Quantikine® HS ELISA

Human IFN-γ Immunoassay

Catalog Number HSDIF0

For the quantitative determination of human IFN-γ concentrations in 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) is an important immunomodulatory cytokine, affecting both the innate and adaptive immune systems. It was discovered in 1965 as a soluble anti-viral factor and has since been shown to promote host defense against a wide variety of pathogens (1, 2). Additionally, it has been shown to promote autophagy and apoptosis, and to have anti-proliferative, anti-angiogenic, and anti-tumorigenic properties (1, 3, 4). IFN-γ is primarily secreted by natural killer (NK) cells (5-7), activated $CD8^+$ T cells (8), Th1 $CD4^+$ T cells (9), NKT cells (10, 11), and macrophages (12-16), but it has also been shown to be produced by a number of other cell types including dendritic cells (17), γδ T cells (18), group 1 ILCs (19), keratinocytes (20), neutrophils (21), mast cells (22), and neurons (23).

The biologically active form of IFN-γ is a non-covalently linked homodimer (24), which binds with high affinity to IFN-γ R1/CD119 and subsequently recruits IFN-γ R2 to form the functional heterotetrameric receptor complex. Formation of this complex leads to phosphorylation and activation of the Janus kinases, Jak1 and Jak2, which in turn phosphorylate and activate STAT1. STAT1 homodimerizes and translocates to the nucleus where it binds to IFN-γ-activated sequence (GAS) elements in the promoters of target genes to regulate their transcription. Many of the IFN-γ/STAT1 target genes are transcription factors that then drive the expression of secondary response genes. Additionally, IFN-γ signaling has been shown to activate MAPK, PI 3-K/Akt, and the NF-κB signaling pathways, leading to the expression of multiple other genes. IFN-γ signaling plays a key role in host defense by promoting macrophage activation, upregulating the expression of antigen processing and presentation molecules, driving the development and activation of Th1 cells, enhancing natural killer cell activity, regulating B cell functions, and inducing the production of chemokines that promote effector cell trafficking to sites of inflammation.

Due to its immunoregulatory activities, IFN-γ has been used as a therapeutic agent for treating a range of bacterial, fungal, helminth, protozoan, and viral infections, immunodeficiency syndromes, multi-drug resistant tuberculosis (MDR-TB), and sepsis (1, 25-34). Additionally, it has been used as an anti-tumor agent to improve patient survival in a number of different types of cancer due to its pro-apoptotic and anti-angiogenic effects (3, 35). In contrast, IFN-γ has been suggested to be involved in the progression of cardiac diseases as elevated levels of this cytokine have been detected in the serum of patients with chronic heart failure, as well as in atherosclerotic lesions and in myocardial tissues of patients with Chagas' cardiomyopathy (1, 36, 37). Similarly, high levels of IFN-γ have been found in the serum and/or cerebrospinal fluid of patients with neurodegenerative diseases such as Amyotrophic lateral sclerosis and Parkinson's disease (38, 39), suggesting that IFN-γ may also be involved in neurodegenerative disease progression and serve as a clinical biomarker. Additionally, there is recent evidence suggesting that IFN-γ may also have context-dependent proliferative and pro-tumorigenic effects (3).

The Quantikine® HS Human IFN-γ Immunoassay is a 4.0 hour solid-phase ELISA designed to measure human IFN-γ in serum and plasma. It contains HEK293-expressed recombinant human IFN-γ and antibodies raised against the recombinant protein. Results obtained using natural human IFN-γ 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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal 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, a biotinylated monoclonal antibody specific for human IFN-γ is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin is added to the wells. After washing away any unbound streptavidin 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.
- 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-γ HS Microplate	899179	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human IFN-γ HS Standard	899181	2 vials of recombinant human IFN-γ in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard for each assay. Discard after use.
Human IFN-γ HS Conjugate	899180	21 mL of a monoclonal antibody specific for human IFN-γ 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 RD5P	895151	21 mL of a buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
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 2 N 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
- 100 mL and 1000 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Polypropylene** test tubes for dilution of standards and samples
- Human IFN-γ HS Controls (optional; R&D Systems®, Catalog # QC280)

PRECAUTIONS

IFN-γ is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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.

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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *IFN-γ is found in saliva. Wear face mask and gloves to protect kit reagents from contamination.*

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.

