QuantiGlo™ ELISA

Human IL-6 Immunoassay

Catalog Number Q6000B
SQ6000B
PQ6000B

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 Rα) 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 QuantiGlo™ Human IL-6 Immunoassay is a solid phase chemiluminescent ELISA that specifically measures human IL-6 in less than 5.5 hours. This kit is designed to measure IL-6 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-6 and antibodies raised against the recombinant factor. It has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-6 showed linear curves that were parallel to the standard curves obtained using the QuantiGlo kit standards. These results indicate that this kit can be used to determine relative mass values for human IL-6.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells and light is produced in proportion to the amount of IL-6 bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted.

LIMITATIONS OF THE PROCEDURE & TECHNICAL HINTS

- 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, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, luminometers, incubation time or temperature, and kit age can cause variations 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 QuantiGlo™ Immunoassay, the possibility of interference cannot be excluded.
- 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.
- Relative light units (RLUs) may differ among luminometers. Adjust settings as recommended by the instrument manufacturer.
- Relative light units may vary within the 15 minute reading window.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low, beginning with the high standard in wells A1 and A2.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # Q6000B	CATALOG # SQ6000B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-6 Microplate	892814	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-6.	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 IL-6 Conjugate	890516	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-6 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-6 Standard	890517	1 vial	6 vials	Recombinant human IL-6 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-83	895875	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *Contains a precipitate. Mix well before and during use.*	
Calibrator Diluent RD6-10	895468	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895222	1 vial	6 vials	100 mL/vial of a 10-fold concentrated solution of buffered surfactant with preservatives.	
Glo Reagent A	895868	1 vial	6 vials	4 mL/vial of stabilized enhanced luminol.	
Glo Reagent B	895869	1 vial	6 vials	8 mL/vial of stabilized hydrogen peroxide.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

Q6000B contains sufficient materials to run an ELISA on one 96 well plate.
SQ6000B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PQ6000B). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human IL-6 Microplate	892814	50 plates
Human IL-6 Conjugate	890516	50 vials
Human IL-6 Standard	890517	25 vials
Assay Diluent RD1-83	895875	50 vials
Calibrator Diluent RD6-10	895468	50 vials
Wash Buffer Concentrate	895222	50 bottles
Glo Reagent A	895868	50 vials
Glo Reagent B	895869	50 vials
Plate Sealers	N/A	100 sheets
Package Inserts	751184	2 booklets

OTHER SUPPLIES REQUIRED

- Luminometer set with the following parameters: 1.0 minute lag time; 0.5 second/well read time; summation mode; auto gain on; or equivalent
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 1000 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
- Human IL-6 Controls (optional; R&D Systems®, Catalog # QC193)

PRECAUTIONS

Calibrator Diluent RD6-10 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

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. Some samples may require dilution.

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, heparin, or citrate 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.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

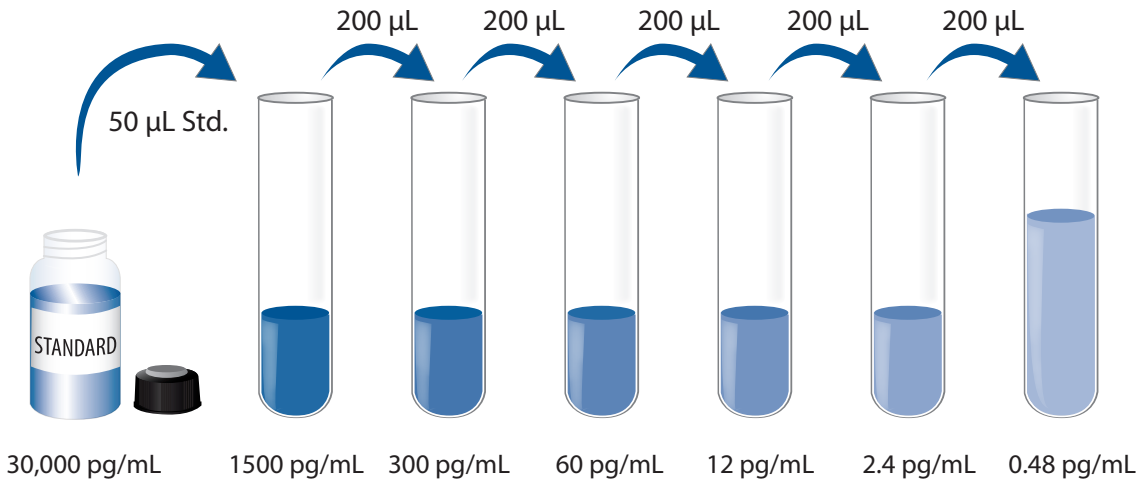
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to 900 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

Working Glo Reagent - Mix together 1 part Glo Reagent A (4 mL) and 2 parts Glo Reagent B (8 mL) 15 minutes to 4 hours prior to use in a capped plastic container and protected from light. 100 µL of the resultant mixture is required per well.

