



TiterZyme[®] EIA

mouse IFN- γ

Enzyme Immunometric Assay Kit

Catalog No. 900-137

96 Well Kit

Table of Contents

Description	Page	2
Introduction		2
Precautions		2
Materials Supplied		3
Storage		3
Materials Needed but Not Supplied		3
Sample Handling		4
Procedural Notes		5
Reagent Preparation		5
Assay Procedure		6
Calculation of Results		7
Typical Results		7
Typical Standard Curve		8
Calibration		8
Performance Characteristics		9
Sample Dilution Recommendations		11
References		11
Limited Warranty		12

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Description

Assay Designs' mouse IFN- γ TiterZyme[®] Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determination of mouse IFN- γ in biological fluids. Please read the complete kit insert before performing this assay. The kit uses an antibody to mouse IFN- γ immobilized on a microtiter plate to bind the mouse IFN- γ in the standards or sample. A recombinant mouse IFN- γ Standard is provided in the kit. After a short incubation, a biotinylated antibody to mouse IFN- γ is added. This antibody binds to the mouse IFN- γ captured on the plate. After a short incubation the excess standards, samples and antibody are washed out and streptavidin conjugated to Horseradish peroxidase is added, which binds to the biotinylated mouse IFN- γ antibody. Excess conjugate is washed out and substrate is added. The substrate reacts with the conjugate bound to the IFN- γ captured on the plate. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of mouse IFN- γ in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard¹ or Tijssen².

Introduction

Mouse Interferon-gamma (IFN- γ) is a polypeptide of 136 amino acids, containing four exons and three introns. The structural gene for murine IFN- γ is on chromosome 10.^{3,4} IFN- γ is alternatively known as antigen-induced interferon, immune interferon (IIF), Type-2 interferon, T interferon, mitogen-induced interferon, and pH2-labile interferon.⁵ IFN- γ plays an important role in immune response. It is secreted by Th1 cells which in turn activate macrophage through the CD40 surface ligand. This secretion also inhibits the development of Th2 cells.⁶ Human and mouse IFN- γ share only a 40% homology on the amino acid level.⁷

Precautions

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1. Stop Solution is a 0.18 M sulfuric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
4. The mouse IFN- γ Standard provided, Catalog No. 80-1297, should be handled with care because of the known and unknown effects of IFN- γ .

Materials Supplied

1. **mouse IFN- γ Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1296**
A strip microtiter plate coated with antibody specific to mouse IFN- γ .
2. **mouse IFN- γ Antibody , 8 mL, Catalog No. 80-1298**
A solution of biotinylated monoclonal antibody to mouse IFN- γ .
3. **mouse IFN- γ Standard Diluent, 20 mL, Catalog No. 80-1299**
Phosphate buffered saline containing proteins and antibiotics.
4. **mouse IFN- γ Streptavidin-HRP Concentrate, 100 μ L, Catalog No. 80-1300**
A concentrated solution of streptavidin conjugated to Horseradish peroxidase.
5. **mouse IFN- γ Streptavidin-HRP Dilution Buffer, 14 mL, Catalog No. 80-1301**
6. **Wash Buffer Concentrate, 50 mL, Catalog No. 80-1253**
Tris buffered saline containing detergents.
7. **mouse IFN- γ Standard, 2 vials, Catalog No. 80-1297**
Two vials of lyophilized recombinant mouse IFN- γ .
8. **mouse IFN- γ TMB Substrate, 13 mL, Catalog No. 80-1302**
A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. **Protect from prolonged exposure to light.**
9. **mouse IFN- γ Stop Solution, 14 mL, Catalog No. 80-1303**
A 0.18 M solution of sulfuric acid in water. Keep tightly capped. Caution: **Caustic.**
10. **mouse IFN- γ Assay Layout Sheet, 1 each, Catalog No. 30-0213**
11. **Plate Sealer, 6 each, Catalog No. 30-0012**

Storage

All components of this kit are stable at 4 °C until the kit's expiration date.

Materials Needed but Not Supplied

1. Deionized or distilled water. No difference in assay results is seen with distilled water.
2. Precision pipets for volumes between 50 μ L and 1,000 μ L.
3. Repeater pipet for dispensing 50 and 100 μ L.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. Adsorbent paper for blotting.
7. Microcentrifuge to prepare Streptavidin-HRP Solution.
8. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
9. Log-log graph paper for plotting the standard curve.

Sample Handling

Assay Designs' TiterZyme® Enzyme Immunometric Assay is compatible with mouse IFN- γ samples in a wide range of matrices.

