

# Quantikine<sup>®</sup> ELISA

## Human IFN- $\alpha$ All Subtype Immunoassay

Catalog Number DFNAS0

For the quantitative determination of human Interferon alpha (IFN- $\alpha$ ) 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

There are 3 major classes of interferons (IFNs): Type I, Type II and Type III. Interferon alpha (IFN- $\alpha$ ), along with IFN- $\beta$ , IFN- $\delta$ , IFN- $\epsilon$ , IFN- $\kappa$ , IFN- $\omega$  and IFN- $\tau$  are all Type I IFNs (1). The sole type II IFN is IFN- $\gamma$ . Type III IFNs include IFN- $\lambda$ 1, IFN- $\lambda$ 2, IFN- $\lambda$ 3 and IFN- $\lambda$ 4 (2). As a part of the innate immune response, Type I IFNs are rapidly induced in response to viral nucleic acids such as double stranded DNA or RNA (dsDNA, dsRNA) and single stranded RNA (ssRNA), viral glycoproteins, microbial cytosine-phosphate-guanosine (CpG) DNA, DNA damage, and chromosomal instability (3,4).

IFN- $\alpha$  subtypes are well described (5-9). There are 15 human IFN- $\alpha$  subtypes with 80% amino acid identity (10). The number of IFN- $\alpha$  subtypes varies by species with 6 equine subtypes, 17 porcine subtypes, 14 bovine subtypes, and 9 canine subtypes known currently. Human IFN- $\alpha$  subtypes include: IFN- $\alpha$ 1a, IFN- $\alpha$ 1b, IFN- $\alpha$ 2a, IFN- $\alpha$ 2b, IFN- $\alpha$ 4a, IFN- $\alpha$ 4b, IFN- $\alpha$ 5, IFN- $\alpha$ 6, IFN- $\alpha$ 7, IFN- $\alpha$ 8, IFN- $\alpha$ 10, IFN- $\alpha$ 14, IFN- $\alpha$ 16, IFN- $\alpha$ 17, and IFN- $\alpha$ 21. Although there is one known heterodimeric IFN- $\alpha$  receptor (IFN- $\alpha$ R, described below), each IFN- $\alpha$  subtype has been correlated with differing biological activities (7). Variability in biological responses can be attributed to differences in binding affinity and duration, receptor density, feedback responses and intracellular characteristics (11). IFN- $\alpha$  responses have been described as robust, especially in the context of viral infection responsiveness by all cells or tunable in a cell type specific manner.

IFN- $\alpha$  signaling is well characterized (2, 5, 11, 12, 13). IFN- $\alpha$  is a ligand for IFN $\alpha$ R, which includes two subunits IFN $\alpha$ R1 and IFN $\alpha$ R2. IFN- $\alpha$  ligand binding to the ubiquitously expressed IFN $\alpha$ R1 triggers a conformational change which allows for the heterodimerization of IFN $\alpha$ R1 and IFN $\alpha$ R2 (10). Heterodimerization results in the cross phosphorylation of the Janus-activated Kinase 1 (JAK1) on IFN $\alpha$ R2 and tyrosine kinase 2 (TYK2) on IFN $\alpha$ R1 respectively, as well as the intracellular domain of IFN $\alpha$ R1 and IFN $\alpha$ R2 (9). The transcription factors Signal transducer and activator of transcription (STAT) 1 and 2 are subsequently recruited to IFN $\alpha$ R via their Src homology 2 (SH2) domain and phosphorylated. In the canonical IFN- $\alpha$  signaling pathway, phosphoSTAT1/STAT2 heterodimers associate with Interferon Regulatory Factor 9 (IRF9) to form Interferon Stimulated Gene Factor 3 (ISGF3), which translocates into the nucleus to bind to gamma-activated sequences (GASs) or interferon-stimulated response elements (ISREs). This stimulates the transcription of interferon-stimulated genes (ISGs). STAT 2 homodimers and monomers can also associate with IRF-9 to form ISGF3-like complex, which also binds to ISRE to stimulate the transcription of ISGs (9).

Although IFN- $\alpha$  is most commonly associated with viral infections, it has been associated with other pathological events. Type 1 interferons represent a standard of care for suppressing Hepatitis B (HBV) or C (HCV) (14). However, it has been associated with neuropsychiatric symptoms such as depression, anhedonia, anxiety and cognitive impairment (15). The role of IFN- $\alpha$  in cancer is complex as well. For example, in the context of inflammatory breast cancer, IFN- $\alpha$  is upregulated. Although IFN- $\alpha$  has been correlated with cellular senescence and apoptosis, some subtypes have been correlated with increased cellular migration and drug resistance.

