Quantikine® ELISA

Human LDL R Immunoassay

Catalog Number DLDLR0

For the quantitative determination of human Low-Density Lipoprotein Receptor (LDL R) concentrations in cell culture supernates, cell lysates, serum, heparin 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

The Low-Density Lipoprotein Receptor (LDL R) is the founding member of the LDL R family of widely expressed cell surface scavenger receptors (1-6). Although members of the family were originally identified as endocytic receptors, they also co-regulate adjacent cell surface signaling molecules (3, 5). Many proteins in the LDL R family are cleaved by extracellular proteases to release soluble forms into the circulation, and many of these soluble forms are active (1, 7). A 140 kDa soluble form of LDL R that includes most or all of the extracellular domain (ECD) can be released from PMA-treated cell lines, such as the liver cell line HepG2 (7). This release is downregulated by inhibiting protein kinase C and/or metalloproteinases. Additionally, an antiviral 28 kDa soluble form of LDL R consisting of the ligand binding portion of the ECD is reportedly released from the cell surface in response to interferons (8, 9).

Mature LDL R is a 160 kDa type I transmembrane glycoprotein that contains cysteine-rich complement-like repeats (class A LDL R domains), calcium-binding EGF repeats, and β-propeller structures (class B LDL R domains) in the ECD (4-6, 10). Class A domains bind apolipoproteins and class B LDL repeats are likely involved in intracellular LDL release by interacting with class A domains at low pH (5, 8). A membrane-proximal Ser/Thr-rich region shows extensive O-linked glycosylation. In other family members, glycosylation of this region can inhibit ECD shedding (5, 6, 11). A cytoplasmic NPxY motif links the LDL R to clathrin pits for endocytosis and binds to select adaptor proteins (1, 11, 12). The human LDL R ECD shares 78% and 76% amino acid identity with mouse and rat LDL R, respectively.

LDL R is constitutively and widely expressed. Its expression can be downregulated by LDL and upregulated by hormones such as insulin and estradiol, and by many cytokines (7, 13). Its ECD binds ApoB and ApoE, the proteins that predominate in low- and very low-density lipoproteins (LDL and VLDL), respectively (1, 2, 5, 14). Hepatocyte LDL R is responsible for endocytosis and clearing of most plasma LDL cholesterol (2, 14). LDL R binds the ApoB portion of LDL and brings it into endocytic vesicles. It dissociates at the low pH of the vesicle, allowing LDL to be degraded while LDL R is recycled back to the cell surface (5, 10, 13, 15). Lack of LDL R expression or function causes familial hypercholesterolemia (FH); over 1100 FH-related human LDL R mutations have been identified (5, 13-16). Overexpression or hyperfunction of the protease PCSK9 can also cause increased plasma cholesterol by lowering cell surface LDL R expression (16-18). PCSK9 alters the pH-induced LDL R conformation change in the endocytic vesicle, thus promoting LDL R degradation rather than recycling to the cell surface (17-19).

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human LDL R has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LDL R present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human LDL R 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 LDL R 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 LDL R Microplate	894744	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human LDL R.	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 LDL R Conjugate	894745	21 mL of a polyclonal antibody specific for human LDL R conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human LDL R Standard	894746	2 vials of recombinant human LDL R in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-64	895355	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-25	895356	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 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 and samples.
- Human LDL R Controls (optional; available from R&D Systems).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 2 (R&D Systems, Catalog # 895347)
- PBS

PRECAUTIONS

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.

Cell Lysates - Cells must be lysed prior to assay as directed 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 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: *EDTA plasma is not suitable for use in this assay.*
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, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and heparin plasma samples require a 40-fold dilution. A suggested 40-fold dilution is 10 µL of sample + 390 µL of Calibrator Diluent RD5-25.

CELL LYSIS PROCEDURE

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

1. Wash cells three times in cold PBS.
2. Resuspend cells at 1 x 10^7 cells/mL in Cell Lysis Buffer 2.
3. Incubate with gentle agitation for up to 60 minutes at room temperature.
4. Centrifuge at 8000 x g for 10 minutes to remove cell debris.
5. Assay immediately or aliquot the lysis supernates and store at ≤ -70 °C until ready for use.

