



promising results™

ATGTTCA ACCAAGCT ACTTACTACC TTTATTTTAT GTTACTCTTT TATAGAT



131 Ethel Road West, Suite 6
Piscataway, NJ 08854 USA

Telephone: +1.732.777.9123 • Facsimile: +1.732.777.9141
Toll Free: 1.877.PBL.8881 • email: info@interferonsource.com

ATGTTCA ACCAAGCT ACTTACTACC TTTATTTTAT GTTACTCTTT TATAGAT

VeriKine™ Cynomolgus/Rhesus IFN- α Serum ELISA Kit

Product # 46100

Detection Range: 25-1600 pg/mL
Store all components at 2-8 °C

Sold under license from Pestka Biomedical Laboratories, Inc. d/b/a PBL InterferonSource. For research use only. Not for diagnostic or clinical use in, or administration to, humans. Not for resale in original or any modified form, including inclusion in a kit, for any purpose. Not for use in the preparation of any commercial product.

©Copyright 2009 Pestka Biomedical Laboratories, Inc. All right reserved.

Specifications: This kit quantitates Cynomolgus and Rhesus monkey interferon alpha in buffers, sera and tissue culture media using a sandwich immunoassay.^{1,2} 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-secondary antibody and a streptavidin horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. The assay is based on the PBL Rhesus/Cynomolgus IFN- α 2 (PBL 14110), which has been calibrated in reference to the International Standard to Human Interferon Alpha-2a.³

Speed: Incubation time, 3 hr 15 min

Specificity: Cynomolgus (*Macaca fascicularis*) and Rhesus (*Macaca mulatta*) IFN- α 2. Strong cross-reactivity was observed with human IFN- α 2. No cross reactivity with human IFN- γ . No cross reactivity with mouse or rat IFN- α .

Storage Conditions/Comments: For retention of activity, all reagents should be kept at 2-8° C in the dark. Diluent reagents should be warmed at room temperature before use.

Please note that the concentrations of the Detecting Antibody 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 Assay 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.

SUBMIT YOUR REFERENCE

GET FREE T-SHIRT*



HERE'S HOW:

PBL is in the process of compiling an extensive list of references on the use of Interferon and Interferon-related products. This reference list, including selected abstractions, will be made available to the scientific community through the PBL web site: www.interferonsource.com. If you have a published scientific paper, scientific poster or other scientific publication which mentioned the use of a PBL InterferonSource product, we will send you a free Interferon T-shirt in return for your citation.

Send the reference (electronic or hard copies appreciated), abstract (please included meeting and year) or other information to:

reference@interferonsource.com

Or mail to Technical Support, PBL InterferonSource, 131 Ethel Road West, Suite 6, Piscataway, NJ 08854, USA.

Please include your T-shirt size, and the address where you would like to receive your free T-shirt.

T-shirt colors and graphics may vary from design shown here. *Limit one per publication.

by highly pathogenic influenza virus”, *Proc Natl Acad Sci.* 106(9):3455-60.

8. Wagner TL, Horton VL, Carlson GL, Myhre PE, Gibson SJ, Imbertson LM, Tomai MA [1997] “Induction of cytokines in cynomolgus monkeys by the immune response modifiers, imiquimod, S-27609 and S-28463”, *Cytokine*, 9(11):837-45.

9. Kandimalla ER, Bhagat L, Li Y, Yu D, Wang D, Cong YP, Song SS, Tang JX, Sullivan T, Agrawal S. [2005] “Immunomodulatory oligonucleotides containing a cytosine-phosphate-2'-deoxy-7-deazaguanosine motif as potent toll-like receptor 9 agonists”, *Proc Natl Acad Sci.* 102(19):6925-30.

10. ICH Guidance for Industry, S8 Immunotoxicity Studies for Human Pharmaceuticals 2006, S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals 1997.

11. Rubinstein, M., Levey W.P., Moschera, J.A., Lai, C.-Y., Hershberg, R.D., Bartlett, R.T., and Pestka [1981] Human Leukocyte Interferons: Isolation and Characterization of Several Molecular Forms,” *Arch Biochem. Biophys.* 210, 307-318;

Introduction:

Interferons (IFN) are a group of cytokines which exhibit pleiotropic activities and which 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 ⁴.

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* ⁵.

