Quantikine™ ELISA

Mouse IFN-α All Subtype Immunoassay

Catalog Number MFNAS0

For the quantitative determination of mouse Interferon alpha (IFN-α) 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

At present, there are three major classes of interferons (IFNs), Type I, Type II, and Type III. Interferon alpha (IFN-α) is a Type I IFN (1). Although IFN-α is most known for its antiviral effects, it also has anti-proliferative and anti-tumor effects. IFN-α modulates host innate and adaptive immune responses (2). IFN-α is secreted predominantly by plasmacytoid dendritic cells (pDCs) after cellular engulfment of materials containing single-stranded RNA (ssRNA) or unmethylated CpG DNA, in certain contexts (3, 4). IFN-α transcription is activated by signaling cascades that are stimulated, in turn by nucleic acids. Nucleic acids are detected by endosomal innate immune recognition receptors (I2R2s, also known as pattern recognition receptors, PRRs) (3), including Toll-like receptors (TLRs) 7, 8, 9, retinoic acid-inducible gene 1 (RIG-1) and melanoma-differentiation-associated gene 5 (MDA 5). Activation of downstream TLR signaling cascades results in the serine phosphorylation of IFN regulatory factor 7 (IRF-7) or IRF-3. IRF-7 but not IRF 3 forms a complex with myeloid differentiation primary response 88 (MYD88) and TNF-associated factor 6 (TRAF 6) (5). MDA 5 and RIG-1 signal via IRF-3 (6). Phosphorylated IRF-7 can also homodimerize or heterodimerize with IRF-3 before translocating into the nucleus to bind to viral response elements (VREs). Also known as positive regulatory domain-like elements (PRD-LEs), VREs are upstream of the IFN-α TATA box and transcription start site. IRF activation of VREs varies as a function of affinity and expression. IFN-α expression is modulated by a positive feedback loop that requires de novo synthesis of IRF-7. pDC cells constitutively express IRF-7 at high levels and have a robust protein synthesis and secretion pathway, thus producing high levels of IFN-α (7).

Fourteen intronless murine IFN-α subtype genes have been identified on chromosome 4. They include α1, α2, α4, α5, α6T, α7/10, α8/6, α9, α11, α12, α13, α14, αA(α3) and αB (8). All bind to the Interferon α/β receptor (IFNAR) but have divergent effects, that correlate with variable binding affinity for IFNAR subtypes 1 and 2. IFN-α signaling is well characterized (2,9-13). IFN-α ligand binding to the ubiquitously expressed (7) IFNAR1 triggers a conformational change which allows for the heterodimerization of IFNAR1 and IFNAR2. Receptor subunit heterodimerization triggers the cross phosphorylation of the Janus-activated Kinase 1 (JAK1) on IFNAR2 and tyrosine kinase 2 (TYK2) on IFNAR1, respectively. The intracellular domains of IFNAR1 and IFNAR2 are also phosphorylated during this process (14). The transcription factors Signal Transducer and Activator of Transcription (STAT) 1 and 2 are subsequently recruited to IFNAR 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), which stimulates the transcription of interferon-stimulated genes (ISGs). Chronic IFN-α signaling, associated with chronic inflammation, stimulates the "non-canonical" IFN-α signaling pathway. STATs are not phosphorylated in this pathway. The unphosphorylated STAT (USTAT) heterodimer consisting of USTAT 1 and USTAT 2 associates with IRF-9 to form active unphosphorylated ISGF3 (U-ISGF3), which can stimulate gene transcription via ISRE and GAS. STAT 2 homodimers and monomers can also associate with IRF-9 to form an ISGF3-like complex, which also binds to ISRE to stimulate the transcription of ISGs (14).

