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R&D SYSTEMS

Quantikine™ QuicKit™ ELISA

Human IFN-γ Immunoassay

Catalog Number QK285

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) 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 antiangiogenic 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™ QuicKit™ Human IFN-γ Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human IFN-γ levels in cell culture supernates, 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 QuicKit 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. 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-γ. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of IFN-γ 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 from other lots or sources.
- If samples generate values higher than the highest standard, 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-γ Standard	899255	2 vials of recombinant human IFN-γ 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-γ Capture Ab Concentrate	899253	Lyophilized tagged monoclonal antibody specific for human IFN-γ.	May be stored for up to 1 month at 2-8 °C.*
Human IFN-γ Detection Ab Concentrate	899254	400 µL of a monoclonal antibody specific for human IFN-γ conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-19	895467	11 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
- 50 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human IFN-γ Controls (optional; R&D Systems®, Catalog # QC290)

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.*

SAMPLE PREPARATION

Serum, plasma, and cell culture supernates can be tested neat. Some supernates may require dilution due to high endogenous levels.

Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human IFN-γ Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IFN-γ Capture Ab Concentrate with Assay Diluent RD1-19. 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 Ab stock can be stored for 4 weeks at 2-8 °C.

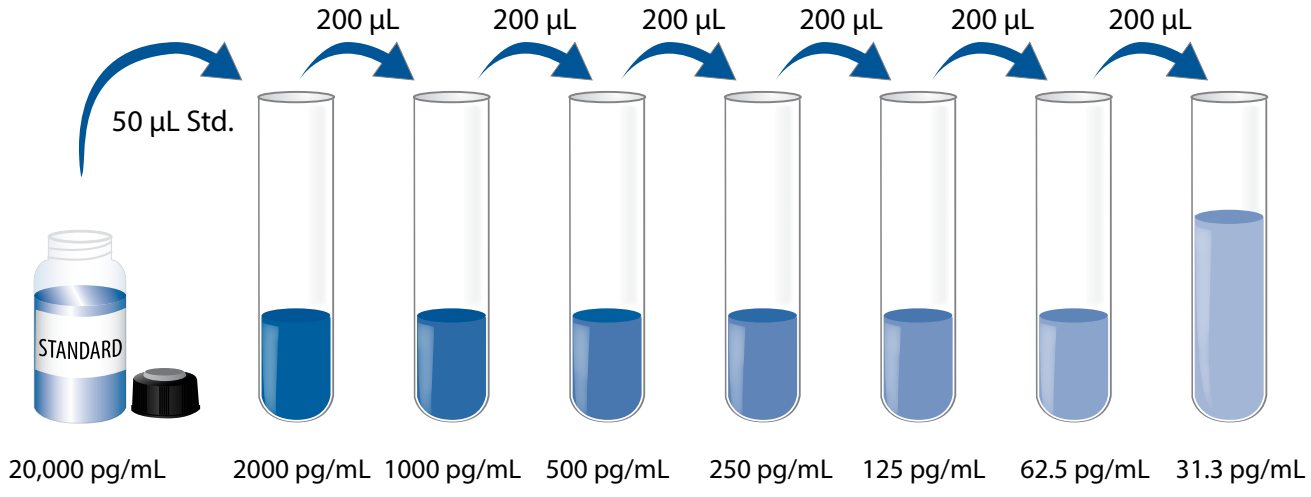
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-19. For a full plate, add 300 µL of reconstituted Human IFN-γ Capture Ab Concentrate and 300 µL of Human IFN-γ Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-19.

