Mouse IFN-γ ELISA Kit

For the quantitative determination of mouse interferon-γ (IFN-γ) concentrations in serum sample and cell culture supernatant

Catalogue Number: MEC1002

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (01) mIFN-γ

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INTENDED USE

This Mouse IFN-γ ELISA kit is to be used for the *in vitro* quantitative determination of mouse interferon-γ concentrations in mouse serum and cell culture supernatant. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

IFN-© is a multifunctional cytokine, possessing important immune-modulation, anti-viral and anti-tumor properties. It is produced by natural killer cells and natural killer T cells, plays an important part in innate immunity. $CD4^+$ T helper 1 cells, CD8 cytotoxic T lymphocyte (CTL) and effecter T cells also produce IFN-γ when acquired immunity develops.

IFN-© stimulates its own expression and up-regulates other genes through the Jak-Stat signaling pathway. IFN-γ released after viral infection stimulates the production of large amount of protein kinase R, which phosphorylates transcription initiation factor eIF in response to viral infection. As a results, enzymes critical to mRNA replication is reduced and mRNA replication inhibited.

Experiment with murine cytomegalovirus infected mice (1) showed that natural killer cells are the main source of IFN-γ. In natural killer cell depleted mice, viral hepatitis and viral replication levels were higher than control group. The experiment also demonstrated that inhibition of viral replication is mediated by IFN-©, since inhibition is independent of cytolytic activity. In the same study, administration of IL-12 to the MCMV infected mice was found to induce the production of IFN-© by natural killer cells. Previously (3), interleukin IL-12 has been identified, by Kobayashi *et al,* as natural killer cell stimulatory factor released from Epstein-Barr-virus infected B cells.

IFN-γ influences the emergence of cellular immunity through stimulating the maturation of CD4+ T helper 1 cells (Th1) and cytotoxic CD8+ cells (2). IFN-γ suppresses T helper 2 differentiation and humoral immunity, and was also found to regulate mouse subclass switching (5).

IFN-γ is a potent macrophage activator. It binds to macrophage in conjugation with CD40, causing the macrophage to produce elevated amounts of MHC molecules. It also stimulates the production of antigen-processing associated transporters and enzymes. The accumulation of macrophages and T helper 1 cells around *M. tuberculosis* infected cells is believed to the mechanism for granuloma formation in TB patients. IFN-γ was found to limit infection (4) in chronic granulomatous diseases (CGD). A form of this cytokine has been approved by FDA for treatment of CGD.

Inhibition to cancer growth by IFN-γ is well documented. Recombinant IFN-γ has been used for treatment of various cancer types in combination with chemotherapy. Recent

experiment with mouse skin cancer model showed that IFN-γ plasmid DNA had inhibitory effects in skin carcinogenesis (6), indicating IFN-γ DNA could be useful for cancer gene therapy.

This mouse IFN-γ ELISA is a 4.5 hour solid phase immunoassay readily applicable to measure IFN-γ levels in mouse serum, cell culture supernatant, and other biological fluids in the range of 0 to 3200pg/mL. It is expected to be useful for investigations into the relationship between IFN-γ and various diseases.

PRINCIPLE OF THE ASSAY

This mouse IFN-γ enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal specific for mouse IFN-γ. Standards or samples are then added to the appropriate microtiter plate wells and incubated. In order to quantitatively determine the amount of mouse IFN-γ present in the sample, a biotin-conjugated antibody specific for mouse IFN-γ is added to each well to "sandwich" the IFN-γ immobilized during the first incubation. The microtiter plate then undergoes a second incubation and the wells are thoroughly washed to remove all unbound Biotin-conjugated antibodies. Avidin can bind to biotin with high affinity and amplify the biotin signal. A preparation of avidin conjugated HRP is added to each well and incubated. The avidin HRP conjugate will bind to wells containing biotin. After washing the wells, the unbounded avidin-HRP conjugate is removed. A TMB (3,3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain IFN-γ and biotin-conjugated antibody, avidin conjugated HRP will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm ± 2nm.

