pbl assay science

VeriKine™ Cynomolgus/ Rhesus IFN Alpha Serum ELISA Kit

Catalog No. 46100

Assay Range: 25 – 1600 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.

Two nonhuman primate species, Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) macaques, are sufficiently genetically similar to humans that they are emerging as highly relevant animal models for studying varying aspects of human physiology.[3] For example, macaques provide valuable surrogate models for examining the pathogenicity of human viral infections such as the 1918 pandemic influenza virus.[4] Furthermore, macaques are included in the evaluation of many new therapeutic agents aimed at modulating host immunity to either enhance or dampen immune responses.[5,6]

These primates also serve as important immunotoxicological models in the testing of human pharmaceuticals.[7]

The Verkine™ Cynomolgus/Rhesus IFN-α Serum ELISA kit will enable determination of IFN-α levels in tissue culture media, serum, and plasma. As such, it should prove an important tool in virology, immunomodulation, and immuntoxicology studies conducted in non-human primates.

MATERIALS PROVIDED

- Pre-coated microtiter plate
- Plate sealers
- Wash Solution Concentrate
- Rhesus/Cyno Interferon Alpha 2 Standard, 10,000 pg/ml
- Standard Diluent
- Sample Buffer
- Antibody Concentrate
- HRP Conjugate Concentrate
- Concentrate Diluent
- TMB Substrate
- Stop Solution

ADDITIONAL MATERIALS REQUIRED *(NOT PROVIDED)*

- Microplate reader capable of reading an OD at a wavelength of 450 nm
- Variable volume microtiter pipettes
- Adjustable multichannel pipette (50-300 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)
- Plate shaker

Specifications: This kit quantitates Cynomolgus and Rhesus monkey interferon alpha in buffers, sera and tissue culture media using a sandwich immunoassay.[8,9] The kit can measure concentrations as low as 25 pg/ml of Cynomolgus and Rhesus monkey interferon alpha in a sample. The kit is based on an ELISA with a biotinylated anti-detection antibody and a streptavidin horseradish peroxidase (HRP). Tetramethylbenzidine (TMB) is the substrate. The assay is based on PBL's Rhesus/Cynomolgus IFNA-α2 (PBL 14110), which has been calibrated in reference to the International Standard to Human Interferon Alpha-2a.[10]

Speed: Incubation time, 3 hr 15 min

Specificity: Cynomolgus (*Macacca fascicularis*) and Rhesus (*Macacca mulatta*) IFN-α2. Strong cross-reactivity was detected with human IFN-α2. No cross reactivity was detected with human IFN-γ, mouse or rat IFN-α.

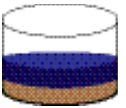
Storage Conditions/Comments: For retention of full activity, all reagents should be kept at 2-8ºC in the dark when not in use. Diluent reagents should be warmed to room temperature (RT), 22-25°C, before use.

Please note that the concentrations of the Antibody Concentrate and HRP 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 their preparation.

CAUTION: Sample Buffer, Standard Diluent, Wash Solution Concentrate and Concentrate 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 material safety data sheet for Kathon CG/ICP.

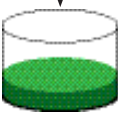
ASSAY PROCEDURE - QUICK REFERENCE

Total Time:
3 hr, 15 min



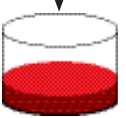
1) Add **50 µl** Sample Buffer
2) Add **50 µl** Standard, Blank or Sample

Incubate **1 hr** (shake at 450 rpm)
Aspirate and Wash **2x**



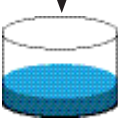
Add **100 µl** Diluted Ab Solution

Incubate **1 hr** (shake at 450 rpm)
Aspirate and Wash **3x**



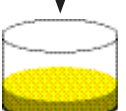
Add **100 µl** Diluted HRP Solution

Incubate **1 hr** (shake at 450 rpm)
Aspirate and Wash **4x**



Add **100 µl** TMB Substrate

Incubate **15 min** in the dark
Do not seal, shake or wash.



Add **100 µl** Stop Solution
Read plate within 5 min
(450 nm)

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

PREPARATION OF REAGENTS

Before starting the assay, the plate, Wash Solution Concentrate, applicable dilution matrices (e.g. tissue culture media, serum or plasma), Standard Diluent, Sample Buffer, Concentrate Diluent, Stop Solution and samples should be equilibrated to room temperature (RT), 22-25°C. The TMB Substrate should be equilibrated to RT (22-25°C) during step 3 of the Assay Procedure. Supplied Rhesus/Cyno IFN-α2 Standard, Antibody Concentrate and HRP Conjugate Concentrate should be kept on ice (4°C).

