Quantikine® ELISA

Human Attractin Immunoassay

Catalog Number DATRN0

For the quantitative determination of human Attractin concentrations in cell culture supernates, serum, plasma, saliva, 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

Attractin, also known as DPPT-L and Mahogany, is a transmembrane glycoprotein with functions in axon myelination, energy balance regulation, immune activation, and hair pigmentation (1, 2). Mature human Attractin consists of a 1196 amino acid (aa) extracellular domain (ECD), a 21 aa transmembrane segment, and a 129 aa cytoplasmic domain (3). Within the ECD, it shares 95% aa sequence identity with mouse and rat Attractin. Alternative splicing generates an approximately 175 kDa secreted soluble isoform that corresponds to the ECD of the transmembrane form and is expressed by T cells and undifferentiated cortical neurons (4-6). Attractin is additionally expressed in monocytes, hair follicle melanocytes hypothalamus, hippocampus, substantia nigra, and adipose tissue (3, 7-10). Activated T cell-derived soluble Attractin can co-stimulate the proliferation of T cells (3, 4, 11). Attractin also functions with Melanocortin 1R as a coreceptor for Agouti and, as a soluble molecule, can neutralize the bioactivity of Agouti on hair follicle melanocytes (8, 9, 12, 13). Similarly, Attractin suppresses the diet-induced obesity which is characteristic of Agouti overexpressing mice (1, 8). In the CNS, Attractin plays a role in axon myelination, neurite branching on differentiating neurons, and protection from neurotoxins (1, 10, 14). It is elevated in the CSF in high grade malignant astrocytoma and promotes glioma cell migration (6, 15). It is also elevated in the synovial fluid of osteoarthritis patients (16).

The Quantikine Human Attractin Immunoassay is a 4.5 hour solid phase ELISA designed to measure Attractin levels in cell culture supernates, serum, plasma, saliva, and urine. It contains CHO cell-expressed recombinant human Attractin and antibodies raised against the recombinant protein. Results obtained for naturally occurring human Attractin showed linear curves that were parallel to the standard curves obtained using the Quantikine Human Attractin Immunoassay standards. These results indicate that this kit can be used to determine relative mass values for natural human Attractin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Attractin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Attractin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Attractin 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 Attractin 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.

PRECAUTIONS

Attractin 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 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.

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 Attractin Microplate	894770	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Attractin.	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 Attractin Standard	894772	2 vials of recombinant human Attractin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
Human Attractin Conjugate	894771	21 mL of a monoclonal antibody specific for human Attractin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-19	895467	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human Attractin Controls (optional; R&D Systems, Catalog # QC202).

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.*
Grossly hemolyzed samples are not suitable 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, 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, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 1000-fold dilution. A suggested 1000-fold dilution can be achieved by adding 10 µL of sample to 490 µL of Calibrator Diluent RD5P (diluted 1:5)*. Complete the 1000-fold dilution by adding 10 µL of the diluted sample to 190 µL Calibrator Diluent RD5P (diluted 1:5).

Saliva and urine samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5P (diluted 1:5).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

