Quantikine® QuicKit™ ELISA

Human IL-6 Immunoassay

Catalog Number QK206

For the quantitative determination of human Interleukin 6 (IL-6) 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 6 (IL-6) is a pleiotropic, α-helical, 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression (1-5). Mature human IL-6 is 183 amino acids (aa) in length and shares 39% aa sequence identity with mouse and rat IL-6 (6). Alternative splicing generates several isoforms with internal deletions, some of which exhibit antagonistic properties (7-10). Cells known to express IL-6 include $CD8^+$ T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, B1 B cells and pancreatic islet beta cells (2, 11-33). IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids (2). Normal human circulating IL-6 is in the 1 pg/mL range, with slight elevations during the menstrual cycle, modest elevations in certain cancers, and large elevations after surgery (34-38).

IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6 R alpha) and a signal transducing subunit (gp130). IL-6 binds to IL-6 Rα, triggering IL-6 Rα association with gp130 and gp130 dimerization (39). gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM (40). Soluble forms of IL-6 Rα are generated by both alternative splicing and proteolytic cleavage (5). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 Rα elicit responses from gp130-expressing cells that lack cell surface IL-6 Rα (5). Trans-signaling enables a wider range of cell types to respond to IL-6, as the expression of gp130 is ubiquitous, while that of IL-6 Rα is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (2, 5). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 Rα but not from other cytokines that use gp130 as a co-receptor (5, 41).

IL-6, along with TNF-α and IL-1, drives the acute inflammatory response. IL-6 is almost solely responsible for fever and the acute phase response in the liver, and it is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease (1-5). When dysregulated, it contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, arthritis, and sepsis (2, 5). IL-6 modulates bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis through its promotion of Th17 cell development and activity (1). It contributes to atherosclerotic plaque development and destabilization as well as the development of inflammation-associated carcinogenesis (1, 2). IL-6 can also function as an anti-inflammatory molecule, as in skeletal muscle where it is secreted in response to exercise (2). In addition, it enhances hematopoietic stem cell proliferation and the differentiation of memory B cells and plasma cells (42).

The Quantikine® QuicKit™ Human IL-6 Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human IL-6 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-6 and antibodies raised against the recombinant protein. Results obtained using natural human IL-6 showed linear curves that were parallel to the standard curves obtained using the recombinant QuicKit™ standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-6.

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 polyclonal detection antibody, specific for human IL-6. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 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 assay 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 zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IL-6 Standard	899082	2 vials of recombinant human IL-6 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 IL-6 Capture Ab Concentrate	899080	Lyophilized tagged monoclonal antibody specific for human IL-6. *Refer to the vial label for reconstitution volume.*	May be stored for up to 1 month at 2-8 °C.*
Human IL-6 Detection Ab Concentrate	899081	400 µL of a polyclonal antibody specific for human IL-6 conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-38	895301	12 mL of a buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
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.
- 500 mL and 100 mL graduated cylinders.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 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 IL-6 Controls (optional; R&D Systems®, Catalog # QC267).

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 require a 10-fold dilution due to a matrix effect. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Assay Diluent RD1-38 (diluted 1:4)*.

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 Assay Diluent RD1-38 (diluted 1:4)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Assay Diluent RD1-38 (diluted 1:4) - Add 10 mL of Assay Diluent RD1-38 to 30 mL of deionized or distilled water to prepare 40 mL of Assay Diluent RD1-38 (diluted 1:4).

Human IL-6 Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-6 Capture Ab Concentrate with Assay Diluent RD1-38 (diluted 1:4). 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 up to 4 weeks at 2-8 °C.