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-γ 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 20,000 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 2000 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 2000 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, 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 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

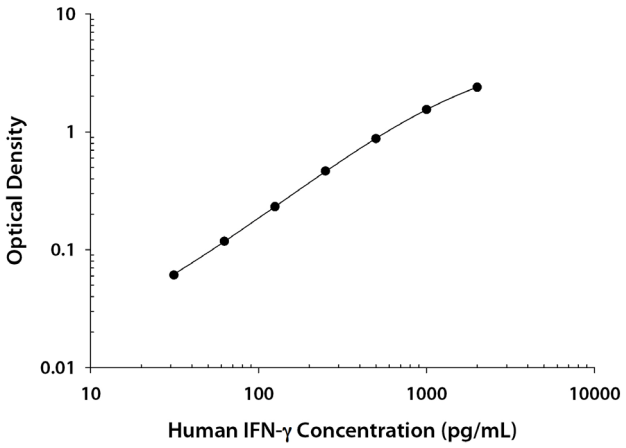
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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	0.005 0.006	0.006	——
31.3	0.065 0.068	0.067	0.061
62.5	0.121 0.126	0.124	0.118
125	0.235 0.241	0.238	0.232
250	0.470 0.472	0.471	0.465
500	0.880 0.882	0.881	0.875
1000	1.520 1.586	1.553	1.547
2000	2.369 2.417	2.393	2.387

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)	198	1156	211	1233
Standard deviation	3.76	20.2	20.4	56.9
CV (%)	1.9	1.8	9.6	4.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	92	83-102%
Serum (n=4)	93	74-114%
EDTA plasma (n=4)	107	88-132%
Heparin plasma (n=4)	105	88-115%

LINEARITY

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

		Cell culture supernates (n=7)	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)
1:2	Average % of Expected	98	89	97	93
	Range (%)	92-108	81-97	90-104	83-103
1:4	Average % of Expected	100	94	94	93
	Range (%)	95-107	87-101	84-104	80-106
1:8	Average % of Expected	102	104	95	96
	Range (%)	95-113	89-118	75-114	76-117
1:16	Average % of Expected	102	95	84	86
	Range (%)	95-109	79-112	68-100	67-105

SENSITIVITY

Sixteen assays were evaluated and the minimum detectable dose (MDD) of human IFN-γ ranged from 0.332-2.56 pg/mL. The mean MDD was 0.964 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 HEK293-expressed recombinant human IFN-γ produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Ten serum and 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. All samples measured less than the lowest standard, 31.3 pg/mL.

Cell Culture Supernates:

Peripheral blood mononuclear cells (multiple donors) (seeded at 1 x 10^6/mL) 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 left untreated or treated with 10 µg/mL of PHA for 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-γ.

$CD4^+$ T cells were isolated from human PBMCs (single donor) using the MagCellect™ Human $CD4^+$ T cell Isolation Kit (R&D Systems, Catalog # MAGH102). $CD4^+$ T cells were then seeded at 5 x 10^5/mL and cultured in ExCellerate Human T Cell Expansion Media, Xeno-Free (R&D Systems, Catalog # CCM030). T cells were left untreated or treated for 5 days. Treated T cell media was supplemented with 10 ng/mL GMP recombinant human (rh) IL-7 (R&D Systems, Catalog # 207-GMP) and 10 ng/mL GMP rhIL-15 (R&D Systems, Catalog # 247-GMP). TCR stimulation was mediated using either (1) immobilized Human CD3 epsilon Antibody (R&D Systems, Catalog # MAB100, coated at 1 µg/mL) with 5 µg/mL soluble Human CD28 Antibody (R&D Systems, Catalog # MAB342) or (2) 25 µL Cloudz CD3/28 particles (Cloudz™ T Cell Activation Kit-CD3/CD28, R&D Systems, Catalog # CLD001) per mL of culture media. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-γ.

Condition	(pg/mL)
Unstimulated	ND
Stimulated PBMCs	111,232
Unstimulated $CD4^+$ T Cell	ND
$CD4^+$ T Cell CD3/CD28	52,293
$CD4^+$ T Cell Cloudz	191,034

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

Recombinant human:

IFN-α1a
IFN-α1b
IFN-α2
IFN-α2a
IFN-α4a
IFN-α4b
IFN-α5
IFN-α6
IFN-α7
IFN-α8
IFN-α10
IFN-α14
IFN-α16
IFN-α17
IFN-α21
IFN-α2a + IFN-α1b complex
IFN-β1
IFN-γ R1
IFN-γ R2
IL-28A
IL-28B
IL-29

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

Recombinant rhesus macaque IFN-γ cross-reacts approximately 1.43% in this assay.

