Quantikine® ELISA

Human sCD14 Immunoassay

Catalog Number DC140

For the quantitative determination of human soluble CD14 (sCD14) 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.

TABLE OF CONTENTS

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INTRODUCTION

CD14 is a glycoprotein that mediates the interaction of lipopolysaccharide (LPS, endotoxin) with cells, thereby signaling the presence of gram-negative bacteria (1-3). CD14 is either soluble (sCD14) (4, 5) or membrane-bound (mCD14) by a glycosylphosphatidylinositol (GPI) anchor (6, 7). mCD14 is a 55 kDa glycoprotein (1), while sCD14 varies from about 43 to 53 kDa, depending on the degree of glycosylation and whether it was synthesized without the anchor or was shed by phospholipase cleavage of the anchor or by proteolysis (12-14). There is no evidence for different mRNAs for m- and sCD14. There is no apparent sequence homology with other proteins. The sequence of human CD14 is 63-73% identical to that of mouse, rat, or rabbit CD14 (15).

mCD14 is expressed primarily on myeloid cells, such as monocytes, macrophages and neutrophils (1-3), the cells most sensitive to LPS, and to a lesser extent on other cells, such as B cells (8) and a circulating dendritic cell progenitor (9). sCD14 appears to mediate LPS stimulation of cells that do not express mCD14 (10, 11), such as endothelial, epithelial and smooth-muscle cells. sCD14 is found in both serum and urine (5).

The binding of LPS to CD14 requires an acute phase protein, LPS-binding protein (LBP) (16). The relationship of mCD14, sCD14, LPS and LBP is complicated. At low concentrations of LPS, LBP is essential for the binding of LPS to CD14, but at high concentrations, LBP may actually inhibit binding of LPS to CD14. In addition, sCD14 may compete with mCD14 for LPS (17) and may serve to help clear LPS (18). These four factors thus appear to participate in a complex feedback mechanism of immune regulation involving both up-regulation and down-regulation of the inflammatory process triggered by LPS. It is loss of control of this mechanism that appears to lead to septic shock. LPS-bound CD14 signals production of inflammatory cytokines and other inflammatory proteins, but the mechanism of signal transduction is unclear. Since a GPI anchor is not transmembrane, there presumably is another transmembrane protein on cells through which LPS-bound CD14 transmits a signal (19).

In addition to its well known role in gram-negative infections, CD14 likely serves other functions as well. It recognizes soluble peptidylglycan from gram-positive cell walls (20), and it has been reported to bind apoptotic cells and induce their phagocytosis (21).

The Quantikine Human soluble CD14 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human soluble CD14 in cell culture supernates, serum, and plasma. It contains recombinant human CD14 expressed from CHO cells and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained measuring natural human sCD14 showed dose-response curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this kit can be used to determine relative mass values for natural human sCD14.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sCD14 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sCD14 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for sCD14 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of sCD14 bound in the initial step. 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 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 soluble receptors, binding proteins, and 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.

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
sCD14 Microplate	890639	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against sCD14.	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.*
sCD14 Conjugate	890640	21 mL of polyclonal antibody against sCD14 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
sCD14 Standard	890641	80 ng of recombinant human sCD14 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate (5X)	895151	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.	
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 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human sCD14 Controls (optional; available from R&D Systems).

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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 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, citrate, 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.

SAMPLE PREPARATION

Serum and plasma samples require at least a 200-fold dilution into Calibrator Diluent RD5P (1X). A suggested 200-fold dilution is 10 µL sample + 1990 µL Calibrator Diluent RD5P (1X).

Cell culture supernate samples may require dilution.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

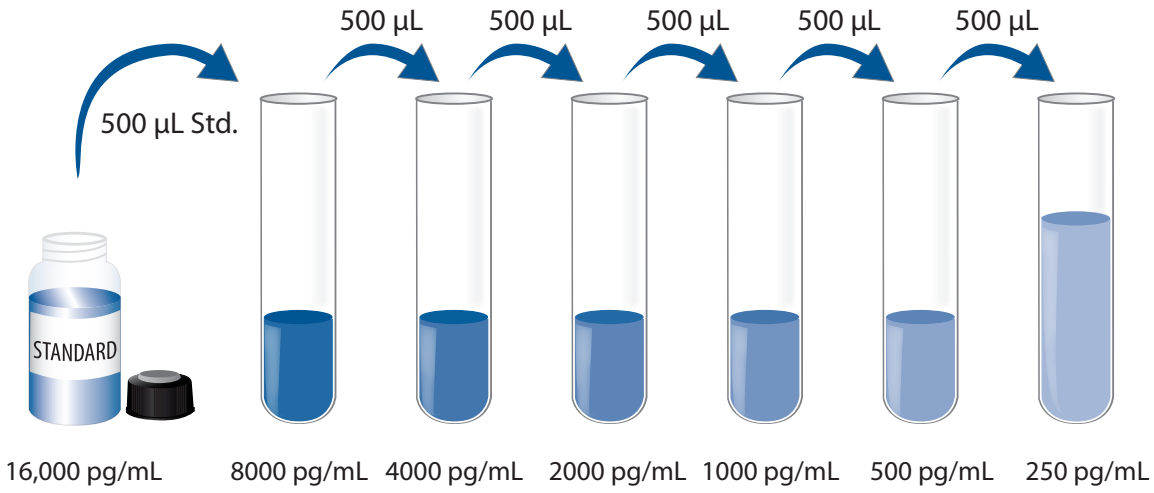
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (1X) - Dilute 20 mL of Calibrator Diluent RD5P (5X) into deionized or distilled water to yield 100 mL of Calibrator Diluent RD5P (1X).