REAGENT PREPARATION

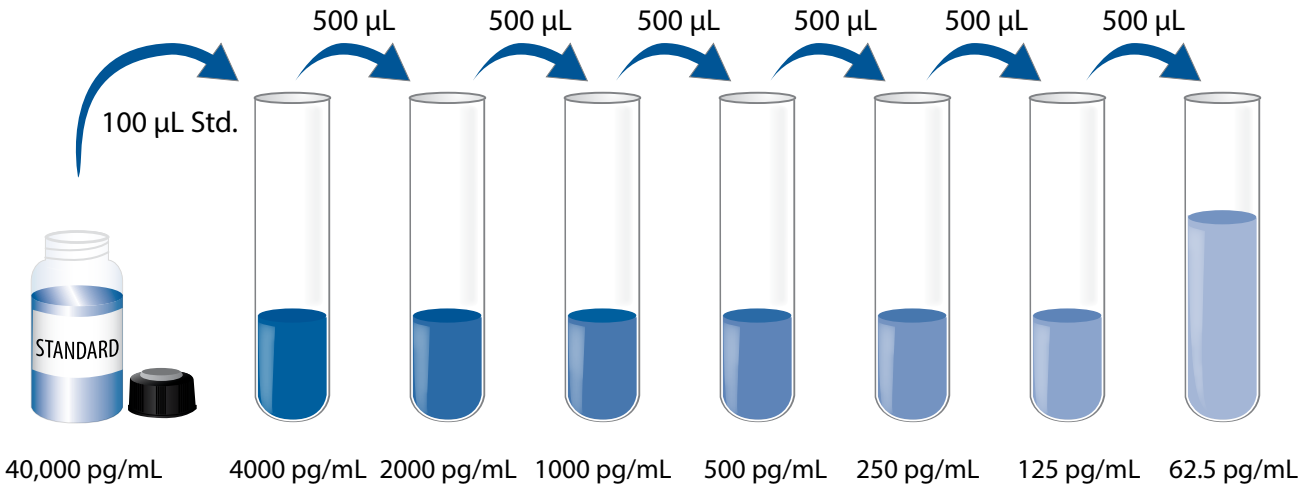
Bring all reagents to room temperature before use.

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 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 LDL R Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human LDL R Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-25 into the 4000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-25 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, controls, and standards 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 50 µL of Assay Diluent RD1-64 to each well.
4. Add 50 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 LDL R 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 the Sample Preparation section.

CALCULATION OF RESULTS

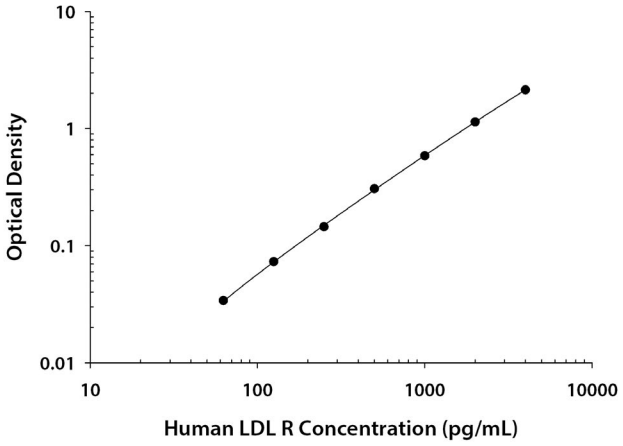
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 LDL R 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	0.017 0.018	0.018	—
62.5	0.051 0.053	0.052	0.034
125	0.090 0.091	0.091	0.073
250	0.163 0.163	0.163	0.145
500	0.318 0.329	0.324	0.306
1000	0.601 0.604	0.603	0.585
2000	1.145 1.169	1.157	1.139
4000	2.155 2.158	2.157	2.139

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 (pg/mL)	260	787	1598	287	823	1602
Standard deviation	5.68	17.5	32.4	15.6	40.0	74.4
CV (%)	2.2	2.2	2.0	5.4	4.9	4.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	92-106%
Urine (n=4)	94	91-99%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human LDL R were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Cell lysates (n=4)	Serum (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	103	100	101	100	103
	Range (%)	102-107	94-105	98-103	99-101	97-107
1:4	Average % of Expected	103	99	103	102	101
	Range (%)	99-107	94-106	100-106	101-103	95-105
1:8	Average % of Expected	101	100	104	105	100
	Range (%)	93-107	95-107	100-108	101-111	97-106
1:16	Average % of Expected	95	99	103	105	97
	Range (%)	92-97	93-104	97-110	100-112	86-104

SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of human LDL R ranged from 2.52-10.3 pg/mL. The mean MDD was 4.72 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.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human LDL R manufactured at R&D Systems.

SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=35)	52,609	18,116-102,018	25,072
Heparin plasma (n=35)	50,689	18,337-102,361	24,302

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=11)	291	91	ND-732

Cell Culture Supernates/Cell Lysates:
MCF-7 human breast cancer cells were cultured in DMEM/Kaighn's F-12 (50/50) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin until confluent. Aliquots of the cell culture supernates were removed and assayed for human LDL R. Cells were lysed and assayed for human LDL R. Results were normalized to total protein concentration.