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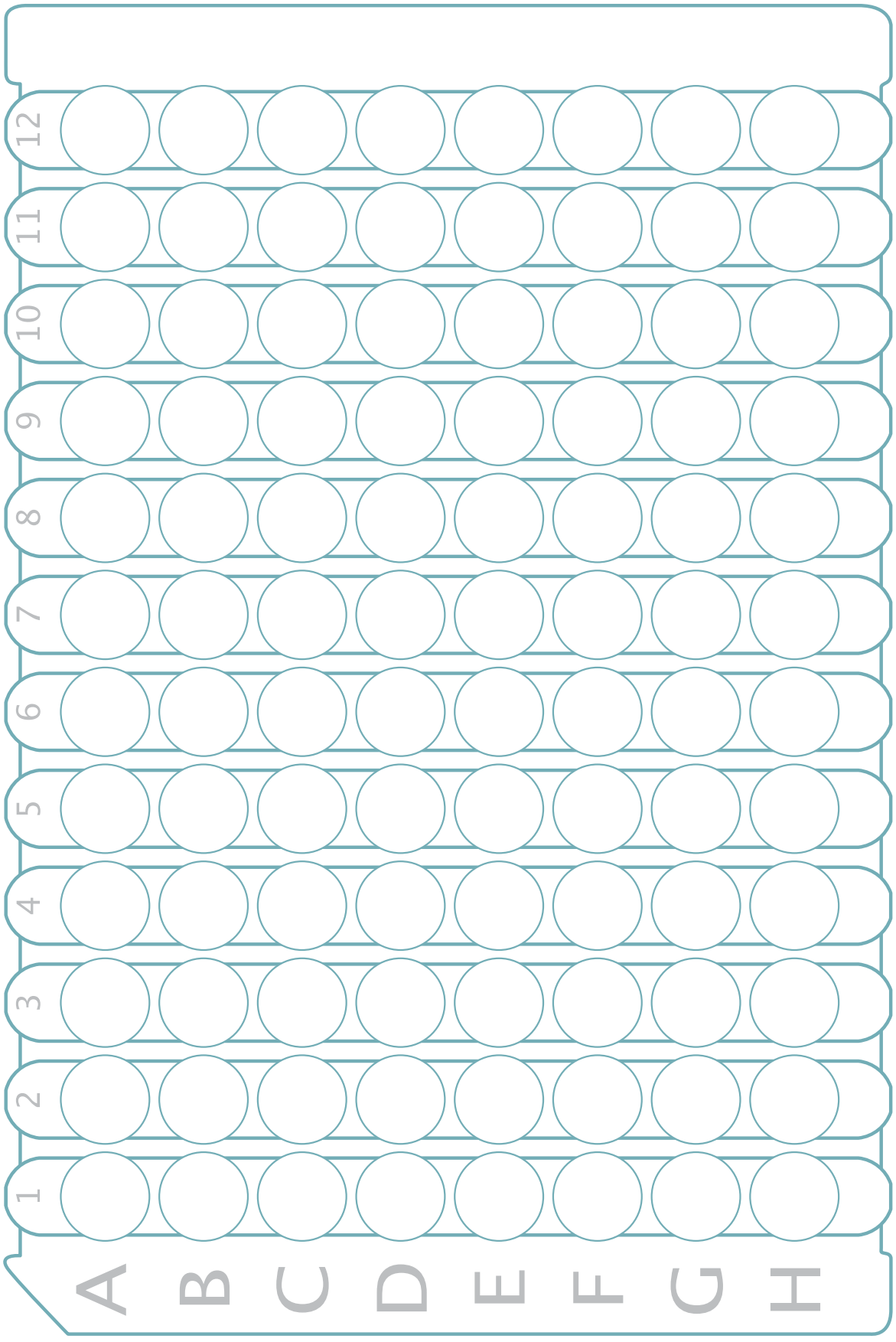
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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