sCD14 Standard - Reconstitute the sCD14 Standard with 5.0 mL of Calibrator Diluent RD5P (1X). This reconstitution produces a stock solution of 16,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD5P (1X) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 16,000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (1X) 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 samples, standards, and controls 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 RD1W to each well.
4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature. A plate layout is provided as a record of samples and standards 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 sCD14 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. 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.
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.

*Serum and plasma samples require dilution. Some cell culture samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

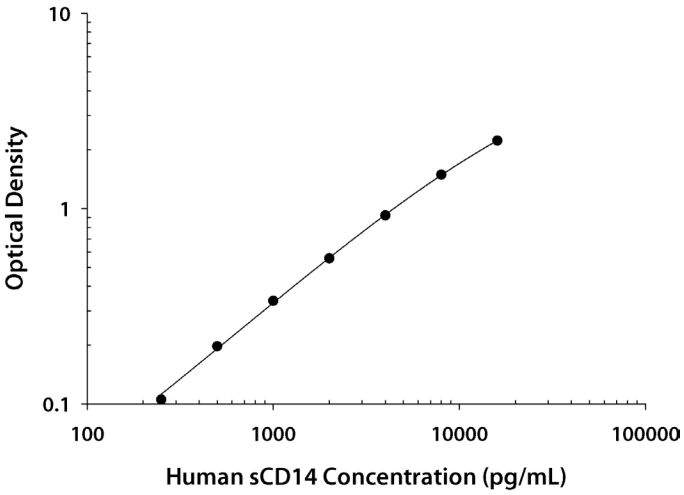
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 sCD14 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	0.037 0.043	0.040	—
250	0.145 0.145	0.145	0.105
500	0.238 0.236	0.237	0.197
1000	0.379 0.375	0.377	0.337
2000	0.597 0.595	0.596	0.556
4000	0.965 0.958	0.962	0.922
8000	1.530 1.530	1.530	1.490
16,000	2.275 2.262	2.268	2.228

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 forty separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	1111	2158	4187	1159	2298	4346
Standard deviation	71	104	216	86	153	209
CV (%)	6.4	4.8	5.2	7.4	6.7	4.8

RECOVERY

The recovery of sCD14 spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	91	85-105%
Serum* (n=5)	98	84-107%
EDTA plasma* (n=5)	98	90-107%
Heparin plasma* (n=5)	96	91-104%
Citrate plasma* (n=5)	102	88-110%

*Samples were first diluted 1:400 and then spiked.

SENSITIVITY

The minimum detectable dose (MDD) of sCD14 is typically less than 125 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing or spiked with high concentrations of sCD14 were diluted with Calibrator Diluent RD5P (1X) to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum* (n=5)	EDTA plasma* (n=5)	Heparin plasma* (n=5)	Citrate plasma* (n=5)
1:2	Average % of Expected	98	100	100	97	106
	Range (%)	91-102	99-102	97-102	89-110	104-109
1:4	Average % of Expected	99	107	99	100	109
	Range (%)	91-104	103-116	96-103	95-114	105-118
1:8	Average % of Expected	99	109	100	99	110
	Range (%)	87-107	104-118	95-109	90-105	103-118
1:16	Average % of Expected	96	103	98	102	105
	Range (%)	87-105	96-116	90-111	88-111	97-111

*Samples were diluted as directed in the Sample Preparation section.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human sCD14 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of sCD14 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=66)	1900	800-3200
EDTA plasma (n=34)	1800	1200-2600
Heparin plasma (n=34)	1900	1200-3100
Citrate plasma (n=34)	2000	1500-3000

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernate were removed on days 1, 3, and 5 and assayed for levels of natural sCD14.

Sample Type	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated	5070	9457	7762
Stimulated	12,287	22,343	24,720

SPECIFICITY

This assay recognizes natural and recombinant human sCD14.