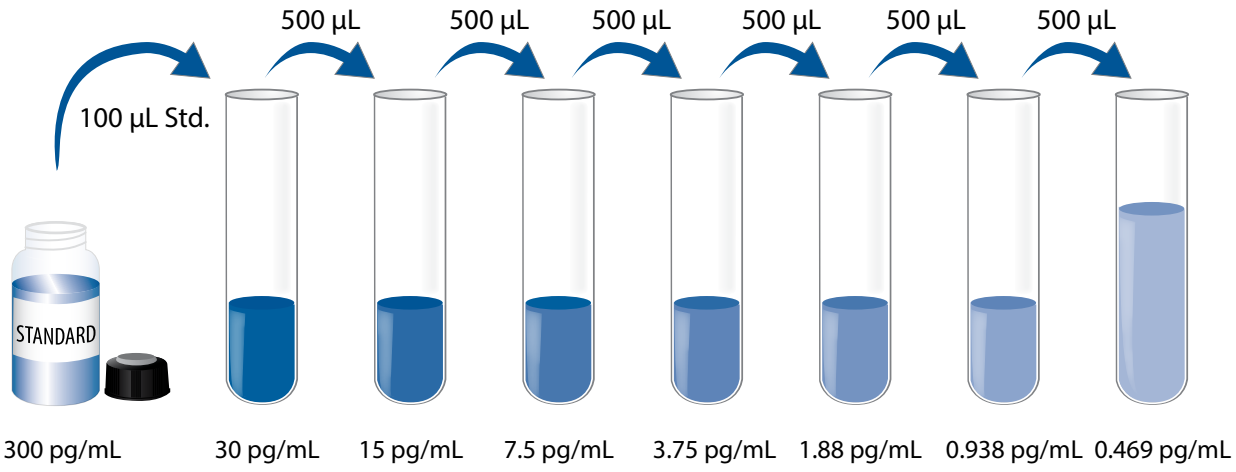
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

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

Human IFN-γ HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IFN-γ Standard with deionized or distilled water. This reconstitution produces a stock solution of 300 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. **Note:** *Do not use vortex.*

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) into the 30 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The standard 30 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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.

Note: *IFN-γ is found in saliva. Wear a face mask and gloves to protect kit reagents from contamination.*

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 100 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-γ 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.

CALCULATION OF RESULTS

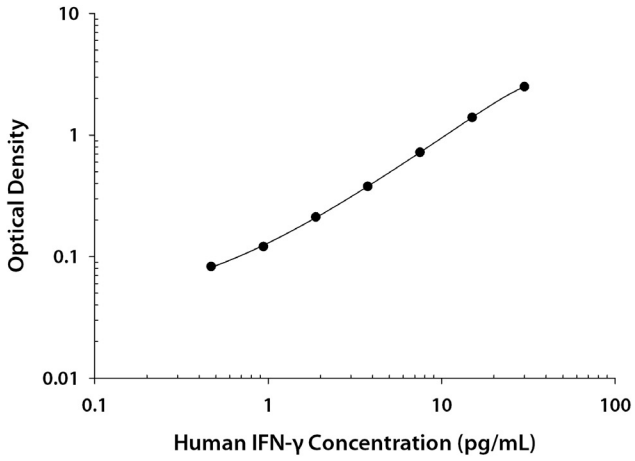
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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	0.039 0.043	0.041	—
0.469	0.077 0.089	0.083	0.042
0.938	0.115 0.126	0.121	0.080
1.88	0.208 0.215	0.212	0.171
3.75	0.376 0.381	0.379	0.338
7.50	0.719 0.724	0.722	0.681
15.0	1.389 1.411	1.400	1.359
30.0	2.454 2.549	2.502	2.461

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)	1.83	6.10	12.8	2.15	6.33	12.4
Standard deviation	0.084	0.187	0.253	0.210	0.421	0.898
CV (%)	4.6	3.1	2.0	9.8	6.7	7.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum (n=4)	97	85-109%
EDTA plasma (n=4)	99	90-108%
Heparin plasma (n=4)	99	92-109%

LINEARITY

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

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	104	103	101
	Range (%)	101-106	98-107	98-105
1:4	Average % of Expected	111	105	104
	Range (%)	97-121	98-111	98-112
1:8	Average % of Expected	113	110	108
	Range (%)	104-124	102-121	98-124
1:16	Average % of Expected	112	109	106
	Range (%)	103-119	100-122	96-119

SENSITIVITY

Twenty-four assays were evaluated and the minimum detectable dose (MDD) of human IFN-γ ranged from 0.025-0.173 pg/mL. The mean MDD was 0.078 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 highly purified HEK293-expressed recombinant human IFN-γ produced at R&D Systems®.