INTRODUCTION *CONTINUED*

Responses to IFN-α include enhanced Natural Killer (NK) cell proliferation, cytotoxicity, and IFN-γ secretion. Other responses to IFN-α include the upregulation of major histocompatibility complex (MHC) 1 and 2 on antigen-presenting cells. IFN-α also facilitates dendritic cell cross-presentation of viral antigens to $CD8^+$ T cells. Although IFN-α is commonly associated with viral infections, it has also been correlated with neuropsychiatric symptoms such as depression, anhedonia, anxiety, and cognitive impairment (15). IFN-α's role in cancer is complex. Although IFN-α has been associated with cellular senescence and apoptosis, some subtypes have been associated with increased cellular migration and drug resistance.

The Quantikine™ Mouse IFN-α All Subtype immunoassay is a 3.5 hour solid-phase ELISA designed to measure IFN-α in mouse cell culture supernates, serum, and plasma. It contains HEK293-expressed recombinant mouse IFN-α and has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IFN-α showed dose response curves that were parallel to the standard curves obtained using the Quantikine mouse kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IFN-α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IFN-α has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IFN-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse IFN-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IFN-α bound in the initial step. The sample values are then read off the standard curve.

This assay recognizes the natural and recombinant mouse IFN-α subtypes listed below.

Recombinant mouse:

IFN-α1
IFN-α4
IFN-α6
IFN-α7
IFN-α9
IFN-α11
IFN-α12
IFN-α13
IFN-α15/IFN-αA
IFN-α16
IFN-αB

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 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.

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.

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
Mouse IFN-α All Subtype Microplate	899344	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IFN-α.	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.*
Mouse IFN-α All Subtype Standard	899346	2 vials of recombinant mouse IFN-α 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.
Mouse IFN-α All Subtype Conjugate	899345	12 mL of a monoclonal antibody specific for mouse IFN-α conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse IFN-α All Subtype Control	899347	2 vials of recombinant mouse IFN-α in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1W	895117	11 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5Y	895201	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	895174	23 mL of diluted hydrochloric 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 and samples

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 20 minutes at 2000 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

Cell culture supernates may require dilution due to high endogenous levels.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

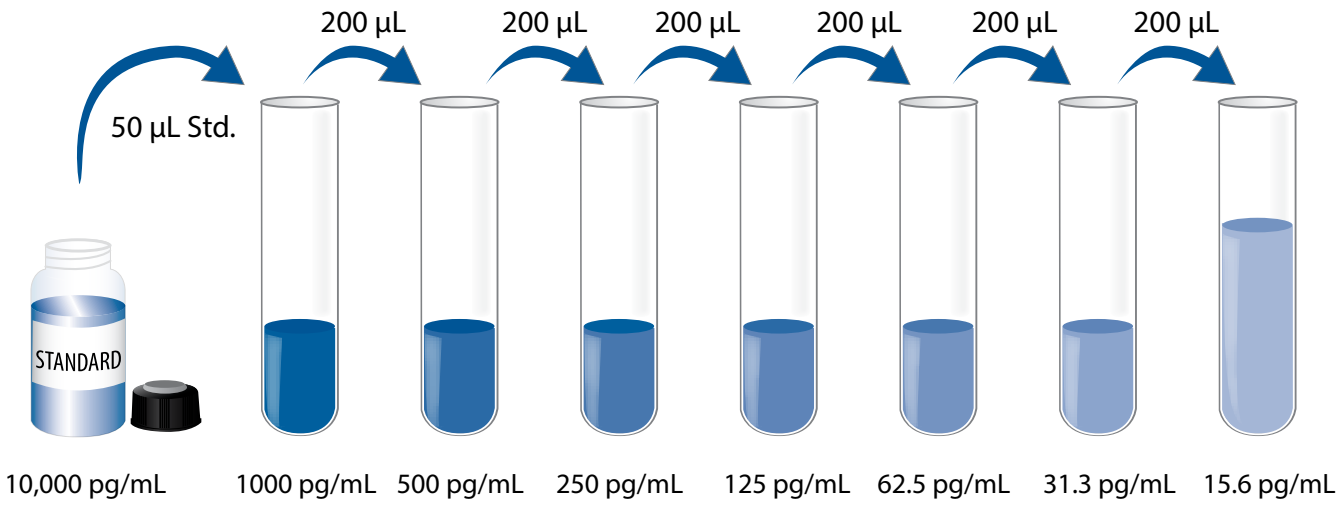
Mouse IFN-α All Subtype Control - Reconstitute the control with 1 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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.

