Quantikine® ELISA

Human Kallikrein 3/PSA Immunoassay

Catalog Number DKK300

For the quantitative determination of human Kallikrein 3/Prostate Specific Antigen (KLK3/PSA) concentrations in cell culture supernates, 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

Human tissue Kallikrein 3 (KLK3), commonly known as prostate specific antigen (PSA), is a serine protease of the human tissue kallikrein family (1). It is produced primarily by the epithelial cells lining the prostate gland and secreted into the lumen as an inactive enzyme (proPSA). In seminal plasma, its concentration ranges from 0.5-2.0 mg/mL (2). proPSA is a 28 kDa protein with 244 amino acids. By removing 7 amino acids from the N-terminus, it becomes activated and displays chymotrypsin-like enzymatic activity (3). The major physiological function of KLK3 is to cleave the gel-forming proteins in semen, seminogelin I, and seminogelin II, leading to liquefaction of semen clogs after ejaculation (4). KLK3 has also been identified in many other tissues and biological fluids, such as breast and saliva. Its levels in these tissues, however, are over 104 times lower than in the prostate and its biological roles in these tissues remain to be elucidated (5).

About 70-90% of the KLK3 circulating in serum complexes with α1-anti-chymotrypsin (ACT)/serpin A3, with minor amounts binding to α_2-macroglobulin or α1-antitrypsin/serpin A1. The remaining 10-30% exists as unbound inactive enzyme (free PSA) (6). When measured with available immunoassays, only free PSA and PSA-ACT are detectable and, traditionally, their sum is termed total PSA. If prostatic tissue damage occurs, such as in prostate cancer and benign hyperplasia, excess amounts of PSA will leak into the circulation, resulting in increased serum total PSA levels. Ratios between total and free PSA are frequently found to be lower in prostate cancer than benign hyperplasia (7).

The expression of KLK3 is regulated by androgen due to the presence of androgen response elements in its gene promoter (8). Measuring KLK3 levels in various biological fluids and cell culture supernates has thus been used to indicate the integrity of the androgen receptor signaling pathway (9, 10).

The Quantikine® Human Kallikrein 3/PSA Immunoassay is a 4.5 hour solid phase immunoassay designed to measure human KLK3/PSA in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human KLK3/PSA, and antibodies raised against the recombinant protein. Natural human KLK3/PSA showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards, indicating that this kit can be used to determine relative levels of natural human KLK3/PSA.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human KLK3/PSA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any KLK3/PSA present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human KLK3/PSA 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 KLK3/PSA 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 KLK3/PSA Microplate	892940	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human KLK3/PSA.	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 KLK3/PSA Conjugate	892941	21 mL of a polyclonal antibody specific for human KLK3/PSA conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human KLK3/PSA Standard	892942	Recombinant human KLK3/PSA in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Assay Diluent RD1X	895121	11 mL of a buffered protein solution with preservatives. *For serum/plasma samples. May contain a precipitate. Warm to room temperature and mix well before and during use.*	
Calibrator Diluent RD5-19	895344	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent QD6-5	895227	21 mL of a buffered animal serum with preservatives. *For 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 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 KLK3/PSA Controls (optional; R&D Systems®, Catalog # QC23).

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 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 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.*

