Quantikine® ELISA

Human Apolipoprotein A-I/ApoA1 Immunoassay

Catalog Number DAPA10

For the quantitative determination of human Apolipoprotein A1 (ApoA1) concentrations in cell culture supernates, serum, plasma, and urine.

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

Apolipoprotein A1 (ApoA1) is a 28 kDa glycoprotein that is the major protein component of high density lipoprotein (HDL) particles. HDL particles play a central role in the reverse transport of cholesterol from peripheral tissues to the liver. HDL is known as "good cholesterol" due to its ability to facilitate the removal of cholesterol from macrophage foam cells in atherosclerotic plaques and thereby retard the progression of disease (1-4). ApoA1 is secreted from hepatocytes with variable amounts of lipidation (5). Disc-like HDL particles increase in size and adopt a spherical shape as they are loaded with additional lipids and cholesterol by ABC family transporters (6, 7). HDL particle interaction with lipid-supplying cells is mediated by ApoA1 binding to the scavenger receptor SR-A1 (8). HDL particles are further modified by the circulating enzymes LCAT (which converts cholesterol to cholesteryl esters), CETP (which transfers cholesteryl esters to LDL/VLDL particles), and PLTP (which transfers phospholipids from HDL to LDL/VLDL particles) (2). Upon HDL particle return to the liver, ApoA1 binds to the scavenger receptor SR-B1 and the beta chain of ATP synthase on hepatocytes (9, 10). Hepatocytes internalize the particles and pass the cholesterol into bile for excretion. Polymorphisms of ApoA1 are associated with dysregulation of HDL levels and cholesterol homeostasis (1, 11). ApoA1 is catabolized in the kidney following its binding to Cubulin on renal proximal tubule epithelial cells (12). ApoA1, either as a free molecule or in lipidated particulate form, induces the release of insulin from pancreatic islets in a process that is dependent on ABCA1 and SR-B1 (13). Mature human ApoA1 shares 65% and 62% amino acid sequence identity with mouse and rat ApoA1, respectively.

The Quantikine Human Apolipoprotein A-I/ApoA1 Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human ApoA1 in cell culture supernates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant human ApoA1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ApoA1 showed linear 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 naturally occurring human ApoA1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ApoA1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ApoA1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ApoA1 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 ApoA1 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 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
Human ApoA1 Microplate	894879	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ApoA1.	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 ApoA1 Standard	894881	2 vials of recombinant human ApoA1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a fresh standard for each assay.
Human ApoA1 Conjugate	894880	21 mL of a polyclonal antibody specific for human ApoA1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	4 vials (21 mL/vial) of a concentrated buffered protein base with preservatives. *Use as a concentrate in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. *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 and samples.
- Human ApoA1 Controls (optional; R&D Systems, Catalog # QC214).

PRECAUTIONS

ApoA1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® 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. Please 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 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 heparin or EDTA 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.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 20,000-fold dilution. A suggested 20,000-fold dilution can be achieved by adding 20 µL of sample to 480 µL of Calibrator Diluent RD5P Concentrate. Then add 20 µL of the diluted sample to 480 µL of Calibrator Diluent RD5P Concentrate. Complete the 20,000-fold dilution by adding 20 µL of the diluted sample to 620 µL of Calibrator Diluent RD5P Concentrate.

Urine samples require a 2-fold dilution due to the matrix effect. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5P Concentrate.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

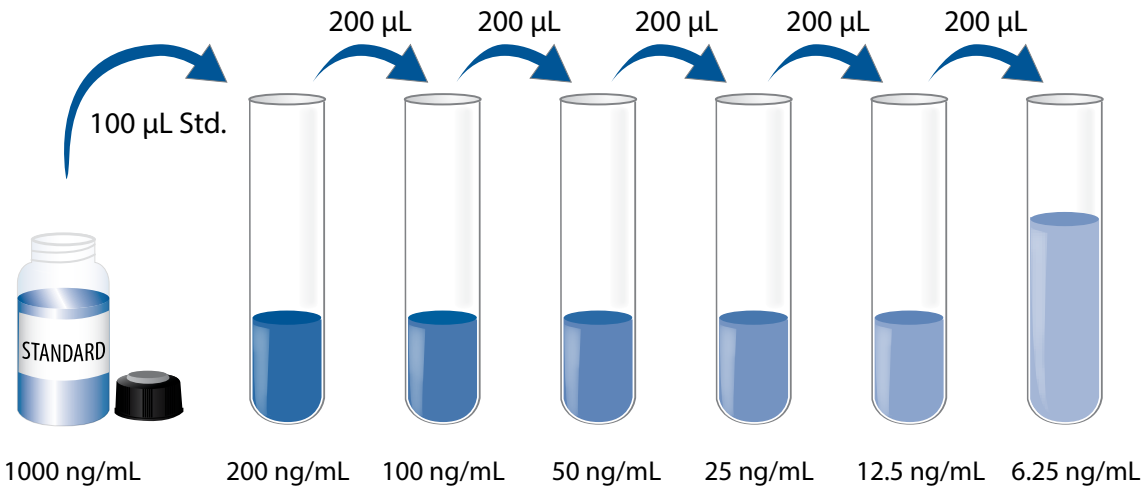
Note: *ApoA1 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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.