Culture fluids, serum and EDTA, heparin and sodium citrate plasma are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of tissue culture media can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Standard Diluent. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media, or Standard Diluent to calculate concentrations of mouse IFN- γ in the appropriate matrix.

Samples must be stored frozen to avoid loss of bioactive mouse IFN- γ . If samples are to be run within 24 hours, they may be stored at 4 °C. Otherwise, samples must be stored frozen at or below -70 °C to avoid loss of bioactive mouse IFN- γ . Up to three freeze/thaw cycles of serum has been shown to have no effect on mouse IFN- γ levels. Nonetheless, excessive freeze/thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and gently mixed by hand. Do not thaw samples in a 37 °C incubator. Do not vortex or sharply agitate samples.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards must be prepared in polypropylene or polyethylene tubes. Do not use polystyrene, polycarbonate or glass tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses plates with removable strips. Unused strips must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
8. **Prior to addition of conjugate and substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**
9. **It is important that the matrix for the standards and samples be as similar as possible. Mouse IFN- γ samples diluted with Standard Diluent should be run with a standard curve diluted in the same buffer. Serum and plasma samples should be evaluated against a standard curve run in Standard Diluent while Tissue Culture samples should be read against a standard curve diluted in the same complete but non-conditioned media. See Reagent Preparation, step #2.**

Reagent Preparation

1. Wash Buffer

Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 1,450 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. mouse IFN- γ Standards

Reconstitute standard with deionized water. Reconstitution volume is stated on the standard vial label. Let it sit at room temperature for 5 minutes. Mix gently. This solution contains 9,000 pg/mL mouse IFN- γ . When testing serum or plasma samples, use the Standard Diluent provided to prepare standard curve serial dilutions. When using cell culture supernatants use tissue culture media to prepare the standard curve serial dilutions.

Label five 12x75 mm test tubes #1 through #5. Pipet 150 μ L of Standard Diluent or tissue culture media into tubes #1 through #5. Add 75 μ L of the 9,000 pg/mL Standard to tube #1. Vortex thoroughly. Add 75 μ L of tube #1 to tube #2 and vortex thoroughly. Add 75 μ L of tube #2 to #3 and vortex thoroughly. Continue this for tubes #4 and #5.

The concentration of mouse IFN- γ in tubes #1 through #5 will be 3,000, 1,000, 333, 111 and 37 pg/mL respectively. See mouse IFN- γ Assay Layout Sheet for dilution details.

Diluted standards should be used within 60 minutes of preparation. Do not store reconstituted standards.

3. Streptavidin-HRP Solution

Prepare Streptavidin-HRP solution **immediately before use**. Do not store prepared Streptavidin-HRP solution. Use a plastic tube to prepare Streptavidin-HRP solution. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom. For each strip used, mix 2.5 μ L of Streptavidin-HRP Concentrate with 1 mL of Streptavidin-HRP Dilution Buffer.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards, controls and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of strips to be used and put any remaining strips with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 50 μ L of standard diluent or Tissue Culture Media into the S0 (0 pg/mL standard) wells.
3. Pipet 50 μ L of Standards #1 through #5 into the appropriate wells.
4. Pipet 50 μ L of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents, and seal with the plate sealer provided.
6. Incubate the plate at room temperature for 2 hours. Do not wash or decant the plate.
7. Pipet 50 μ L of the Biotinylated Antibody into each well, except the Blank.
8. Seal the plate and incubate at room temperature for 1 hour.
9. Empty the contents of the wells and wash by adding 400 μ L of wash solution to every well. Repeat the wash 2 more times for a total of **3 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
10. Add 100 μ L of the freshly prepared Streptavidin-HRP Conjugate to each well, except the Blank.
11. Seal the plate and incubate at room temperature for 30 minutes.
12. Empty the contents of the wells and wash by adding 400 μ L of wash solution to every well. Repeat the wash 2 more times for a total of **3 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
13. Pipet 100 μ L of Substrate Solution into each well.
14. Incubate for 30 minutes at room temperature in the dark.
15. Pipet 100 μ L Stop Solution to each well.
16. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Several options are available for the calculation of the concentration of mouse IFN- γ in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of mouse IFN- γ can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average Blank OD}$$

2. Using linear graph paper, plot the Average Net OD for each standard versus concentration of mouse IFN- γ in each standard. Approximate a straight line through the points. The concentration of mouse IFN- γ in the unknowns can be determined by interpolation.

