pbl assay science

VeriKine Human IFN Gamma Receptor 1 ELISA Kit

Catalog No. 41580

Standard Diluent Assay Range: 9.38 – 300 pg/ml

Cell Lysates Assay Range: 9.38 – 300 pg/ml

Serum Assay Range: 234.38 – 7500 pg/ml

Tissue Culture Media Assay Range: 37.5 – 1200 pg/ml

Store **all** components at 2 - 8°C

INTRODUCTION

The receptor for interferon gamma (IFN-gamma) is a membrane bound heterodimer of the IFNGR1 and IFNGR2 chain with IFNGR1 providing the high affinity binding site.[1] IFNGR1 is also known as CD119 and Interferon Gamma Receptor alpha (IFNGRa). Binding of IFN-gamma to the receptor leads to activation of a number of genes and multiple biological activities. The receptor density can vary from 1,000 - 40,000 copies per cell depending upon the cell line.[2,3,4]

IFNGR1 can be present in two forms, the classical membrane bound form and a soluble form. Unlike IFNAR2 no alternatively spliced secreted transcript has been identified for IFNGR1 but is thought to be cleaved at the membrane by proteases in a manner similar to other cytokine receptors. There appears to be a basal level in the serum of healthy donors. The soluble IFNGR1 may be elevated in certain disease states such as Rheumatoid Arthritis and Graft vs. Host Disease.[5,6]

PBL Assay Science's VeriKine Human Interferon Gamma Receptor 1 ELISA kit allows for the measurement of IFNGR1 in cell lysates, tissue culture media (TCM), serum and plasma and may prove a useful tool in elucidating the mechanism of expression, shedding and to identify diseases where this molecule is regulated.

41580 Rev. 00

MATERIALS PROVIDED

- Pre-coated microtiter plate(s)
- Plate sealers
- Wash Solution Concentrate
- Human IFN Gamma Receptor 1 Standard, 500,000 pg/ml
- Sample Diluent
- Antibody Concentrate
- Antibody Diluent
- HRP Conjugate Concentrate
- HRP Diluent
- TMB Substrate
- 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 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)
- Timer
- Graduated Cylinder

Specifications: This kit quantitates Human IFN Gamma Receptor 1 (IFNGR1) in serum, plasma, tissue culture media (TCM) and cell lysates by sandwich enyzme linked immunosorbent assay (ELISA). IFNGR1 binds to plates coated with antibody and detection is accomplished using a detection antibody followed by streptavidin conjugated to horseradish peroxidase (HRP). This ELISA kit utilizes Tetramethyl-benzidine (TMB) as the substrate. The standard provided is recombinant Human IFNGR1 expressed in mammalian cells.

Speed: Incubation time, 3 hr 15 min

Specificity: No measurable IFNGR1 was observed when using this kit with sera of cynomolgus, rhesus, pig, bovine, rabbit, mouse, sheep, or donkey. No cross reactivity was detected with mouse IFNGR1-Fc.

Over two hundred different lots of pooled human serum were screened for endogenous Human IFNGR1.

Precision & Recovery: Human IFNGR1 was spiked into a single lot of normal human serum at three different concentrations and analyzed:
Intra-Assay CV - 16 replicates of each concentration on a plate
Inter-Assay CV - 9 independent assays run by same operator
Average Recovery - 27 independent assays

Concentration (pg/ml)	375	1500	5000
Intra-Assay CV	2.5%	1.6%	2.8%
Inter-Assay CV	1.6%	3.2%	5.1%
Average Recovery	95%	93%	96%

Storage Conditions/Comments: For retention of full activity, all reagents should be kept in the dark at 2-8°C when not in use. Deionized or distilled water should be used for preparation of all reagents. All dilutions should be made with polypropylene tubes and pipette tips. Pipette tips should be changed between each dilution tube. All measurements for standards and samples should be performed in duplicate. At least two control wells (wells with Sample Diluent only) should be used for each assay; these control values should be subtracted from all readings prior to any calculations or plots of the data.