In order to measure the concentration of mouse IFN-γ in the samples this kit contains two calibration diluents. (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant). According to the testing system, the provided standard is 2 x fold serial diluted with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus mouse IFN-γ concentration (pg/mL). The concentration of mouse IFN-γ in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **Mouse IFN-γ MICROTITER PLATE** (Part MEC02-1) ***96 wells***
   Pre-coated with anti-Mouse IFN-γ monoclonal antibody.
2. **Mouse IFN-γ BIOTIN CONJUGATE** (Part MEC02-2) ***6 mL***
   Anti-Mouse IFN-γ antibody conjugated to biotin.
3. **AVIDIN HRP CONJUGATE** (Part MEC02-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.
4. **Mouse IFN-γ STANDARD** (Part MEC02-4) ***2 vials***
   Recombinant Mouse IFN-γ (3.2 ng/vial) in a buffered protein base, lyophilized.
5. **CALIBRATOR DILUENT I** (Part MEC02-5) ***25 mL***
   Buffered PBS with BSA and preservative. *For serum/plasma testing.*
6. **CALIBRATOR DILUENT II** (Part MEC02-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant/urine testing.*
7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.
8. **SUBSTRATE A** (Part MEC02-7) ***10 mL***
   Buffered solution with H_2O_2.
9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.
10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. 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.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Mouse serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from Mouse blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate Mouse viruses.
   Solid Wastes: Autoclave 60 min. at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming up to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING, AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C), and serum extracted.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, and cell culture supernatant should be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Substrate 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.

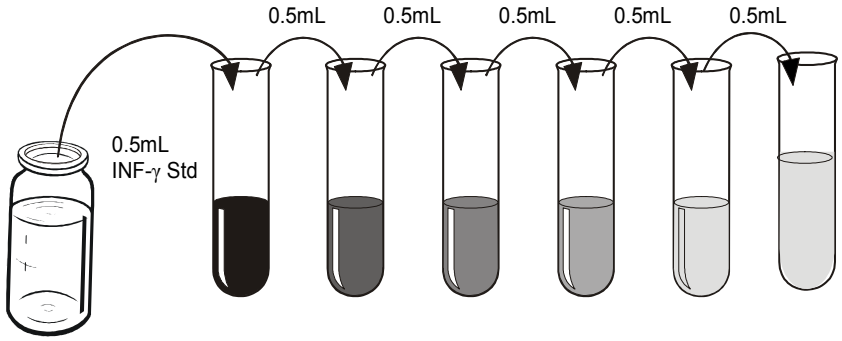
Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **Mouse IFN-γ Standard:**

a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant/urine testing. Reconstitute the IFN-γ Standard with either 1.0mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (1x) (for cell culture supernatant/urine testing). This reconstitution produces a stock

solution of 3200 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IFN-γ standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles. Aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (50 pg/mL to 3200 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IFN-γ Standard will serve as the high standard (3200 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



IFN-γ 3200 pg/mL	1600 pg/ml	800 pg/ml	400 pg/ml	200 pg/ml	100 pg/ml	50 pg/ml

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and mouse IFN- γ Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 – **0 pg/mL** (S1)	**2A, 2B**	Standard 5 - **400 pg/mL** (S5)
1C, 1D	Standard 2 - **50 pg/mL** (S2)	**2C, 2D**	Standard 6 - **800 pg/mL** (S6)
1E, 1F	Standard 3 – **100 pg/mL** (S3)	**2E, 2F**	Standard 7 - **1600 pg/mL** (S7)
1G, 1H	Standard 4 – **200 pg/mL** (S4)	**2G, 2H**	Standard 7 - **3200 pg/mL** (S8)
3A – 12H	Samples		

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 100 μL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate. Add 50μL Anti-mouse INF-r Biotin conjugate to each wells. Mix well. Cover and incubate for 3 hours at room temperature.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Dispense 100μl of Avidin HRP conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.

5. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

6. Repeat wash procedure as described in Step 3.

7. Add 100μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IFN-γ in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IFN-γ concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IFN-γ in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IFN-γ concentration. **If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.**

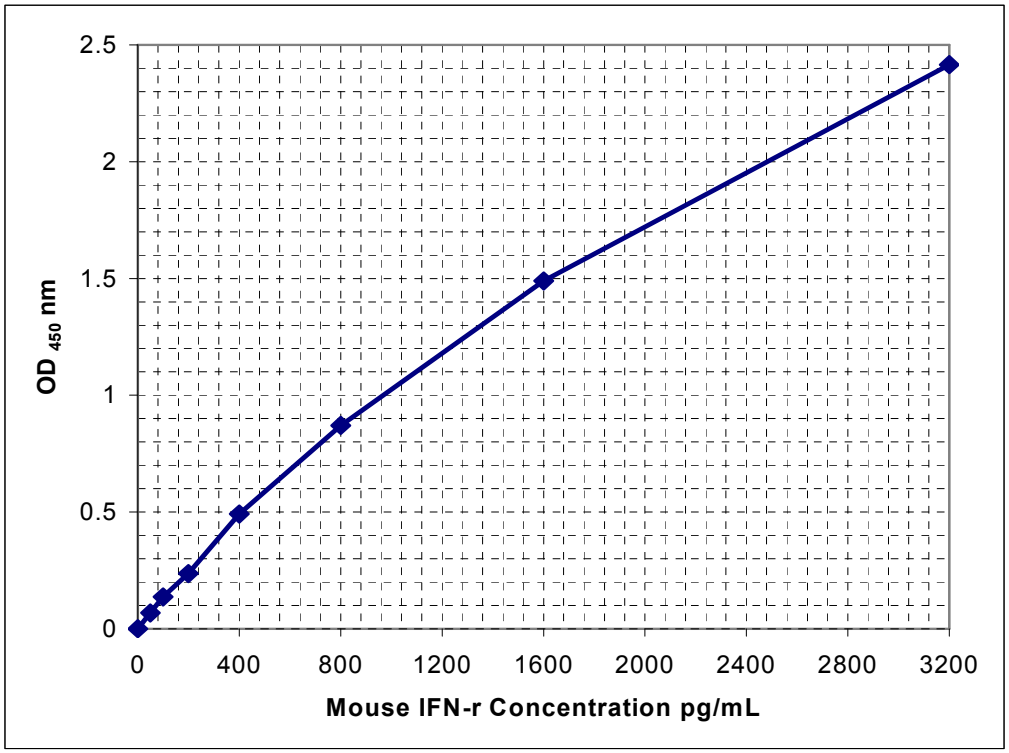
TYPICAL DATA

Results of a typical standard run of mouse IFN-γ ELISA is shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain own standard curve

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

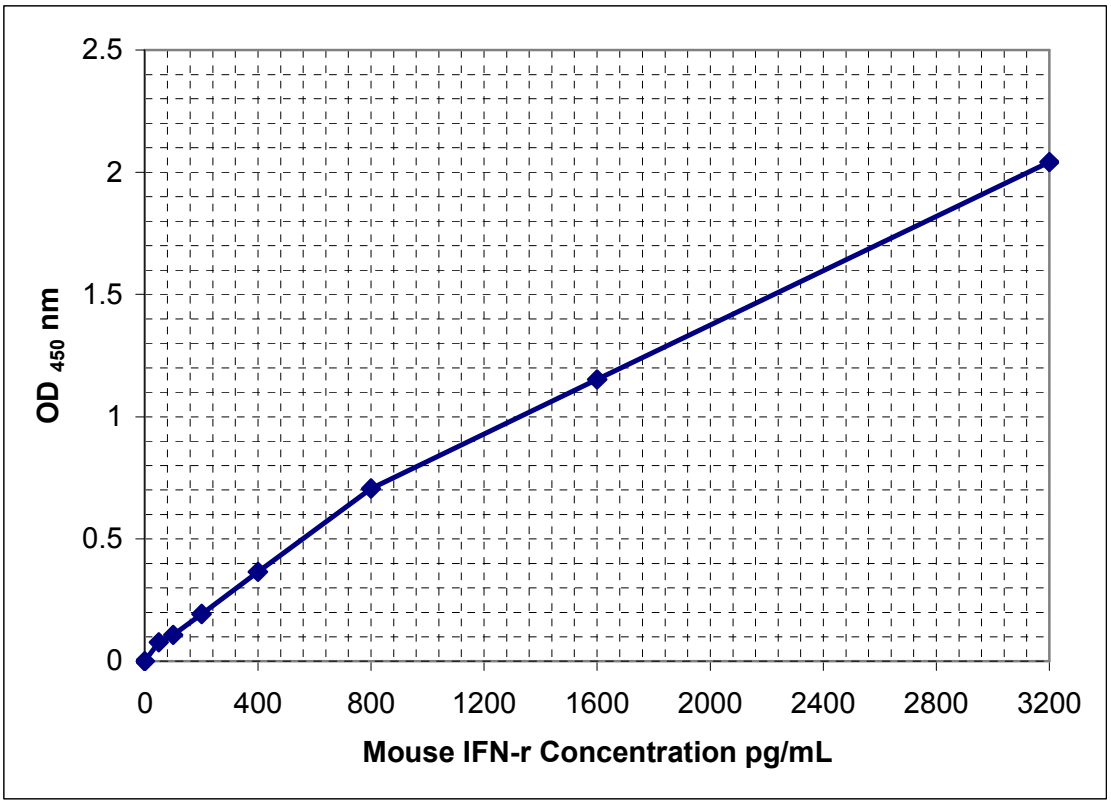
Standard (pg/mL)	O.D. (450 nm)	Zero Standard Subtracted
0	0.043	0
50	0.110	0.067
100	0.179	0.136
200	0.278	0.235
400	0.535	0.492
800	0.913	0.870
1600	1.532	1.489
3200	2.426	2.419