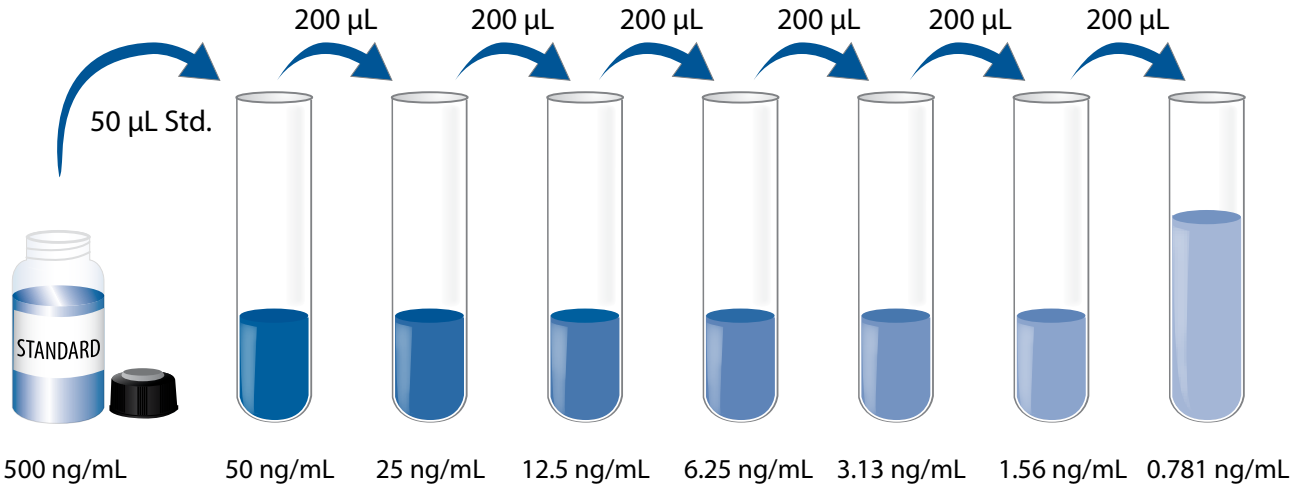
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human Attractin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Attractin 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 with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 50 ng/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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 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 100 µL of Assay Diluent RD1-19 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 Attractin 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 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

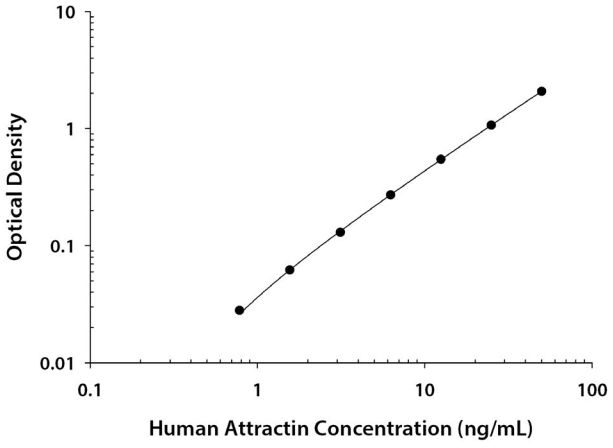
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Attractin 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.



(ng/mL)	O.D.	Average	Corrected
0	0.007 0.007	0.007	—
0.781	0.034 0.036	0.035	0.028
1.56	0.069 0.069	0.069	0.062
3.13	0.136 0.138	0.137	0.130
6.25	0.277 0.278	0.278	0.271
12.5	0.550 0.558	0.554	0.547
25	1.070 1.079	1.075	1.068
50	2.081 2.092	2.087	2.080

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 (ng/mL)	4.16	12.7	26.2	4.61	13.7	27.5
Standard deviation	0.132	0.324	0.893	0.498	1.10	1.54
CV (%)	3.2	2.6	3.4	10.8	8.0	5.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	91	87-97%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Attractin were diluted with 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	107	101	103	105	97	106
	Range (%)	105-110	96-104	99-106	102-111	95-102	103-109
1:4	Average % of Expected	110	101	102	105	98	107
	Range (%)	107-112	96-106	96-106	101-110	96-103	103-110
1:8	Average % of Expected	112	101	103	106	97	110
	Range (%)	110-116	93-106	96-108	97-112	94-101	105-113
1:16	Average % of Expected	110	101	101	103	95	112
	Range (%)	102-115	95-106	93-106	98-109	93-98	108-114

*Samples were dilluted prior to assay.

SENSITIVITY

Twenty-nine assays were evaluated and the minimum detectable dose (MDD) of human Attractin ranged from 0.018-0.217 ng/mL. The mean MDD was 0.055 ng/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 highly purified recombinant human Attractin produced at R&D Systems.