The NIBSC/WHO Human IFN-γ International Standard 82/587 (Lectin-stimulated human leukocyte derived) was evaluated in this kit. The dose response curve in this International Standard parallels the Quantikine® HS standard curve.

To convert sample values obtained with the Quantikine® HS Human IFN-γ kit to approximate NIBSC/WHO 82/587 International units, use the equation below.

NIBSC/WHO (82/587) approximate value (IU/mL) = 0.0141 x Quantikine® HS Human IFN-γ value (pg/mL)

Based on data generated from January 2020.

SAMPLE VALUES

Serum/Plasma - 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.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=30)	0.658	23	ND-1.00
EDTA plasma (n=30)	0.697	23	ND-1.07
Heparin plasma (n=30)	0.691	23	ND-1.04

ND=Non-detectable

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-α1a
IFN-α1b
IFN-α2a
IFN-α2
IFN-α2a/IFNα-1b Heterodimer
IFN-α4a
IFN-α4b
IFN-α5
IFN-α6
IFN-α7
IFN-α8
IFN-α10
IFN-α14
IFN-α16
IFN-α17
IFN-α21
IFNβ
IFN-γ R1/IFN-γ Rα Heterodimer
IFN-γ R1
IFN-γ R2
IL-28A
IL-28B
IL-29

Other recombinants:

bovine IFN-γ
canine IFN-γ
cotton rat IFN-γ
equine IFN-γ
feline IFN-γ
mouse IFN-γ
mouse IFN-γ R1
porcine IFN-γ
rat IFN-γ

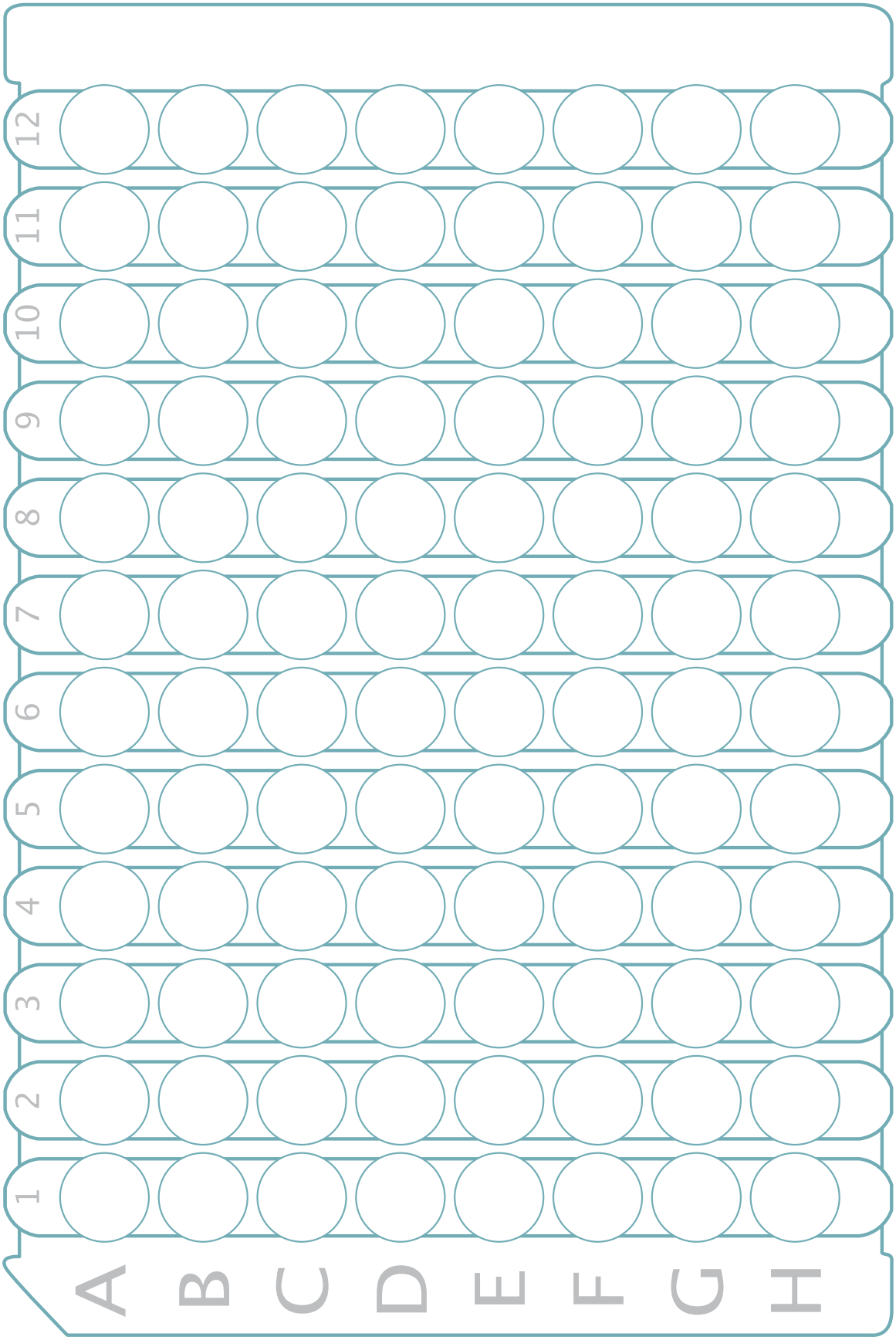
Recombinant rhesus macaque IFN-γ cross-reacts approximately 0.18% in this assay with interferences at concentrations > 1.25 ng/mL.

