Quantikine® ELISA

Human IFN-γ Immunoassay

Catalog Number DIF50
SIF50
PDIF50

For the quantitative determination of human interferon gamma (IFN-γ) concentrations in cell culture supernates, serum, and plasma.

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 human IFN-γ shares 90% amino acid (aa) sequence identity with rhesus IFN-γ, 59-64% with bovine, canine, equine, feline, and porcine IFN-γ, and 37-43% aa identity with cotton rat, mouse, and rat 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 a 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® Human IFN-γ Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IFN-γ levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IFN-γ and antibodies raised against the recombinant factor. Results obtained for naturally occurring human IFN-γ samples showed linear 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 human IFN-γ.

The presence of a naturally occurring form of a receptor for IFN-γ has been reported in normal human urine (23). Until the soluble IFN-γ receptor has been tested in the Quantikine® Human IFN-γ Immunoassay, the possiblity of interference cannot be excluded.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human IFN-γ has been pre-coated onto a microplate. Standards 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 human 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 and color develops in proportion to the amount of IFN-γ bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 calibrator diluent and repeat the assay. If cell culture supernate samples require a large dilution, perform an intermediate dilution with culture media and the final dilution in calibrator diluent.
- Any variation in standard diluent, 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DIF50	CATALOG # SIF50	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IFN-γ Microplate	890582	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human IFN-γ.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IFN-γ Standard	890210	1 vial	6 vials	Recombinant human IFN-γ in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C for up to 1 month in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human IFN-γ Conjugate	890583	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IFN-γ conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-51	895342	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives and blue dye.	
Calibrator Diluent RD6-21	895261	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DIF50 contains sufficient materials to run an ELISA on one 96 well plate.
SIF50 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDIF50). 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.
- 500 mL graduated cylinder.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- **Polypropylene** test tubes for dilution of standards.
- Human IFN-γ Controls (optional; R&D Systems®, Catalog # QC01-1).

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

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. **Heparin and citrate plasma samples cannot be used in this assay.**

Note: *Hemolyzed samples are not suitable for the measurement of human IFN-γ with this assay. Samples with high levels of rheumatoid factor may interfere with the measurement of human IFN-γ in this assay.*

REAGENT PREPARATION

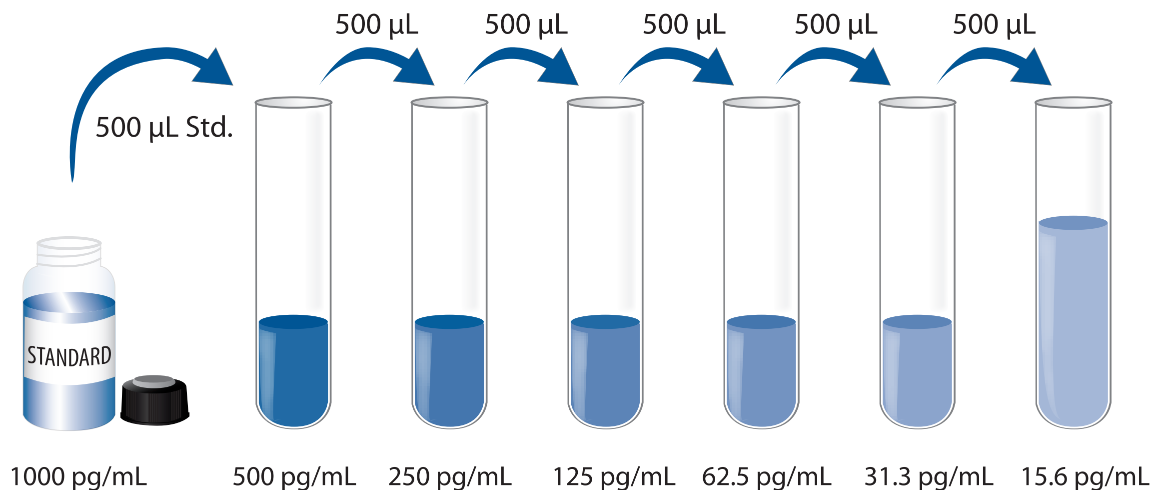
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. 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. 200 µL of the resultant mixture is required per well.