SAMPLE VALUES

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

Sample Type	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum (n=36)	17.9	10.6-25.1	3.28
EDTA plasma (n=36)	15.9	10.9-22.8	3.20
Heparin plasma (n=36)	16.7	9.71-25.7	3.13

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Saliva (n=10)	52.5	22.8-118	28.0
Urine (n=10)	52.3	22.8-112	26.4

Cell Culture Supernates:
SW13 human adrenal cortex adenocarcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine and grown until confluent. An aliquot of the cell culture supernate was removed, assayed for human Attractin, and measured 5.65 ng/mL.

Human Th17 cells were generated by isolating $CD4^+$ T cells from human peripheral blood mononuclear cells. $CD4^+$ T cells were seeded at 1.0-1.5 x 10^6 cells/mL and cultured in RPMI supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 20 ng/mL recombinant human (rh) IL-2, 20 ng/mL rhIL-23, 10 ng/mL rhIL-1β, 40 ng/mL rhIL-6, and 5 µg/mL anti-human CD28 for 6 days. Cells were then stimulated overnight with 10 ng/mL PMA and 500 ng/mL Ionomycin. An aliquot of the cell culture supernate was removed, assayed for human Attractin, and measured 2.18 ng/mL

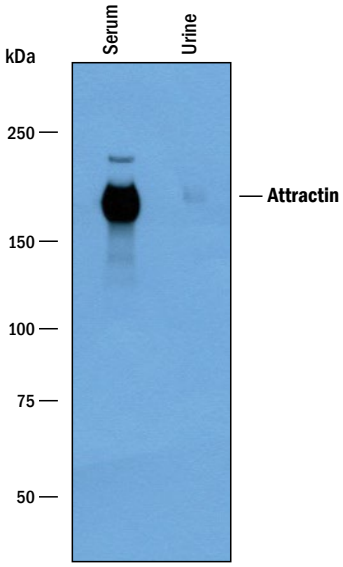
SPECIFICITY

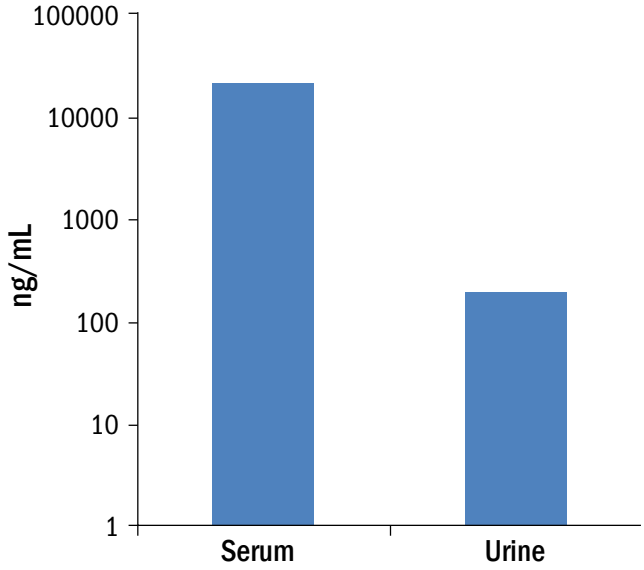
This assay recognizes natural and recombinant human Attractin.

The factors listed below were prepared at 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant human Attractin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CLEC1	CLEC14A
CLEC2	CLECSF8
CLEC-2A	E-Selectin
CLEC3B	L-Selectin
CLEC9A	Polycystin
CLEC10A	P-Selectin
CLEC12B	Thrombomodulin





Human serum and urine samples from apparently healthy volunteers were analyzed by Western blot and Quantikine ELISA. Samples were prepared by diluting the serum sample 1:200 while the urine samples were a 1:2 dilution. Samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody in this kit. The Western blot shows a direct correlation with the ELISA value.

