (CoA) for reagent preparation.

CAUTION: Wash Solution Concentrate, Dilution Buffer, Assay Buffer, Antibody Concentrate, and Antibody Diluent contain 0.1% Kathon CG/ICP as a preservative; they should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

For laboratory research use only. Not for use in human diagnostic or therapeutic procedures.

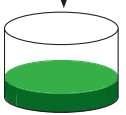
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
3 hr, 15 min



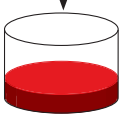
1. Add **90 µl** Assay Buffer
2. Add **10 µl** Standard, Blank, or Sample

Incubate **1 hr** (shake at 650 rpm) @ RT (22-25°C)
Aspirate and Wash **3x**



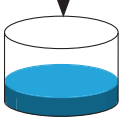
Add **100 µl** Diluted Ab Solution

Incubate **1 hr** (shake at 650 rpm) @ RT (22-25°C)
Aspirate and Wash **3x**



Add **100 µl** Diluted HRP Solution

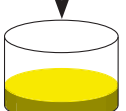
Incubate **1 hr** (shake at 650 rpm) @ RT (22-25°C)
Aspirate and Wash **3x**



Add **100 µl** TMB Substrate

Incubate **15 min** in the dark @ RT (22-25°C)
Do not seal, shake, or wash.

Note: ALL incubations are at room temperature (22-25°C)



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

PREPARATION OF REAGENTS

Before starting the assay, the Wash Solution Concentrate and Stop Solution should be equilibrated to room temperature (RT), 22-25°C. All other supplied components should be kept on ice (4°C) throughout the assay, aside from the TMB Substrate Solution, which should be equilibrated to RT (22-25°C) during step 3 of the Assay Procedure.

Wash Solution: The Wash Solution Concentrate may contain crystals; place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:20 working wash solution (e.g. add 50 ml of the Wash Solution Concentrate to 950 ml of distilled or deionized water). Mix thoroughly before use. The diluted Wash Solution can be stored at RT (22-25°C) when not in use.

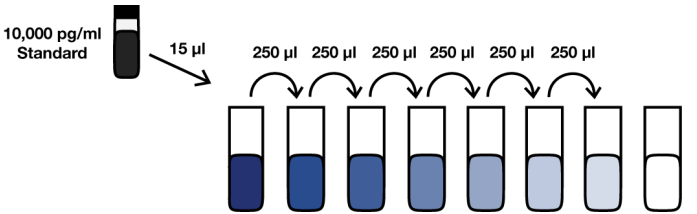
Pig Interferon Alpha Solution: Dilute the Pig IFN Alpha Standard, provided at 10,000 pg/ml, in the same matrix as the test samples. In the event that the sample matrix is not available, the Dilution Buffer may be used to prepare the standard curve.

Standard Curve Preparation:

Construct a standard curve 2.34 – 150 pg/ml.

a) Label seven polypropylene tubes (S1-S7).
b) Add indicated volumes of Sample Matrix or Dilution Buffer to the labeled tubes as indicated in Figure 1.
c) Using polypropylene tips, add the indicated volume of Pig IFN-α 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 (4°C)** until use in step 1 of the Assay Procedure.

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



Sample Matrix Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Matrix Vol. (µl)	985	250	250	250	250	250	250	250
IFN-α Conc. (pg/ml)	150	75	37.5	18.75	9.37	4.68	2.34	0

Test Sample Preparation: Prepare test samples of unknown IFN concentration using Dilution Buffer as required. Measurements in duplicate are recommended. Keep on ice (4°C) until use in step 1 of the Assay Procedure.

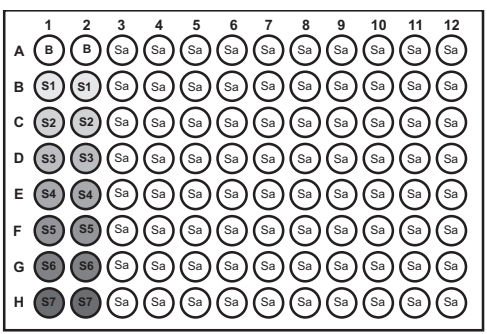
Antibody Solution: Dilute Antibody Concentrate in the volume of Antibody Diluent recommended in the lot specific Certificate of Analysis (CoA). Prepare 15 minutes prior to use in step 2 of the Assay Procedure and keep on ice (4°C).