REAGENT PREPARATION

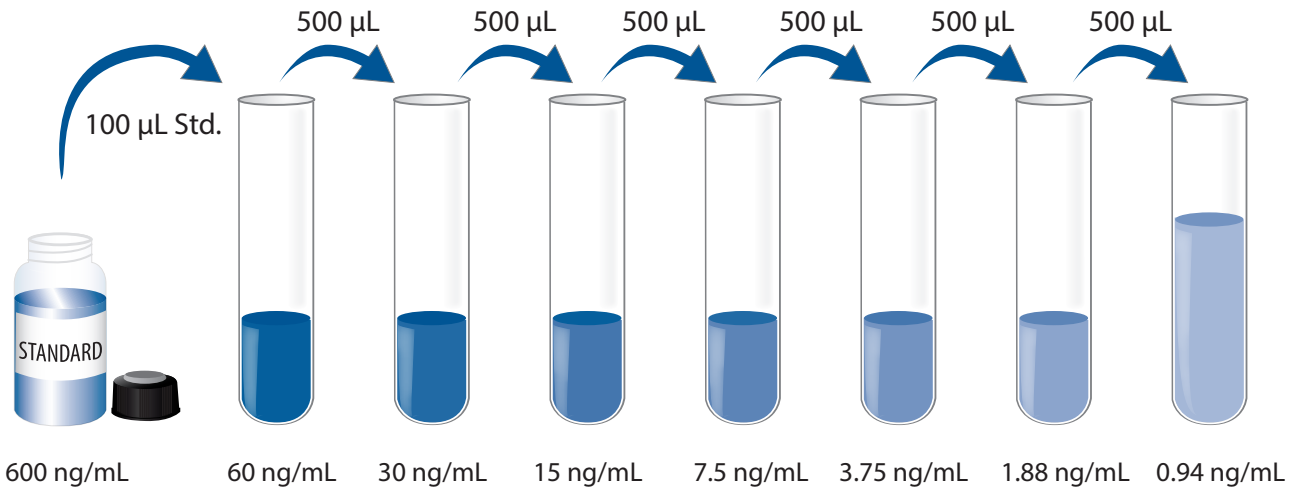
Bring all reagents to room temperature before use.

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 KLK3/PSA Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human KLK3/PSA Standard with deionized or distilled water. This reconstitution produces a stock solution of 600 ng/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 RD5-19 (*for cell culture supernate samples*) or Calibrator Diluent QD6-5 (*for serum/plasma samples*) into the 60 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 60 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.

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 for cell culture samples or Assay Diluent RD1X for serum/plasma samples to each well. *Assay Diluent RD1X may contain a precipitate. Warm to room temperature and mix well before and 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 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 KLK3/PSA 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.).

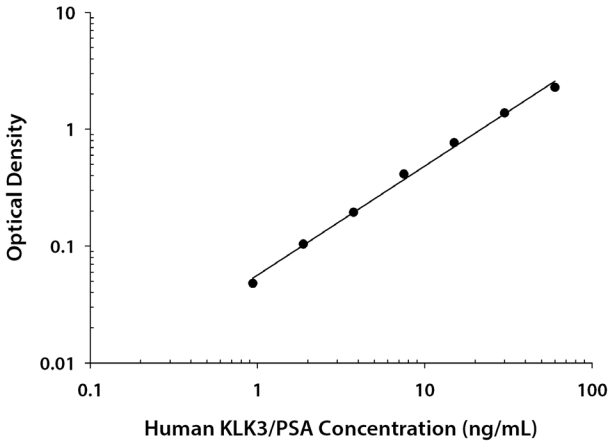
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human KLK3/PSA concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

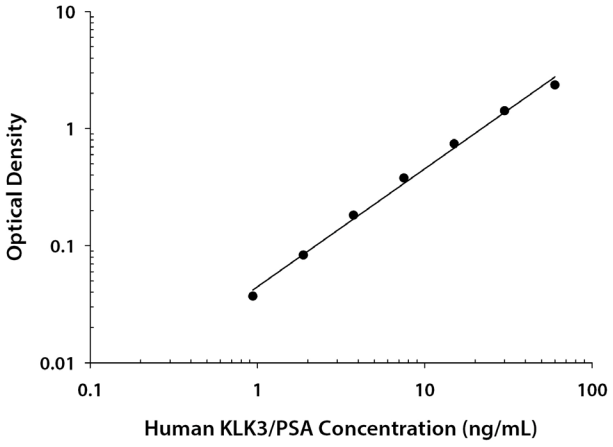
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
0.94	0.056 0.060	0.058	0.048
1.88	0.112 0.115	0.114	0.104
3.75	0.203 0.205	0.204	0.194
7.5	0.419 0.426	0.423	0.413
15	0.763 0.787	0.775	0.765
30	1.345 1.422	1.384	1.374
60	2.254 2.318	2.286	2.276