Lipopolysaccharide was prepared at 1 µg/mL, mouse CD14 was prepared at 50 ng/mL, and the other factors listed below were prepared at 160 ng/mL in Calibrator Diluent RD5P (1X) and assayed for cross-reactivity. Preparations of the following factors prepared as described above in a mid-range recombinant human sCD14 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
AR
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
G-CSF
GM-CSF
sgp130
GROα
GROβ
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-1 sRI
IL-1 sRII
IL-2
IL-2 sRα
IL-3
IL-3 sRα
IL-4
IL-4 sR
IL-5
IL-5 sRβ
IL-6
IL-6 sR
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
KGF
LAP (TGF-β1)
LIF
M-CSF
MCP-1
MIP-1α
MIP-1β
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β2
TGF-β3
TGF-β sRII
TNF-α
TNF-β
sTNF RI
sTNF RII
VEGF

Recombinant mouse:

CD14
CD14/Fc Chimera
GM-CSF
IL-1α
IL-1β
IL-3
IL-4
IL-5
IL-5 sRα
IL-6
IL-7
IL-9
IL-10
IL-13
LIF
MIP-1α
MIP-1β
SCF
TNF-α

Recombinant amphibian:

TGF-β5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1

Other:

Lipopolysaccharide

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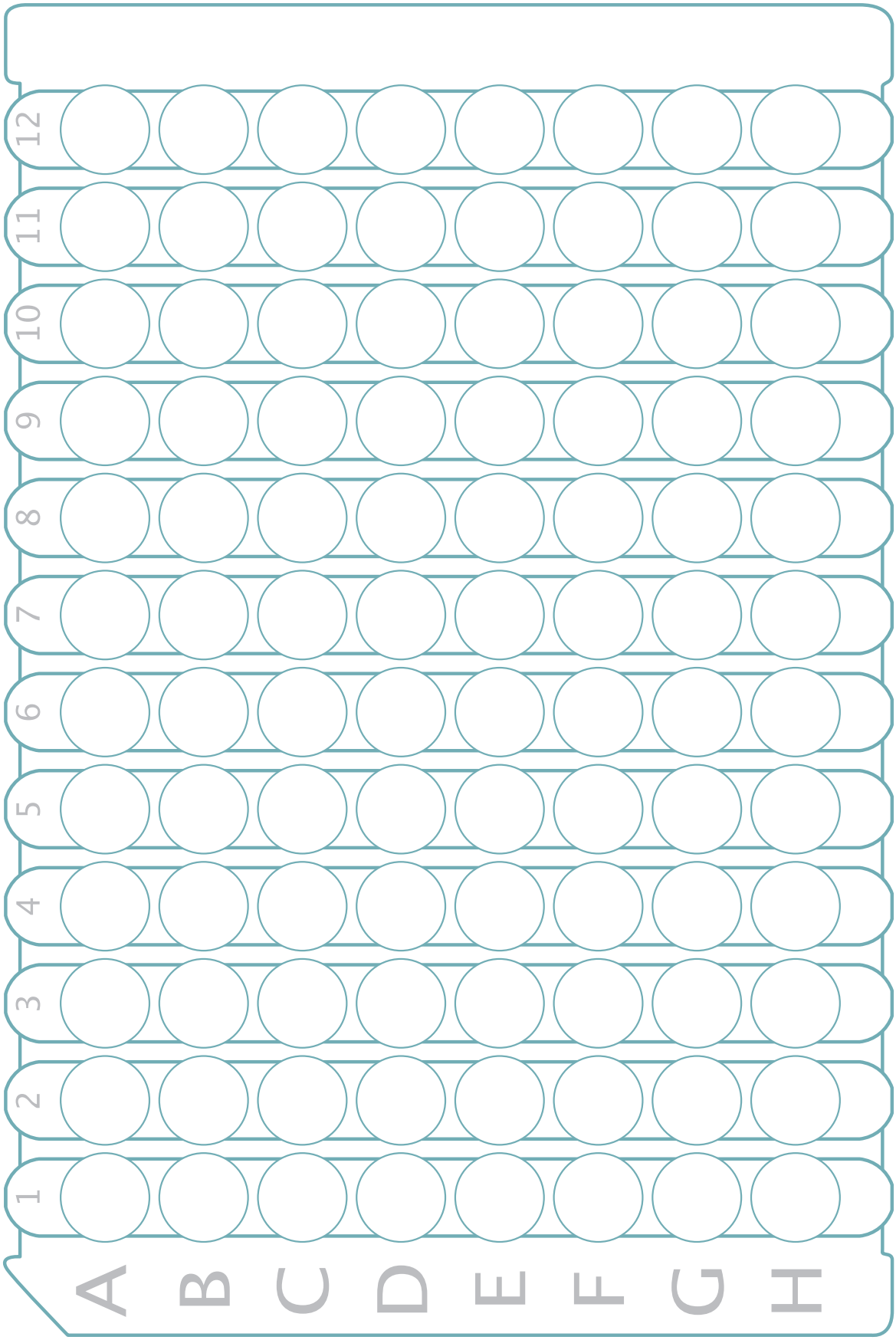
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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