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 until confluent. Aliquots of the cell culture supernates were removed and assayed for human LDL R. Cells were lysed and assayed for human LDL R. Results were normalized to total protein concentration.

Cell Line	Cell Culture Supernate Value (pg/mL)	Cell Lysate Value (pg/mg)
MCF-7	1224	3219
HepG2	7500	26,341

SPECIFICITY

This assay recognizes natural and recombinant human LDL R. This assay also recognizes recombinant human LDL R/PCSK9 complex.

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 LDL R control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Apolipoprotein B
Apolipoprotein B_{100}
Apolipoprotein E
LDL
LRP-12
PCSK9

Recombinant mouse:

VLDL R

Recombinant mouse LDL R cross-reacts approximately 0.30% in this assay.

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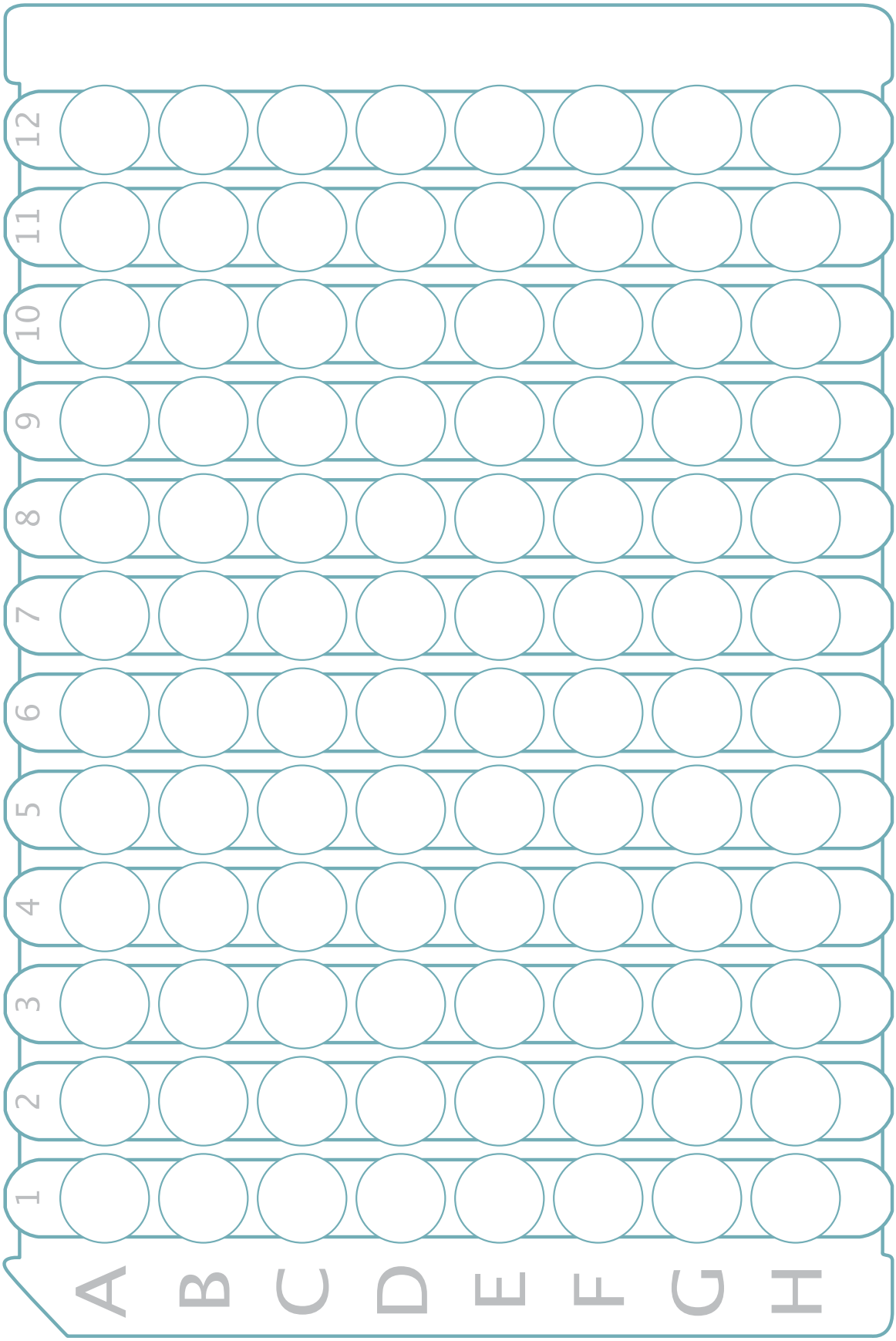
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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