Quantikine® ELISA

Human Pentraxin 3/TSG-14 Immunoassay

Catalog Number DPTX30

For the quantitative determination of human Pentraxin 3 concentrations in serum-free cell culture supernates, plasma, and saliva.

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

Pentraxin 3 (PTX3), also known as Tumor necrosis factor-Stimulated Gene 14 (TSG-14), is a long pentraxin belonging to the pentraxin superfamily. (1, 2). The secreted human PTX3 is a 45 kDa glycoprotein with a 162 amino acid (aa) N-terminal extension and a 202 aa C-terminal pentraxin domain. The structure of the pentraxin domain is similar to that of the pentraxin domains found in classical short pentraxins (CRP and SAP) that are produced in the liver (1-3). Pentraxins are multi-functional pattern-recognition proteins that have a characteristic pentagonal discoid arrangement. PTX3 forms decamers and higher-order multimers through the use of interchain disulfide bonds (4). PTX3 is induced in response to pro-inflammatory stimuli and Toll-like Receptor (TLR) engagement (1-7). It is secreted by a variety of cell types including macrophages, neutrophils, myeloid-derived dendritic cells, ovarian granulosa cells, endothelial cells, fibroblasts, adipocytes, renal mesangial cells, synovial cells, smooth muscle cells, alveolar epithelium, and glial cells (1-6, 8, 9). PTX3 is an acute phase protein in mice and humans and increases rapidly in plasma during inflammatory and infectious conditions (1, 2). PTX3 is present in atherosclerotic lesions and is sometimes increased in plasma following myocardial infarction (10).

High affinity binding of PTX3 to its soluble ligands plays an important role in several physiological conditions, ranging from innate immunity to female fertility (1-15). Through its interaction with TSG-6, an extracellular matrix hyaluronan (HA)-binding protein, PTX3 is involved in the assembly of the HA-rich extracellular matrix of the cumulus oophorus, which is essential for female fertility (8). PTX3 has a dual role in the regulation of the innate immune response. Via its C-terminal pentraxin domain, immobilized PTX3 will bind complement component C1q to induce classical complement activation. Conversely, fluid-phase PTX3 inhibits classical complement activation (11). PTX3 interacts with selected viral, fungal, and bacterial components, providing protection from infection and may act as an opsonin (6, 7, 12, 13). It binds the outer membrane protein A (OmpA) of *Enterobacteriaceae* (e.g. KpOmpA from *Klebsiella pneumoniae*), helping to amplify an ongoing inflammatory response (12). While PTX3 enhances C1q binding on apoptotic cells and facilitates the complement-mediated clearance of these cells, it can also bind apoptotic cells and inhibit their uptake by dendritic cells, preventing the onset of an autoimmune response (14, 15). Finally, PTX3 may play a role in vascular inflammation (16). The N-terminal extension of PTX3 has been shown to bind FGF basic and inhibit FGF basic-dependent angiogenesis (9).

The Quantikine® Human Pentraxin 3/TSG-14 Immunoassay is a 5.0 hour solid-phase ELISA designed to measure human Pentraxin 3 in serum-free cell culture supernates, plasma, and saliva. It contains NS0-expressed recombinant human Pentraxin 3 and has been shown to accurately quantitate the recombinant factor. Results obtained using pretreated natural human Pentraxin 3 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 Pentraxin 3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A streptavidin-coated plate is incubated with a biotinylated monoclonal antibody specific for human Pentraxin 3. Plates are washed, and pretreated standards and samples are added to the wells. Any Pentraxin 3 present is bound by the immobilized biotinylated antibody. After washing away any unbound substances, an enzyme-linked conjugate specific for human Pentraxin 3 is added to the wells. Following a wash to remove any unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of Pentraxin 3 bound. 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, pretreat the samples, 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
Streptavidin Microplate	890649	96 well polystyrene microplate (12 strips of 8 wells) coated with Streptavidin.	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 Pentraxin 3 Standard	893050	Recombinant human Pentraxin 3 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Untreated standard may be stored for up to 1 month at 2-8 °C.* Discard excess pretreated standard after use.
Human Pentraxin 3 Biotinylated Antibody	893272	21 mL of a monoclonal antibody specific for human Pentraxin 3 conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Pentraxin 3 Conjugate	893049	21 mL of a monoclonal antibody specific for human Pentraxin 3 conjugated to horseradish peroxidase with preservatives.	
Pretreatment D	895578	1 mL of pretreatment solution.	
Assay Diluent RD1-56	895102	17 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-24	895325	21 mL of a buffered protein base with preservatives. *Use diluted 1:2 in this assay.*	
Wash Buffer Concentrate	895003	2 vial (21 mL/vial) 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	8 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.
- 50 mL and 500 mL graduated cylinders.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human Pentraxin 3 Controls (optional; R&D Systems®, Catalog # QC151).