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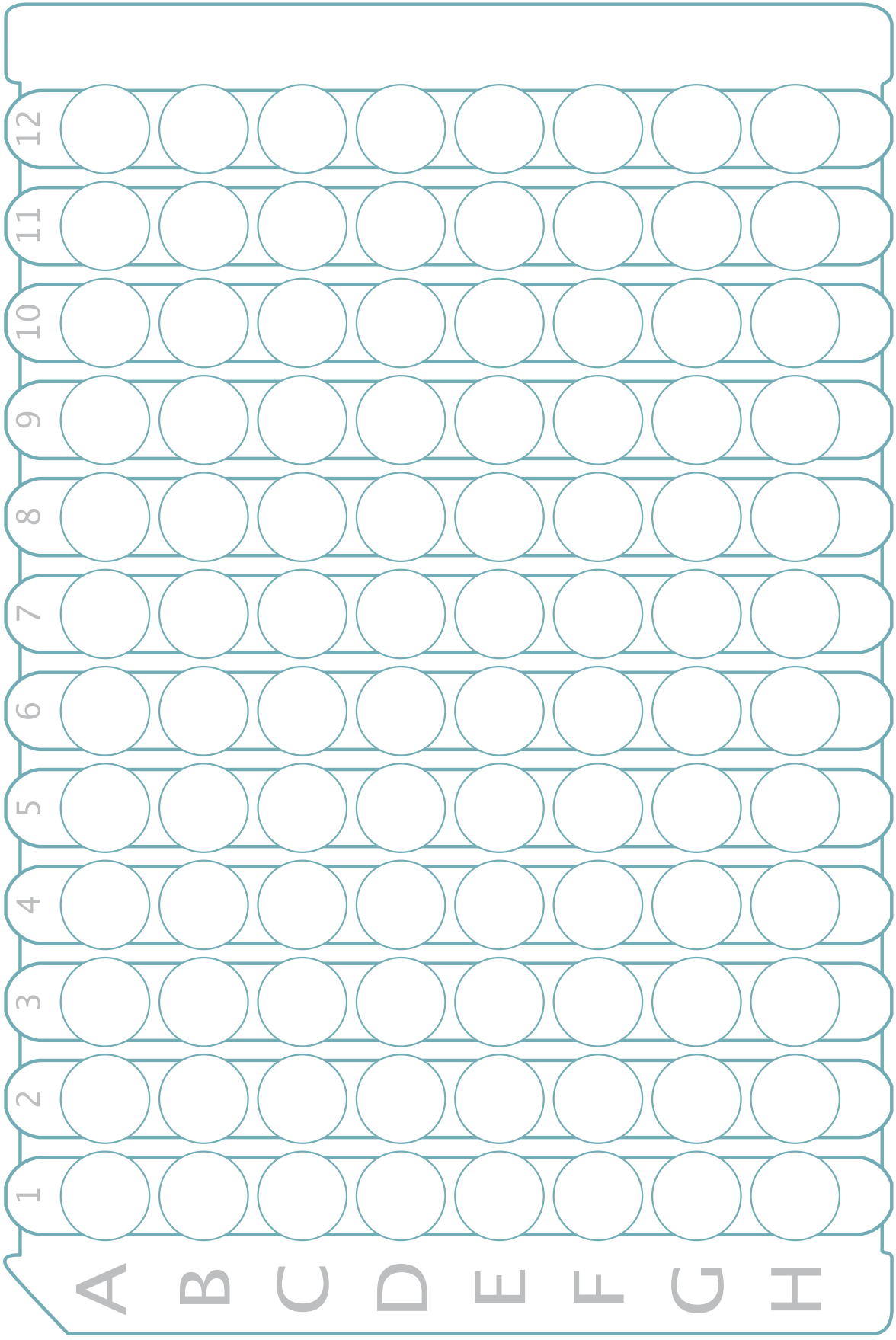
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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