Following expression and secretion, IFN- α binds to a heterodimeric 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, antiproliferative 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 physiology⁶. For example, macaques provide valuable surrogate models of human viral infections including examination of the pathogenicity of the 1918 pandemic influenza virus ⁷. Furthermore, macaques are included in the evaluation of many new therapeutic agents aimed at modulating host immunity to either enhance or dampen immune responses ^{8,9}.

These primates also serve as important immunotoxicological models in the testing of human pharmaceuticals¹⁰.

The *Verkine*[™] Cynomolgus/Rhesus IFN- α Serum ELISA kit (PBL# 46100) 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 immunotoxicology studies conducted in non-human primates.

References:

1. Staehelin, T., Stähli, C., Hobbs, D.S., and Pestka, S. [1981] "A Rapid Quantitative Assay of High Sensitivity for Human Leukocyte Interferon with Monoclonal Antibodies," in *Methods in Enzymology*, Vol. 79 [S. Pestka, ed.], Academic Press, New York, 589-595.
2. Kelder, B., Rashidbaigi, A., and Pestka, S. [1986] "A Sandwich Radioimmunoassay for Human IFN β ," in *Methods in Enzymology*, Vol. 119 [S. Pestka, ed.], Academic Press, New York, 582-587.
3. Human IFN α international reference standard provides by NIH, reference no. GXA01-901-535. Pestka [1986] "Interferon standards and general Abbreviation," in *Methods in Enzymology*, Vol. 119 [S. Pestka, ed.] Academic Press New York 14-23
4. 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.
5. 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.
6. Carlsson HE, Schapiro SJ, Farah I, Hau J [2004] "Use of primates in research: a global overview", *Am J Primatol* 63:225-237.
7. Baskin CR, Bielefeldt-Ohmann H, Tumpey TM, Sabourin PJ, Long JP, García-Sastre A, Tolnay AE, Albrecht R, Pyles JA, Olson PH, Aicher LD, Rosenzweig ER, Murali-Krishna K, Clark EA, Kotur MS, Fornek JL, Proll S, Palermo RE, Sabourin CL, Katze MG. [2009] "Early and sustained innate immune response defines pathology and death in nonhuman primates infected

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 8 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)

Materials Provided:

- Pre-coated micro-titer stripwell plate (s)
- Plate sealers
- Wash Solution Concentrate (10x)
- Rhesus/Cyno IFN- α 2 Standard (10,000pg/ml)
- Standard Diluent
- Sample Buffer
- Antibody Concentrate
- HRP Conjugate Concentrate
- Assay Diluent
- TMB Substrate
- Stop Solution
- Standard Diluent

Additional Materials Required:

- Micro-titer plate reader capable of reading a wavelength of 450nm
- Variable volume micro-titer pipettes
- Adjustable multi-channel pipette (50-300 μ l)
- Reagent reservoirs
- Wash bottle or plate washing system
- Distilled or deionized water
- Serological pipettes (1, 5, 10 or 25ml)
- Disposable pipette tips (polypropylene)
- Plate Shaker

Assay Procedure

All incubations should be performed at room temperature (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 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.

1. Wash buffer: Prepare the wash buffer by diluting 50ml of 10X Wash Solution Concentrate with 450ml of distilled or deionized water. Mix thoroughly before use. The diluted wash buffer can be stored at 2-25°C.

2. Standard Diluent, Sample Buffer and Assay Diluent should be brought to room temperature (RT) before use in the assay.

3. Preparation of Rhesus/Cyno IFN- α 2 Standard curve:

Note: 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.

Dilute the Rhesus/Cyno IFN- α 2 Standard, provided at 10,000pg/ml, in your sample matrix as indicated on page 7 in the standard curve. Construct this IFN- α 2 reference standard curve of 25-1600 pg/ml by:

- a. Labeling seven polypropylene tubes (S1-S7).
- b. Fill tubes with Sample Matrix (e.g. buffer, tissue culture media, serum) as indicated.

