Quantikine® ELISA

Mouse IFN-γ Immunoassay

Catalog Number MIF00
SMIF00
PMIF00

For the quantitative determination of mouse Interferon gamma (IFN-γ) concentrations in cell culture supernates and serum.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Interferon-gamma (IFN-γ, also known as type II interferon) is an important immunoregulatory cytokine that was originally identified through its anti-viral activity (1, 2). It plays key roles in host defense by exerting anti-viral, anti-proliferative, and immunoregulatory activities (3, 4). On many cell types, IFN-γ induces the production of cytokines and upregulates the expression of various membrane proteins including class I and II MHC antigens, Fc receptors, leukocyte adhesion molecules, and B7 family antigens. IFN-γ is a potent activator of macrophage effector functions. It directs the synthesis, class switching, and secretion of immunoglobulins by B cells. IFN-γ also influences T-helper cell phenotype development by inhibiting Th2 differentiation and stimulating Th1 development (3, 4). IFN-γ plays a central role in the progression of inflammatory diseases such as autoimmunity and atherosclerosis (5, 6).

Biologically active IFN-γ consists of a noncovalently linked homodimer of 20-25 kDa variably glycosylated subunits (7). Mature mouse IFN-γ shares 86% amino acid (aa) sequence identity with rat IFN-γ, and 38-44% aa identity with bovine, canine, cotton rat, equine, feline, human, porcine, and rhesus IFN-γ. IFN-γ dimers bind to transmembrane IFN-γ RI (alpha subunits) which then interact with transmembrane IFN-γ RII (beta subunits) to form the functional receptor complex of two α and two β subunits (8, 9). Inclusion of IFN-γ RII in the receptor complex increases the ligand binding affinity as well as the efficiency of signal transduction (9, 10). Whereas the α-chain is expressed constitutively on many cell types, the cellular regulation of the β-chain correlates with an IFN-γ responsive state and is tightly regulated (8).

IFN-γ is produced by a number of cell types under inflammatory conditions, including dendritic epidermal/γδ T cells (11), keratinocytes (12), peripheral blood γδ T cells (13), mast cells (14), neurons (15), $CD8^+$ T cells (16), macrophages (17), B cells (18), neutrophils (19), NK cells (20), $CD4^+$ T cells (21), and testicular spermatids (22).

The Quantikine® Mouse IFN-γ immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IFN-γ in cell culture supernates and mouse serum. It contains *E. coli*-expressed recombinant mouse IFN-γ and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant mouse IFN-γ accurately. Results obtained using natural mouse IFN-γ showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IFN-γ.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Monoclonal antibody specific for mouse IFN-γ has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IFN-γ present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IFN-γ is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IFN-γ bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # MIF00	CATALOG # SMIF00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IFN-γ Microplates	890475	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IFN-γ.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IFN-γ Standard	890477	3 vials	9 vials	Recombinant mouse IFN-γ in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new Standard and Control for each assay.
Mouse IFN-γ Control	890478	3 vials	9 vials	Recombinant mouse IFN-γ in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse IFN-γ Conjugate	892666	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse IFN-γ conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5Y	895201	1 vial	3 vials	21 mL/vial of a buffered protein solution with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-12	895214	1 vial	3 vials	21 mL/vial of a buffered protein solution with preservatives. *For serum samples.*	
Wash Buffer Concentrate	895003	2 vials	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

MIF00 contains sufficient materials to run ELISAs on two 96 well plates.
SMIF00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PMIF00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