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.

Human ApoA1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ApoA1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 1000 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 400 µL of Calibrator Diluent RD5P Concentrate into the 200 ng/mL tube. 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 200 ng/mL standard serves as the high standard. Calibrator Diluent RD5P Concentrate 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, samples, and controls be assayed in duplicate.

Note: *ApoA1 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 50 µL of Standard, control, or sample* per 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 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 ApoA1 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

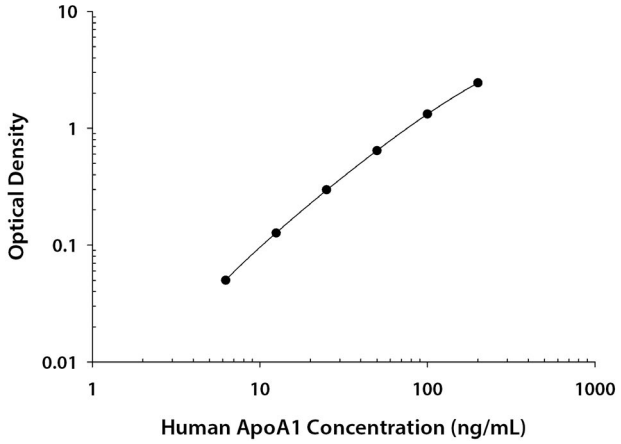
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 ApoA1 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.



(ng/mL)	O.D.	Average	Corrected
0	0.008 0.011	0.010	—
6.25	0.059 0.061	0.060	0.050
12.5	0.136 0.137	0.137	0.127
25	0.305 0.308	0.307	0.297
50	0.650 0.656	0.653	0.643
100	1.325 1.336	1.331	1.321
200	2.438 2.469	2.454	2.444

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)	30.3	60.2	120	35.5	67.9	129
Standard deviation	1.43	2.17	4.05	1.92	4.65	9.82
CV (%)	4.7	3.6	3.4	5.4	6.8	7.6

RECOVERY

The recovery of human ApoA1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	90-114%
Urine* (n=4)	99	83-109%

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

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human ApoA1 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)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	102	104	105	103	100
	Range (%)	100-105	101-106	99-111	101-105	95-106
1:4	Average % of Expected	102	105	104	107	99
	Range (%)	100-102	97-111	98-111	103-111	95-106
1:8	Average % of Expected	101	106	104	109	100
	Range (%)	99-103	93-113	94-115	103-115	91-111
1:16	Average % of Expected	94	111	106	110	98
	Range (%)	90-99	106-114	101-114	109-112	93-104

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

SENSITIVITY

Thirty-two assays were evaluated and the minimum detectable dose (MDD) of human ApoA1 ranged from 0.097-0.603 ng/mL. The mean MDD was 0.275 ng/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.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human ApoA1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human ApoA1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (mg/mL)	Range (mg/mL)	Standard Deviation (mg/mL)
Serum (n=36)	2.21	1.48-3.20	0.466
EDTA plasma (n=36)	1.97	1.40-2.87	0.405
Heparin plasma (n=36)	1.99	1.19-2.97	0.466

Sample Type	Mean of Detectable (µg/g Creatinine)	% Detectable	Range (ng/mL)
Urine (n=10)	14.9	30	ND-28.3

ND=Non-detectable

Cell Culture Supernates:
Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were unstimulated or stimulated with 10 µg/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for human ApoA1. No detectable levels were observed.

HepG2 human hepatocellular carcinoma cells were cultured in MEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 1 mM sodium pyruvate. An aliquot of the cell culture supernates were removed, assayed for human ApoA1, and measured 2160 ng/mL.

Hep3B human hepatocellular carcinoma cells were cultured in DMEM:F12 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernates were removed, assayed for human ApoA1, and measured 672 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human ApoA1.

The factors listed below were prepared at 2500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 2500 ng/mL in a mid-range recombinant human ApoA1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

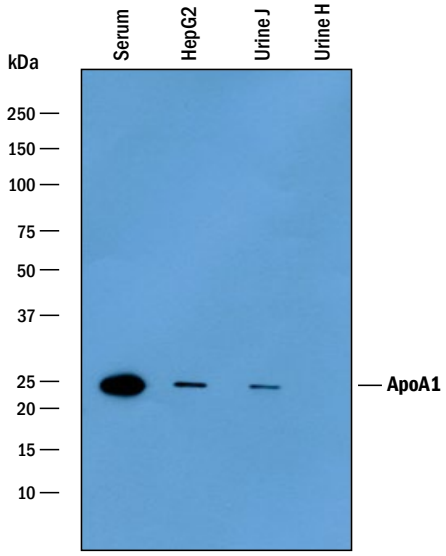
ApoA2
ApoB
ApoB100
ApoC1
ApoC2
ApoD
ApoE
ApoER2
ApoH
ApoM
APP
Cubulin
LRP-1C2
LRP-1C4
LDL R
MSR

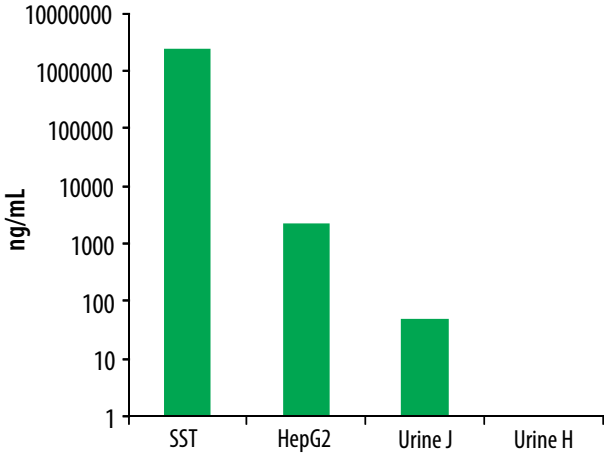
Recombinant mouse:

LDL R

Natural proteins:

human ApoA2





Human serum, HepG2 conditioned media, and urine were analyzed by Western blot and Quantikine ELISA. For the Western blot, diluted samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody used in this kit. The Western blot shows a direct correlation with the ELISA value for these samples.

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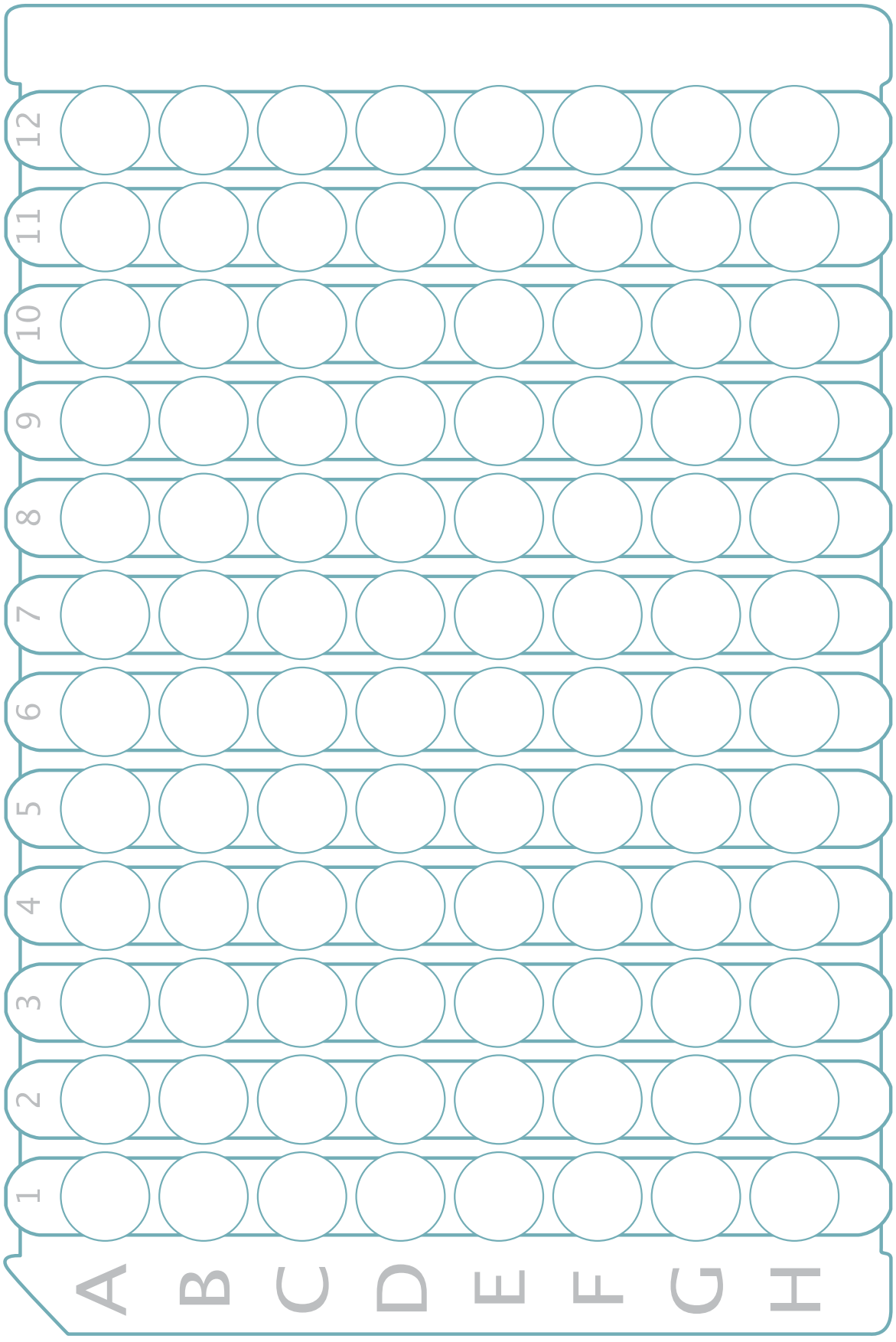
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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