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R&D SYSTEMS

Quantikine® ELISA

Human Uteroglobin Immunoassay

Catalog Number DUGB00

For the quantitative determination of human Uteroglobin concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

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

Uteroglobin, also called CCSP, CCPBP, CC10, or CC16 (Clara cell secretory protein, phospholipid binding protein, 10 kDa or 16 kDa) is a small, non-glycosylated secreted protein of the secretoglobin superfamily, (designated 1A, member 1) (1-4). Its name is derived from its very high expression in the preimplantation uterus. It is produced by the non-ciliated, non-mucous secretory cells that predominate in lung bronchioles (Clara cells) and other non-ciliated epithelia that communicate with the external environment (1-3). Expression is induced by steroid hormones such as estrogen and enhanced by the non-steroid hormone prolactin (1). Mature human Uteroglobin is a 70 amino acid (aa) secreted protein that is found in blood, urine, and other body fluids (1). It shares 53-56% aa identity with mouse, rat, bovine, canine, equine, and rabbit Uteroglobin and is active in mice (5). Uteroglobin is found as a disulfide-linked head-to-tail homodimer of 16 kDa, but it runs anomalously at 10 kDa in non-reducing SDS-PAGE (2, 6). Formation of the dimer is thought to create a binding pocket that binds hydrophobic ligands such as phospholipids, progesterone, and retinols (6). In the lungs, Uteroglobin also sequesters tumorigenic molecules such as polychlorinated bisphenols and carcinogens in cigarette smoke (7, 8).

Uteroglobin participates in several immunoregulatory activities. It sequesters prostaglandins and leukotrienes, limiting their inflammatory effects, while also inhibiting the activity of phospholipase A2, an enzyme in their synthesis pathway (3, 4). It can inhibit dendritic cell migration by binding the chemotaxis-related formyl peptide receptor, FPR2 (9). It can also inhibit T cell differentiation to the Th2 phenotype, and thus downregulate the production of Th2 cytokines (5, 10, 11). Mice deficient in Uteroglobin show exaggerated response to allergens and pollutants. Since most circulating Uteroglobin is produced by lung Clara cells, low plasma Uteroglobin can indicate low Clara cell production that is typical of airway injury due to asthma, acute lung injury, or cumulative environmental insults such as cigarette smoking (12-15). Conversely, disruption of the lung epithelial barrier function, for example, by ozone exposure, can cause leakage that increases circulating uteroglobin (13).

Uteroglobin is a ligand for some transmembrane or extracellular matrix molecules. It can bind the adhesion molecule fibronectin and may be crosslinked to fibronectin by another ligand, transglutaminase (1, 3, 16). Studies in mice indicate that Uteroglobin/fibronectin complex formation in the lungs may limit formation of fibronectin/IgA complexes that migrate to the kidney in IgA nephropathy (17-20). Uteroglobin is also a potent inhibitor of thrombin-induced platelet aggregation, possibly via interacting with integrins or transglutaminase (1). Binding of Uteroglobin to the lipocalin-1 receptor (LRBM1L) has been reported to suppress motility of cancer cells (21, 22).

The Quantikine® Human Uteroglobin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Uteroglobin in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains *E. coli*-expressed recombinant human Uteroglobin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Uteroglobin 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 Uteroglobin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Uteroglobin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Uteroglobin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Uteroglobin 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 Uteroglobin 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, dilute the samples with the appropriate 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Uteroglobin Microplate	893748	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Uteroglobin.	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 Uteroglobin Conjugate	893749	21 mL of a monoclonal antibody specific for human Uteroglobin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Uteroglobin Standard	893750	Recombinant human Uteroglobin in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-45	895146	11 mL of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a buffered protein base with preservatives. *Use undiluted for saliva/urine/ human milk samples. Use diluted 1:10 for cell culture supernates/serum/plasma samples.*	
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/vial 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.
- Test tubes for dilution of standards.
- Human Uteroglobin Controls (optional; R&D Systems®, Catalog # QC181).