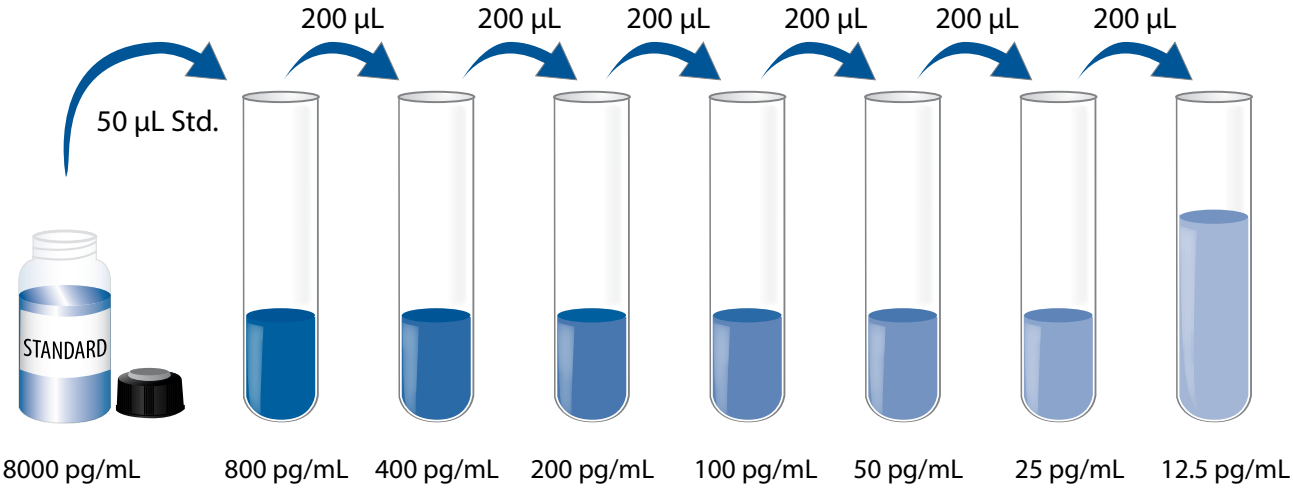
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-38 (diluted 1:4). For a full plate, add 300 µL of reconstituted Human IL-6 Capture Ab stock and 300 µL of Human IL-6 Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-38 (diluted 1:4).

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 IL-6 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-6 Standard with Assay Diluent RD1-38 (diluted 1:4) This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Assay Diluent RD1-38 (diluted 1:4) into the 800 pg/mL tube. Pipette 200 µL into each remaining tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 800 pg/mL standard serves as the high standard. The Assay Diluent RD1-38 (diluted 1:4) 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, 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. A plate layout is provided to record standards and samples assayed.
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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

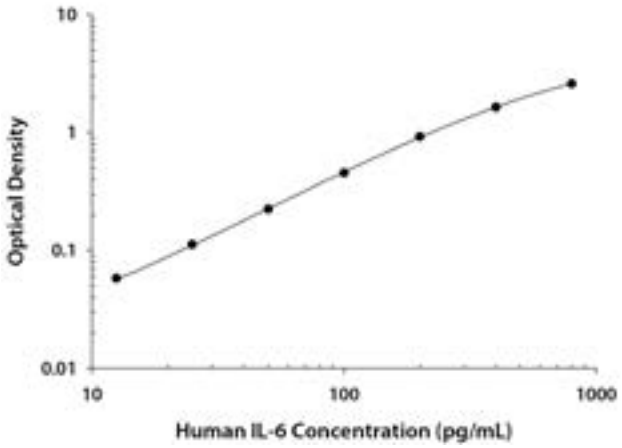
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 IL-6 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.

Since 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	0.033		——
	0.041	0.037	
12.5	0.092		
	0.097	0.095	0.058
25	0.145		
	0.152	0.149	0.112
50	0.257		
	0.264	0.261	0.224
100	0.487		
	0.494	0.491	0.454
200	0.952		
	0.961	0.957	0.920
400	1.648		
	1.700	1.674	1.637
800	2.631		
	2.642	2.637	2.600

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)	91.9	497	87.9	487
Standard deviation	2.15	14.3	7.49	38.6
CV (%)	2.3	2.9	8.5	7.9

RECOVERY

The recovery of human IL-6 spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated. Sample were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	119	114-126%
Serum (n=2)	106	96-115%
EDTA plasma (n=2)	117	112-122%
Heparin plasma (n=2)	111	107-114%

SENSITIVITY

Ten assays were evaluated and the minimum detectable dose (MDD) of human IL-6 ranged from 0.741-2.95 pg/mL. The mean MDD was 1.70 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-6 in various matrices and diluted to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed the Sample Preparation section.

		Cell culture supernates (n=4)	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)
1:2	Average % of Expected	94	106	101	101
	Range (%)	90-100	99-113	97-104	98-104
1:4	Average % of Expected	92	106	96	101
	Range (%)	89-96	97-115	92-101	99-103
1:8	Average % of Expected	92	103	95	97
	Range (%)	85-95	94-112	93-97	96-98
1:16	Average % of Expected	94	99	89	90
	Range (%)	90-100	95-103	89-90	89-91

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-6 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 IL-6 in this assay. All samples measured less than 25 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBMCs) (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were left unstimulated or stimulated with 1.0 µg/mL LPS for 24 hours. Aliquots of the culture supernates were removed, aassayed for levels of human IL-6, and were not detectable or measured 6356 pg/mL, respectively.

