Quantikine™ HS ELISA

Human IFN-α2 Immunoassay

Catalog Number HSIFNA2

For the quantitative determination of human Interferon alpha 2 (IFN-α2) 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.

TABLE OF CONTENTS

SECTION PAGE

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

There are 3 major classes of interferons (IFNs): Type I, Type II and Type III. Interferon alpha (IFN-α), along with IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-ω and IFN-τ are all Type I IFNs (1). The sole type II IFN is IFN-γ. Type III IFNs include IFN-λ1, IFN-λ2, IFN-λ3 and IFN-λ4 (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-α subtypes are well described (5-9). There are 16 human IFN-α subtypes with 80% amino acid identity (10). The number of IFN-α subtypes varies by species with 6 equine subtypes, 17 porcine subtypes, 14 bovine subtypes, and 9 canine subtypes known currently. Human IFN-α subtypes include: IFN-α1a, IFN-α1b, IFN-α2a, IFN-α2b, IFN-α2c, IFN-α4a, IFN-α4b, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α14, IFN-α16, IFN-α17, and IFN-α21. Although there is one known heterodimeric IFN-α receptor (IFN-αR, described below), each IFN-α 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-α 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-α signaling is well characterized (2, 5,11,12,13). IFN-α is a ligand for IFNαR, which includes two subunits IFNαR1 and IFNαR2. IFN-α ligand binding to the ubiquitously expressed IFNαR1 triggers a conformational change which allows for the heterodimerization of IFNαR1 and IFNαR2 (10). Heterodimerization results in the cross phosphorylation of the Janus-activated Kinase 1 (JAK1) on IFNαR2 and tyrosine kinase 2 (TYK2) on IFNαR1 respectively, as well as the intracellular domain of IFNαR1 and IFNα2 (9). The transcription factors Signal transducer and activator of transcription (STAT) 1 and 2 are subsequently recruited to IFNαR via their Src homology 2 (SH2) domain and phosphorylated. In the canonical IFN-α 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).

INTRODUCTION *CONTINUED*

Although IFN-α 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). It has been associated with neuropsychiatric symptoms such as depression, anhedonia, anxiety and cognitive impairment (15). The role of IFN-α in cancer is complex as well. For example, in the context of inflammatory breast cancer, IFN-α is upregulated. Although IFN-α has been correlated with cellular senescence and apoptosis, some subtypes have been correlated with increased cellular migration and drug resistance.

The Quantikine™ HS Human IFN-α2 Immunoassay is a 4.0 hour solid phase ELISA designed to measure human IFN-α2 (IFN-α2a, IFN-α2b, IFN-α2c) in cell culture supernates, serum, and plasma. It contains HEK293-expressed recombinant human IFN-α2a and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IFN-α2 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-α2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IFN-α2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN-α2 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated monoclonal antibody specific for human IFN-α2 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin polymer is added to the wells. After washing away any unbound streptavidin polymer-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IFN-α2 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.
- 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-α2 HS Microplate	899479	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IFN-α2.	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-α2 HS Standard	899481	2 vials of recombinant human IFN-α2 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IFN-α2 HS Conjugate	899480	21 mL of a monoclonal antibody specific for human IFN-α2 conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-63	895352	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-17	895512	21 mL of a buffered protein base with preservatives.	
Streptavidin Polymer-HRP Diluent	898387	21 mL of a solution with preservatives.	
Streptavidin Polymer-HRP (100X)	898350	0.3 mL of Streptavidin Polymer-HRP in a buffer with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) 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 2N sulfuric acid.	
Plate Sealers	N/A	8 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.
- 1000 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 and samples
- Human IFN-α2 HS Controls (optional; R&D Systems®, Catalog # QC297)

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.

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 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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