Note: *If running the assay in less than 96 wells, mix appropriate amounts of Glo Reagent A and Glo Reagent B. To assay half a plate (48 wells), mix 2 mL of Glo Reagent A with 4 mL of Glo Reagent B. Working Glo Reagent should be discarded after use.*

Human IL-6 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-6 Standard with deionized or distilled water. This reconstitution produces a stock solution of 30,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 950 µL of Calibrator Diluent RD6-10 into the 1500 pg/mL tube. Pipette 800 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1500 pg/mL standard serves as the high standard. Calibrator Diluent RD6-10 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 100 µL of Assay Diluent RD1-83 to each well. *Assay Diluent RD1-83 contains a precipitate. Mix well before and during use.*
4. Add 100 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 200 µL of Human IL-6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 3 hours at room temperature on the shaker.

   Note: *Prepare Working Glo Reagent at this time.*
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Working Glo Reagent to each well. Incubate for 5-20 minutes at room temperature **on the benchtop. Protect from light.**
9. Determine the RLU of each well using a luminometer set with the following parameters: 1.0 minute lag time; 0.5 second/well read time; summation mode; auto gain on, or equivalent.

CALCULATION OF RESULTS

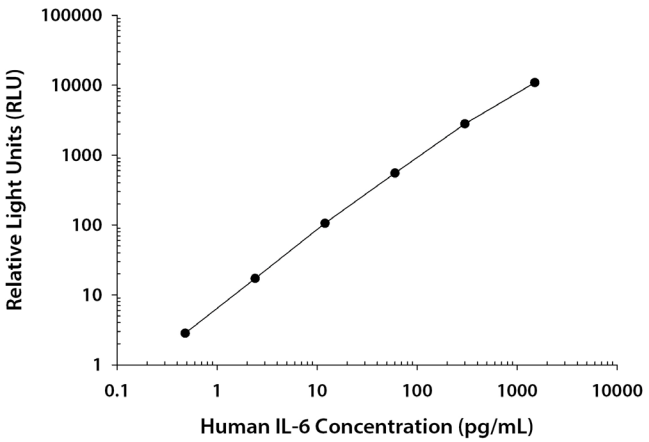
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard relative light units (RLU).

Create a standard curve by reducing the data using computer software capable of generating a cubic-spline or quadratic curve fit.

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)	RLU	Average	Corrected
0	4.15 4.32	4.24	—
0.48	6.90 7.22	7.06	2.82
2.4	21.20 21.53	21.37	17.13
12	108.9 111.1	110.0	105.8
60	542.8 565.5	554.2	550.0
300	2695 2892	2793	2789
1500	10,745 10,924	10,835	10,831

PRECISION

Intra-Assay Precision (Precision within an assay)
Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Four 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**	**4**	**1**	**2**	**3**	**4**
n	20	20	20	20	20	20	20	20
Mean (pg/mL)	1.21	10.7	118	766	1.56	13.2	142	752
Standard deviation	0.07	0.32	4.15	37.5	0.15	1.02	8.93	53.7
CV (%)	5.8	3.0	3.5	4.9	9.6	7.7	6.3	7.1

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	98-113%
Serum (n=4)	94	88-106%
EDTA plasma (n=4)	89	82-95%
Heparin plasma (n=4)	88	80-97%
Citrate plasma (n=4)	87	82-96%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-6 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	106	100	105	106	105
	Range (%)	104-107	98-105	101-114	102-112	100-111
1:4	Average % of Expected	107	100	109	108	109
	Range (%)	101-112	98-105	103-117	104-115	106-115
1:8	Average % of Expected	107	102	105	108	107
	Range (%)	106-108	96-107	97-117	101-114	103-112
1:16	Average % of Expected	105	92	102	100	101
	Range (%)	102-107	89-99	92-117	95-109	94-104
1:32	Average % of Expected	104	91	98	97	100
	Range (%)	99-108	88-97	90-109	91-100	95-102

SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of human IL-6 ranged from 0.05-0.35 pg/mL. The mean MDD was 0.16 pg/mL.

The MDD was determined by adding two standard deviations to the mean RLU 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-6 produced at R&D Systems®.

The NIBSC/WHO 1st International Standard 89/548, which was intended as a potency standard, was evaluated in this kit. The standard is a CHO cell-derived recombinant human IL-6. The dose response curve of this international standard parallels the QuantiGlo™ standard curve. To convert sample values obtained with the QuantiGlo Human IL-6 kit to approximately NIBSC 89/548 International Units, use the equation below.

NIBSC (89/548) approximate value (IU/mL) = 0.131 x QuantiGlo Human IL-6 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-6 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=38)	1.35	61	ND-5.84
EDTA plasma (n=38)	1.32	45	ND-4.70
Heparin plasma (n=38)	1.30	45	ND-4.90
Citrate plasma (n=20)	1.68	40	ND-4.52

ND = Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 6 and assayed for levels of human IL-6.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	655	425
Stimulated	23,630	43,520

SPECIFICITY

This assay recognizes natural and recombinant human IL-6.

