Rat IFN alpha ELISA Kit

Enzyme Immunoassay for the quantification of rat IFN alpha in serum, plasma, cell culture supernatants and other suitable sample solution

Catalog number: ARG80951

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The protein encoded by this gene is produced by macrophages and has antiviral activity. This gene is intronless and the encoded protein is secreted. [provided by RefSeq, Sep 2011]

Produced by macrophages, IFN-alpha have antiviral activities. Interferon stimulates the production of two enzymes: a protein kinase and an oligoadenylate synthetase. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat IFN-alpha has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IFN-alpha present is bound by the immobilized antibody. A HRP-conjugated antibody specific for IFN-alpha is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a chromogenic substrate solution is added to the wells and color develops in proportion to the amount of IFN-alpha bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of IFN-alpha in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 wells X 8 strips	4°C
Standards A-F (Concentration: 3.12, 6.25, 12.5, 25, 50, 100 pg/ml)	6 vials X 0.5ml	4°C
Sample Diluent	6 ml	4°C
HRP-conjugated Antibody	10 ml	4°C
20X Wash buffer	25 ml	4°C
Chromogen solution A	6 ml	4°C (Protect from light)
Chromogen solution B	6 ml	4°C (Protect from light)
STOP solution	6 ml	4°C
Plate sealer	2	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 37°C incubator
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT until the crystals are completely dissolved.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.
- Haemolytic samples might influence the result, so haemolytic samples should be avoided.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants and other biological fluids- Remove particulates by centrifugation and aliquot & store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C or below. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

c) Serum, plasma, and cell culture fluid samples can be stored at 2-8°C up to 3 days, for long term storage, samples must be stored at -20°C up to 1 months or at -80°C up to 6 months to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. *DO NOT USE HEAT-TREATED SAMPLES.*

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- Samples: If samples give OD readings higher than the highest standard provided, please dilute samples with sample diluent and repeat the assay. For the calculation of the concentrations this dilution factor has to be taken into account. (It is recommended to do pre-test to determine the suitable dilution factor).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 18-25°C) before use. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl of Sample Diluent Buffer in duplicate into wells (zero standard).
3. Add 50 µl of standards and samples in duplicate into appropriate wells.
4. Add 100 µl of HRP-conjugated antibody to each well.
5. Mix well gently, mixing well in this step is important. Cover and incubate the plate for 1 hour at 37°C.
6. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 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 until no moisture appears.

7. Add 50 µl of Chromogen solution A and 50 µl of Chromogen solution B to each well subsequently. Mix thoroughly. Cover wells and incubate for 15 minutes at 37°C. Protect from light.
8. Add 50 µl of Stop Solution to each well. Mix well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. Using linear, semi-log or log-log 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.

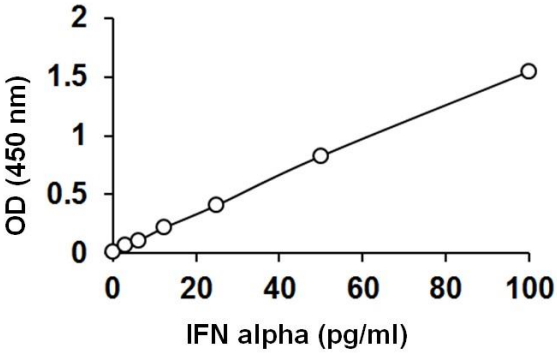
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of IFN-alpha ranged from 3.12-100 pg/ml. The mean MDD was 1.0 pg/ml.