Pig IFN gamma ELISA Kit

Pig IFN-gamma ELISA Kit is an Enzyme Immunoassay kit for the quantification of Pig IFN gamma in serum, plasma and cell culture supernatants.

Catalog number: ARG81161

Package: 2 x 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

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INTRODUCTION

Interferon gamma (IFNγ) is a dimerized soluble cytokine that is the only member of the type II class of interferons. The existence of this interferon, which early in its history was known as immune interferon, was described by E. F. Wheelock as a product of human leukocytes stimulated with phytohemagglutinin, and by others as a product of antigen-stimulated lymphocytes. It was also shown to be produced in human lymphocytes or tuberculin-sensitized mouse peritoneal lymphocytes challenged with PPD; the resulting supernatants were shown to inhibit growth of vesicular stomatitis virus. Those reports also contained the basic observation underlying the now widely employed interferon gamma release assay used to test for tuberculosis. In humans, the IFN γ protein is encoded by the IFNG gene. [Provide by Wikipedia: Interferon gamma]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for Pig IFN-gamma has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Pig IFN-gamma present is bound by the immobilized antibody. After washing away any unbound substances, added antibody-conjugate specific for Pig IFN-gamma to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of Pig IFN-gamma bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Pig IFN-gamma in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	2 plate	4°C
Standards (lyophilized)	4 vial	4°C
Diluent Buffer	100 mL (ready to use)	4°C
Antibody Conjugate (lyophilized)	4 vial	4°C
HRP Conjugate	1.2 mL	4°C
20X Wash Buffer	100 mL	4°C
TMB substrate	20 mL (ready to use)	4°C (protect from light)
STOP solution	20 mL (ready to use)	4°C
Plate Sealer	6 ea	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Individual components of this kit contain no preservatives.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Select the number of coated wells required for the assay. The remaining wells should be placed in the re-sealable pouch with a desiccant. The pouch must be re-sealed to protect from moisture.
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.

- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Cell Culture Supernatants: Centrifuge cell culture media at 1,500 x g for 10 minutes at 4°C to remove particulates.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and store on ice.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Store all samples on ice after preparation and use immediately or aliquot and store at -80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 20X Wash Buffer into 950 mL of distilled water to a final volume of 1 L)
- Antibody Conjugate: Reconstitute 1 vial of Antibody Conjugate in 0.275 mL distilled water, and dilute 1:20 in Diluent Buffer.

  Note: Reconstituted solutions are stable at 20°C for up to 2 month. Avoid repeated freeze-thaw cycles.
- HRP Conjugate: Dilute the HRP Conjugate 1:20 in Diluent Buffer.
- Standards: Reconstitute 1 vial of Standards in 0.220 mL distilled water to a concentration of 1,000 ng/mL. Then dilute in Diluent Buffer to prepare a series diluted Standards according to the table below. Do not store the diluted Standards.

Standard tube	Final Standards conc. (pg/mL)	Volume of Diluent Buffer (µL)	Volume of 1,000 ng/mL Standard (µL)
S1	15,000	985	15
S2	7,500	500	500 of S1
S3	3,750	500	500 of S2
S4	1,875	500	500 of S3
S5	937.5	500	500 of S4
S6	468.75	500	500 of S5
S7	234.375	500	500 of S6
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **200 µL** of **1X Wash Buffer** to each well. Aspirate the wells to remove liquid and wash the plate **3 times** using **300 µL** of 1X Washing Buffer per well. After the last wash, invert plate to remove residual solution and blot on paper towel.
2. Add **100 µL** of **samples, blank and Standards** to the **Antibody Coated microplate**. Cover the plate with the Plate Sealer. Incubate for **at least 2 hour** at **room temperature.**
3. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of the **diluted Antibody Conjugate** per well. Then cover the plate with the Plate Sealer. Incubate for **2 hour** at **room temperature**.
5. Aspirate and wash plate as in step 3.
6. Add **100 µL** of the **diluted HRP Conjugate** per well. Then cover the plate with the Plate Sealer. Incubate for **30 minutes** at **room temperature**.
7. Aspirate and wash plate as in step 3.
8. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **20-30 minutes** at **room temperature** in the dark.
9. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank

wells. The color of the solution should change from blue to yellow.

10. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Subtract the Standard 0 reading from each averaged value above.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

234 pg/mL

Cross Reactivity

When tested at 50 ng/mL the following antigen (recombinant protein) did not exhibit significant cross reactivity.