Quantikine® QuicKit™ ELISA

Human CD14 Immunoassay

Catalog Number QK383

For the quantitative determination of human CD14 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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Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

CD14 is an acute phase glycoprotein that binds lipopolysaccharide endotoxins (LPS) with cells, thereby signaling the presence of gram-negative bacteria (1-4). Its 11 leucine-rich repeats mediate the interaction with LPS. The 55 kDa form, mCD14, is anchored to the membrane via glycosylphosphatidylinositol (GPI) linkage (1, 2, 5-7). Human mCD14 shares 63-73% amino acid sequence identity with mouse, rat or rabbit CD14. Soluble forms of CD14, or sCD14, may be secreted prior to GPI linkage or shed by proteolysis or cleavage of the GPI linkage. This variation, plus variable glycosylation, creates forms that may range from 43 to 53 kDa (8-10). In humans, the N terminal 13 kDa, termed presepsin or sCD14-ST, is a small soluble subtype that is found in plasma during sepsis or local infection (11-13). Membrane CD14 is expressed primarily on the cells that are most sensitive to LPS, including monocytes, macrophages and neutrophils (1-3). Lower amounts are detected on other cells, such as B cells, epithelial cells, endothelial cells, and fibroblasts (1, 14-16). Soluble CD14 is found in serum, urine and body fluids (5).

CD14 cooperates with another acute phase protein, LBP (LPS-binding protein), which binds LPS and transfers it to CD14 (1-3, 17). LPS can be further transferred from CD14 to the TLR4/MD2 complex on the cell surface (1). LPS causes signaling via clustering of LPS-bound CD14/TLR4/MD2 complexes within cholesterol-rich lipid rafts (1). These signals induce production of inflammatory cytokines and other inflammatory proteins (18, 19). When uncoordinated, the signals contribute to septic shock (1). CD14 potentiates TLR-mediated signals triggered by microbe-derived ligands other than LPS, including TLR2 activation by polymeric peptidoglycan, gram-positive bacterial lipoteichoic acid, or mycobacterial lipoarabinomannan (1, 20). It increases uptake and trafficking of the viral TLR3 ligand, poly(I:C) (1, 20). In the lungs, CD14 binds phosphoinositides and surfactant proteins, such as SP-A and SP-D, via interaction of lipids with the CD14 LPS binding site (21, 22). CD14 may also bind ICAM-3 on apoptotic leukocytes and induce phagocytosis (23).

High concentrations of sCD14 may inhibit LPS-mediated responses by competing for binding to mCD14 (24). However sCD14, in the presence of LBP, can also potentiate LPS responses or help cells to clear circulating LPS, whether or not the cells express mCD14 (16-19). At low concentrations of LPS, both sCD14 and mCD14 can mediate endothelial cell responses such as upregulation of E-selectin, while response to intermediate concentrations of LPS requires mCD14 (16). Circulating sCD14, especially presepsin, is increased in sepsis and may correlate with severity (11, 12). sCD14 may be increased as compared to normal circulating sCD14 in some autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, but decreased in others, such as Crohn's disease (25-27). sCD14 can also modulate inflammation-driven insulin resistance (28).

The Quantikine® QuicKit™ Human CD14 Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human CD14 levels in cell culture supernates, serum, and plasma. It contains CHO-expressed recombinant human CD14 and antibodies raised against the recombinant protein. Results obtained for naturally occurring human CD14 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 determine relative mass values for natural CD14.

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 CD14. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of CD14 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the diluted calibrator 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 CD14 Standard	899097	2 vials of recombinant human CD14 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human CD14 Capture Ab Concentrate	899095	Lyophilized tagged monoclonal antibody specific for human CD14.	May be stored for up to 1 month at 2-8 °C.*
Human CD14 Detection Ab Concentrate	899096	400 µL of a polyclonal antibody specific for human CD14 conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a buffered protein base with preservatives.	
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.
- 500 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.
- Human CD14 Controls (optional; R&D Systems®, Catalog # QC263).

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.*

SAMPLE PREPARATION

Serum and plasma samples require at least a 500-fold dilution prior to assay. A suggested 500-fold dilution is 10 µL sample + 490 µL Calibrator Diluent RD5P (diluted 1:10)*. Complete the 500-fold dilution by adding 20 µL of the previously diluted sample to 180 µL Calibrator Diluent RD5P (diluted 1:10)*.

Cell culture supernate samples may require dilution due to high endogenous levels.

*See Reagent Preparation section

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Assay Diluent RD1W (diluted 1:2) - Add 5 mL of Assay Diluent RD1W to 5 mL of deionized or distilled water to prepare 10 mL of Assay Diluent RD1W (diluted 1:2).