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	O.D. (450 nm)	Zero Standard Subtracted
0	0.055	0
50	0.134	0.078
100	0.164	0.108
200	0.249	0.194
400	0.420	0.365
800	0.762	0.707
1600	1.208	1.153
3200	2.096	2.041



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by replicates of 8 in 1 assay.

	Calibrator Diluent I assay			*Calibrator Diluent II assay*		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
N	8	8	8	8	8	8
Mean (pg/mL)	87.8	182.9	395.6	94.7	212.5	451.4
Standard Deviation (pg/mL)	3.89	4.59	5.91	3.63	3.01	3.02

2. INTER-ASSAY PRECISION

	Calibrator Diluent I assay			*Calibrator Diluent II assay*		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
N	6	6	6	6	6	6
Mean (pg/mL)	96.8	182.9	370	94	189	375
Standard Deviation (pg/mL)	8.80	5.99	5.34	9.89	6.59	5.43

3. RECOVERY

The recovery of mouse IFN-γ spiked to 3 different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Cell culture media	Mouse serum
High level	111 %	119%
Medium level	106 %	98%
Low level	95 %	95%

4. SENSITIVITY

The minimum detectable dose of mouse IFN-γ was determined by adding two standard deviations to the mean optical density value of the 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is *13.1 pg/mL* and using Calibrator Diluent II is *7.5 pg/mL*.

REFERENCES

1. Requirement for natural killer cell-produced interferon gamma in defense against murine cytomegalovirus infection and enhancement of this defense pathway by interleukin 12 administration. JS Orange, B Wang, C Terhorst and CA Biron JEM 1995, 182(4):1045-1056
2. Intracellular IFN-gamma expression in natural killer cells precedes Lung CD8+ T cell recruitment respiratory syncytial virus infection. T Hussell and PJ Openshaw. J Gen Virol 1998, 79(11): 2593-2601
3. Identification and purification of Natural Killer stimulatory factor, a cytokine with multiple biologic effects on human lymphocytes. M Kobayashi, L Fitz, M Ryan, RM Hewick, SC Clark, S Chan, R Loudon, F Sherman, B Perussia and G Trinchieri. JEM 1989, 179:827-845
4. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. "The International Chronic Granulomatous Disease Cooperative Study Group." N. Engl. J. Med. 1991, 324 (8):509-16
5. A two-signal model for regulation of immunoglobulin isotype switching. J. Purkerson and P. Isakson. FASEB Journal 1992, 6:3245-3252
6. Inhibitory effects of interferon-gamma plasmid DNA on DMBA-DBA induced mouse skin carcinogenesis. JH Ko, BG Jung, YS Park and BJ Lee Cancer gene therapy 2011 18:646-654