Monocytes were prepared from human PBMCs by adherence to plastic. Unattached cells were removed and attached monocytes were cultured in RPMI supplemented with 10% fetal bovine serum. Cells were left unstimulated or stimulated with 500 ng/mL LPS for 24 hours. Aliquots of the cell culture supernates were removed, assayed for levels of human IL-6, and were not detectable or measured 15,467 pg/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant human IL-6.

The factors listed below were prepared at 50 ng/mL in Assay Diluent RD1-38 (diluted 1:4) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a low level recombinant human IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-6 Rα
IL-6 Rα/gp130

Other recombinants:

mouse IL-6
rat IL-6

Recombinant porcine IL-6 cross-reacts approximately 0.31% and interferes at concentrations > 1.25 ng/mL in this assay.

Recombinant human IL-6 Ra/IL-6 chimera cross-reacts approximately 24%, but does not interfere in this assay.

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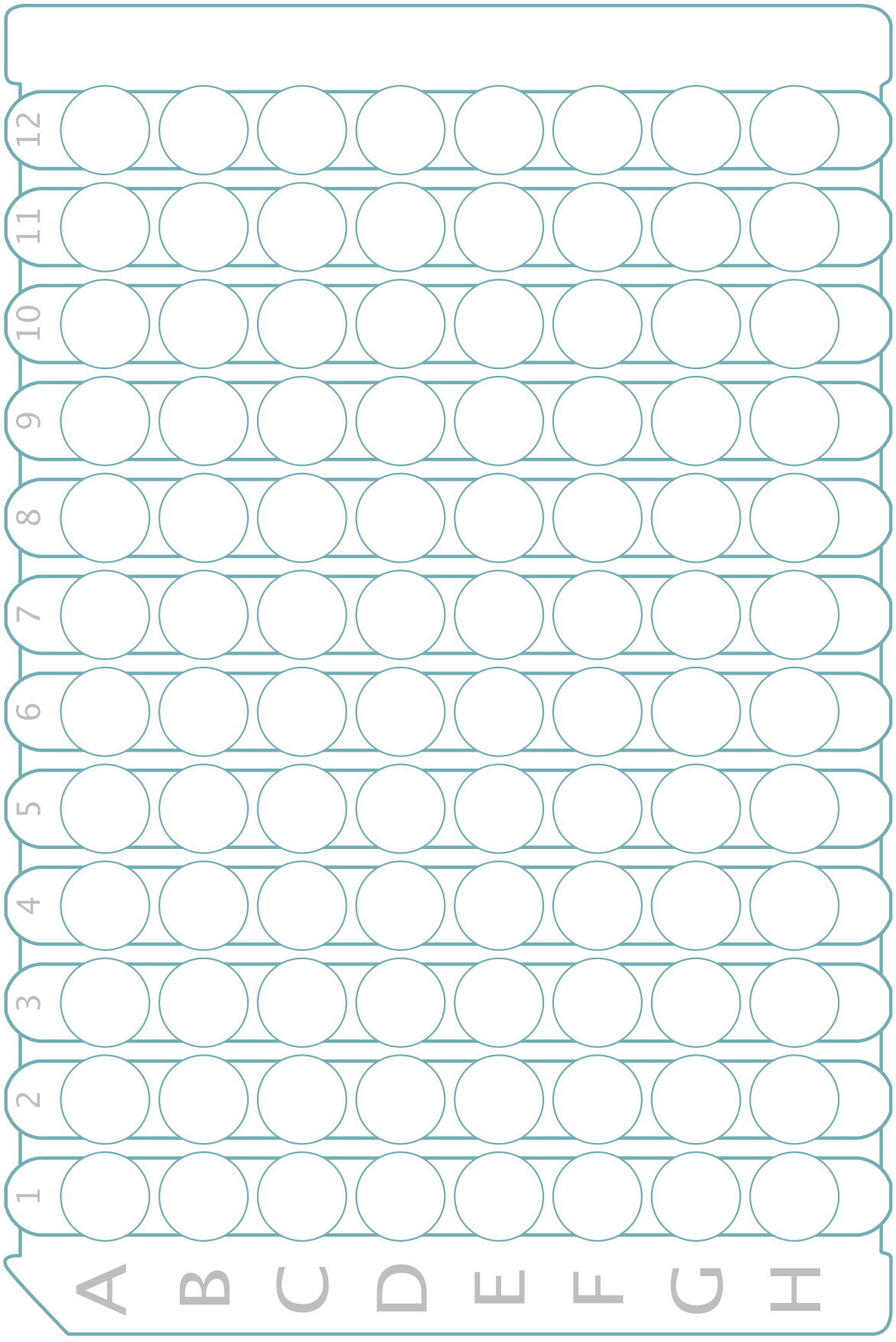
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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