Mouse IFN gamma ELISA Kit

Enzyme Immunoassay for the quantification of Mouse IFN gamma in serum and cell culture supernatants.

Catalog number: ARG81368

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INTRODUCTION

IFN gamma is a soluble cytokine that is a member of the type II interferon class. The encoded protein is secreted by cells of the both the innate and adaptive immune systems. The active protein is a homodimer that binds to the interferon gamma receptor which triggers a cellular response to viral and microbial inflections. Mutations in this gene are associated with an increased susceptibility to viral, bacterial and parasitic infections and to several autoimmune diseases. [provided by RefSeq, Sep 2015]

IFN gamma produced by lymphocytes activated by specific antigens or mitogens. IFN-gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons. [UniProt]

PRINCIPLE OF THE ASSAY

This is an enzyme immunoassay for the quantification of Mouse IFN gamma in serum and cell culture supernatants. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IFN gamma has been pre-coated onto a wells of microtiter plate. Standards or samples are pipetted into the wells and any IFN gamma present is bound by the immobilized antibody. Then a biotin-conjugated antibody specific to IFN gamma is added to each well and incubate. After washing away any unbound substances, an HRP-conjugated avidin is added to each well and incubate. A substrate solution (TMB) is added to the wells and color develops in proportion to the amount of cytokine 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 450nm ±2nm. The concentration of IFN gamma 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	8 X 12 strips	4°C.
Standard (3200 pg/vial)	2 vials (Lyophilized).	4°C
Biotin-conjugated antibody	6 ml (Ready-to-use)	4°C
HRP-conjugated Avidin	12 ml (Ready-to-use)	4°C
Standard Diluent I (for serum samples)	25 ml (Ready-to-use)	4°C
Standard Diluent II (for cell culture supernatant samples)	25 ml (Ready-to-use)	4°C
20X Wash buffer	60 ml	4°C
Substrate A	10 ml (Ready-to-use)	4°C (Protect from light)
Substrate B	10 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	14 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 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.
- Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
- All kit reagents and specimens should be brought to room temperature (20-25°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens. Do not use water baths to thaw samples or reagents.
- If crystals are observed in the 20X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Substrate Solution is easily contaminated. If bluish prior to use, do not use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

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 - Remove particulates by centrifugation and assay immediately or aliquot and 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 10 minutes at 1000 x g at 4°C. Collect serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

When performing the assay slowly bring samples to room temperature.

DO NOT USE HEAT-TREATED SPECIMENS.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer, mix thoroughly. (E.g.: Add 20 ml of 20X Wash buffer into 380 ml of distilled water, mix well) 1X Wash buffer is stable for 1 month at 2-8 °C. Mix well before use.
- TMB Substrate mixture solution

  Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Mixture Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

- Standards: Please select appropriate Diluent buffer for each sample type. Two vials of Standards with Mouse cell expressed recombinant IFN gamma protein are provided in this kit to allow both serum and cell culture supernatant samples. Standard diluent I contains animal serum and PBS for serum samples. Standard diluent II contains animal serum and cell culture medium for cell culture supernatant samples. The standards provided in the kit are for customers to use at own discretion. If samples generate values higher than the highest standard, dilute the

samples with appropriate standard diluent and repeat assay.

High concentration standard stock:

Reconstitute standards with either 1ml Standard diluent I (for serum samples) or Standard diluent II (for cell culture supernatant samples) to obtain high concentration standard stock (3200 pg/ml). Allow solution to sit for 15 minutes with gentle agitation prior to making dilutions. This stock solution can be used within one hour of reconstitution or aliquoted and stored frozen at -20 °C or-70 °C for up to 30 days.

Dilution of standard mixture:

Make sure the standard is dissolved completely before making serial dilutions. The appropriate Standard diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with appropriate Standard diluent (serum samples: Standard diluent I; cell culture supernatant samples: Standard diluent II) as according to the suggested concentration below: 3200 pg/ml, 1600 pg/ml, 800 pg/ml, 400 pg/ml, 200 pg/ml, 100 pg/ml and 50 pg/ml.

The example of the dilution of standards

Standard No.	Standard Conc. (pg/ml)	Standard diluent (µl)	Standard (µl)
S1 (Stock)	3200	0	1000
S2	1600	500	500 µl of Stock (S1)
S3	800	500	500 µl of S2
S4	400	500	500 µl of S3
S5	200	500	500 µl of S4
S6	100	500	500 µl of S5
S7	50	500	500 µl of S6
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls 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 **standards or samples** into wells. Cover the wells and incubate for **1h at room temperature**.
3. Without discarding the content, add **50 µl** of **Biotin-conjugated antibody** into each well, mix well on a microplate shaker. Cover the wells and incubate for **1 hour at room temperature**.
4. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes**. Wash by filling each well with **1× Wash Buffer (350µl)** 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.
5. Add **100 µl** of **HRP-conjugated Avidin** into each well. Gently tap the plate to mix well. Cover the wells and incubate for **1h at room temperature**.
6. Aspirate each well and wash as step 4.
7. Add **100 µl** of **TMB Substrate solution Mixture** to each well. Cover the wells and incubate for **15 minutes at room temperature in dark**.
8. Add **100 µl** of **Stop Solution** to each well. Gently tap the plate to mix well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at **450 nm** immediately. (It is

recommended that read the O.D. within 30 min after adding Stop solution)

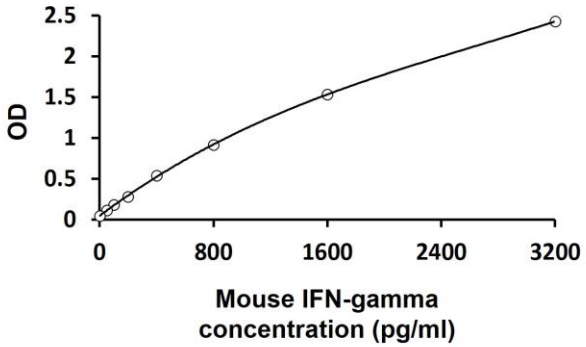
CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using 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.
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. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 500 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

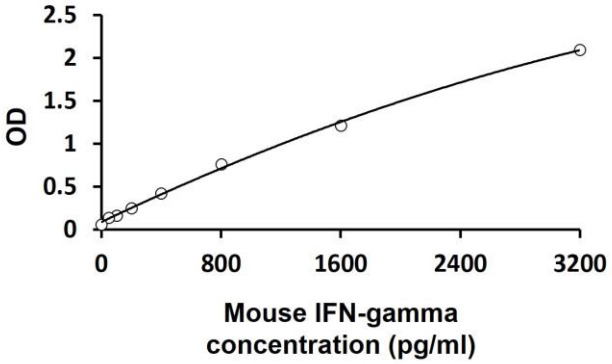
EXAMPLE OF TYPICAL STANDARD CURVE

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

- Standard diluted with CALIBRATOR DILUENT I



- Standard diluted with CALIBRATOR DILUENT II



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Mouse IFN gamma ranged from 50-3200 pg/ml. The mean MDD was 7.5 pg/ml.

Intra-assay and Inter-assay precision

Intra-Assay: <10 %

Inter-Assay: < 10 %

Recovery

95-119%