Human IFN-γ Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IFN-γ Standard with Calibrator Diluent RD6-21. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 500 µL of Calibrator Diluent RD6-21 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human IFN-γ Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD6-21 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 standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents and working standards as directed in 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 100 µL of Assay Diluent RD1-51 to each well.
4. Add 100 µL of standard, sample, or control per well. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 µL of Human 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the well should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, 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

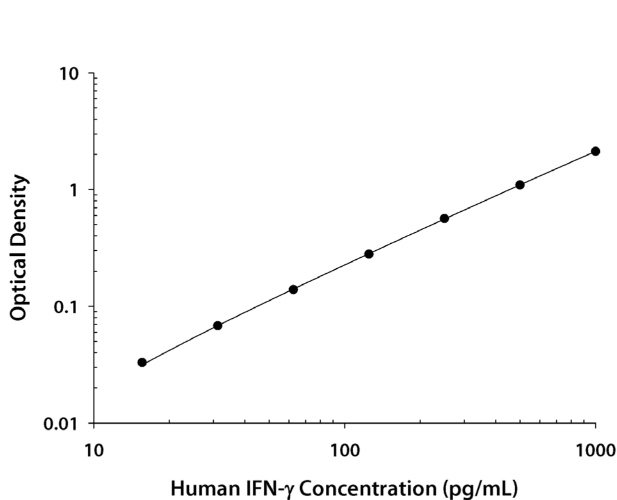
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph. The data may be linearized by plotting the log of the human IFN-γ concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.094 0.093	0.094	—
15.6	0.125 0.129	0.127	0.033
31.3	0.163 0.162	0.162	0.068
62.5	0.231 0.235	0.233	0.139
125	0.373 0.376	0.374	0.280
250	0.644 0.670	0.657	0.563
500	1.190 1.187	1.188	1.094
1000	2.196 2.237	2.216	2.122

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	79.2	214	466	78.7	206	457
Standard deviation	3.7	5.5	13.0	6.1	13.1	17.0
CV (%)	4.7	2.6	2.8	7.8	6.4	3.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	93-109%
Serum (n=5)	102	91-118%
EDTA plasma (n=5)	98	88-111%

SENSITIVITY

The minimum detectable dose (MDD) of human IFN-γ is typically less than 8.0 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.

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IFN-γ in various matrices and diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)
1:2	Average % of Expected	98	100	104
	Range (%)	95-103	98-101	101-109
1:4	Average % of Expected	100	99	107
	Range (%)	93-106	97-103	101-115
1:8	Average % of Expected	98	96	101
	Range (%)	88-109	92-99	94-107
1:16	Average % of Expected	93	89	94
	Range (%)	75-109	84-98	86-99

CALIBRATION

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

The NIBSC/WHO 1st British Standard for human leukocyte IFN-γ (82/587) was evaluated in this kit in July 2008. To convert sample values obtained with the Quantikine® Human IFN-γ kit to approximate NIBSC units, use the equation below:

NIBSC/WHO (82/587) approximate value (U/mL) = 0.017 x Quantikine® Human IFN-γ value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy volunteers were evaluated for the presence of human IFN-γ in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest human IFN-γ standard, 15.6 pg/mL.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL of PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for levels of human IFN-γ.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	453	2651
Stimulated	8360	7851

SPECIFICITY

This assay recognizes natural and recombinant human 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 at 50 ng/mL in a mid-range recombinant human IFN-γ control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
IFN-γ R1

Recombinant mouse:
IFN-γ
IFN-γ R1

Other recombinants:
bovine IFN-γ
canine IFN-γ
cotton rat IFN-γ
equine IFN-γ
feline IFN-γ
porcine IFN-γ
rat IFN-γ
rhesus macaque IFN-γ

Recombinant porcine IFN-γ cross-reacts approximately 0.08% in this assay.

Recombinant rhesus macaque IFN-γ cross-react approximately 0.12% in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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

NOTES

NOTES

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