Assay Procedure– Quick Reference

Step	Reagent	Volume/ well	Incubation	Wash	Comments
5	Sample Buffer	50 µl	60 min	2X	Total volume/ well =100 µl
5	Standard, Blanks, Samples	50 µl			
6 & 7	Diluted Anti- body Solution	100 µl	60 min	3X	
8 & 9	Diluted HRP Solution	100 µl	60 min	4X	During incu- bation, warm TMB to room temp.
10	TMB Substrate	100 µl	15 min	DO NOT WASH	Incubate in the dark; no plate sealer
11	Stop Solution	100 µl	0 min	DO NOT WASH	Read within 5 minutes
12	Read Plate at 450 nm				

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

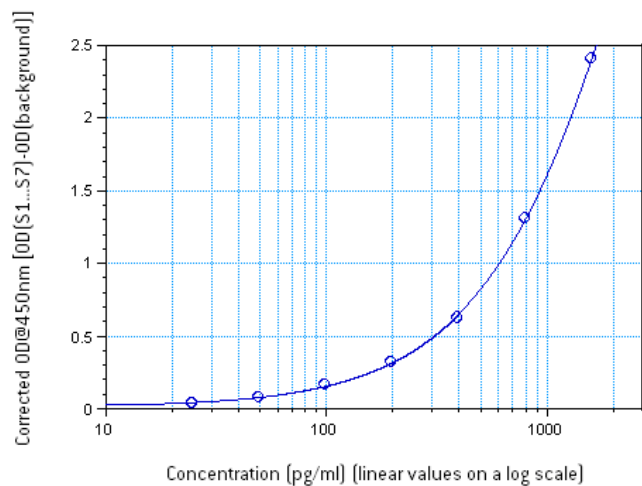
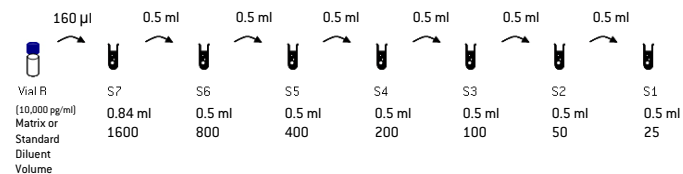


Figure 1: Standard Curve prepared using 4-parameter fit:

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.

- c. Using polypropylene tips add the Rhesus/Cyno IFN- α 2 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.



4. 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.

5. Addition of Standards and Samples: Each standard, blank and sample should be run in duplicate. Determine the number of micro-plate strips required to test the desired number of samples plus the appropriate number of wells needed to run blanks and standards. Remove extra micro-titer strips from the frame, seal in the foil bag provided and store at 2-8°C.

Add 50 μ l Sample Buffer to each well. Add 50 μ l of the Std. curve, blank and test samples to wells containing 50 μ l sample buffer.

Cover and shake (450-500RPM) for 1 hour at RT.

6. Preparation of Antibody Solution: Approximately 15 minutes prior to use, dilute Antibody Concentrate in Assay Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct dilution of Antibody Concentrate to prepare. Store the diluted Antibody at RT.

7. Wash # 1 and Addition of Antibody Solution: After 1 hour, empty the contents of the plate and wash the wells two times with 250 µl diluted wash buffer prepared in step 1. Add 100 µl of diluted antibody solution from step 6 to all wells. Cover and shake for 1 hour at RT.

8. Preparation of HRP Solution: Approximately 15 minutes prior to use, dilute HRP Conjugate Concentrate in Assay Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct dilution of HRP Conjugate Concentrate to prepare. Store the diluted HRP Conjugate at RT.

9. Wash # 2 and Addition of HRP Solution: After 1 hour, empty the contents of the plate and wash the wells three times with 250 µl diluted wash buffer. Add 100 µl of diluted HRP solution from step 8 to all wells. Cover and shake for 1 hour at RT. During this incubation period, warm the TMB Substrate Solution to room temperature [22-25°C].

10. Wash # 3 and Addition of TMB Substrate: After 1 hour, empty the contents of the plate and wash the wells four times with 250 µl diluted wash buffer. Add 100 µl of the TMB Substrate Solution to each well. Incubate, in the dark, for 15 minutes.

DO NOT SHAKE. DO NOT USE a plate sealer during the incubation.

11. Addition of Stop Solution: After the 15 minute incubation of TMB, DO NOT WASH. Add 100 µl of Stop Solution to each well.

12. Read: Using a micro-plate 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 OD's should be subtracted from the standards and sample OD's to eliminate background. The OD s 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 - α 2 where Units are determined by comparison to human interferon alpha 2 international standard¹¹. 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.