Mouse IFN-α All Subtype Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IFN-α All Subtype Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5Y 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 RD5Y 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, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, 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 Assay Diluent RD1W 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 100 µL of Mouse IFN-α All Subtype Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

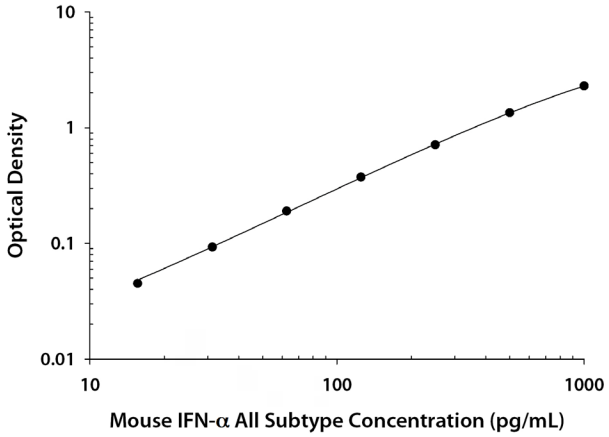
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse IFN-α 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	0.012 0.013	0.013	—
15.6	0.057 0.059	0.058	0.045
31.3	0.104 0.108	0.106	0.093
62.5	0.201 0.205	0.203	0.190
125	0.386 0.387	0.387	0.374
250	0.724 0.725	0.725	0.712
500	1.341 1.378	1.360	1.347
1000	2.286 2.327	2.307	2.294

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 using one lot of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	82.2	590	199	79.3	602	195
Standard deviation	3.04	15.9	3.08	5.11	57.4	7.93
CV (%)	3.7	2.7	1.5	6.4	9.5	4.1

RECOVERY

The recovery of mouse IFN-α spiked to levels throughout the range of the assay in various matrices was evaluated.

	Average % Recovery	Range
Cell culture media (n=4)	98	91-106%
Serum (n=4)	102	86-118%
EDTA plasma (n=4)	105	91-121%
Heparin plasma (n=4)	106	96-120%

LINEARITY

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

		Cell culture supernates* (n=4)	Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	101	96	97	100	100
	Range (%)	99-106	94-98	96-101	97-102	94-107
1:4	Average % of Expected	101	94	97	98	97
	Range (%)	97-107	94-95	94-101	95-100	92-103
1:8	Average % of Expected	103	93	95	97	94
	Range (%)	96-111	93-94	92-100	94-99	83-102
1:16	Average % of Expected	110	91	94	97	93
	Range (%)	103-119	91-92	88-101	92-102	84-103

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-six assays were evaluated and the minimum detectable dose (MDD) of mouse IFN-α ranged from 0.408-3.79 pg/mL. The mean MDD was 1.05 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 mouse IFN-α produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Ten serum and plasma samples were evaluated for the presence of mouse IFN-α in this assay. All samples measured less than the lowest IFN-α standard, 15.6 pg/mL.

Cell Culture Supernates - JAWSII mouse immature dendritic cells were cultured in alpha-MEM supplemented with 20% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 5 ng/mL of recombinant mouse GM-CSF (R&D Systems, Catalog # 415-ML/CF) until near confluency. Cells were then left unstimulated or stimulated with 10 µg/mL of poly I:C (Tocris™, Catalog # 4287) in the presence of Lipofectamine 2000 for 3 days. Aliquots of the cell culture supernates were removed and assayed for mouse IFN-α.

Condition	Value (pg/mL)
Unstimulated	ND
Stimulated	298

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant mouse IFN-α.