PRECAUTIONS

Uteroglobin 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 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 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.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and centrifuge twice more for a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Uteroglobin is detectable 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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

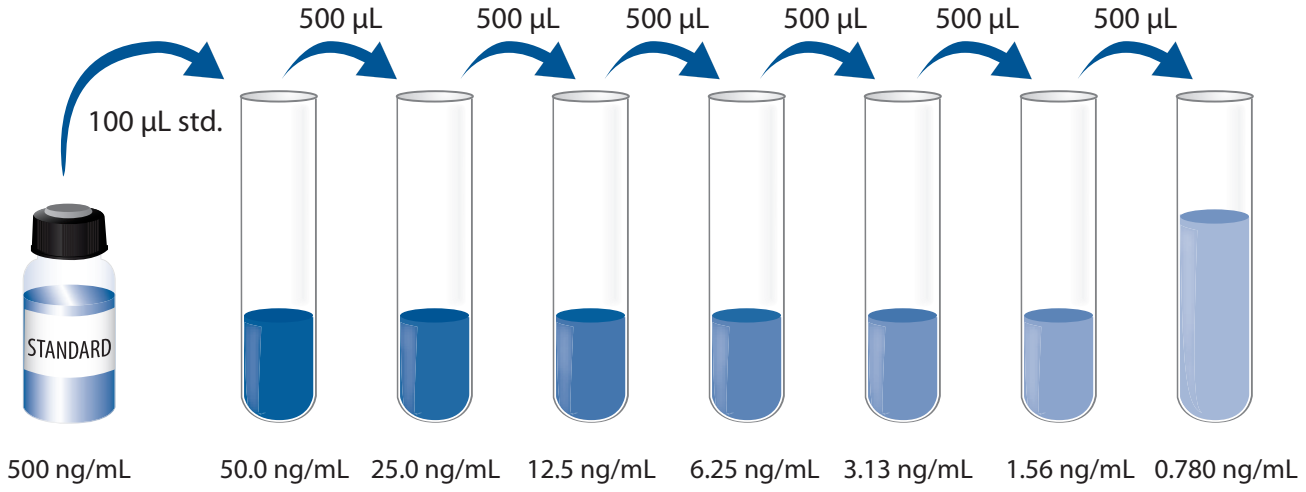
Calibrator Diluent RD5P (diluted 1:10) - For cell culture supernate, serum, and plasma samples. Add 1.0 mL of Calibrator Diluent RD5P to 9.0 mL of deionized or distilled water to prepare 10 mL of Calibrator Diluent RD5P (diluted 1:10).

Note: *Saliva, urine, and human milk samples are to be run with undiluted Calibrator Diluent RD5P.*

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 Uteroglobin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Uteroglobin Standard with deionized or distilled water. This reconstitution produces a stock solution of 500 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 900 µL of Calibrator Diluent RD5P (*for saliva, urine, and human milk samples*) or Calibrator Diluent RD5P (diluted 1:10) (*for cell culture supernate, serum, and plasma samples*) into the 50.0 ng/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50.0 ng/mL standard serves as the high standard. The appropriate calibrator diluent 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: *Uteroglobin is detectable 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 RD1-45 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. 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 Uteroglobin 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.

CALCULATION OF RESULTS

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

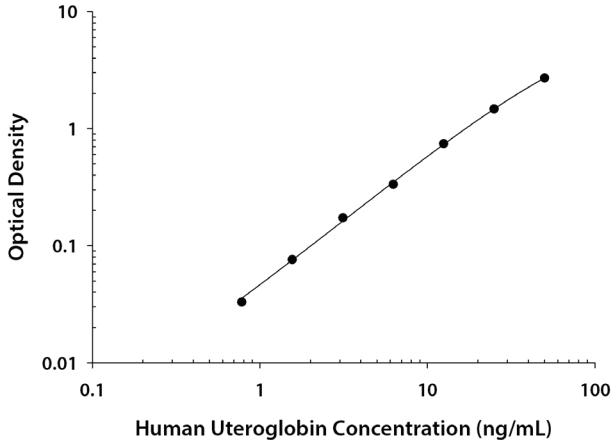
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Uteroglobin 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