Please note that the dilutions 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: Wash Solution Concentrate, Sample Diluent, and Antibody Diluent contain 0.1% Kathon CG/ICP as a preservative. These components 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 diagnostic or therapeutic procedures.

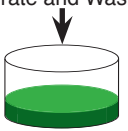
ASSAY PROCEDURE - QUICK REFERENCE

Total Time: 3 hr, 15 min

For serum & plasma samples:
Add **100 µl** Standard, 25x diluted Sample, or Blank

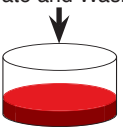
For cell culture samples:
Add **100 µl** Standard, Cell Lysate, 4x diluted Tissue Culture Supernatant, or Blank

Incubate **2 hr** (shake at 650 rpm)
Aspirate and Wash **4x**



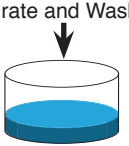
Add **50 µl** Diluted Ab Solution

Incubate **1 hr** (shake at 650 rpm)
Aspirate and Wash **4x**



Add **50 µl** Diluted HRP Solution

Incubate **5 min** (shake at 650 rpm). **Do not seal.**
DO NOT EXCEED 5 MINUTES.
Aspirate and Wash **4x**



Add **100 µl** TMB Substrate

Incubate **10 min** in the dark
Do not seal, shake or wash.

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

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

PREPARATION OF REAGENTS

Before starting the assay, the plate(s), Wash Solution Concentrate, TMB Substrate and Stop Solution should be equilibrated to room temperature (RT), 22-25ºC. All other supplied components should be kept on ice (4ºC) throughout the assay until being used.

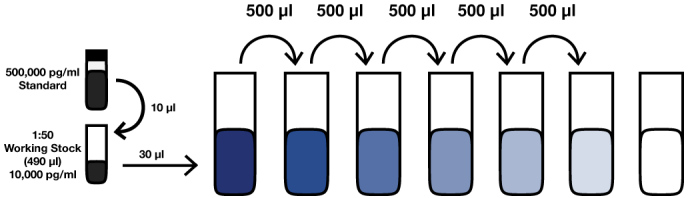
Wash Solution: The Wash Solution Concentrate may contain crystals. Place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:20 working wash solution (e.g. Add 50 ml of Wash Solution Concentrate to 950 ml of distilled or deionized water) and mix thoroughly. The diluted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

Human IFN Gamma Receptor 1 (IFNGR1) Solution: Using the Human IFNGR1 Standard, provided at 500,000 pg/ml, construct a standard curve (9.38 - 300 pg/ml), as shown in figure 1, in Sample Diluent.

Standard Curve Preparation:

a. Prepare 1:50 *working stock* of human IFNGR1 Standard by pipetting 10 µl of IFN Standard into 490 µl of Sample Diluent. Mix thoroughly by gently pipetting up and down 10 times.
b. Label six polypropylene tubes (S1-S6).
c. Fill tubes with Sample Diluent as indicated in Figure 1.
d. Add 30 µl of the working stock of Human IFNGR1 Standard to S6 and mix thoroughly to recover all material adhered to the inside of the pipette tip.
e. Using a pipette set at 500 µl, mix S6 thoroughly by pipetting up and down. Transfer 500 µl of S6 to S5 and mix thoroughly by pipetting up and down. Repeat to complete series to S1.
f. Set aside at RT (22-25ºC) until use in step 1 of the Assay Procedure.

Fig. 1: 6-Point Standard Curve Prepared in Sample Diluent



Sample Dilution Label	S6	S5	S4	S3	S2	S1	Blank
Sample Diluent (µl)	970	500	500	500	500	500	500
IFN-γ R1 Conc. (pg/ml)	300	150	75	37.5	18.75	9.38	0

Test Sample Preparation: Prepare test samples of unknown Human IFNGR1 concentration using Sample Diluent as required. Measurements in duplicate are recommended. It is recommended that, upon collection, test samples be quick frozen and stored at ≤ -70ºC (avoiding multiple freeze-thaw cycles) until being tested. Keep on ice (4ºC) until step 1 of the Assay Procedure.