The factors listed below were prepared at 10 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL in a mid-range mouse IFN-α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IFN-λ
IFN-β1
IFN-ε
IFN-κ
IFN-α/β R1
IFN-α/β R2

Recombinant human:

IFNA-1α
IFNA-1β
IFNA-2α
IFNA-2β
IFNA-4A
IFNA-4B
IFNA-5
IFNA-6
IFNA-7
IFNA-8
IFNA-10
IFNA-14
IFNA-16
IFNA-17
IFNA-21

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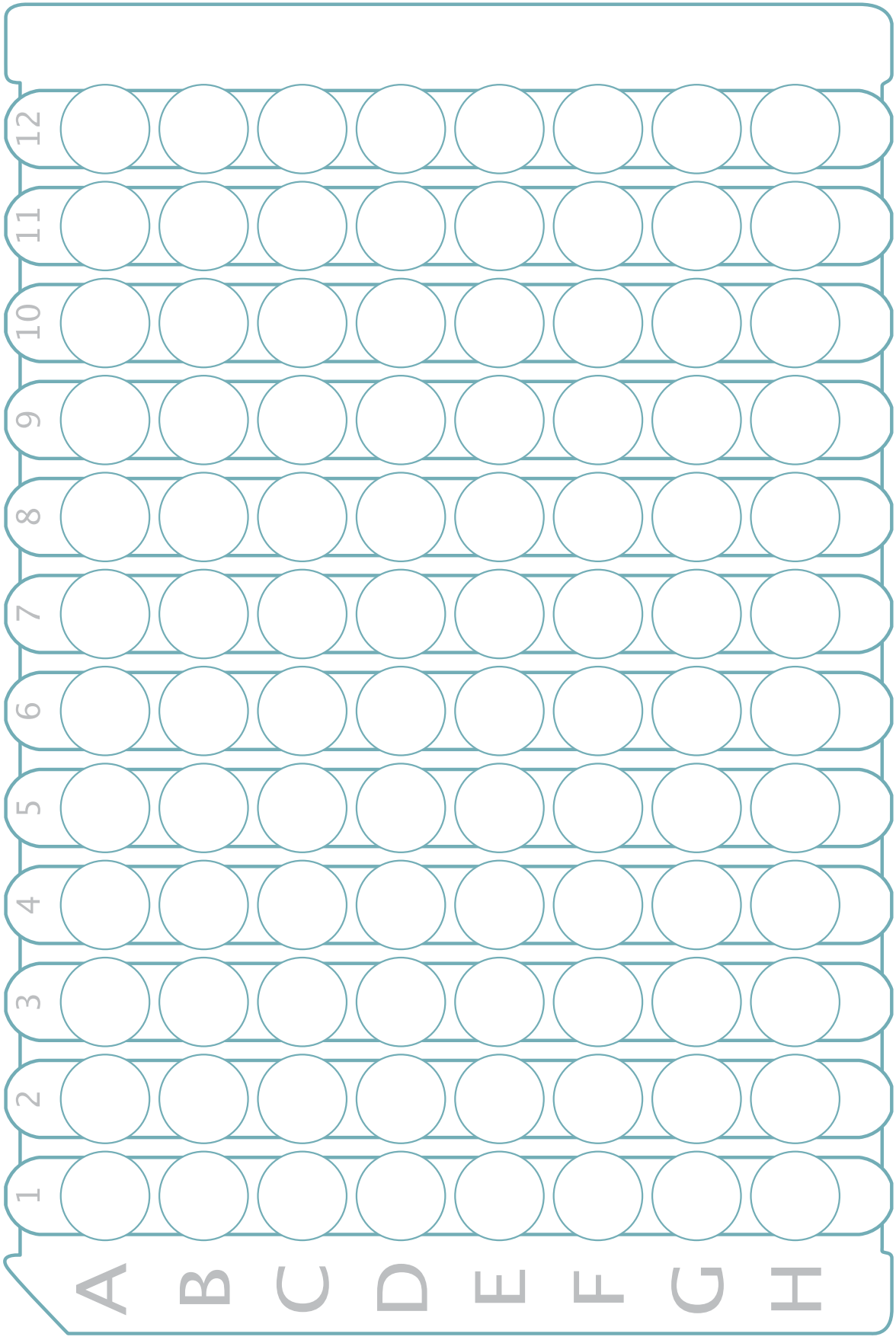
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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