Quantikine® ELISA

Mouse IL-6 Rα Immunoassay

Catalog Number MR600

For the quantitative determination of mouse Interleukin 6 Receptor alpha (IL-6 Rα) concentrations in cell culture supernates, serum, and heparin 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 multifunctional cytokine that displays both pro- and anti-inflammatory properties (1-4). It is produced by a variety of immune and non-immune cells and plays important roles in a wide range of biological activities including immune regulation, inflammation, hematopoiesis, bone metabolism, and cancer progression (3, 5-7). Altered levels of IL-6 have been reported to be associated with a variety of diseases including Crohn's disease, rheumatoid arthritis, Castleman's disease, mesangial proliferative glomerulonephritis, osteoporosis, cardiac ischemia, multiple myeloma, and sepsis (1-4).

Classical signaling of IL-6 is mediated by a cell surface heterodimeric receptor complex that is composed of a ligand binding subunit (IL-6 Rα) and a signal transducing subunit (gp130). IL-6 binds to IL-6 Rα, triggering IL-6 Rα association with gp130 and gp130 dimerization. This leads to activation of the Jak/STAT, ERK, and PI-3 Kinase signal transduction pathways (2, 5). An approximately 50 kDa soluble form of IL-6 Rα (sIL-6 Rα) has been found in human serum and urine (1). This soluble receptor can be generated by ectodomain shedding, which is constitutively mediated by ADAM10 or inducibly mediated by ADAM17, as seen during apoptosis (2, 8-10). IL-6 Rα can also be cleaved by the *Serratia marcescens* metalloprotease and Cathepsin G (11, 12). Additionally, alternative splicing of human IL-6 Rα can generate a soluble form of the receptor by premature truncation upstream of the transmembrane region (2, 9).

sIL-6 Rα and membrane-bound IL-6 Rα bind to IL-6 with comparable affinity, and the IL-6/sIL-6 Rα complex can associate with gp130 to elicit a biological response (1, 2, 4, 9, 13). While IL-6 Rα is expressed on only a small population of cells, such as macrophages, neutrophils, and hepatocytes, gp130 is expressed on all cell types (1, 2, 4, 9, 13). Activation of gp130 by the IL-6/sIL-6 Rα complex, a process called trans-signaling, renders virtually all cells responsive to IL-6. Cells that can only respond to IL-6 in the presence of sIL-6 Rα include smooth muscle cells, neural cells, and embryonic and hematopoietic stem cells (1, 2, 13).

It has been suggested the pro-inflammatory responses of IL-6 are mediated by trans-signaling whereas the regenerative or anti-inflammatory activities of IL-6 are mediated by classical signaling (1, 2). Levels of sIL-6Rα have been shown to be moderately increased in numerous inflammatory and autoimmune diseases including Crohn's and Graves' disease, rheumatoid arthritis, peritonitis and asthma, as well as inflammation-induced colon cancer (4, 9, 10, 14, 15). Additionally, sIL-6 Rα levels are elevated in patients with myeloma, breast cancer, schizophrenia, and HIV infection (16-19).

The Quantikine® Mouse IL-6 Rα Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-6 Rα levels in cell culture supernates, serum, and heparin plasma. It contains NS0-expressed recombinant mouse IL-6 Rα and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse IL-6 Rα accurately. Results obtained using natural mouse IL-6 Rα showed dose-response 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 mouse IL-6 Rα.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-6 Rα has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-6 Rα present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse IL-6 Rα 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 IL-6 Rα bound in the initial step. The sample values are then read off the standard curve.

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 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® 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.
- 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.

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 IL-6 Rα Microplate	894736	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-6 Rα.	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 IL-6 Rα Standard	894738	2 vials of recombinant mouse IL-6 Rα in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard and control for each assay.
Mouse IL-6 Rα Control	894739	2 vials of recombinant mouse IL-6 Rα in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse IL-6 Rα Conjugate	894737	12 mL of a monoclonal antibody specific for mouse IL-6 Rα conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-14	895180	12 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 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	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.
- 100 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 and samples.

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 MSDS 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. 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 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.*
EDTA plasma & grossly lipemic samples are not suitable for use in this assay.