The Quantikine® Human IFN- $\alpha$  All Subtype Immunoassay is a 4.5 hour solid phase ELISA designed to measure all human IFN- $\alpha$  subtypes in cell culture supernates, serum, and plasma. It contains HEK293-expressed recombinant human IFN- $\alpha$ 2a and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IFN- $\alpha$  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- $\alpha$  subtypes.

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IFN- $\alpha$  has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN- $\alpha$  present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IFN- $\alpha$  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- $\alpha$  bound in the initial step. The color development is stopped and the intensity of the color is measured.

This assay recognizes the natural and recombinant human IFN- $\alpha$  subtypes listed below.

### **Recombinant human:**

IFNA-1 $\alpha$

IFNA-1 $\beta$

IFNA-2

IFNA-2 $\beta$

IFNA-4A

IFNA-4B

IFNA-5

IFNA-6

IFNA-7

IFNA-8

IFNA-10

IFNA-14

IFNA-16

IFNA-17

IFNA-21

## 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.
- Standards, controls, and samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with 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® 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IFN- $\alpha$ All Subtype Microplate	899170	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IFN- $\alpha$ .	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- $\alpha$ All Subtype Standard	899172	2 vials of recombinant human IFN- $\alpha$ in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Use a new standard for each assay. Discard after use.
Human IFN- $\alpha$ All Subtype Conjugate	899171	21 mL of a polyclonal antibody specific for human IFN- $\alpha$ conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-4	895435	21 mL of a concentrated buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
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  $\pm$  50 rpm
- Test tubes for dilution of standards and samples
- Human IFN- $\alpha$  Controls (optional; R&D Systems®, Catalog # QC278)

## 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  $\leq -20$  °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  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay.*

## SAMPLE PREPARATION

Cell culture supernate samples may require dilution due to high endogenous levels.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

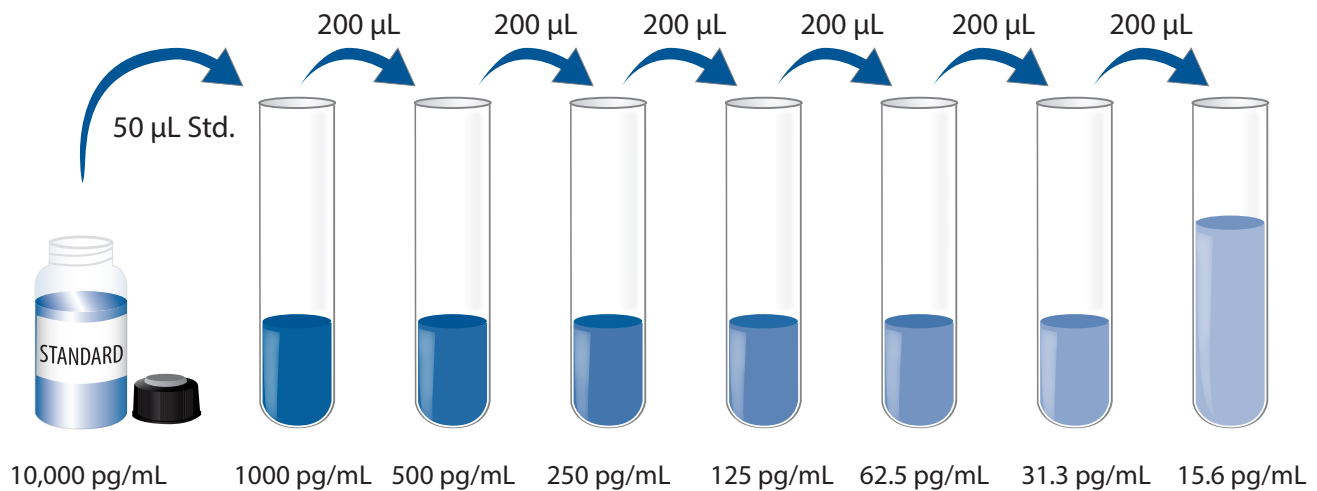
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

**Human IFN- $\alpha$  All Subtype Standard - Refer to the vial label for reconstitution volume.**

Reconstitute the Human IFN- $\alpha$  All Subtype Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Pipette 450  $\mu$ L of Calibrator Diluent RD5-4 into the 1000 pg/mL tube. Pipette 200  $\mu$ L 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 RD5-4 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, working standards, and samples 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  $\mu\text{L}$  of Assay Diluent RD1W to each well.
4. Add 50  $\mu\text{L}$  of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm  $\pm$  50 rpm. A plate layout is provided to record samples and standards 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  $\mu\text{L}$ ) 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  $\mu\text{L}$  of Human IFN- $\alpha$  All Subtype Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50  $\mu\text{L}$  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.
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

