Quantikine® ELISA

Human Kallikrein 5 Immunoassay

Catalog Number DKK500

For the quantitative determination of human Kallikrein 5 (KLK5) concentrations in cell culture supernates, serum, plasma, saliva, 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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USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Kallikrein 5 (KLK5, hK5), also known as SCTE (stratum corneum tryptic enzyme) and previously called KLK-L2, is a 33 kDa secreted glycoprotein that is a member of the human tissue kallikrein family of serine proteases (1-6). KLK4, 5, 6, and 7 form a subgroup with restricted expression, and structural and functional similarities (3, 5). Like other kallikreins, KLK5 is synthesized with a signal peptide amino acid (aa) 1-22, a pro region (aa 23-66) and an enzyme domain (aa 67-293) (1, 2). It is secreted as an inactive proprotein that is subsequently activated by trypsin-like cleavage. KLK5 is constitutively expressed in skin, but has also been found in breast, brain, kidney, pancreas, salivary gland, esophagus, intestine, uterus, placenta, and testis (1, 2, 5, 7). It can be produced by keratinocytes, pancreatic acinar cells, and ovarian cancer cells (5-7). KLK5 is present in body fluids such as plasma, sweat, breast milk, ovarian cancer ascites, seminal plasma, follicular fluid, amniotic fluid, saliva, and cerebrospinal fluid (4-6, 8, 9).

KLK5 is especially involved in cutaneous immunology. It can activate KLK6, 7, 14, and itself in the stratum corneum (3, 4, 10, 11). Unlike most kallikreins, KLK5 is active at low pH and is postulated to initiate protease cascades during local acidosis of wounded skin (4). KLK5 is one of several KLK family members that can activate antimicrobial peptides such as defensin-1α and cathelicidins that are found with KLK5 in skin and sweat, thus contributing to defense against infection (4, 10, 12). Skin KLK5 is inhibited by products of SPINK5 and SPINK9 genes (LEKTI and LEKTI2, respectively), but this inhibition is less effective at low pH (4, 10, 13-15). In the Netherton syndrome, a SPINK5 deficiency which causes severe ichthyosis (enhanced desquamation), KLK5 induces atopic dermatitis by initiating a cascade that begins with activating KLK14 and PAR2 (3, 4, 8, 10, 14). Several components of corneodesmosomes and the extracellular matrix, such as desmoglein-1, gelatin, collagens I-IV, laminin, fibronectin, and vitronectin are good KLK5 substrates, consistent with KLK5 involvement in cell detachment and breaching of skin barriers (3, 4, 9, 14, 16). Other pro-kallikreins (KLK2, 3, 11, and 12) as well as meprins, fibrinogen, uPA, plasminogen, kininogen, and IGFBP1-5 have also been reported as substrates (3, 4, 9-11, 14-18). Overexpression of KLK5 correlates with poor prognosis in ovarian cancer (4, 6, 9, 16, 19-21). KLK5 has also been proposed as a cancer marker in breast, prostate, or oral squamous cell cancers (9, 16, 19, 22-24).

The Quantikine® Human Kallikrein 5 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human KLK5 in cell culture supernates, serum, plasma, saliva, and human milk. It contains NS0-expressed recombinant human KLK5 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human KLK5 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 KLK5.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human KLK5 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any KLK5 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human KLK5 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 KLK5 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.
- Samples must be pipetted within 15 minutes.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human KLK5 Microplate	893896	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human KLK5.	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 KLK5 Standard	893898	2 vials of recombinant human KLK5 in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human KLK5 Conjugate	893897	21 mL of a monoclonal antibody specific for human KLK5 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-27	895245	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-10	895468	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.
- Test tubes for dilution of standards.
- Human KLK5 Controls (optional; R&D Systems®, Catalog # QC109).

PRECAUTIONS

KLK5 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Calibrator Diluent RD6-10 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 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.*

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.

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.

SAMPLE PREPARATION

Saliva and human milk samples require at least a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD6-10.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