For measuring IFNGR1 in Serum and Plasma:

- A pre-dilution of 1:25 in Sample Diluent is recommended.

For measuring IFNGR1 in Cell Culture:

- For tissue culture media (TCM) samples, centifuge supernatant at 500Xg for 5 minutes and then carefully collect the clear supernatant. A pre-dilution of 1:4 in Sample Diluent is recommended.
- For cell lysates, prepare samples by directly suspending cells in the provided Sample Diluent. Detailed 'Test Sample Preparation' instructions are available on the PBL website.

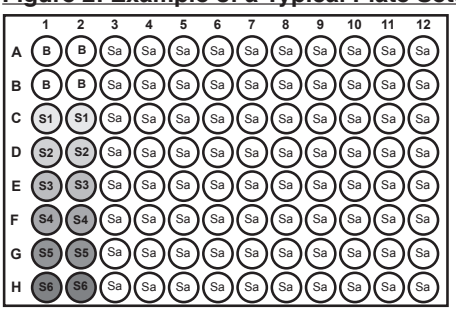
Antibody Solution: Dilute Antibody Concentrate in the volume of Antibody Diluent recommended in the lot specific Certificate of Analysis (CoA). Prepare 5 minutes prior to use in step 2 of the Assay Procedure and keep at RT (22-25ºC).

HRP Solution: Dilute HRP Concentrate in the volume of HRP Diluent recommended in the lot specific Certificate of Analysis (CoA). Prepare prior to use in step 3 of the Assay Procedure. Prepare while the plate soaks in the 4th wash in step 2 of the Assay Procedure for 5 minutes. Then keep at RT (22-25ºC).

ASSAY PROCEDURE

All incubations should be performed at room temperature (RT), 22-25ºC, keeping the plate away from drafts and other temperature fluctuations. Use plate sealers to cover the plate(s) 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 250 µl of diluted Wash Solution for each wash step. Refer to Preparation of Reagents for details on dilution of concentrated solutions.

Figure 2: Example of a Typical Plate Setup



B = Blank
S1-S6 = 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 the IFNGR1 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 100 µl of Standard, Test Sample, or Blank.
For serum/plasma samples: Add 100 µl of 25x diluted Sample
For TCM samples: Add 100 µl of 4x diluted Sample
For cell lysate samples: Add 100 µl of Sample

Cover with plate sealer and shake plate at 650 rpm at RT (22-25°C) for 2 hours.

After 2 hours, empty the contents of the plate and wash the wells four times with at least 250 µl of working Wash Solution (refer to Preparation of Reagents).

2. **Antibody Solution:** Add 50 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 four times with at least 250 µl of working Wash Solution. During the 4th wash, allow the plate to soak for 5 minutes while preparing the HRP Solution.

3. **HRP:** Add 50 µl of diluted HRP Solution (refer to Preparation of Reagents) to each well. Do not use a plate sealer and shake plate at 650 rpm at RT (22-25°C) for 5 minutes.

DO NOT EXCEED 5 MINUTES WITH HRP.

At the end of 5 minutes, empty the contents of the plate and wash the wells four times with at least 250 µl of working 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 10 minutes. Do not use a plate sealer and do not shake during the incubation.

