Quantikine® QuicKit™ ELISA

Human Total IL-18/IL-1F4 Immunoassay

Catalog Number QK318

For the quantitative determination of human free and complexed Interleukin 18 (Total IL-18) 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

Interleukin-18 (IL-18) is a proinflammatory cytokine in the IL-1 family that exerts distinct immune effects depending on the local cytokine environment. It is expressed as a 24 kDa precursor by endothelial and epithelial cells, keratinocytes, γδT cells, and phagocytes. The precursor is activated intracellularly by Caspase-1 mediated proteolysis to release the 17 kDa mature cytokine. The precursor can also be released by necrotic cells for extracellular cleavage by multiple proteases. IL-18 activation is induced by infection or tissue damage and contributes to disease pathology in chronic inflammation (1-3). IL-18 binds to the widely expressed IL-18 Rα which recruits IL-18 Rβ to form the signaling receptor complex (4, 5). Its bioactivity is negatively regulated by interactions with IL-18 binding proteins and virally encoded IL-18BP homologs (6). In the presence of IL-12 or IL-15, IL-18 enhances anti-viral Th1 immune responses by inducing IFN-γ production and the cytolytic activity of $CD8^+$ T cells and NK cells (7, 8). In the absence of IL-12 or IL-15, however, IL-18 promotes production of the Th2 cytokines IL-4 and IL-13 by $CD4^+$ T cells and basophils (9, 10). In the presence of IL-1β or IL-23, IL-18 induces the antigen-independent production of IL-17 by γδ T cells and $CD4^+$ T cells (11). IL-18 also promotes myeloid dendritic cell maturation and triggers neutrophil respiratory burst (12, 13). In cancer, IL-18 exhibits diverse activities including enhancing anti-tumor immunity, inhibiting or promoting angiogenesis, and promoting tumor cell metastasis (14). Mature human IL-18 shares approximately 63% amino acid sequence identity with mouse and rat IL-18 (15). Alternative splicing in human ovarian cancer generates an isoform that is resistant to Caspase-1 activation (16). A cell surface form can be expressed on M-CSF induced macrophages and released in response to bacterial endotoxin (17).

The Quantikine® QuicKit™ Human Total IL-18/IL-1F4 Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human IL-18 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-18 and has been shown to accurately quantitate the recombinant factor free and in complex with IL-18 BPa. Results obtained using natural human IL-18 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 IL-18.

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 IL-18. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of IL-18 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 with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard 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 Total IL-18 Standard	899085	2 vials of recombinant human IL-18 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 Total IL-18 Capture Ab Concentrate	899083	Lyophilized tagged monoclonal antibody specific for human IL-18.	May be stored for up to 1 month at 2-8 °C.*
Human Total IL-18 Detection Ab Concentrate	899084	400 µL of a monoclonal antibody specific for human IL-18 conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-73	895541	11 mL of a buffered protein base with preservatives. *Use diluted 1:6 in this assay.*	
Calibrator Diluent RD6-46	895883	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:1.5 for cell culture supernate. Use undiluted for serum/plasma samples.*	
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.
- 100 mL and 1000 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 and samples.
- Human Total IL-18 Controls (optional; R&D Systems®, Catalog # QC260).

PRECAUTIONS

IL-18 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.

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 dilution due to high endogenous levels.

Serum and plasma samples require a 2-fold dilution due to a matrix effect. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD6-46.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Assay Diluent RD1-73 (diluted 1:6) - Add 5.0 mL of Assay Diluent RD1-73 to 25 mL of deionized or distilled water to prepare 30 mL of Assay Diluent RD1-73 (diluted 1:6).

Human Total IL-18 Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-18 Capture Ab Concentrate with Assay Diluent RD1-73 (diluted 1:6). 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 antibody 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 RD1-73 (diluted 1:6). For a full plate, add 300 µL of reconstituted Human Total IL-18 Capture Ab stock and 300 µL of Human Total IL-18 Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-73 (diluted 1:6).