PRECAUTIONS

Pentraxin 3 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.

Serum-Free Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Samples containing serum are not suitable sample types in this assay.*

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge at 2-8 °C. 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: *Serum and heparin plasma are not suitable for use in this assay.*
Citrate plasma has not been validated for use in this assay.

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of Pentraxin 3 are found 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 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 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 RD5-24 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD5-24 to 20 mL deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-24 (diluted 1:2).

Human Pentraxin 3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Pentraxin 3 Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 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 pretreatment.

MICROPLATE PREPARATION

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Pipette 200 µL of the Human Pentraxin 3 Biotinylated Antibody into all wells. Securely cover and incubate for 15-60 minutes at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.

   Hint: *Perform the Standard and Sample Pretreatment during this incubation.*
3. Aspirate each well and wash, repeating the process for a total of two 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.
4. Proceed to the Assay Procedure immediately after the wash. Do not allow the wells to dry.

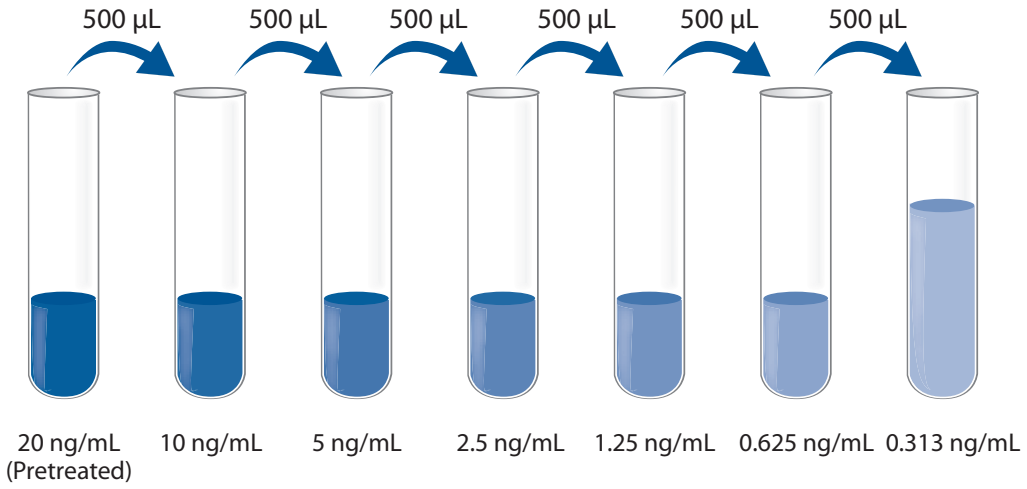
STANDARD & SAMPLE PRETREATMENT

All standards, controls, and samples must be treated before being added to the microplate.

1. Add 900 µL of Calibrator Diluent RD5-24 (diluted 1:2) to a **polypropylene tube** containing 100 µL of reconstituted Human Pentraxin 3 Standard. This prepares the 20 ng/mL high standard.
2. Add 100 µL of Pretreatment D to the 20 ng/mL tube from step 1. Add 10 µL of Pretreatment D to 100 µL of each sample in a **polypropylene tube.**
3. Vortex gently and incubate for 10-30 minutes at room temperature. Prepare standards as described below. Assay immediately and discard any excess pretreated standard and samples.

STANDARD CURVE PREPARATION

1. Label six additional **polypropylene tubes**, starting at 10 ng/mL, 5 ng/mL, etc. (as indicated below). Add 500 µL of Calibrator Diluent RD5-24 (diluted 1:2) into each tube.
2. Transfer 500 µL from the **pretreated** 20 ng/mL standard tube (from step 2 above) to produce a dilution series (below). Mix each tube thoroughly before the next transfer.
3. The 20 ng/mL standard serves as the high standard. Calibrator Diluent RD5-24 (diluted 1:2) 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.