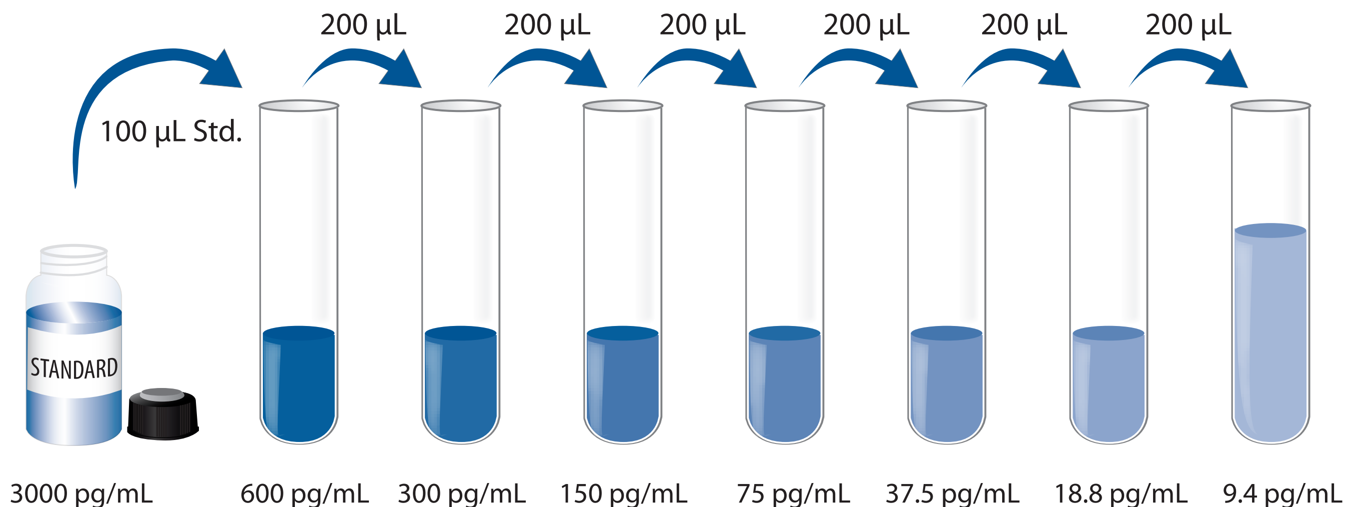
Mouse IFN-γ Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 μL of the resultant mixture is required per well.

Mouse IFN-γ Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IFN-γ Standard with Calibrator Diluent RD5Y (*for cell culture supernate samples*) or Calibrator Diluent RD6-12 (*for serum samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 400 μL of Calibrator Diluent RD5Y *(for cell culture supernate samples)* or Calibrator Diluent RD6-12 *(for serum samples*) into the 600 pg/mL tube. Pipette 200 μL of the appropriate calibrator diluent in the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 600 pg/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and control be assayed in duplicate.

1. Prepare reagents, samples, and standards as directed by the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of standard, control, or sample to each well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided as a record of samples and standards assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse IFN-γ Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

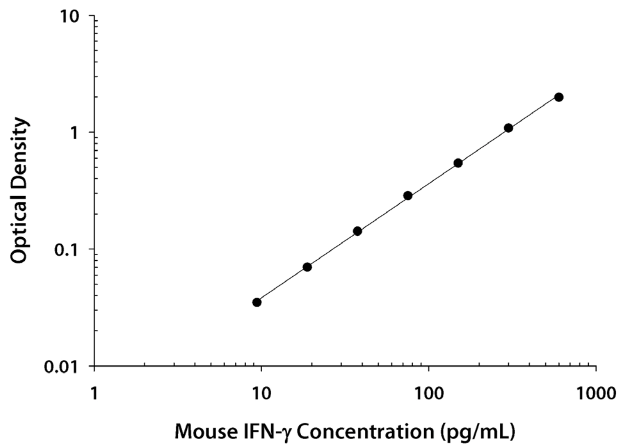
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the mouse IFN-γ concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

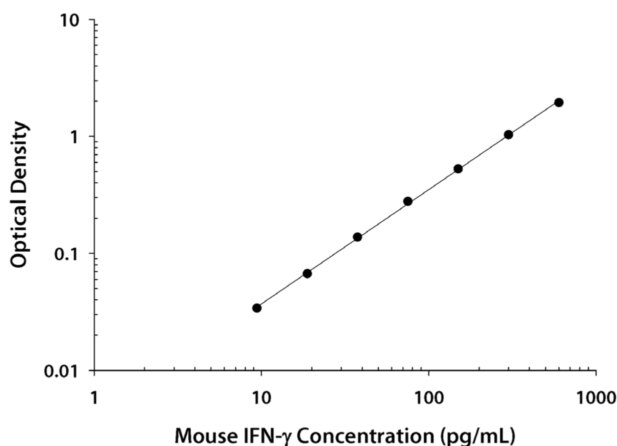
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.042 0.043	0.042	—
9.4	0.077 0.077	0.077	0.035
18.8	0.113 0.112	0.112	0.070
37.5	0.183 0.186	0.184	0.142
75	0.327 0.329	0.328	0.286
150	0.580 0.594	0.587	0.545
300	1.130 1.124	1.127	1.085
600	2.020 2.042	2.031	1.989