REFERENCES

1. Kak, G. *et al.* (2018) Biomol Concepts. **9**:64.
2. Wheelock, E.F. (1965) Science **146**:310.
3. Castro, F. *et al.* (2018) Front. Immunol. **9**:847.
4. Burke, J.D. & H.A. Young (2019) Semin. Immunol. **43**(101280).
5. Scharton, T.M. & P. Scott (1993) J. Exp. Med. **178**:567.
6. Bancroft, G.J. *et al.* (1987) J. Immunol. **139**:1104.
7. Sher, A. *et al.* (1993) J. Immunol. **150**:3982.
8. Denton, A. E. *et al.* (2011) Proc. Natl. Acad. Sci. **108**:15306.
9. Swanson, M.A. *et al.* (2001) J. Immunol. **166**:232.
10. Brigl, M. *et al.* (2003) Nat. Immunol. **4**:1230.
11. Crowe, N.Y. *et al.* (2002) J. Exp. Med. **196**:119.
12. Fultz, M.J. (1993) Int. Immunol. **5**:1383.
13. Munder, M. *et al.* (1998) J. Exp. Med. **187**:2103.
14. Wang, J. *et al.* (1999) J. Clin. Invest. **103**:1023.
15. Robinson, C.M. & G.J. Nau. (2008) J. Infect Dis. **198**:359.
16. Robinson, C.M. *et al.* (2010) J. Innate Immun. **2**:56.
17. Pan, J. *et al.* (2004) Immunol. Lett. **94**:141.
18. Gao, Y. *et al.* (2003) J. Exp. Med. **198**:433.
19. Colonna, M. (2018) Immunity **48**: 1104.
20. Howie, S.E. *et al.* (1996) J. Invest. Dermatol. **106**:1218.
21. Ethuin, F. *et al.* (2004) Lab Invest. **84**:1363.
22. Ackermann, L. *et al.* (1999) Br. J. Dermatol. **140**:624.
23. Neumann, H. *et al.* (1997) J. Exp. Med. **186**:2023.
24. Gray, P.W. & D.V. Goeddel (1982) Nature **298**:859.
25. Rhein, B.A. *et al.* (2015) PLoS Pathogens **11**:e1005263.
26. Delsing, C.E. *et al.* BMC Infect. Dis. **14**:166.
27. Skerrett, S.J. & T.R. Martin (1994) Am. J. Respir. Crit. Care Med. **149**:50.
28. Segal, B.H. & T.J. Walsh (2006) Am. J. Respir. Crit. Care Med. **173**:707.
29. Malmvall, B.E. & P. Follin (1993) Scan. J. Infect. Dis. **25**:61.
30. Badaro, R. *et al.* (1990) New. Engl. J. Med. **322**:16.
31. Milanes-Virelles, M.T. *et al.* (2008) BMC Infect. Dis. **8**:17.
32. Hashemi, H. *et al.* (2017) J. Res. Med. Sci. **22**:53.
33. Condos, R. *et al.* (1997) Lancet **349**:1513.
34. Vincent, J-L. *et al.* (2002) Clin. Infect. Dis. **34**:1084.
35. Saleiro, D. & L.C. Platanias (2019) Semin. Immunol. **43**(101299).
36. Voloshyna, I. *et al.* (2014) Trends Cardiovasc. Med. **24**:45.
37. Levick, S.P. (2014) Heart Fail. Rev. **19**:227.
38. Liu, J. *et al.* (2015) PLoS One **10**:e0136937.
39. Mount, M.P. *et al.* (2007) J. Neurosci. **27**:3328.

PLATE LAYOUT

Use this plate layout to record standards, controls, and samples assayed.



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