Note: *High concentrations of Pentraxin 3 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare the microplate, all reagents, pretreated standards, and pretreated samples as directed in the previous sections.
2. Add 100 µL of Assay Diluent RD1-56 to each well.
4. Add 20 µL of pretreated 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 Pentraxin 3 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

CALCULATION OF RESULTS

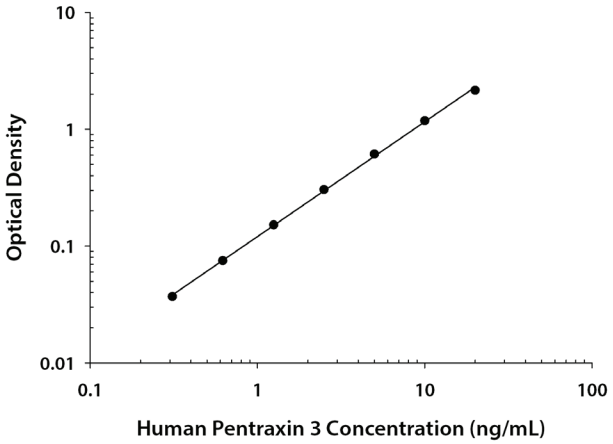
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Pentraxin 3 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

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	0.006 0.012	0.009	—
0.313	0.045 0.047	0.046	0.037
0.625	0.083 0.085	0.084	0.075
1.25	0.161 0.161	0.161	0.152
2.5	0.311 0.316	0.313	0.304
5	0.618 0.625	0.621	0.612
10	1.171 1.213	1.192	1.183
20	2.131 2.201	2.166	2.157

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)	2.61	7.72	14.1	2.75	7.94	14.5
Standard deviation	0.10	0.28	0.62	0.17	0.39	0.60
CV (%)	3.8	3.6	4.4	6.2	4.9	4.1

RECOVERY

The recovery of human Pentraxin 3 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Serum-free cell culture media (n=4)	101	95-108%
EDTA plasma (n=4)	99	83-106%

LINEARITY

To assess the linearity of the assay, pretreated samples containing and/or spiked with high concentrations of human Pentraxin 3 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum-free cell culture supernates (n=4)	EDTA plasma (n=4)	Saliva (n=4)
1:2	Average % of Expected	102	103	108
	Range (%)	97-107	100-105	103-114
1:4	Average % of Expected	101	107	107
	Range (%)	96-105	104-113	103-111
1:8	Average % of Expected	100	101	107
	Range (%)	92-104	96-106	100-115
1:16	Average % of Expected	95	99	98
	Range (%)	84-102	97-102	90-107

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human Pentraxin 3 ranged from 0.007-0.116 ng/mL. The mean MDD was 0.025 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 Pentraxin 3 produced at R&D Systems®.

SAMPLE VALUES

Plasma/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human Pentraxin 3 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)
EDTA plasma (n=35)	0.65	83	ND-1.18
Saliva (n=10)	4.95	90	ND-9.63

ND-Non-detectable

Serum-Free Cell Culture Supernates:
WS-1 human fetal skin fibroblasts (1 x 10^6 cells/mL) were cultured in MEM supplemented with 10% fetal bovine serum, NEAA, and 2 mM L-glutamine until confluent. Cells were transferred to serum-free media and stimulated with 10 ng/mL of recombinant human IL-1β, 10 ng/mL of recombinant human TNF-α, or 100 ng/mL of LPS for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human Pentraxin 3.

Stimulant	Pentraxin 3 (ng/mL)
IL-1β	20.8
TNF-α	21.4
LPS	2.86

IMR-90 human lung fibroblasts (1 x 10^6 cells/mL) were cultured in MEM supplemented with 10% fetal bovine serum, NEAA, and 2 mM L-glutamine until confluent. Cells were transferred to serum-free media and were unstimulated or stimulated with 10 ng/mL of recombinant human TNF-α for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human Pentraxin 3.

Condition	Pentraxin 3 (ng/mL)
Unstimulated	4.40
Stimulated	28.6

SPECIFICITY

This assay recognizes natural and recombinant human Pentraxin 3.