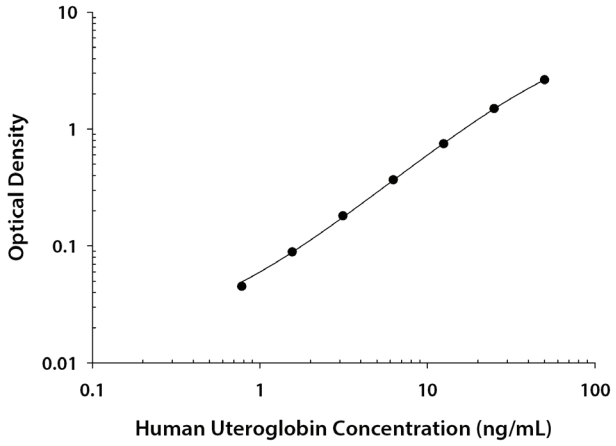
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

SALIVA/URINE/HUMAN MILK ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.012 0.012	0.012	—
0.780	0.044 0.045	0.045	0.033
1.56	0.082 0.094	0.088	0.076
3.13	0.184 0.186	0.185	0.173
6.25	0.341 0.349	0.345	0.333
12.5	0.745 0.763	0.754	0.742
25.0	1.447 1.514	1.481	1.469
50.0	2.662 2.776	2.719	2.707

CELL CULTURE SUPERNATE/SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.016 0.019	0.018	—
0.780	0.062 0.064	0.063	0.045
1.56	0.106 0.107	0.107	0.089
3.13	0.197 0.200	0.199	0.181
6.25	0.375 0.397	0.386	0.368
12.5	0.761 0.769	0.765	0.747
25.0	1.504 1.509	1.507	1.489
50.0	2.648 2.652	2.650	2.632

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.

SALIVA/URINE/HUMAN MILK ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	5.1	15.4	31.3	5.2	15.4	29.9
Standard deviation	0.29	0.34	1.04	0.31	0.72	2.25
CV (%)	5.7	2.2	3.3	6.0	4.7	7.5

CELL CULTURE SUPERNATE/SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	5.2	15.8	31.7	5.0	14.7	28.8
Standard deviation	0.21	0.49	1.16	0.28	0.92	2.09
CV (%)	4.0	3.1	3.7	5.6	6.3	7.3

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	99-109%
Serum (n=4)	95	85-104%
EDTA plasma (n=4)	95	85-107%
Heparin plasma (n=4)	95	86-109%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Uteroglobin were serially diluted with the 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)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	102	105	104	103	105	106
	Range (%)	99-106	99-112	95-109	101-105	102-108	104-111
1:4	Average % of Expected	101	105	107	105	105	108
	Range (%)	98-106	98-112	95-112	100-113	102-108	100-117
1:8	Average % of Expected	102	105	110	106	98	102
	Range (%)	98-109	97-111	99-118	101-110	78-117	95-108
1:16	Average % of Expected	106	100	103	102	96	99
	Range (%)	102-110	95-107	90-115	93-109	93-99	90-114

SENSITIVITY

Forty-seven assays were evaluated and the minimum detectable dose (MDD) of human Uteroglobin ranged from 0.014-0.217 ng/mL. The mean MDD was 0.070 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 *E. coli*-expressed recombinant human Uteroglobin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Uteroglobin 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=34)	19.9	5.12-40.5	9.37
EDTA plasma (n=34)	18.9	4.91-38.1	8.73
Heparin plasma (n=34)	18.9	4.92-38.0	8.58

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Saliva (n=15)	25.8	53%	ND-114
Urine (n=10)	17.9	60%	ND-45.2

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed, assayed for human Uteroglobin, and measured below the lowest standard, 0.780 ng/mL.