SERUM ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.035 0.037	0.036	—
9.4	0.069 0.071	0.070	0.034
18.8	0.099 0.107	0.103	0.067
37.5	0.170 0.179	0.174	0.138
75	0.309 0.318	0.314	0.278
150	0.551 0.576	0.564	0.528
300	1.053 1.081	1.067	1.031
600	1.950 2.005	1.978	1.942

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	44.8	156	518	43.3	144	509
Standard deviation	2.2	5.0	14.2	3.6	14.7	48.6
CV (%)	4.9	3.2	2.7	8.3	10.2	9.5

SERUM ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	45.1	143	528	42.8	139	518
Standard deviation	2.1	4.4	11.8	3.6	13.6	46.5
CV (%)	4.7	3.1	2.2	8.4	9.8	9.0

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=7)	105	98-115%
Serum (n=5)	97	91-106%

SENSITIVITY

The minimum detectable dose (MDD) of mouse IFN-γ is typically less than 2 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

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LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of mouse IFN-γ in each matrix were diluted with the appropriate calibrator diluent and then assayed.

		Cell culture supernates (n=4)	Serum (n=4)
1:2	Average % of Expected	101	97
	Range (%)	99-104	92-101
1:4	Average % of Expected	101	95
	Range (%)	98-104	92-98
1:8	Average % of Expected	104	94
	Range (%)	100-110	91-96
1:16	Average % of Expected	100	92
	Range (%)	95-105	90-93

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse IFN-γ produced at R&D Systems®.

The NIH reference preparation mouse IFN-γ Gg02-901-533 which was intended as a bioassay standard, was evaluated in this kit. Each ampule contains a nominal 1 µg of natural mouse IFN-γ and was assigned an arbitrary unitage of 1000 Units/ampule.

NIH Gg02-901-533: 1 Unit of standard = 230 pg of Quantikine® Mouse IFN-γ

SAMPLE VALUES

Serum - Forty individual mouse serum samples were evaluated for the presence of mouse IFN-γ in this assay. Thirty-eight samples measured less than the lowest Mouse IFN-g Standard, 9.4 pg/mL. Two samples read 24 pg/mL and 60 pg/mL, respectively.

Cell Culture Supernates:
Mouse splenocytes (2 x 10^6 cells/mL) were cultured for 3 days in RPMI plus 10% fetal bovine serum and stimulated with 10 μg/mL PHA. An aliquot of cell culture supernate was removed, assayed for mouse IFN-γ and measured 32 ng/mL.

EL-4 mouse lymphoblast cells (9 x 10^5 cells/mL) were cultured for 2 days in DMEM plus 10% fetal bovine serum and stimulated with 10 μg/mL PHA and 10 ng/mL PMA. An aliquot of cell culture supernate was removed, assayed for mouse IFN-γ and measured 4 ng/mL.

Mouse lung conditioned media (1 lung, 1-2 mm pieces in 10 mL of medium) was collected after culturing for 5 days in RPMI plus 10% fetal bovine serum. An aliquot of cell culture supernate was removed, assayed for mouse IFN-γ and measured 198 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IFN-γ.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant mouse IFN-γ control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:			Recombinant human:
C10	IL-4	KC	IFN-γ
G-CSF	IL-5	LIF	
GM-CSF	IL-6	M-CSF	
IFN-γ RI	IL-7	MIP-1α	
IFN-γ RII	IL-9	MIP-1β	
IFN-κ	IL-10	MIP-2	
IL-1α	IL-10 R	SCF	
IL-1β	IL-12	TNF-α	
IL-2	IL-13	Tpo	
IL-3	JE	VEGF	

A sample containing 66 ng/mL of recombinant rat IFN-γ measured 27 pg/mL in this assay (0.04% cross-reactivity). Upon dilution, the dose-curve of the recombinant rat IFN-γ was parallel to the mouse IFN-γ standard curve.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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NOTES

NOTES

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