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

Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

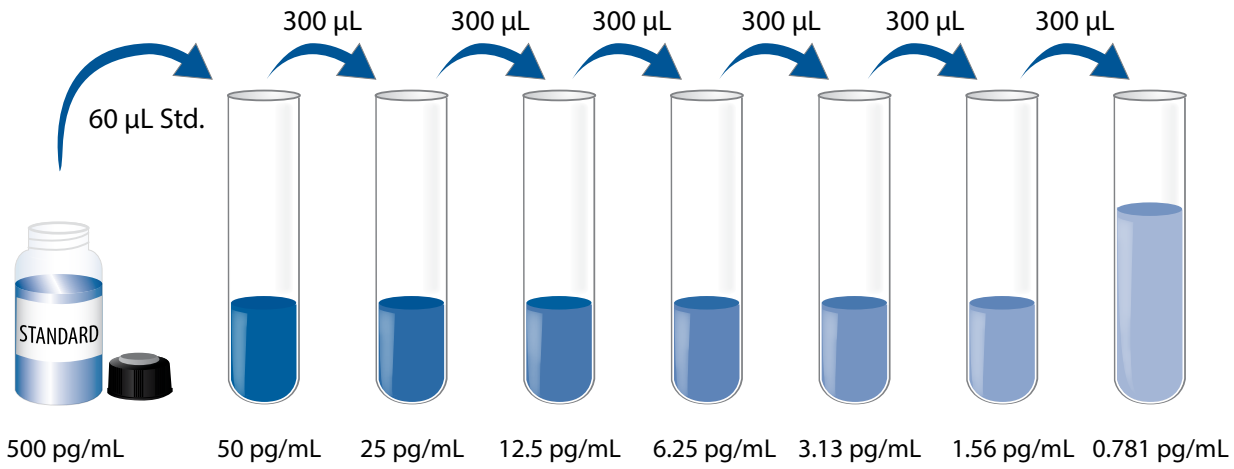
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

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.

Streptavidin Polymer-HRP (1X) - Add 0.215 mL of Streptavidin Polymer-HRP (100X) directly to the Streptavidin Polymer-HRP Diluent. Mix well.

Human IFN-α2 HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IFN-α2 HS Standard with deionized or distilled water. This reconstitution produces a stock solution of 500 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 540 µL of Calibrator Diluent RD5-17 into the 50 pg/mL tube. Pipette 300 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 pg/mL standard serves as the high standard. Calibrator Diluent RD5-17 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 100 µL of Assay Diluent RD1-63 to each well.
4. Add 50 µL 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 ± 50 rpm. 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-α2 HS Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 200 µL of Streptavidin Polymer-HRP (1X) to each well. Cover with a new adhesive strip. Incubate for **30 minutes** at room temperature on the shaker.
9. Repeat the wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for **30 minutes** at room temperature **on the benchtop. Protect from light.**
11. 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.
12. 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.

*Sample may require dilution.

CALCULATION OF RESULTS

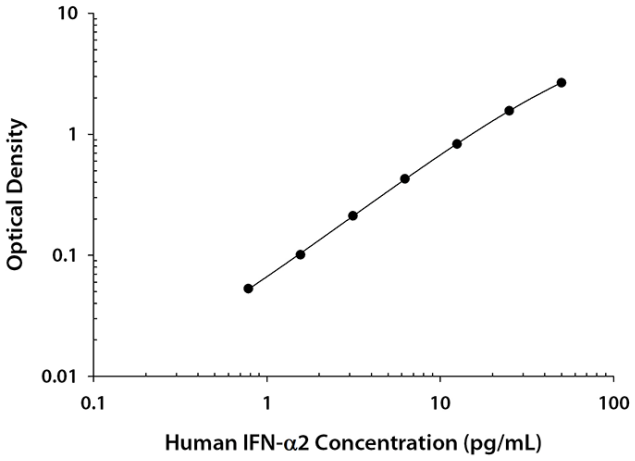
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-α2 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	0.041 0.042	0.042	—
0.781	0.093 0.097	0.095	0.053
1.56	0.141 0.145	0.143	0.101
3.13	0.250 0.257	0.254	0.212
6.25	0.458 0.481	0.470	0.428
12.5	0.870 0.875	0.873	0.831
25	1.593 1.618	1.606	1.564
50	2.703 2.710	2.707	2.665

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	2.38	15.1	32.3	2.65	15.5	29.7
Standard deviation	0.082	0.686	1.36	0.150	0.965	1.69
CV (%)	3.4	4.5	4.2	5.7	6.2	5.7

RECOVERY

The recovery of human IFN-α2 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	90-108%
Serum (n=4)	86	75-99%
EDTA plasma (n=4)	89	75-101%
Heparin plasma (n=4)	86	75-92%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IFN-α2 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates* (n=9)	Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	100	98	103	106	98
	Range (%)	93-108	95-103	94-110	102-110	87-109
1:4	Average % of Expected	103	99	109	111	107
	Range (%)	95-114	95-105	102-118	107-115	98-119
1:8	Average % of Expected	101	99	111	110	110
	Range (%)	94-112	94-106	103-120	97-116	102-119
1:16	Average % of Expected	100	101	118	116	118
	Range (%)	95-109	95-109	111-121	114-119	112-122

*Samples were diluted prior to assay.