The factors listed below were prepared at ≥ 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at ≥ 200 ng/mL in a mid-range recombinant human Pentraxin 3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
CRP
Pentraxin 2/SAP

Recombinant rat:
CRP
Pentraxin 2/SAP

Recombinant mouse:
CRP
Pentraxin 3/TSG-14

Other:
dBiotin

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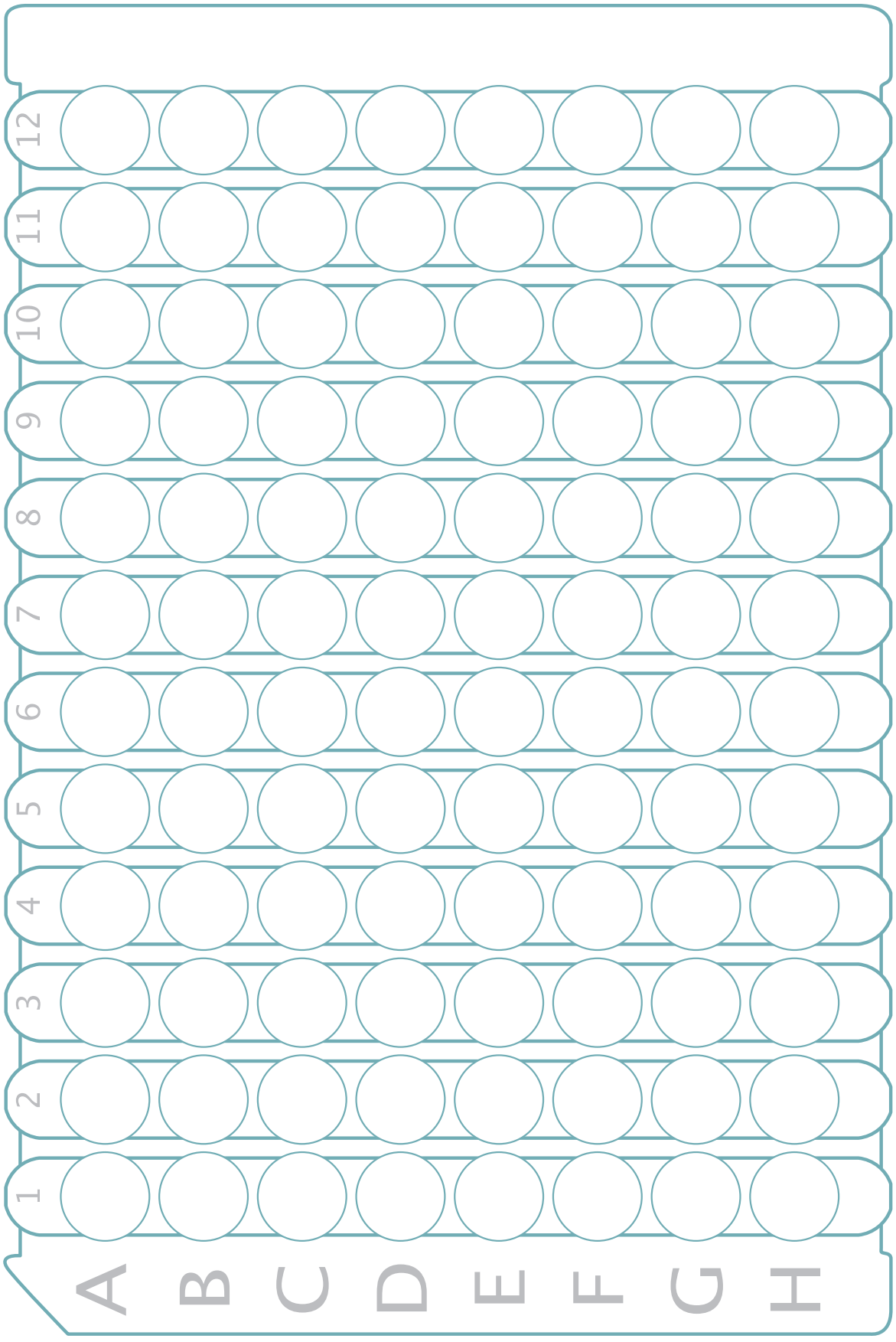
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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