SAMPLE PREPARATION

Serum and heparin plasma samples require a 5-fold dilution due to sample matrix effects. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse IL-6 Rα Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

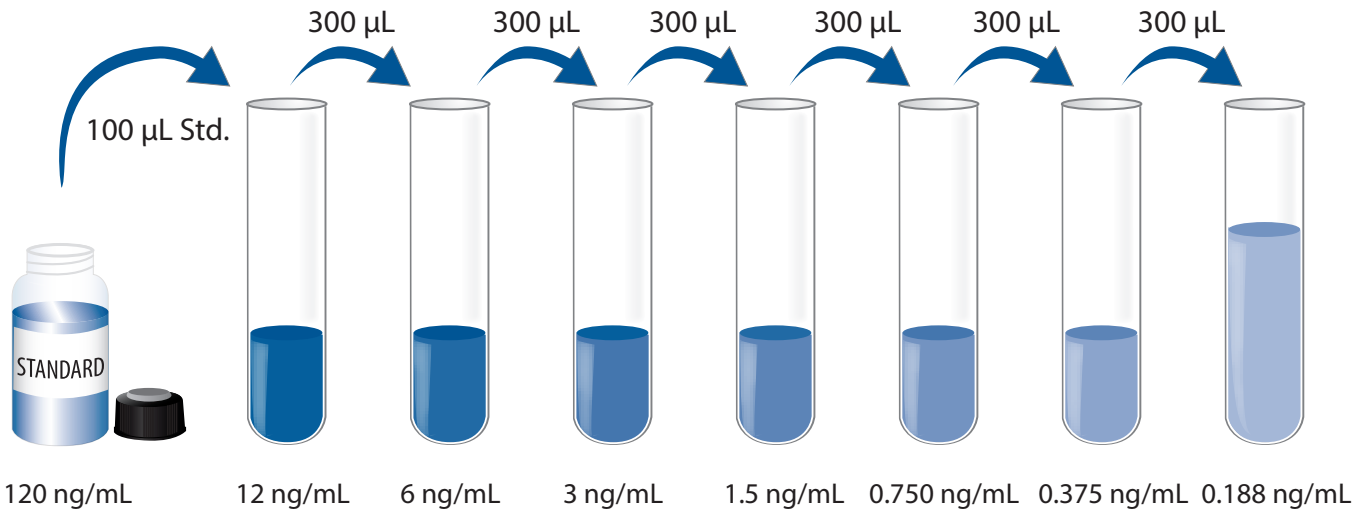
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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

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 IL-6 Rα Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-6 Rα Standard with deionized or distilled water. This reconstitution produces a stock solution of 120 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) into the 12 ng/mL tube. Pipette 300 µL into the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The 12 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/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, working standards, 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 RD1-14 to each well. *Assay Diluent RD1-14 may contain a precipitate. Mix well before and during use.*
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.
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 IL-6 Rα Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours 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

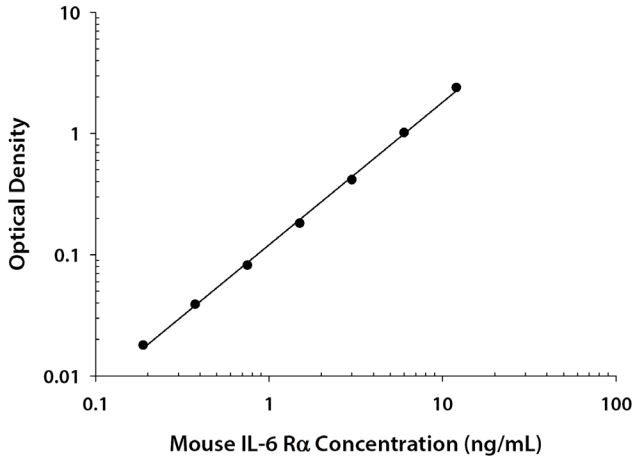
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the mouse IL-6 Rα concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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.