CALIBRATION

This immunoassay is calibrated against a highly purified HEK293-expressed recombinant human IFN-α2a produced at R&D Systems®.

The following NIBSC/WHO International Standard materials were evaluated. The dose response curves of the reference reagents parallel the Quantikine™ standard curve. To convert sample values obtained with the Quantikine Human IFN-α2 HS kit to approximate NIBSC/WHO Units, use the appropriate equations below.

94/784 - The NIBSC/WHO Human IFN-α International Standard (Human leukocyte-derived) was evaluated in this kit.

NIBSC/WHO (94/784) approximate value (IU/mL) = 0.9577 x Quantikine Human IFN-α2 HS value (pg/mL)

95/650 - The NIBSC/WHO Human IFN-α2a International Standard (*E. coli*-expressed) was evaluated in this kit.

NIBSC/WHO (95/650) approximate value (IU/mL) = 0.1980 x Quantikine Human IFN-α2 HS value (pg/mL)

95/566 - The NIBSC/WHO Human IFN-α2b International Standard (*E. coli*-expressed) was evaluated in this kit.

NIBSC/WHO (95/566) approximate value (IU/mL) = 0.1183 x Quantikine Human IFN-α2 HS value (pg/mL)

95/580 - The NIBSC/WHO Human IFN-α2c International Standard 95/580 (*E. coli*-expressed) was evaluated in this kit.

NIBSC/WHO (95/580) approximate value (IU/mL) = 0.1431 x Quantikine Human IFN-α2 HS value (pg/mL)

Note: *Based on data generated in November 2021.*

SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of human IFN-α2 ranged from 0.038-0.107 pg/mL. The mean MDD was 0.070 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.

SAMPLE VALUES

Serum/Plasma - Ten serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human IFN-α2 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest IFN-α2 standard, 0.781 pg/mL.

Cell Culture Supernates:
Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) from individual donors were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were untreated or treated with 10 ug/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-α2.

Condition	Donor 1 (pg/mL)	Donor 2 (pg/mL)	Donor 3 (pg/mL)
Untreated	ND	ND	ND
Treated	469	363	521

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human (rh) IFN-α2.

The human factors listed below were prepared at 200 pg/mL and 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 200 pg/mL and 50 ng/mL in a mid-range recombinant human IFN-α2 control were assayed for interference. No significant cross-reactivity or interference was observed.

The mouse 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-α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human (200 pg/mL):	Recombinant human (50 ng/mL):	Recombinant mouse:
IFN-α1a	IFN-α/β Rα	IFN-α1
IFN-α1b	IFN-α/β R1	IFN-α2
IFN-α4a	IFN-α/β R2	IFN-α4
IFN-α4b	IFN-β1	IFN-α6
IFN-α5	IFN-ε	IFN-α7
IFN-α6	IFN-γ	IFN-α9
IFN-α7	IFN-λ4	IFN-α11
IFN-α8	IκBK-α	IFN-α12
IFN-α10	IκBK-β	IFN-α13
IFN-α14	IL-28A	IFN-α15/IF-αA
IFN-α16	IL-28B	IFN-α16
IFN-α17		IFN-αB
IFN-α21		IFN-β1