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.007 0.007	0.007	—
0.94	0.043 0.045	0.044	0.037
1.88	0.088 0.091	0.090	0.083
3.75	0.187 0.191	0.189	0.182
7.5	0.373 0.397	0.385	0.378
15	0.745 0.751	0.748	0.741
30	1.407 1.441	1.424	1.417
60	2.337 2.384	2.361	2.354

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.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	6.96	15.5	30.0	7.38	16.7	33.0
Standard deviation	0.5	0.7	0.9	0.5	0.9	1.6
CV (%)	7.2	4.5	3.0	6.8	5.4	4.8

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	12.3	24.3	44.1	11.8	24.2	43.8
Standard deviation	0.7	1.3	2.0	0.9	0.5	0.8
CV (%)	5.7	5.3	4.5	7.6	2.1	5.5

RECOVERY

The recovery of human KLK3/PSA spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	93-105%
Serum (n=4)	102	97-109%
EDTA plasma (n=4)	100	93-103%
Heparin plasma (n=4)	101	97-109%

SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of human KLK3/PSA ranged from 0.015-0.069 ng/mL. The mean MDD was 0.030 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.

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of human KLK3/PSA were diluted with the appropriate 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)	EDTA plasma (n=4)
1:2	Average % of Expected	110	105	108	106
	Range (%)	105-112	103-107	105-112	104-108
1:4	Average % of Expected	110	104	105	109
	Range (%)	104-115	97-115	101-111	104-111
1:8	Average % of Expected	106	98	103	99
	Range (%)	104-107	95-102	94-109	95-101
1:16	Average % of Expected	95	90	92	90
	Range (%)	89-98	85-97	85-100	85-93

CALIBRATION

This immunoassay utilizes a highly purified NS0-expressed recombinant human KLK3/PSA produced at R&D Systems® that is directly calibrated to the NIBSC/WHO 1st International Standard (96/670) for PSA (total).

The NIBSC/WHO 1st International Standard (96/668) for PSA (free) was also evaluated in this immunoassay. This standard parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Kallikrein3/PSA kit to the approximate NIBSC/WHO 96/668 concentration, use the equation below.

NIBSC/WHO 96/668 approximate value (ng/mL) = 0.505 x Quantikine® Human KLK3/PSA value (ng/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human KLK3/PSA in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Serum (n=35)	2.77	8	ND-3.99
EDTA plasma (n=35)	3.30	8	ND-4.98
Heparin plasma (n=35)	2.25	8	ND-3.09

ND=Non-detectable

Cell Culture Supernates:
Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 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 supernate were removed and assayed for levels of human KLK3/PSA. No detectable levels were observed.

LNCap human prostate cancer cells were cultured in RPMI supplemented with 10% fetal bovine serum, 10 mM HEPES and 1 nM sodium pyruvate. An aliquot of the cell culture supernate was removed, assayed for human KLK3/PSA, and measured 25.2 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human KLK3/PSA.

Each of the factors listed below were prepared at 200 ng/mL in calibrator diluent and assayed. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human KLK3/PSA control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Azurocidin/CAP37
Coagulation Factor 11/Thrombin
Complement component C1r
Complement component C1s
Enteropeptidase/Enterokinase
Factor D
Granzyme
HGFA
Kallikrein 1
Kallikrein 4
Kallikrein 5
Kallikrein 7
Kallikrein 8
Kallikrein 11
Kallikrein 13
Kallikrein 14
Kallikrein 15
Kininogen (Hka)
MASP3
Maspin
MPN
SerpinA1
SerpinA3
SerpinA4/Kallistatin
SerpinA5
SerpinC1
SerpinD1
SerpinE1/PAI-1
SerpinF2
Tryptase γ-1/TPSG1
uPA

Recombinant mouse:

Serpin C1
Spinesin

Natural proteins:

α_2-Macroglobulin

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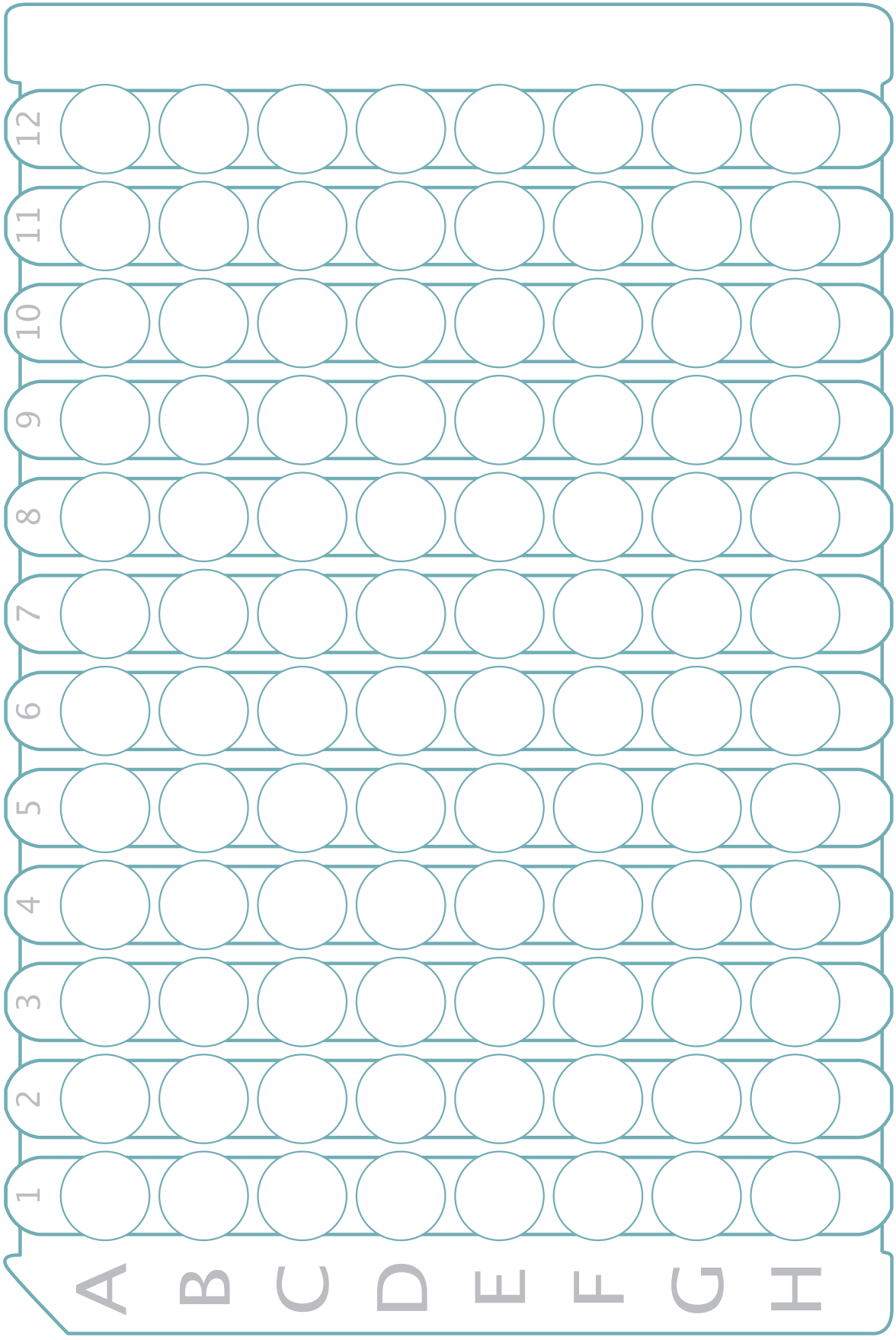
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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