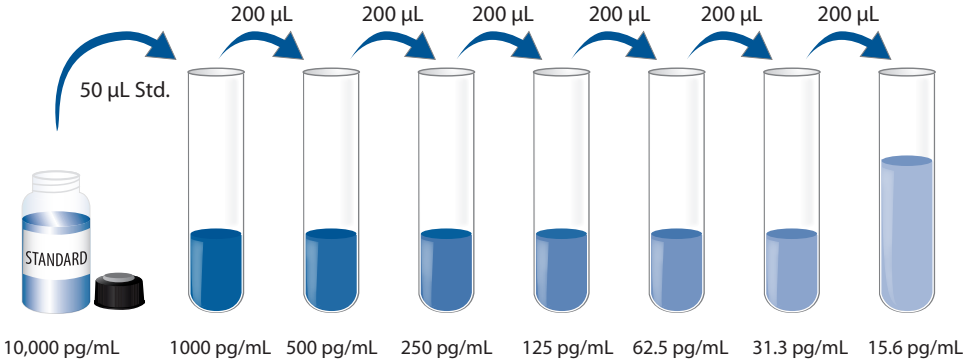
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD6-46 (diluted 1:1.5) - **For cell culture supernate samples only.** Add 10 mL of Calibrator Diluent RD6-46 to 5.0 mL of deionized or distilled water to prepare 15 mL of Calibrator Diluent RD6-46 (diluted 1:1.5).

Human Total IL-18 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Total IL-18 Standard with Calibrator Diluent RD6-46 (diluted 1:1.5) (*for cell culture supernate samples*) or Calibrator Diluent RD6-46 (*for serum/plasma samples*). 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 15 minutes with gentle agitation prior to making dilutions. Use stock solution within 60 minutes of reconstitution.

Pipette 450 µL of Calibrator Diluent RD6-46 (diluted 1:1.5) (*for cell culture supernate samples*) or Calibrator Diluent RD6-46 (*for serum/plasma samples*) into the 1000 pg/mL tube. Pipette 200 µL of the appropriate calibrator diluent 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. The appropriate calibrator diluent 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: *IL-18 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, samples, and working standards 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.
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.).

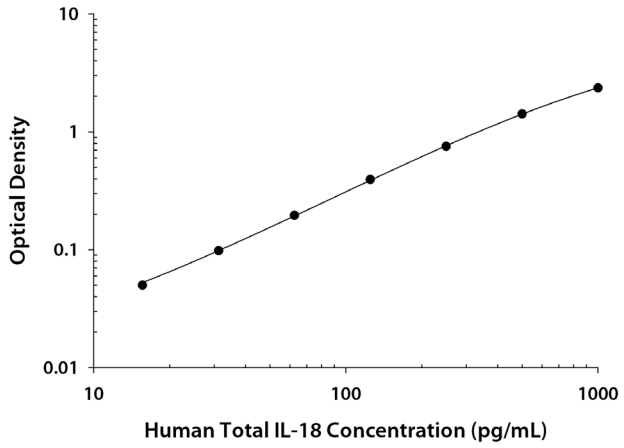
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Total IL-18 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

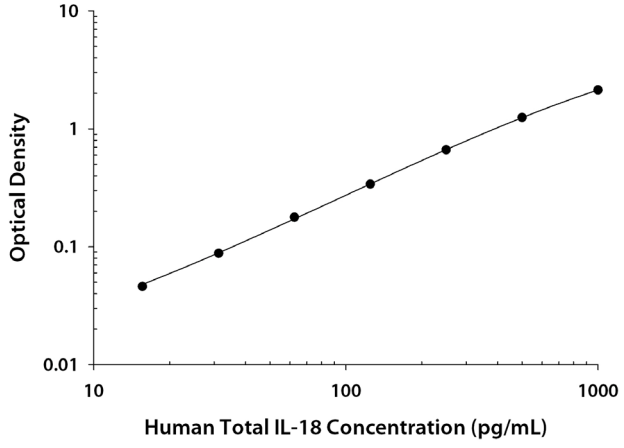
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.014		—
	0.016	0.015	
15.6	0.063		
	0.066	0.065	0.050
31.3	0.112		
	0.113	0.113	0.098
62.5	0.210		
	0.212	0.211	0.196
125	0.405		
	0.415	0.410	0.395
250	0.750		
	0.785	0.768	0.753
500	1.412		
	1.455	1.434	1.419
1000	2.371		
	2.380	2.376	2.361