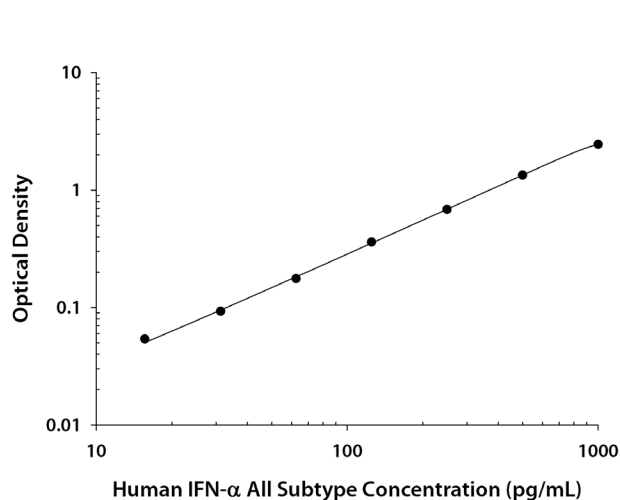
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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- $\alpha$  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	0.013 0.015	0.014	—
15.6	0.054 0.054	0.054	0.040
31.3	0.093 0.093	0.093	0.079
62.5	0.172 0.180	0.176	0.162
125	0.340 0.362	0.351	0.337
250	0.685 0.686	0.686	0.672
500	1.319 1.366	1.343	1.329
1000	2.433 2.479	2.456	2.442

## 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.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	178	340	698	185	354	737
Standard deviation	3.62	8.35	20.9	9.50	20.5	41.0
CV (%)	2.0	2.5	3.0	5.1	5.8	5.6

## RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	96-103%
Serum (n=4)	89	83-94%
EDTA plasma (n=4)	92	89-96%
Heparin plasma (n=4)	91	88-96%

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IFN- $\alpha$  were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=5)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	103	100	102
	Range (%)	95-101	100-107	98-102	98-107
1:4	Average % of Expected	99	106	104	107
	Range (%)	98-102	102-108	103-107	103-112
1:8	Average % of Expected	101	110	107	110
	Range (%)	98-104	103-112	105-108	103-121
1:16	Average % of Expected	102	111	108	113
	Range (%)	99-106	107-114	105-114	104-121

\*Samples were diluted prior to assay.

## SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of human IFN- $\alpha$  ranged from 0.315-4.80 pg/mL. The mean MDD was 1.07 pg/mL.

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

## CALIBRATION

This immunoassay is calibrated against a highly purified HEK293 expressed recombinant human IFN- $\alpha$  produced at R&D Systems®.

The NIBSC/WHO IFN- $\alpha$  International Standard 94/784 (Human leukocyte-derived) was evaluated in this kit. The dose response curve of the reference reagent 94/784 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human IFN- $\alpha$  All Subtype kit to approximate NIBSC/WHO 94/784 Units, use the equation below.

NIBSC/WHO (94/784) approximate value (IU/mL) = 0.355 x Quantikine® Human IFN- $\alpha$  All Subtype value (pg/mL)

**Note:** Based on data generated in April 2020.

## SAMPLE VALUES

**Serum/Plasma** - Ten serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human IFN- $\alpha$  in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest IFN- $\alpha$  standard, 15.6 pg/mL.

### Cell Culture Supernates:

Human peripheral blood mononuclear cells ( $5 \times 10^6$  cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. Cells were untreated or treated with 10  $\mu$ g/mL of poly I:C in the presence of Lipofectamine 2000 for 24 hours. Aliquots of the culture supernates were removed and assayed for levels of human IFN- $\alpha$ .

Condition	Donor 1 (pg/mL)	Donor 2 (pg/mL)	Donor 3 (pg/mL)
Untreated	ND	ND	ND
Treated	887	350	1826

ND=Non-detectable

## SPECIFICITY

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- $\alpha$  control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

IFNA- $\alpha$ / $\beta$  R $\alpha$   
IFNA- $\alpha$ / $\beta$  R1  
IFNA- $\alpha$ / $\beta$  R2  
IFNB-1  
IFNE  
INFG  
IFNL-4  
IL-28A  
IL-28B  
IL-29  
TYK-2

### Recombinant mouse:

IFNA-4  
IFNA-11  
IFNA-13

### Other recombinant:

cotton rat IFN- $\alpha$

The mouse IFN- $\alpha$  subtypes listed below cross-react in this assay at < 6%. No interference was observed.

### Recombinant mouse:

IFNA-1  
IFNA-2  
IFNA-6  
IFNA-7  
IFNA-15/IFNAA heterodimer  
IFNA-16  
IFNAB

Recombinant mouse IFNA-9 cross-reacts approximately 0.75% and interferes at concentrations > 5,000 pg/mL in this assay.

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# PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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

# NOTES

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