Quantikine™ QuicKit™ ELISA

Human IFN-β Immunoassay

Catalog Number QK410

For the quantitative determination of human Interferon beta (IFN-β) concentrations in cell culture supernates.

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 beta (IFN-β), also known as fibroblast IFN, is a secreted, approximately 22 kDa member of the type I interferon family of molecules (1). Mature human IFN-β shares 47% and 46% amino acid sequence identity with the mouse and rat proteins, respectively. Fibroblasts are the major producers of IFN-β, but it can also be produced by dendritic cells, macrophages, and endothelial cells in response to pathogen exposure (2). It is transcriptionally regulated by TRAF3, IRF3, IRF7, and NFκB (3, 4). It has also been shown that the RIPK1 and RIPK3 kinases play a role in LPS-induced upregulation of IFN-β in mice (5). Following secretion, IFN-β signals through the heterodimeric IFN-α/β Receptor and activates the JAK/STAT signaling pathway (6-9). IFN-β appears to have a complex role in the regulation of inflammasomes. It has been shown to directly inhibit NLRP1 and NLRP3 inflammasomes in a STAT1-dependent manner and increase the susceptibility of mice to *C. albicans* infection (10). In contrast, *L. monocytogenes* has been shown to activate the NLRP3 inflammasome in an IFN-β-dependent manner (11). Viral infection of human mini-gut organoids induces IFN-β which leads to upregulation of Viperin and IFIT1 IFN-stimulated genes (12).

IFN-β-deficient mice show increased susceptibility to experimental autoimmune encephalomyelitis (EAE), a disease model of human multiple sclerosis (MS) (13). Furthermore, IFN-β has been shown to suppress the Th17 cell response in both MS and EAE and has commonly been used as a treatment for MS (14-18). Low levels of IFN-β have been associated with the hyporesponsive state of monocytes from sepsis patients, suggesting that IFN-β may have a role in restoring monocyte function and reversing immunosuppression (19). Type I IFNs (IFN-α and IFN-β) appear to have a role in the polarization of neutrophils in cancer. Inflammation, along with functional type I IFN signaling, was shown to alter neutrophil polarization towards anti-tumor phenotype (20).

The Quantikine™ QuicKit™ Human IFN-β Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human IFN-β levels in cell culture supernates. It contains CHO cell-expressed recombinant human IFN-β and antibodies raised against the recombinant protein. Results obtained using natural human IFN-β showed linear curves that were parallel to the standard curves obtained using the QuicKit standards. These results indicate that this kit can be used to determine relative mass values for natural human IFN-β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked monoclonal detection antibody, specific for human IFN-β. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of IFN-β bound. 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, further dilute the samples with the calibrator diluent and repeat the assay.
- Any variation in 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™ QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuicKit™ Coated Microplate	899063	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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.*
Human IFN-β Standard	899201	2 vials of recombinant human IFN-β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IFN-β Capture Ab Concentrate	899199	Lyophilized tagged monoclonal antibody specific for human IFN-β.	May be stored for up to 1 month at 2-8 °C.*
Human IFN-β Detection Ab Concentrate	899200	400 µL of a monoclonal antibody specific for human IFN-β conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-38	895301	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

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
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human IFN-β Controls (optional; R&D Systems®, Catalog # QC284)

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 SDS 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.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human IFN-β Capture Ab Concentrate - Refer to the vial label for reconstitution volume. Reconstitute the Human IFN-β Capture Ab Concentrate with Assay Diluent RD1-38. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