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.013		—
	0.014	0.014	
15.6	0.058		
	0.061	0.060	0.046
31.3	0.101		
	0.102	0.102	0.088
62.5	0.189		
	0.195	0.192	0.178
125	0.351		
	0.357	0.354	0.340
250	0.673		
	0.682	0.678	0.664
500	1.264		
	1.257	1.261	1.247
1000	2.147		
	2.152	2.150	2.136

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.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision		Inter-Assay Precision	
Sample	**1**	**2**	**1**	**2**
n	20	20	10	10
Mean (pg/mL)	105	507	114	552
Standard deviation	4.15	12.8	6.12	43.8
CV (%)	4.0	2.5	5.4	7.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision		Inter-Assay Precision	
Sample	**1**	**2**	**1**	**2**
n	20	20	10	10
Mean (pg/mL)	120	591	131	638
Standard deviation	4.23	22.0	6.90	38.1
CV (%)	3.5	3.7	5.3	6.0

RECOVERY

The recovery of human IL-18 spiked to three different level throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Serum (n=2)	101	85-117%
EDTA plasma (n=2)	116	108-123%
Heparin plasma (n=2)	101	93-112%
Cell culture media (n=4)	117	109-128%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-18 were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)	Cell culture media (n=4)
1:2	Average % of Expected	97	92	96	89
	Range (%)	92-102	91-92	95-98	86-92
1:4	Average % of Expected	97	91	94	86
	Range (%)	94-101	91-91	92-96	85-87
1:8	Average % of Expected	96	89	94	87
	Range (%)	92-99	88-90	93-95	84-89
1:16	Average % of Expected	96	89	94	85
	Range (%)	88-105	87-91	89-99	81-89

SENSITIVITY

Fifty-six assays were evaluated and the minimum detectable dose (MDD) of human total IL-18 ranged from 0.520-4.57 pg/mL.

The mean MDD was 1.99 pg/mL for RD6-46 (*serum/plasma assay*) and 4.57 pg/mL for RD6-46 (*cell culture supernate assay)*.

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 *E. coli*-expressed recombinant human IL-18 manufactured at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Total IL-18 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	190	121-277	46.3
EDTA plasma (n=10)	235	137-351	59.0
Heparin plasma (n=10)	198	117-298	52.0

Cell Culture Supernates - THP-1 human acute monocytic leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until they reached a cell density of approximately $1x10^6$ cells/mL. Cells were left unstimulated or stimulated with 1 µg/mL LPS for 4 hours followed by 20 µM Nigericin for 1 hour. Aliquots of the cell culture supernates were removed, assay for levels of human total IL-18, and measured 27.7 pg/mL and 387 pg/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant human IL-18.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD6-46 (undiluted) and RD6-45 (diluted 1:1.5) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a low level-range recombinant human total IL-18 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IFN-β
IFN-γ
IL-1α
IL-1β
IL-4
IL-10
IL-11
IL-12
IL-13
IL-15
IL-16
IL-17
IL-17E
IL-17F
IL-18 BPa/Fc Chimera
IL-18 R
IL-18 Rβ/IL-1 R7 Fc Chimera
IL-19
IL-23

Other recombinants:

mouse IL-18
rat IL-18

Recombinant rhesus macaque IL-18 cross-reacts approximately 71% and interferes at concentrations > 50 pg/mL in this assay.

Recombinant human IL-18BP/rhIL-18 cross-reacts approximately 31% and interferes at concentrations > 100 pg/mL in this assay.