HRP Solution: Dilute HRP Conjugate Concentrate in the volume of Concentrate Diluent recommended in the lot specific Certificate of Analysis (CoA). Prepare 15 minutes prior to use in step 3 of the Assay Procedure and keep on ice (4°C).

ASSAY PROCEDURE

All incubations should be performed at RT (22-25°C) keeping the plate away from drafts and other temperature fluctuations. Use plate sealers to cover the plate as directed. During all wash steps, remove contents of plate by inverting and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate. Wash each well with a minimum of 300 µl of diluted Wash Solution for each wash step. Refer to Preparation of Reagents for details on dilution of concentrated solutions. Any alteration of the described procedures can directly affect assay performance.

Figure 2: Example of a Typical Plate Setup



B: Blank
S1-S7: Standard Curve
Sa: Sample

1. **Standards and Test Samples:** Determine the number of microplate strips required to test the desired number of samples plus the appropriate number of wells needed to run blanks and standards. We recommend running both the IFN-α standard, blanks, and samples in duplicate or triplicate (see Figure 2 for example plate setup). A standard curve is required for each assay. Remove extra microplate strips from the frame, seal in the foil bag provided, and store at 2-8°C. Unused strips can be used in later assays.

Add 90 µl of Assay Buffer to each well.
Add 10 µl of Standard, Test Sample, or Blank*
(For Blank, add Dilution Buffer or relevant dilution matrix.)
(Total volume = 100 µl/well)

***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 plate at 650 rpm at RT (22-25°C) for 1 hour.

After 1 hour, empty the contents of the plate and wash the wells three times with 300 µl of diluted Wash Solution (refer to Preparation of Reagents).

2. **Antibody Solution:** Add 100 µl of diluted Antibody Solution (refer to Preparation of Reagents) to each well. Cover with plate sealer and shake plate at 650 rpm at RT (22-25°C) for 1 hour.

After 1 hour, empty the contents of the plate and wash the wells three times with 300 µl of diluted Wash Solution.

3. **HRP:** Add 100 µl of diluted HRP Solution (refer to Preparation of Reagents) to each well. Cover with plate sealer and shake plate at 650 rpm at RT (22-25°C) for 1 hour. During this time, warm the TMB Substrate Solution to RT (22-25°C).

After 1 hour, empty the contents of the plate and wash the wells three times with 300 µl of diluted Wash Solution.

4. **TMB Substrate:** Add 100 µl of the TMB Substrate Solution to each well. Incubate, in the dark, at RT (22-25°C), for 15 minutes. Do not use a plate sealer and do not shake during the incubation.

5. **Stop Solution:** After the 15 minute incubation of TMB, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add 100 µl of Stop Solution to each well.

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

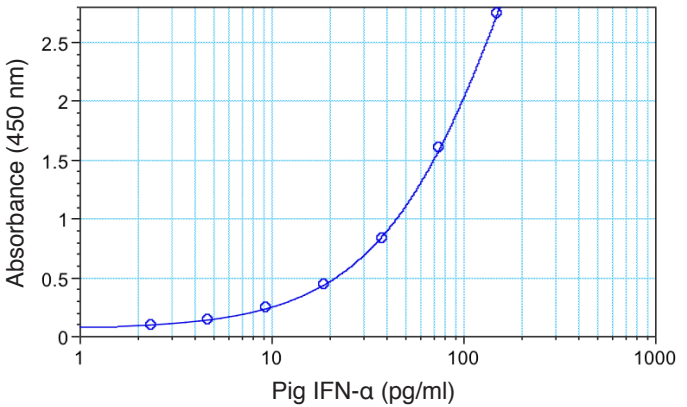
CALCULATION OF RESULTS

By plotting the optical densities (OD) using a 4-parameter logistic fit for the standard curve, the interferon titer in the samples can be determined. Based on user preference, blank ODs may be subtracted from the standard and sample ODs to eliminate background.