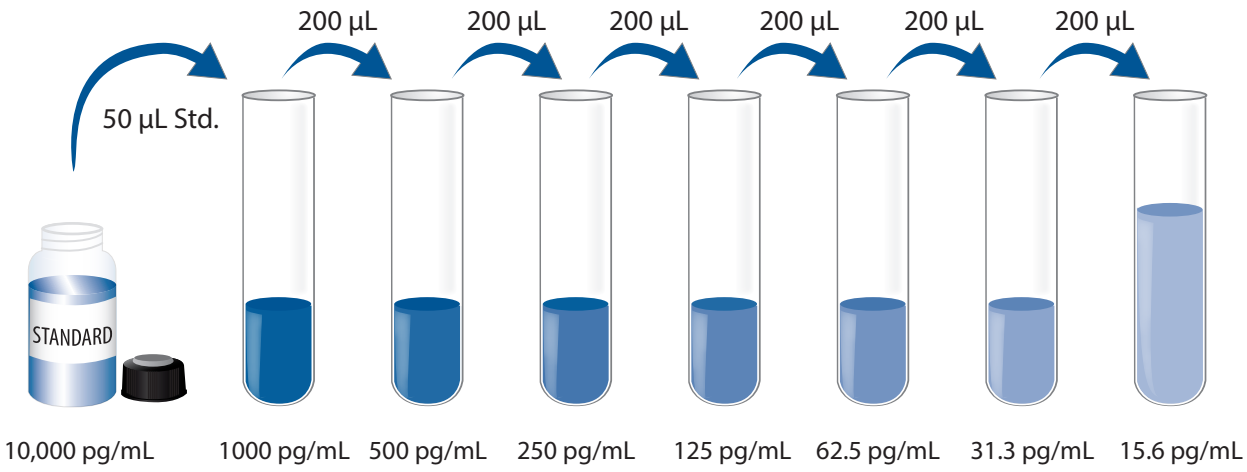
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-38. For a full plate, add 300 µL of reconstituted Human IFN-β Capture Ab stock and 300 µL of Human IFN-β Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-38 to get 6 mL of Human IFN-β Antibody Cocktail.

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 480 mL of 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.

Human IFN-β Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human IFN-β Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-5 into the 1000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-5 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, controls, and samples be assayed in duplicate.

1. Prepare all reagents, samples, 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 50 µL of standard, control, or sample per well.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. 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

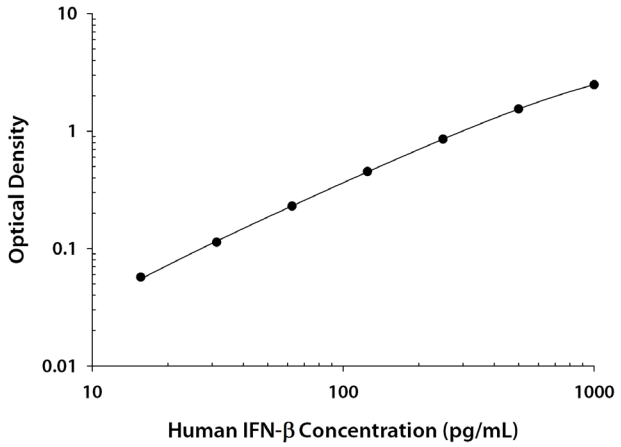
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-β 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	0.006 0.006	0.006	—
15.6	0.056 0.057	0.057	0.051
31.3	0.112 0.113	0.113	0.107
62.5	0.227 0.232	0.230	0.224
125	0.450 0.456	0.453	0.447
250	0.842 0.865	0.854	0.848
500	1.516 1.572	1.544	1.538
1000	2.474 2.495	2.485	2.479

PRECISION

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

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	**1**	**2**	**1**	**2**
n	20	20	10	10
Mean (pg/mL)	101	613	121	669
Standard deviation	3.82	30.1	6.48	33.3
CV (%)	3.8	4.9	5.4	5.0

RECOVERY

The recovery of human IFN-β spiked to three levels in samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=6)	104	90-117 %

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human IFN-β were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=5)
1:2	Average % of Expected	96
	Range (%)	93-98
1:4	Average % of Expected	97
	Range (%)	95-100
1:8	Average % of Expected	100
	Range (%)	96-103
1:16	Average % of Expected	104
	Range (%)	97-115

SENSITIVITY

Eighteen assays were evaluated and the minimum detectable dose (MDD) of human IFN-β ranged from 0.270-2.88 pg/mL. The mean MDD was 0.925 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.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human IFN-β produced at R&D Systems®.

SAMPLE VALUES

Cell Culture Supernates:
PBMCs were isolated from a single donor over a Ficoll-Paque PLUS density gradient. PBMCs were seeded at 5 x 10^6/mL and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were left untreated or treated with 10 µg/mL of poly I:C in the presence of Lipofectamine 2000 for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-β.

Condition	pg/mL
Untreated	ND
Treated	323

ND=Non-detectable

A549 adenocarcinomic human alveolar basal epithelial cells were cultured in Kaighn's Nutrient Mixture F-12 supplemented with 10% fetal bovine serum until nearly confluent. The cells were left untreated or treated with 10 µg/mL of poly I:C in the presence of Lipofectamine 2000 for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-β.

Condition	pg/mL
Untreated	ND
Treated	1470

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IFN-β.

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 low level recombinant human IFN-β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IFN-α/β R1
IFN-α/β R2
IFNA-2
IFN-γ
IL-6

Recombinant mouse:

IFN-β1

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