Human CD14 Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CD14 Capture Ab Concentrate with Assay Diluent RD1W. 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 RD1W (diluted 1:2). For a full plate, add 300 µL of reconstituted Human CD14 Capture Ab stock and 300 µL of Human CD14 Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1W (diluted 1:2).

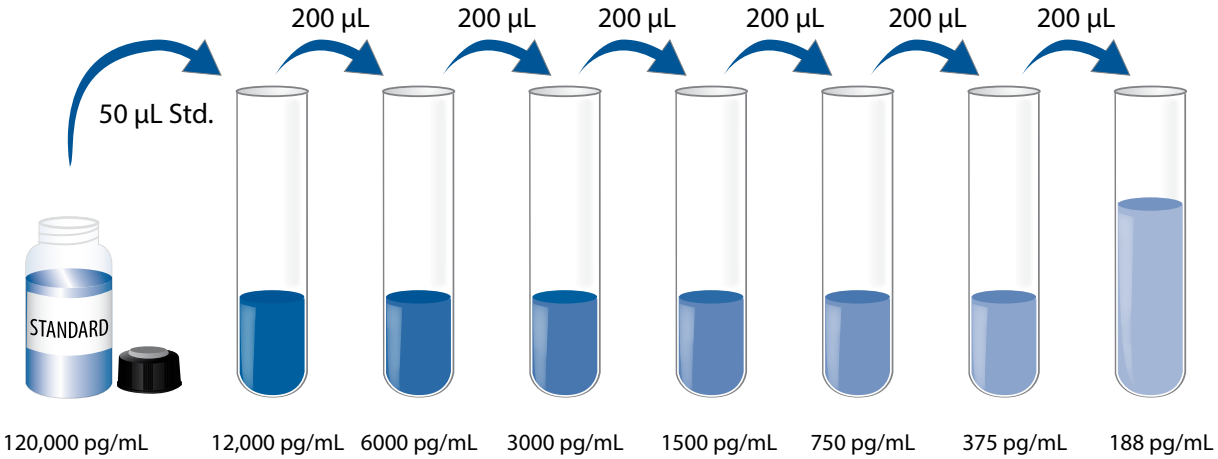
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:10) - Add 5 mL of Calibrator Diluent RD5P to 45 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:10).

Human CD14 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human CD14 Standard with Calibrator Diluent RD5P (diluted 1:10). This reconstitution produces a stock solution of 120,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:10) into the 12,000 pg/mL. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 12,000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:10) 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, 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. A plate layout is provided to record standards and samples assayed.
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

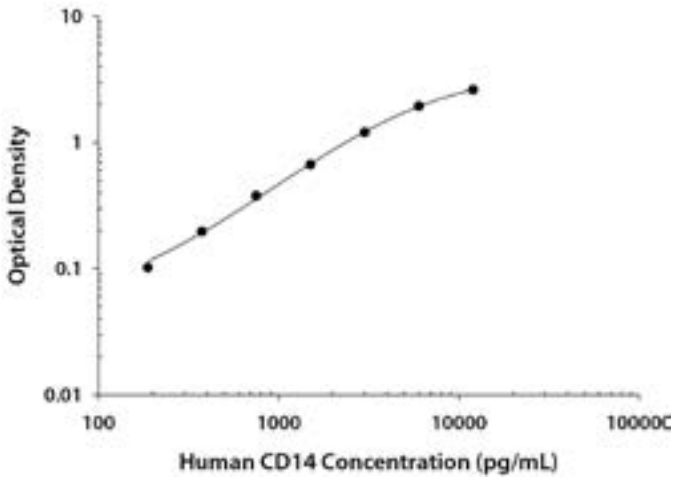
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 CD14 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	0.123		—
	0.124	0.124	
188	0.225		
	0.226	0.226	0.102
375	0.320		
	0.321	0.321	0.197
750	0.500		
	0.504	0.502	0.378
1500	0.790		
	0.796	0.793	0.669
3000	1.322		
	1.329	1.326	1.202
6000	2.030		
	2.099	2.065	1.941
12,000	2.724		
	2.757	2.741	2.617

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)	1228	8177	1282	8360
Standard deviation	28.3	262	77.4	339
CV (%)	2.3	3.2	6.0	4.1

RECOVERY

The recovery of human CD14 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=4)	97	92-100%

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of human CD14 ranged from 7.12-18.8 pg/mL. The mean MDD was 12.2 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 high concentrations of human CD14 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum * (n= 4)	EDTA plasma* (n= 4)	Heparin plasma* (n= 4)
1:2	Average % of Expected	102	97	97	99
	Range (%)	101-104	91-104	92-104	90-105
1:4	Average % of Expected	104	98	98	99
	Range (%)	100-112	91-107	92-108	86-108
1:8	Average % of Expected	105	98	99	99
	Range (%)	98-115	94-106	94-105	87-109
1:16	Average % of Expected	101	95	97	94
	Range (%)	95-111	92-98	89-100	82-98

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

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human CD14 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human CD14 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=10)	1901	1457-2260
EDTA plasma (n=10)	1534	1040-2015
Heparin plasma (n=10)	1678	1330-2480

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. Aliquots of the cell culture supernates were removed after 1 day, assayed for human CD14, and measured 6561 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human CD14.