(ng/mL)	O.D.	Average	Corrected
0	0.028 0.029	0.029	—
0.188	0.046 0.047	0.047	0.018
0.375	0.068 0.068	0.068	0.039
0.750	0.110 0.112	0.111	0.082
1.5	0.208 0.213	0.211	0.182
3	0.444 0.446	0.445	0.416
6	1.031 1.065	1.048	1.019
12	2.404 2.445	2.425	2.396

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 two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.456	1.07	2.94	0.474	1.08	2.82
Standard deviation	0.036	0.039	0.148	0.060	0.080	0.200
CV (%)	7.9	3.6	5.0	12.7	7.4	7.1

RECOVERY

The recovery of mouse IL-6 Rα spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	90-110%
Serum* (n=4)	102	93-110%
Heparin plasma* (n=4)	99	92-106%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-6 Rα were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	98	91	94
	Range (%)	95-103	84-96	92-97
1:4	Average % of Expected	95	87	95
	Range (%)	93-97	75-98	93-96
1:8	Average % of Expected	97	83	96
	Range (%)	92-105	77-92	92-102
1:16	Average % of Expected	98	85	100
	Range (%)	94-103	79-95	94-109

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-nine assays were evaluated and the minimum detectable dose (MDD) of mouse IL-6 Rα ranged from 0.008-0.070 ng/mL. The mean MDD was 0.027 ng/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 NS0-derived recombinant mouse IL-6 Rα produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse IL-6 Rα in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=11)	7.32	4.87-9.80	1.72
Heparin plasma (n=5)	6.22	4.55-7.79	1.31

Cell Culture Supernates:
J774A.1 mouse reticulum cell sarcoma macrophage cells (5 x 10^4 cells/mL) were cultured for 2 days in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for mouse IL-6 Rα, and measured 0.473 ng/mL.

Livers from mice were rinsed with PBS and homogenized with a tissue homogenizer. Tissues were cultured for 1 day in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for mouse IL-6 Rα, and measured 0.885 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-6 Rα.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant mouse IL-6 Rα control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

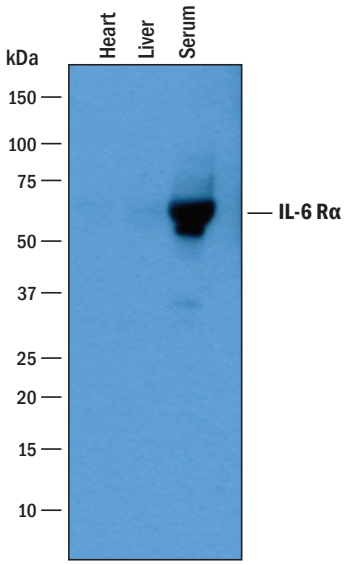
gp130
IL-6
IL-11 Rα
IL-27
IL-27 p28

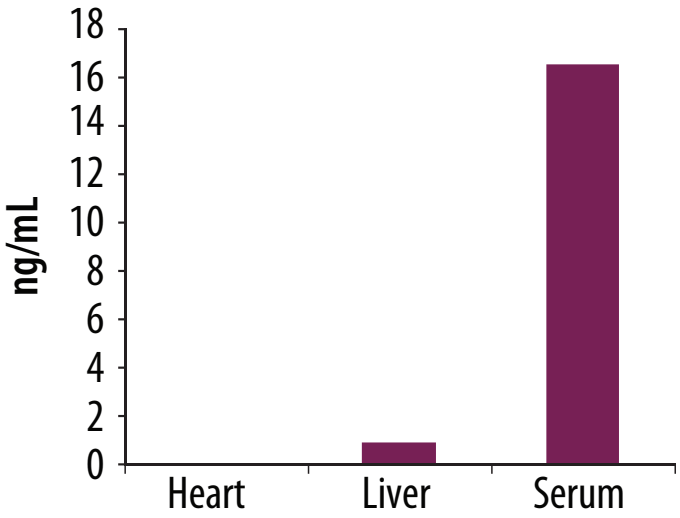
Recombinant rat:

CNTF Rα
IL-6 Rα

Recombinant human:

IL-6 Rα





Conditioned media and serum samples from NSA mice were analyzed by Western Blot and Quantikine® ELISA. The conditioned media and serum samples were diluted 1:20 and 1:200, respectively, for the Western Blot. Samples were resolved under reducing SDS-PAGE conditions, transferred to a PVDF membrane, and immunoblotted with goat anti-mIL-6Rα (R&D Systems®, Catalog # AF1830). The Western Blot shows a direct correlation with the ELISA value for those samples, with the mouse liver conditioned media sample below the sensitivity of the Western Blot.