5. **Stop Solution:** After the 10 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

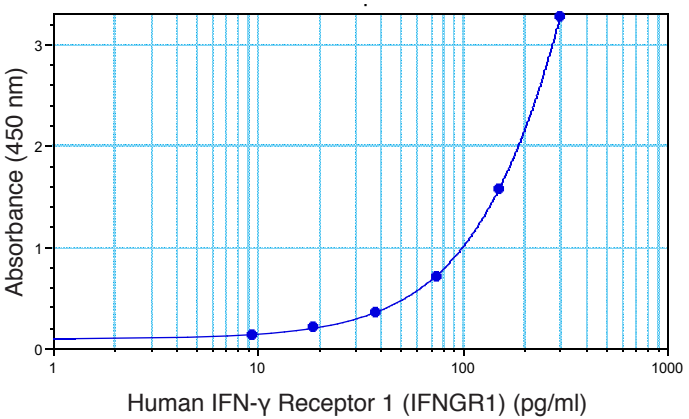
By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the IFNGR1 titer in the samples can be determined. Based on user preference, blank ODs may be subtracted from the standard and sample ODs to eliminate background.

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

Results of a typical standard curves 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.

Figure 3: Typical Standard Curve in Sample Diluent



REFERENCES

1. Bach, E. A., Aguet, M., and Schreiber, R. D. (1997) “The IFN-γ receptor: a paradigm for cytokine receptor signaling.” *Annu. Rev. Immunol.* 15:563.

2. Hawkyard, S. J., et al. (1992) “.The expression of interferon γ receptors on bladder cancer cells: does it correlate with biological response?” *Urological Research.* 20:229-232.

3. Merlin, G., Falcoff, E., and Aguet, M. (1985) “I-labelled Human Interferon Alpha, Beta and Gamma: Comparative Receptor-binding Data.” *J. Gen. Virol.* 66:1149-1152.

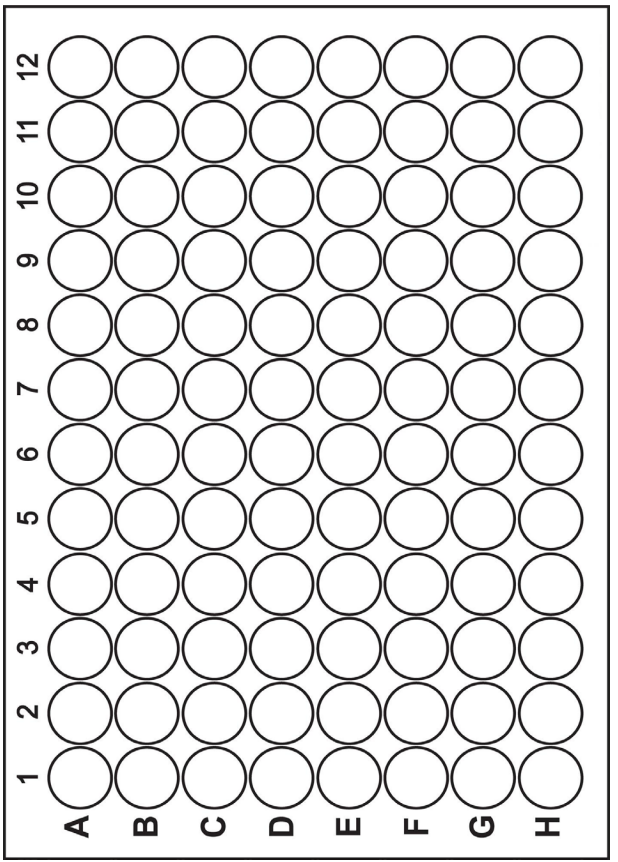
4. Celada, A., et al. (1985) “Demonstration and partial characterization of the interferon-gamma receptor on human mononuclear phagocytes.” *J. Clin. Invest.* 76:2196.

5. Bello, I., et al. (1998) “High levels of soluble IFN gamma receptor alpha chain in the plasma of rheumatoid arthritis patients.” *Biotherapy.* 11(1):53-57.

6. Toren, A., et al. (1997) “Soluble interferon-gamma receptor and interferon-gamma in patients undergoing allogeneic bone marrow transplantation for hematological malignancies.” *Cytokines Cell Mol Ther.* 3(3):153-158.

PLATE LAYOUT

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



NOTES

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