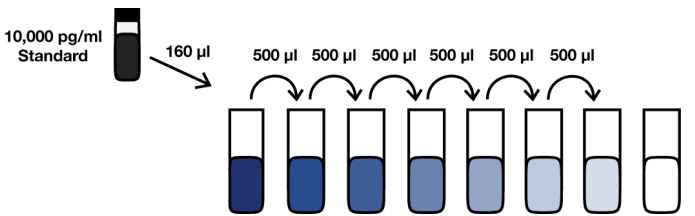
Wash Solution: The Wash Solution Concentrate may contain crystals. Place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution by adding 50 ml of Wash Solution Concentrate to 450 ml of distilled or deionized water. Mix thoroughly before use. Diluted Wash Solution can be stored at RT (22-25°C) when not in use.

Rhesus/Cynomolgus Interferon Alpha 2 Solution: Using the Rhesus/Cyno IFN-α2 Standard, provided at 10,000 pg/ml, construct a standard curve (25-1600 pg/ml), as shown in Figure 1, in your sample matrix. *In certain situations, "test" samples such as samples in tissue culture media or serum may contain substances that can interfere with assay results. Therefore, it is recommended to run the IFN standard curve diluted in endogenous IFN-free sample matrix.* In the event that the sample matrix is not available, the Standard Diluent may be used to prepare the standard curve.

Standard Curve Preparation:

a) Label seven polypropylene tubes (S1-S7).
b) Fill tubes with sample matrix (e.g. Standard Diluent, tissue culture media, serum, plasma) as indicated.
c) Using polypropylene tips add the Rhesus/Cyno IFN-α2 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.

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



Sample Dilution Label	S7	S6	S5	S4	S3	S2	S1	Blank
Matrix Vol. (µl)	840	500	500	500	500	500	500	500
IFN-α Conc. (pg/ml)	1600	800	400	200	100	50	25	0

Sample Preparation: Prepare test samples of unknown interferon concentration to be tested. Any pre-dilution of the samples to get the anticipated concentration within range of the Standard Curve must be made in the sample matrix and not the supplied Standard Diluent. Measurements in duplicate are recommended.

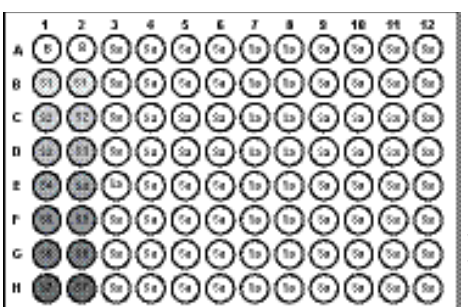
Antibody Solution: Dilute Antibody Concentrate with Concentrate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct amount of Antibody Concentrate to use. Prepare 15 minutes prior to use in step 2 of the Assay Procedure and keep at RT (22-25°C).

HRP Solution: Dilute HRP Conjugate Concentrate with Concentrate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct amount of HRP Conjugate Concentrate to use. Prepare 15 minute prior to use in step 3 of the Assay Procedure and keep at RT (22-25°C).

ASSAY PROCEDURE

All incubations should be performed at room temperature (RT), 22-25°C, keeping the plate away from drafts and other temperature fluctuations. Set plate shaker speed to 450 rpm where indicated. 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. All wells should be filled with a minimum of 250 µl of diluted Wash Solution. Improper washing may result in increased background values and poor coefficient of variation (%CV) values.

Figure 2: Example of a Typical Plate Setup



B = Blank
S1-S7 = Std Curve
Sa = Samples

1. **Standards and Test Samples:** Each standard, blank and sample should be run in duplicate. Determine the number of microtiter plate strips required to test the desired number of

samples plus the appropriate number of wells needed to run blanks and standards. Remove extra microtiter plate strips from the frame, seal in the foil bag provided and store at 2-8°C.

Add 50 µl Sample Buffer to each well. Add 50 µl of standard, blank or test sample to wells containing 50 µl Sample Buffer. Cover with plate sealer and shake plate (450 rpm) at RT (22-25°C) for 1 hour.

After 1 hour, empty the contents of the plate and wash the wells two times with 250 µl diluted Wash Solution.

2. **Antibody Solution:** Add 100 µl of diluted Antibody Solution to each well. Cover with plate sealer and shake plate (450 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 250 µl diluted Wash Solution.

3. **HRP Solution:** Add 100 µl of diluted HRP solution to each well. Cover with plate sealer and shake plate (450 rpm) at RT (22-25°C) for 1 hour. During this incubation period, warm the TMB Substrate Solution to RT (22-25°C).

After 1 hour, empty the contents of the plate and wash the wells four times with 250 µl 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 SHAKE. DO NOT USE a plate sealer 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 5 minutes after the addition of the Stop Solution.