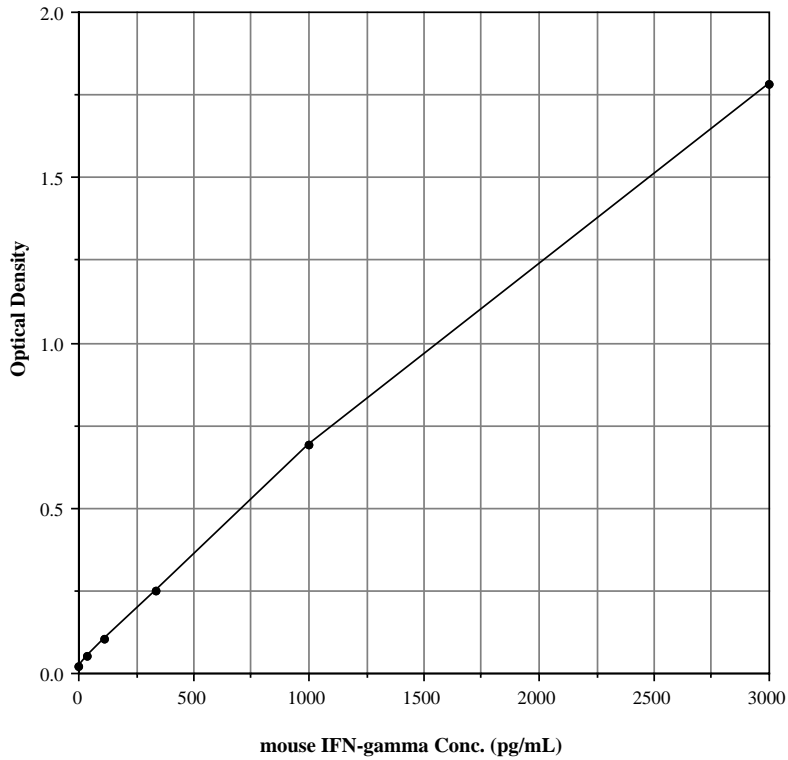
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

<u>Sample</u>	<u>Average OD</u>	<u>Net OD</u>	<u>m IFN-γ (pg/mL)</u>
Blank	(0.010)		
S0	0.029	0.019	0
S1	1.793	1.783	3,000
S2	0.702	0.692	1,000
S3	0.260	0.250	333
S4	0.114	0.104	111
S5	0.062	0.052	37
Unknown 1	0.790	0.780	1,144.8
Unknown 2	0.345	0.335	451.5

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate mouse IFN- γ concentrations; each user must run a standard curve for each assay.



Calibration

The standard in this kit is calibrated to the NIAID/NIH reference standard Gg02-901-533. One (1) pg of Assay Designs' Standard = 0.2 NIAID pg.

Performance Characteristics

Sensitivity: < 10 pg/mL

The sensitivity or Lower Limit of Detection (LLD) is determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Linearity

A sample containing 2,067.2 pg/mL mouse IFN- γ was serially diluted 6 times 1:2 in the standard diluent supplied in the kit and measured in the assay. The data was plotted graphically as actual mouse IFN- γ concentration versus measured mouse IFN- γ concentration.

The line obtained had a slope of 1.091 with a correlation coefficient of 0.994.

Precision

Intra-assay CV: < 10%

Inter-assay CV: < 10%

Cross Reactivities

The TiterZyme[®] mouse IFN- γ EIA Kit is specific for natural and recombinant mouse IFN- γ . It is unaffected by the presence of mouse IL-1 α , IL-1 β , IL-3, IL-4, IL-6, IL-7, IL-10, TNF- α , GM-CSF, human IFN- α or IFN- γ .

Sample Recoveries

Cytokine recovery is determined by spiking 600 pg/mL of natural mouse IFN- γ in to serum and plasma sample pools collected from apparently healthy mice. Values were compared against a Standard Diluent control. Sample values were corrected for any endogenous mouse IFN- γ observed.

<u>Sample Type</u>	<u>Mean</u>	<u>Range</u>
Serum (n=5)	110%	105-115%
Heparin-Plasma (n=5)	106%	95-118%

References

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LIMITED WARRANTY

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Material Safety Data Sheet (MSDS) available on our website or by fax.

**Assay Designs, Inc.
800 Technology Drive
Ann Arbor, MI 48108
U.S.A.**

**Telephone: (734) 668-6113
(800) 833-8651 (USA & Canada only)
Fax: (734) 668-2793
e-mail: info@assaydesigns.com
website: www.assaydesigns.com**

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