A shift in optical densities is typical between users and kit lots. The back fit concentration interpolated from the standard curve is a more accurate determination of the sample titer and performance of the kit. Variations from the typical curve provided can be a result of operator technique, altered incubation time, fluctuations in temperature, and kit age.

Results of a typical standard curve using a 4-parameter logistic fit are provided for demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.

Figure 3: Typical Standard Curve in Dilution Buffer



REFERENCES

1. Krause CD, Pestka S. (2005) “Evolution of the Class 2 cytokines and receptors, and discovery of new friends and relatives,” *Pharmacol Ther*. 106(3):299-346.

2. Fitzgerald-Bocarsly P, Dai J, Singh S. (2008) “Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history,” *Cytokine Growth Factor Rev*. 19(1):3-19.

3. Davies PR. (2012) “One world, one health: the threat of emerging swine diseases. A North American perspective.” *Transbound Emerg Dis*. 59 Suppl 1:18-26.

4. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. “The pig: a model for human infectious diseases.” *Trends Microbiol*. 20(1):50-7.

5. Schook L, Beattie C, Beever J, Donovan S, Jamison R, Zuckermann F, Niemi S, Rothschild M, Rutherford M, Smith D. (2005) “Swine in biomedical research: creating the building blocks of animal models.” *Anim Biotechnol*. 16(2):183-90.

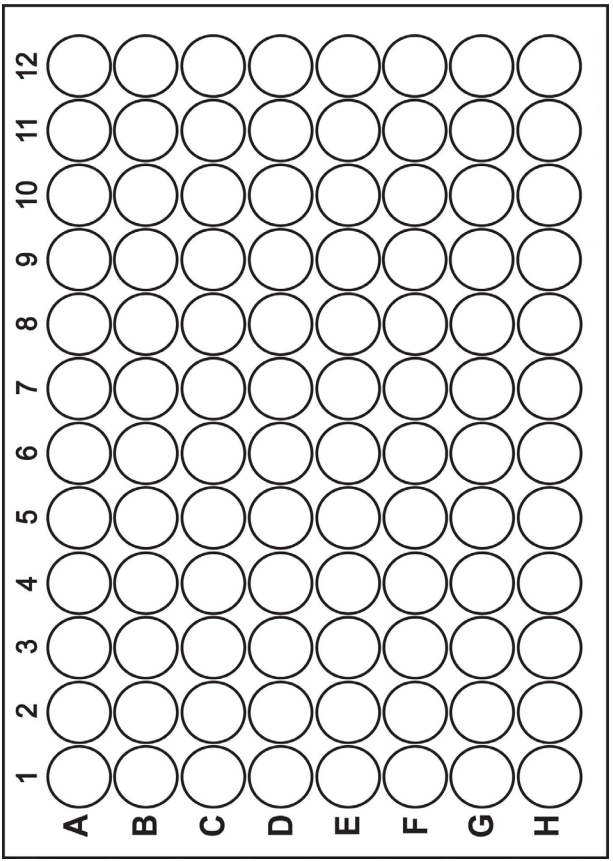
6. Razzuoli E, Villa R, Sossi E, Amadori M. (2011) “Characterization of the interferon-α response of pigs to the weaning stress.” *J Interferon Cytokine Res*. 31(2):237-47.

7. Souza M, Cheetham SM, Azevedo MS, Costantini V, Saif LJ. (2007) “Cytokine and antibody responses in gnotobiotic pigs after infection with human norovirus genogroup II.4 (HS66 strain).” *J Virol*. 81(17):9183-92.

8. Van Reeth, K., Nauwynck, H., Pensaert, M. (1998) “Bronchoalveolar Interferon-α, Tumor Necrosis Factor-α, Interleukin-1, and Inflammation during Acute Influenza in Pigs: A Possible Model for Humans?” *J Infect Dis*. 177(4): 1076-1079.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



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