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 should be subtracted from the standards and sample ODs to eliminate background. The ODs should be plotted against a range of 0-1600 pg/ml.

Note: The lowest limit of quantitation (LLOQ) is 25 pg/ml. Concentrations of unknown samples that measure < 25 pg/ml are suspect.

Because the interferon samples are titrated against the international standard, the values from the curves can be determined in units/ml as well as pg/ml. The conversion factor of about 3 – 5 pg/unit is applicable for Rhesus/Cyno IFN-α2 where units are determined by comparison to human interferon alpha 2 international standard.[11] Nevertheless, this conversion factor is only an approximation.

A shift in optical densities is typical between users and kit lots. The back fit concentration extrapolated 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.

Figure 3: Typical Standard Curve

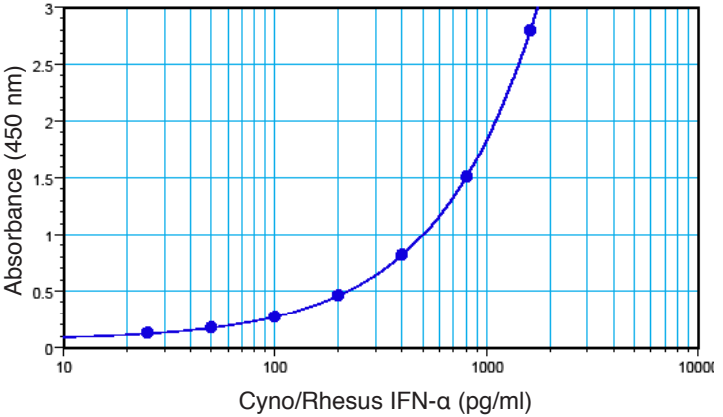


Figure 3 is a typical standard curve prepared using a 4-parameter fit.

Note: Results of a typical standard curve using a 4-parameter 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.

PERFORMANCE CHARACTERIZATION STUDIES

1. **Normal Cynomolgus Monkey Serum screen**

Thirty four lots of normal Cynomolgus Monkey Serum were screened. Only eight lots had detectable signal corresponding to 35-150 pg/ml on a standard curve.

2. **Spike Recovery in Normal Cynomolgus Monkey Serum:**

High, medium and low spikes were prepared in eight normal low background lots of Cynomolgus Monkey sera. The concentrations of the spikes were calculated from a Standard Curve prepared in normal Cynomolgus Monkey Serum.

	Concentration (pg/ml)	% Recovery Range	Average % Recovery
Low Spike	40	75-115%	93%
Med Spike	240	88-119%	109%
High Spike	1440	94-121%	104%

3. **Intra-assay and Inter-assay % CV:**

	Cynomolgus Monkey Serum	Tissue Culture Media (10% FBS)
Intra-Assay CV	3.6% (n=27 assays)	2.5% (n=27 assays)
Inter-Assay CV	14.4% (n=27 assays)	9.2% (n=27 assays)

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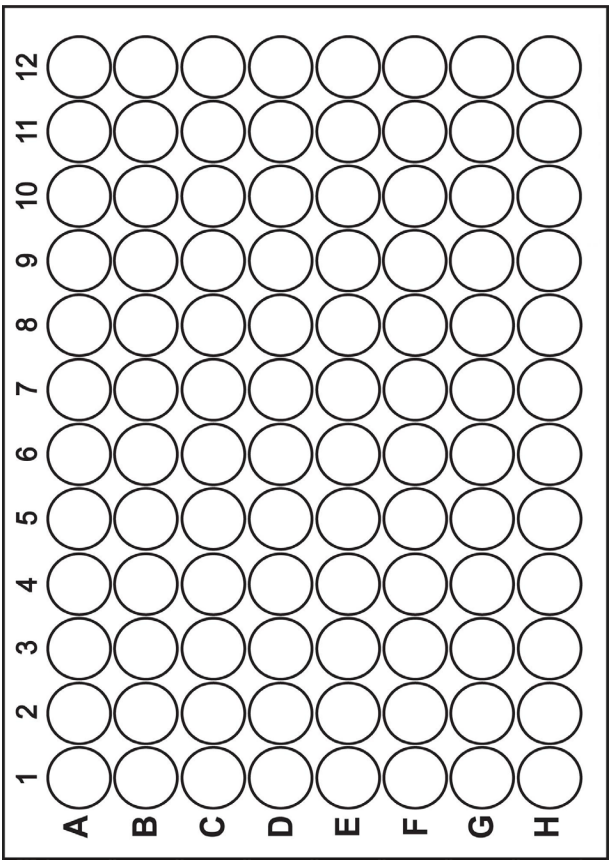
PLATE LAYOUT

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



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