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 mid-range recombinant human IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:	Recombinant rat:	Other recombinants:
CD40 Ligand	IL-6	CNTF	canine IL-6
CT-1	IL-10	G-CSF	cotton rat IL-6
CTLA-4	IL-11	IL-6	
G-CSF	IL-12	OSM	
gp130	IL-12/IL-23 p40		
IL-6 R	IL-13		
IL-6 R/gp130			
IL-9			
IL-10			
IL-11			
IL-12			
IL-12/IL-23 p40			
LIF			
LIF R			
OSM			

Recombinant porcine IL-6 does not interfere but does cross-react approximately 0.03% at concentrations ≥ 10 ng/mL in this assay.

Recombinant human CNTF does not interfere but does cross-react approximately 0.003% at concentrations > 10 ng/mL in this assay.

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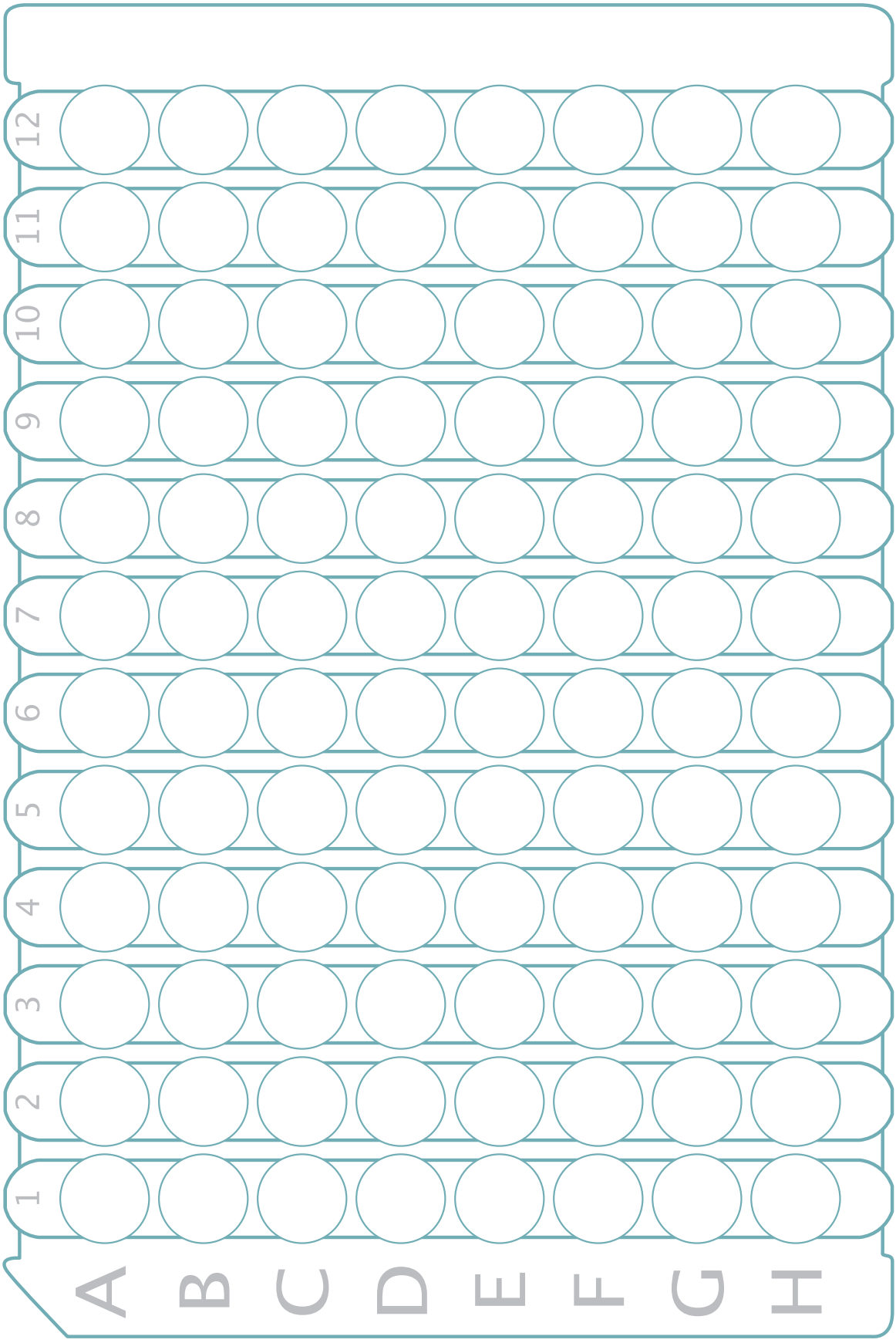
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES