Quantikine® ELISA

Human Proprotein Convertase 9/PCSK9 Immunoassay

Catalog Number DPC900
SPC900
PDPC900

For the quantitative determination of human Proprotein Convertase Subtilisin Kexin 9 (PCSK9) concentrations in cell culture supernates, cell lysates, 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

Proprotein convertase subtilisin kexin 9 (PCSK9), also named neural apoptosis-regulated convertase 1 (NARC-1), is a member of the proteinase K subfamily of subtilisin-related serine endoproteases. The full-length protein has 692 amino acids, including a signal peptide, a pro- domain, and a catalytic domain. PCSK9 is highly expressed in the liver, intestine, and kidney. It is initially synthesized as a soluble 74 kDa precursor protein. In the endoplasmic reticulum, it undergoes autocatalytic intramolecular cleavage to generate a 14 kDa pro- domain and a 60 kDa catalytic domain. These two domains remain associated when PCSK9 is secreted outside the cells (1-3). The primary physiologic function of PCSK9 is to mediate the degradation of low density lipoprotein receptor (LDL R). Early observations indicated that gain-of-function missense mutations in the PCSK9 gene can cause an autosomal dominant form of hypercholesterolemia (4, 5). The expression of PCSK9 was observed to be up-regulated by the sterol regulatory element binding proteins (SREBPs), a family of transcription factors that are responsible for the upregulation of genes involved in cholesterol and fatty acid metabolism, such as the LDL R gene (6, 7). Further experimental evidence revealed that in mice, when the PCSK9 gene was knocked out, the number of LDL R in hepatocytes increased, whereas when PCSK9 was over-expressed, the amount of LDL R protein was reduced in the liver (8, 9). In humans, genetic analyses have shown that individuals who have nonsense or loss-of-function mutations in the PCSK9 gene have significantly lower plasma LDL cholesterol levels (10, 11). These investigations clearly indicated that PCSK9 plays a key role in reducing the hepatic LDL R levels. Recently, the underlying mechanism has been uncovered: under normal physiologic conditions, the LDL R is internalized on the cell surface and directed to the endosomes in order to be recycled back to the cell surface. PCSK9 binds to the EGF domain of the LDL R and prevents LDL R from being sorted to the endosomes. Instead, the PCSK9/LDL R complex is redistributed to the lysosomes for degradation (12-14). As such, PCSK9 regulates the amount of LDL R in the circulation and modulates cholesterol levels. Serum PCSK9 concentrations have been found to be directly associated with cholesterol levels (15, 16). Since individuals with loss-of-function PCSK9 mutations have strikingly reduced risk of coronary heart diseases, PCSK9 has become an attractive drug target in recent years (17, 18). One approach is to generate small molecules that are able to interfere with PCSK9 autoactivation and its interaction with LDL R. Other approaches aiming to reduce the amounts of PCSK9 in the circulation, such as small interfering RNAs (siRNAs), have also shown promise (19, 20).

The Quantikine® Human Proprotein Convertase 9/PCSK9 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human PCSK9 in cell culture supernates, cell lysates, serum, and plasma. It contains NS0-expressed recombinant human PCSK9 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural PCSK9 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 natural human PCSK9.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human PCSK9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PCSK9 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human PCSK9 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 PCSK9 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 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 #	CATALOG # DPC900	CATALOG # SPC900	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human PCSK9 Microplate	893354	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human PCSK9.	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 PCSK9 Standard	893356	1 vial	6 vials	Recombinant human PCSK9 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C.*
Human PCSK9 Conjugate	893355	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human PCSK9 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-9	895167	1 vial	6 vials	11 mL/vial of a buffered protein solution with preservatives. *May contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*	
Calibrator Diluent RD5P	895151	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DPC900 contains sufficient materials to run an ELISA on one 96 well plate.
SPC900 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDPC900). Specific vial counts of each component may vary. 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.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human PCSK9 Microplate	893354	50 plates
Human PCSK9 Conjugate	893355	50 vials
Human PCSK9 Standard	893356	25 vials
Calibrator Diluent RD5P	895151	50 vials
Assay Diluent RD1-9	895167	50 vials
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Wash Buffer Concentrate	895126	9 bottles
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Inserts	751650	2 booklets

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 PCSK9 Controls (optional; R&D Systems®, Catalog # QC235).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 1 (R&D Systems®, Catalog # 890713).

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.

Cell Lysates - Prior to assay, cells must be lysed according to the directions in the Cell Lysis Procedure.

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 a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5P (diluted 1:5)*.

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples.

1. Resuspend cells at 5 x 10^6 cells/mL in 10 mL of Cell Lysis Buffer 1.

2. Incubate with periodic vortexing for 30 minutes at room temperature.

3. Centrifuge for 10 minutes at 12,000 rpm to remove cell debris.

4. Aliquot the lysis supernate and store at ≤ -20 °C until ready for use.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

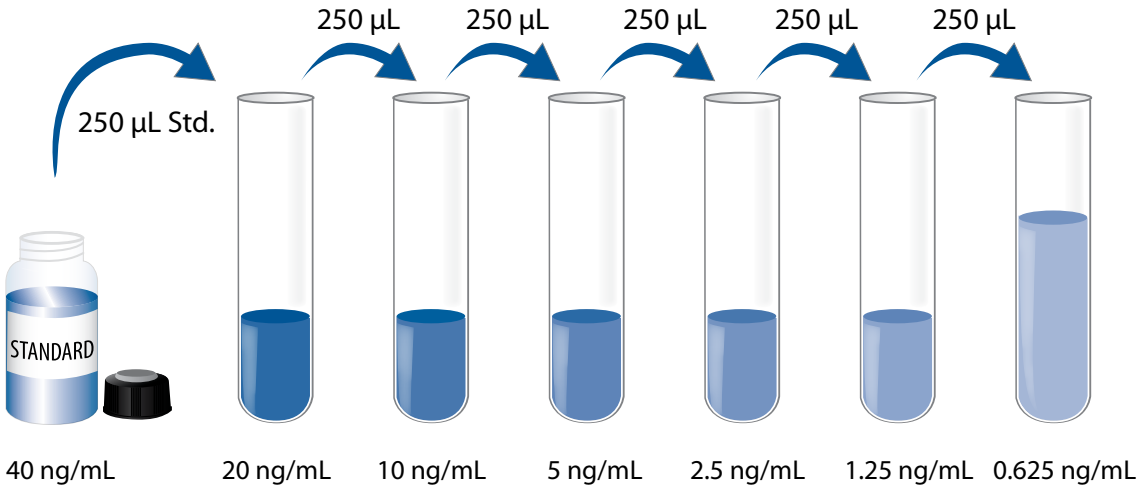
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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

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

Human PCSK9 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human PCSK9 Standard with Calibrator Diluent RD5P (diluted 1:5). This reconstitution produces a stock solution of 40 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum 15 minutes with gentle agitation prior to making dilutions.

Pipette 250 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted PCSK9 Standard (40 ng/mL) 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, 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 RD1-9 to each well. *Assay Diluent RD1-9 may contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well 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. A plate layout is provided to record the 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 PCSK9 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

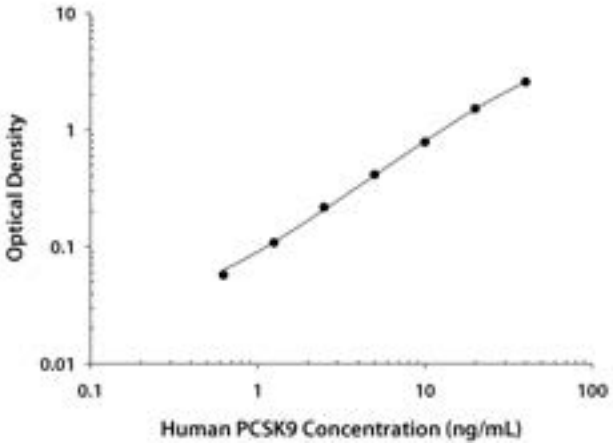
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 PCSK9 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 prior to assay, 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	0.015 0.016	0.016	—
0.625	0.069 0.077	0.073	0.057
1.25	0.121 0.126	0.124	0.108
2.5	0.231 0.235	0.233	0.217
5	0.426 0.435	0.431	0.415
10	0.786 0.817	0.802	0.786
20	1.510 1.542	1.526	1.510
40	2.577 2.578	2.578	2.562

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. 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	40	40	40
Mean (ng/mL)	4.82	14.0	27.7	4.64	14.5	27.9
Standard deviation	0.196	0.795	1.81	0.276	0.629	1.13
CV (%)	4.1	5.7	6.5	5.9	4.3	4.1

RECOVERY

The recovery of human PCSK9 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	107	100-111%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human PCSK9 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=2)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	105	104	104
	Range (%)	99-102	103-107	102-106	102-107
1:4	Average % of Expected	103	106	105	104
	Range (%)	101-104	104-108	101-108	100-109
1:8	Average % of Expected	107	106	105	107
	Range (%)	105-109	102-110	99-115	102-113
1:16	Average % of Expected	112	104	103	106
	Range (%)	110-114	96-109	97-109	102-110

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

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human PCSK9 ranged from 0.030-0.219 ng/mL. The mean MDD was 0.096 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-expressed recombinant human PCSK9 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human PCSK9 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=37)	313	177-460	71.5
EDTA plasma (n=37)	308	160-521	73.3
Heparin plasma (n=37)	315	159-547	81.7

Cell Culture Supernates - HepG2 human hepatocellular carcinoma cells were cultured in DMEM 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 human PCSK9, and measured 47.2 ng/mL.

Cell Lysates - HepG2 human hepatocellular carcinoma cells were prepared as described in the Cell Lysis Procedure. An aliquot of the cell lysate was removed, assayed for human PCSK9, and measured 4.74 ng/mg of total protein.

SPECIFICITY

This assay recognizes free and LDL R-bound PCSK9, and recombinant human PCSK9.

The factors listed below were prepared at 400 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 400 ng/mL in a mid-range recombinant human PCSK9 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

LDLR
PCSK1
PCSK3 (Furin)
PCSK7

Recombinant mouse:

PCSK9

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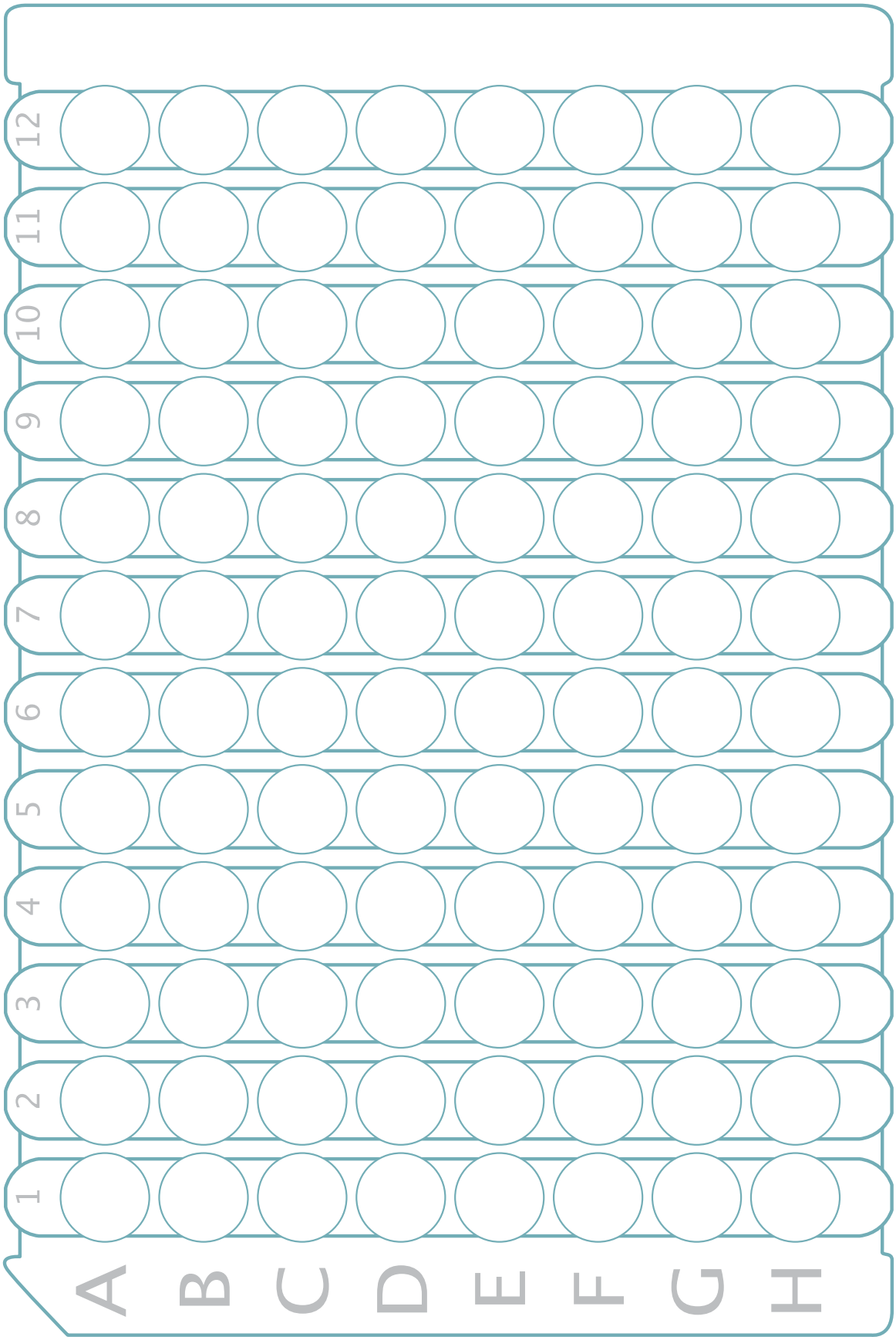
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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