Human Milk - Nine human milk samples were evaluated for detectable levels of human Uteroglobin in this assay. Eight samples measured below the lowest standard, 0.780 ng/mL. One sample measured 0.97 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Uteroglobin.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human Uteroglobin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

HIN-1/SCGB3A1
UGRP1

Recombinant mouse:

HIN-1/SCGB3A1
UGRP1

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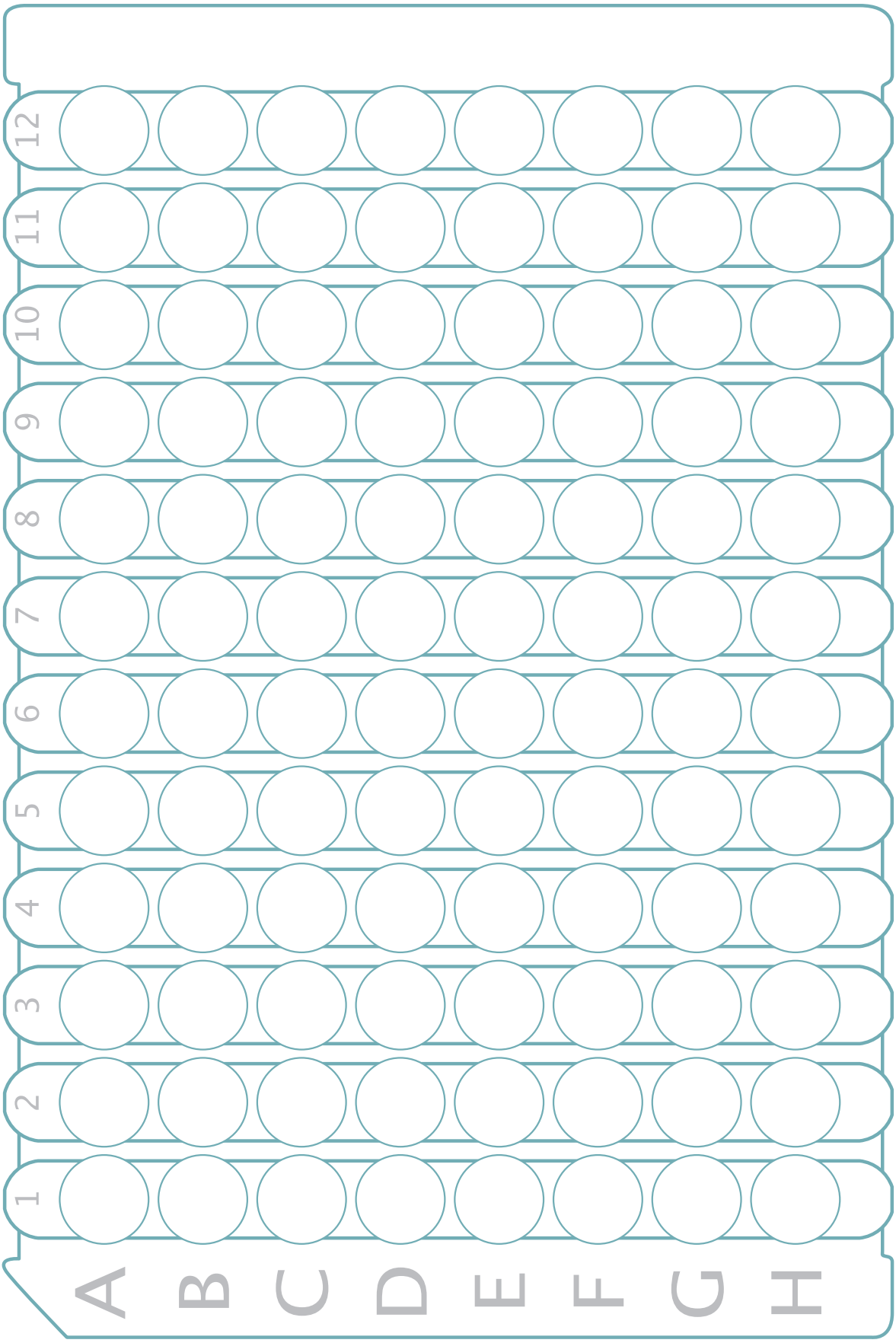
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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