The factors listed below were prepared at 160 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 160 ng/mL in a low level recombinant human CD14 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

FGF acidic
FGF basic
GM-CSF
IL-4
IL-13
LBP
LPA R1
MD2
MyD88
TIRAP
TLR1
TLR4
TLR6
TLR7
TLR9
TNF-α
TRAF-6

Other recombinants:

mouse CD14

Other:

Lipopolysaccharide*

*Lipopolysaccharide was prepared at 1.0 µg/mL.

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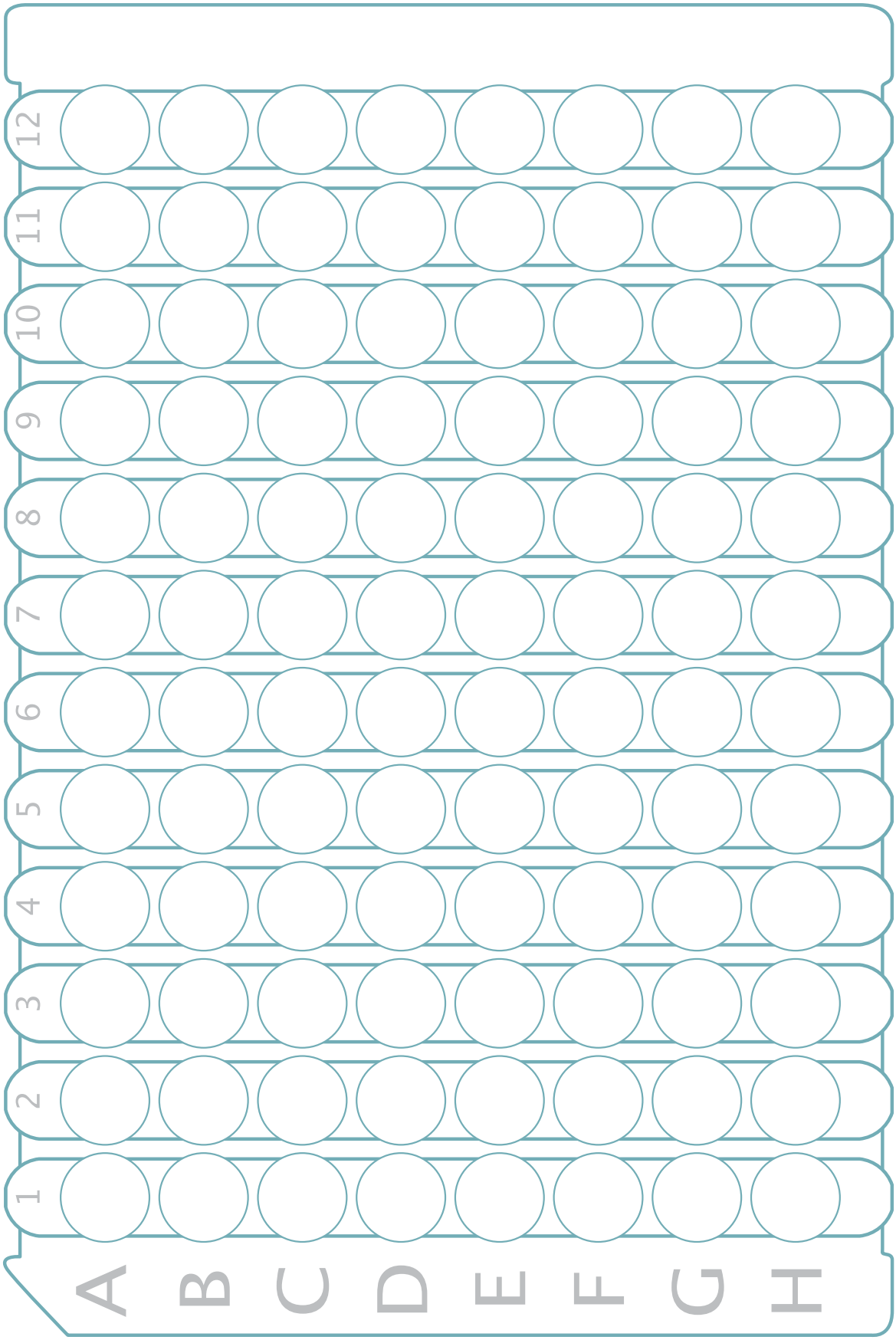
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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