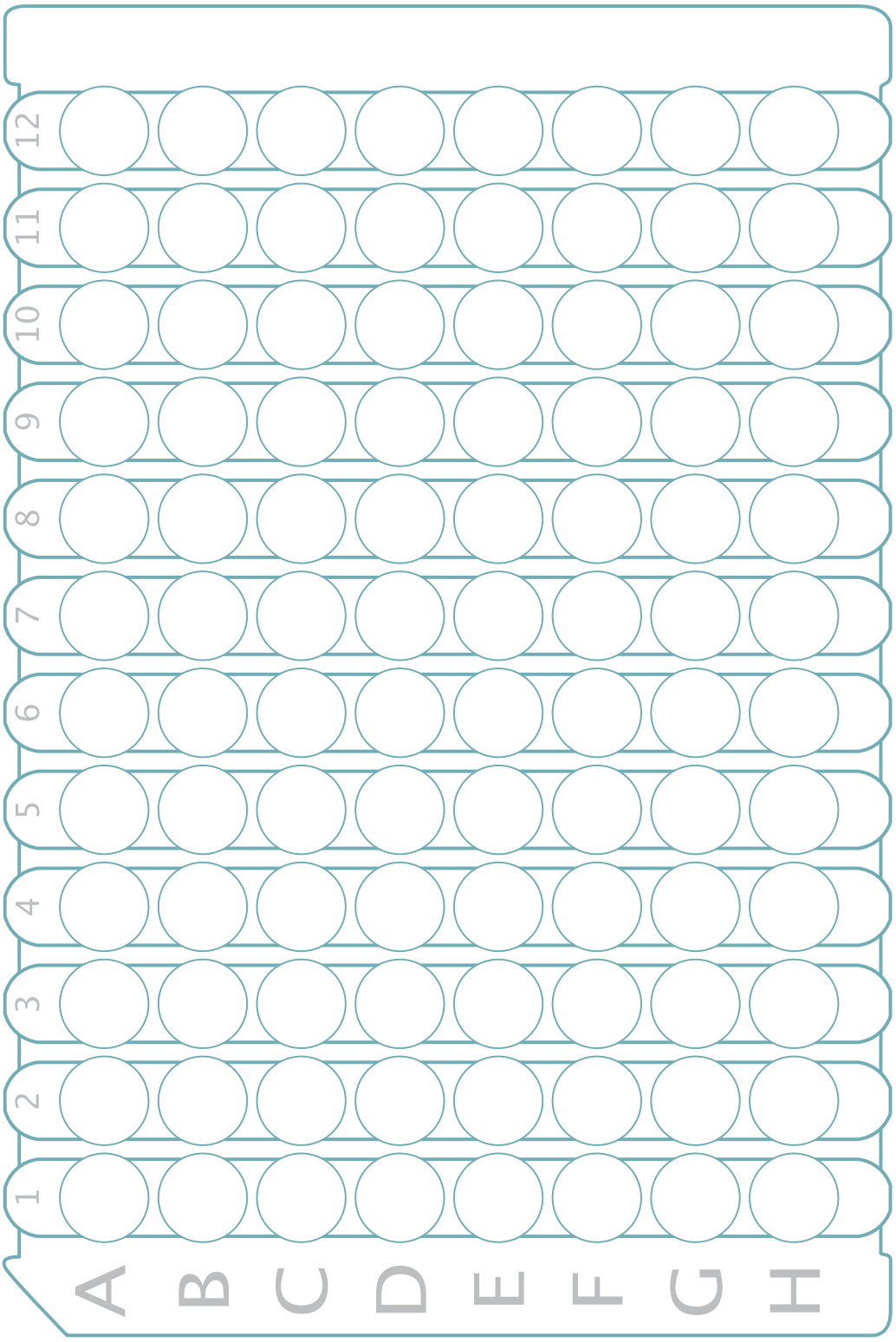
This assay detects rhIFN-α2a, rhIFN-α2b, and rhIFN-α2c.

REFERENCES

1. Murira, A. & A. Lamarre (2016) Front. Immunol. **7**:1
2. Lazear, H. M. *et al.* (2019) Immunity. **50**:909
3. Tomasello, E. *et al.* (2014) Front. Immunol. **5**:1
4. Fuertes, M.B. *et al.* (2013) Trends Immunol. **34**:67
5. Ivashkiv, L. B. *et al.* (2014) Nat. Rev. Immunol. **14**:36
6. Li, S. *et al.* (2018) Cell. Phys. And Biochem. **51**:2377
7. Gibbert, K. *et al.* (2010) Br. J of Pharm. **168**:1048
8. Moll, H. P. *et al.* (2011) Cytokine. **53**:52
9. Provance, O. K. & J. Lewis-Wambi (2019) Breast Cancer Res. **21**:59
10. Paul, F. *et al.* (2015) Gene. **567**:132
11. Schreiber, G. (2017) JBC. **292**:7285
12. Heise, R. *et al.* (2015) PLOS One. **11**:1
13. Blaszczyk, K. *et al.* (2016) Cytokine & Growth Factors Rev. **29**:71
14. Lin, F. and H.A. Young (2014) Cytokines & Growth Factor Rev. **25**:369
15. Murakami, Y. and Y. Imamura (2017) *Pharmacokinetics and Adverse Effects of Drugs*. Intech Open: p366

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



All trademarks and registered trademarks are the property of their respective owners.