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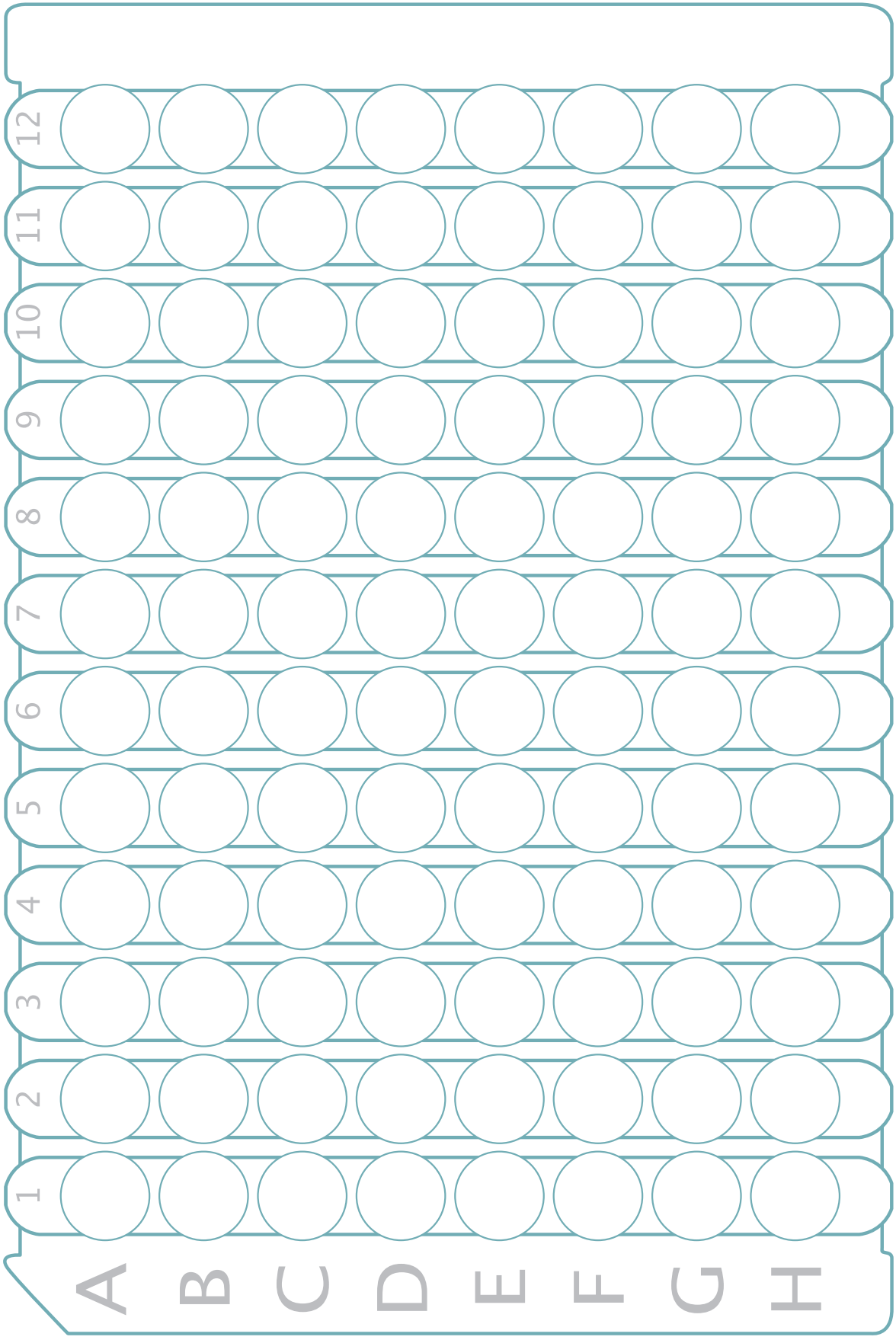
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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