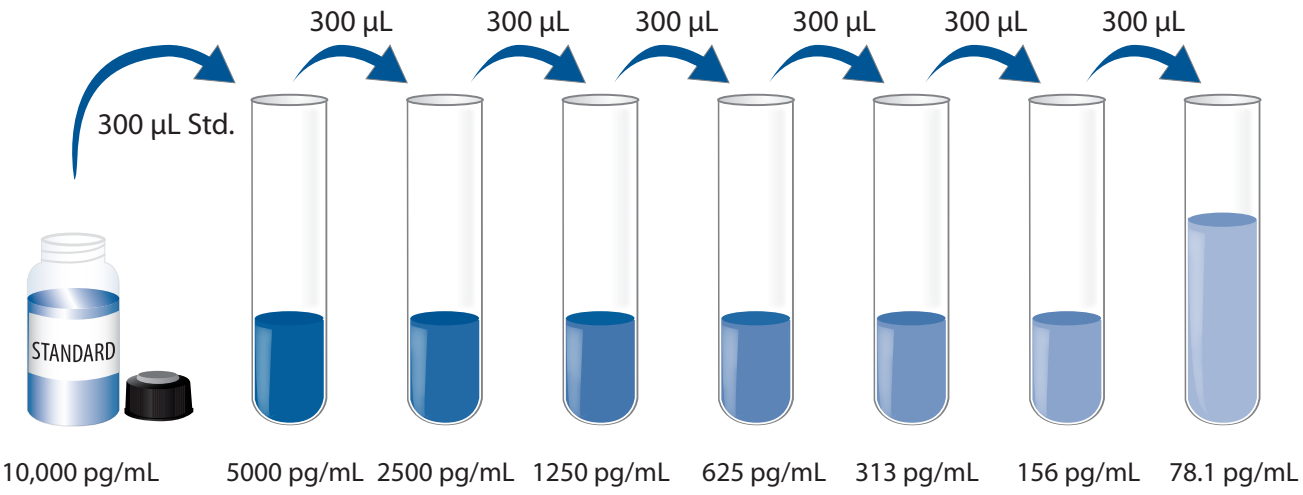
Note: *KLK5 is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 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 KLK5 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human KLK5 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/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 300 µL of Calibrator Diluent RD6-10 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. Calibrator Diluent RD6-10 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, samples, and controls be assayed in duplicate.

Note: *KLK5 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 RD1-27 to each well.
4. Add 50 µL of standard, control, or sample* per well. **Addition of samples must be completed within 15 minutes.** Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
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 KLK5 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

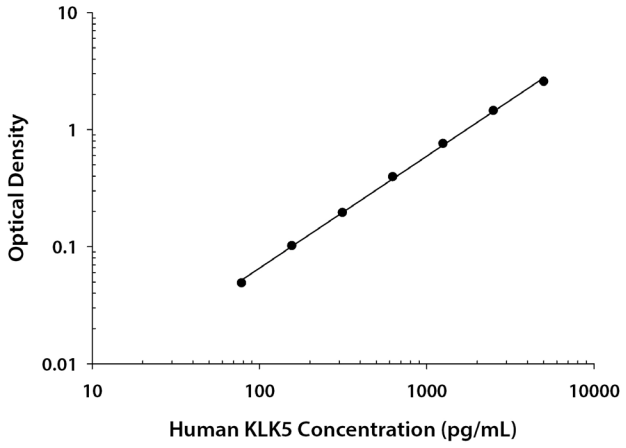
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the human KLK5 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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	0.032 0.033	0.033	—
78.1	0.080 0.083	0.082	0.049
156	0.134 0.136	0.135	0.102
313	0.226 0.232	0.229	0.196
625	0.427 0.431	0.429	0.396
1250	0.793 0.796	0.795	0.762
2500	1.459 1.510	1.485	1.452
5000	2.604 2.621	2.613	2.580

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)	510	1685	3407	577	1941	3852
Standard deviation	35.8	57.2	75.4	63.6	161	278
CV (%)	7.0	3.4	2.2	11.0	8.3	7.2

RECOVERY

The recovery of human KLK5 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	91-109%
Serum (n=4)	93	85-106%
EDTA plasma (n=4)	90	82-95%
Heparin plasma (n=4)	91	86-103%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human KLK5 were serially 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)	Saliva* (n=4)	Human milk* (n=4)
1:2	Average % of Expected	102	110	105	109	98	107
	Range (%)	100-103	108-112	100-109	107-112	96-100	101-110
1:4	Average % of Expected	100	111	110	113	95	110
	Range (%)	95-107	104-116	104-115	109-116	91-100	97-119
1:8	Average % of Expected	98	106	108	114	94	109
	Range (%)	88-106	97-113	103-112	111-116	90-97	103-118
1:16	Average % of Expected	99	104	106	109	89	107
	Range (%)	94-102	94-114	100-115	105-116	85-97	95-118

*Samples were diluted prior to assay.

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human KLK5 ranged from 4.35-30.0 pg/mL. The mean MDD was 13.2 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.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human KLK5 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human KLK5 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=35)	130	69%	ND-214
EDTA plasma (n=35)	132	66%	ND-243
Heparin plasma (n=35)	124	86%	ND-231

ND=Non-detectable

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Saliva (n=9)	4777	612-11,864	4764
Human milk (n=10)	7416	768-24,648	7734

Cell Culture Supernates:
MDA-MB-468 human breast cancer cells were cultured in L-15 medium supplemented with 10% fetal bovine serum, and incubated at 37 °C with no CO_2 until confluent. An aliquot of the cell culture supernate was removed, assayed for human KLK5, and measured 49,024 pg/mL.

MCF 10A human breast epithelial cells were cultured in 50% F-12 and 50% DMEM supplemented with 5% equine serum, 100 ng/mL cholera enterotoxin, 10 µg/mL insulin, 0.5 µg/mL hydrocortisol, and 20 ng/mL EGF until confluent. An aliquot of the cell culture supernate was removed, assayed for human KLK5, and measured 4698 pg/mL.