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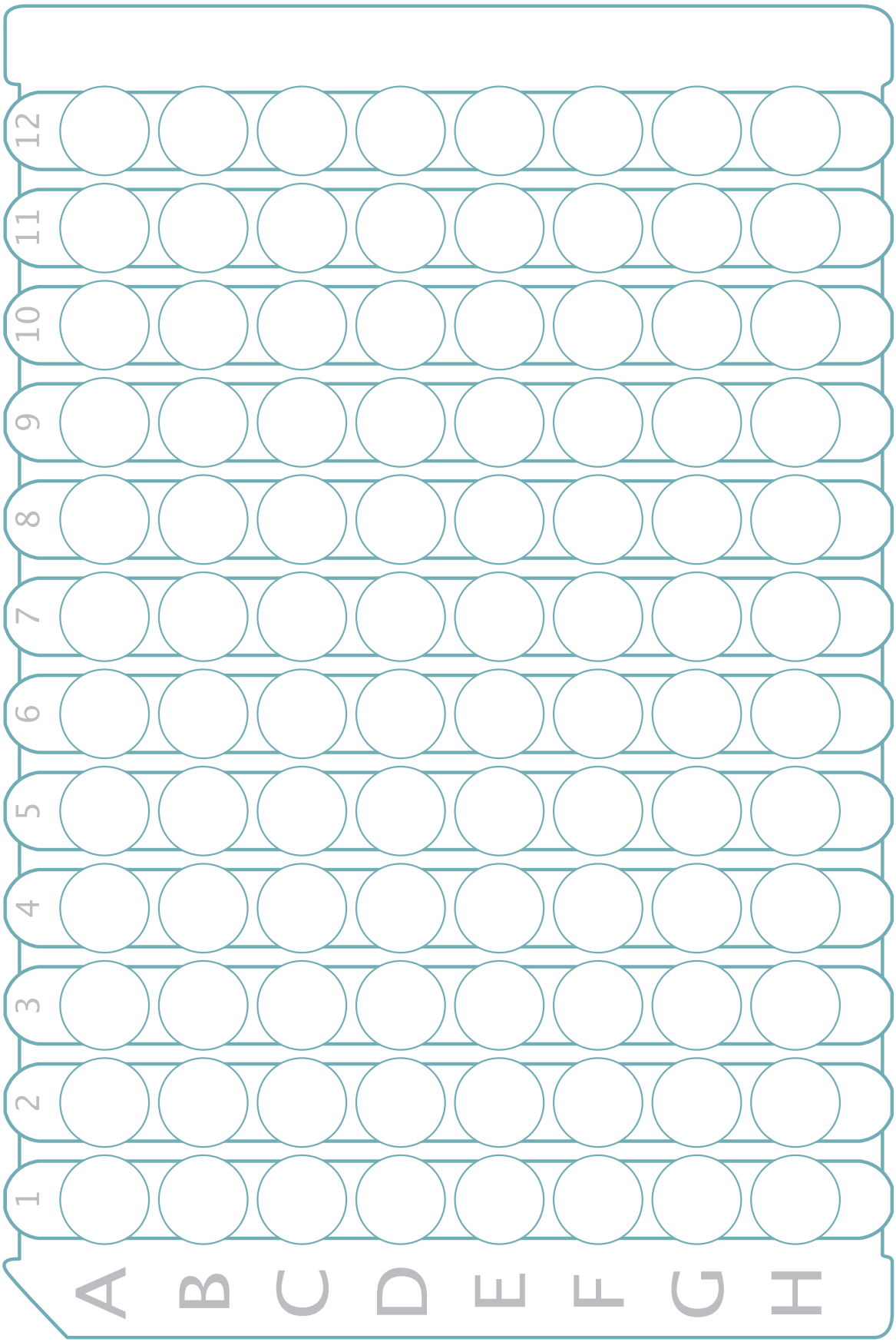
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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