OVCAR-3 human ovarian carcinoma cells were cultured in RPMI supplemented with 20% fetal bovine serum, 10 µg/mL bovine insulin, 10mM HEPES, 1mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate until confluent. An aliquot of the cell culture supernate was removed, assayed for human KLK5, and measured 4006 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human KLK5.

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 KLK5 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Azurocidin/CAP37
C1s
Coagulation Factor II/Thrombin
Complement component C1r
Enteropeptidase/Enterokinase
Factor D
Granzyme H
HGFA
Kininogen (Hka)
KLK1
KLK2
KLK3/PSA
KLK4
KLK6/Neurosin
KLK7
KLK8
KLK9
KLK10
KLK11
KLK12
KLK13
KLK14
KLK15
KLKB1
MASP3
Maspin
MPN
SerpinA1
SerpinA3
SerpinA4/Kallistatin
SerpinA5
SerpinC1
SerpinD1
SerpinE1/PAI-1
SerpinF2
Tryptase γ-1/TPSG1
uPA

Recombinant mouse:

KLB1
KLK5
SerpinC1
Spinesin

Natural proteins:

human α2-Macroglobulin

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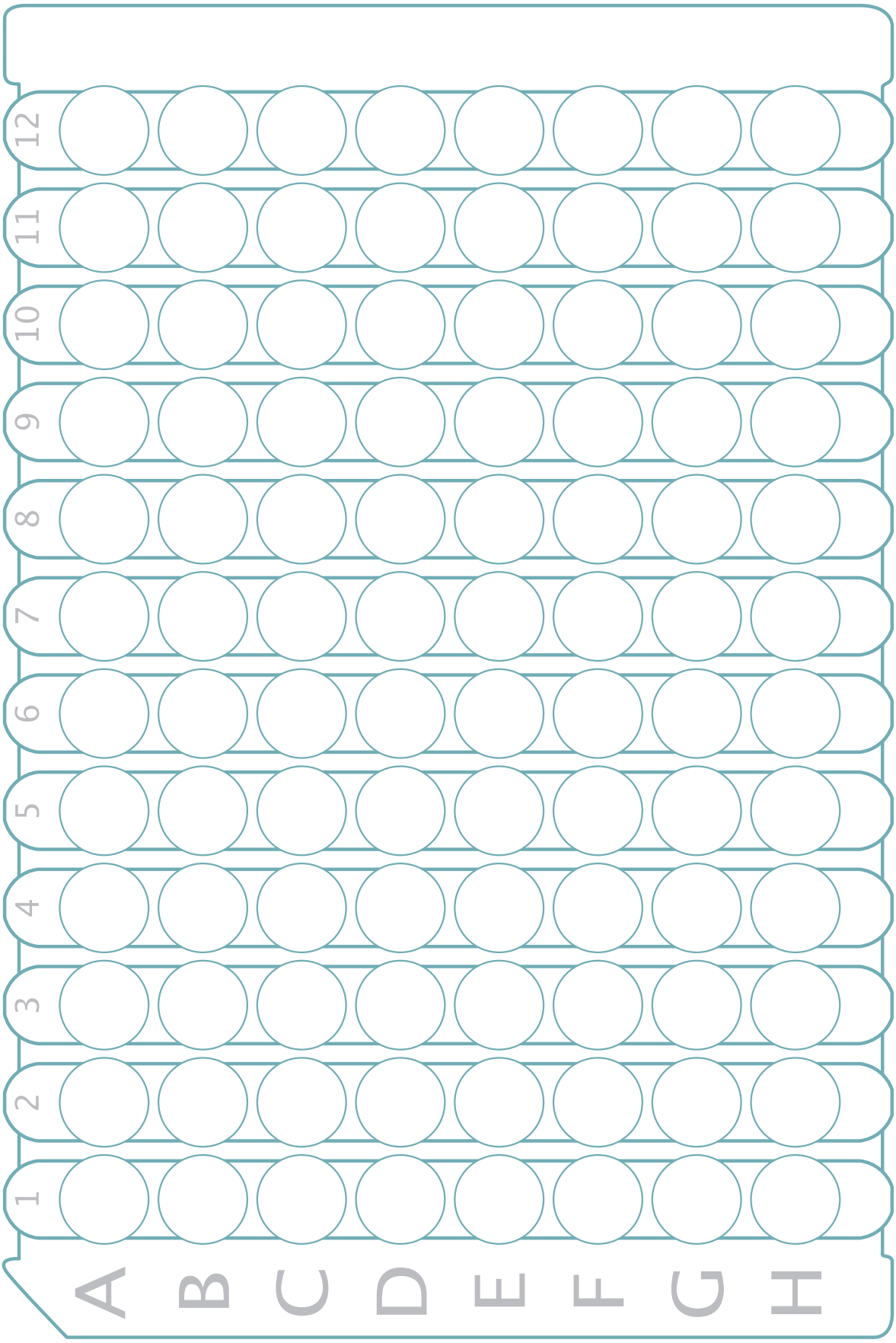
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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