Quantikine® ELISA

Rat Total Adiponectin/Acrp30 Immunoassay

Catalog Number RRP300

For the quantitative determination of rat Adiponectin concentrations in cell culture supernates, tissue lysates, serum, plasma, 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

Adiponectin, also known as Acrp30, apM1, AdipoQ, and GBP28, is a 30 kDa glycoprotein that is secreted primarily by adipocytes and induces wide ranging paracrine and endocrine effects on metabolism and inflammation (1-3). Mature rat Adiponectin consists of a 60 amino acid (aa) N-terminal collagenous region and a 134 aa C-terminal C1q/TNF-α-like globular domain. It shares approximately 85% and 91% aa sequence identity with human and mouse Adiponectin, respectively.

Adiponectin forms 90 kDa homotrimers that contain two disulfide-linked monomers and a third subunit which is non-covalently associated. Two trimers can be covalently linked to create a 180 kDa hexamer which associates into >300 kDa high molecular weight (HMW) Adiponectin (4-6). The various forms of Adiponectin exhibit distinct biological properties, and they do not interconvert in the serum (6-8). Adiponectin is O-glycosylated on four hydroxylated lysines in its collagen domain, a modification which is required for the intracellular formation of HMW Adiponectin and its insulin-sensitizing activity (5, 6). The ratio between different forms of Adiponectin may be biologically significant; a much greater amount of HMW Adiponectin circulates in females compared to males, although the levels of trimeric and hexameric Adiponectin are comparable between genders (6, 9, 10). A cleaved form of Adiponectin, known as gAdiponectin, consists of the globular domains in trimeric complexes (11, 12). Circulating Adiponectin levels are high, comprising approximately 0.01% of total plasma protein (9). Adiponectin exerts its bioactivity through interactions with the 7-transmembrane receptors AdipoR1 and AdipoR2 (13-15). The widely expressed AdipoR1 binds gAdiponectin with high affinity but binds full length Adiponectin with very low affinity (13). AdipoR2 binds both the full length and globular forms with intermediate affinity and is relatively restricted to the liver (13). The various forms of Adiponectin also differentially interact with Cadherin-13 in muscle and with several growth factors (16, 17).

Adiponectin promotes insulin sensitivity through multiple actions on glucose and fatty acid metabolism, frequently in opposition to the actions of TNF-α (18-22). It induces a decrease in serum glucose and triglyceride levels, an increase in serum glucagon, but no change in insulin levels (19, 21, 23). In the liver, Adiponectin enhances the insulin-dependent inhibition of gluconeogenesis (21, 23). In skeletal muscle, Adiponectin promotes fatty acid uptake and oxidation, glucose uptake, and lactate production (11, 18, 19, 24, 25). HMW Adiponectin is the most potent isoform at inducing insulin sensitization in liver, and gAdiponectin is more potent than the full length molecule at inducing metabolic effects in muscle (7, 8, 11, 24-26). The various isoforms of Adiponectin differentially trigger the activation of AMPK and NFκB in liver and muscle (5, 7, 24, 25, 27). In the adult (but not in the fetus), elevated levels of circulating total Adiponectin, and particularly HMW Adiponectin, are negatively correlated with conditions related to metabolic syndrome (9, 28). Decreased plasma HMW Adiponectin levels are associated with upper body obesity, insulin resistance, reduced fatty acid oxidation, dyslipidemia, coronary artery disease, and atherogenesis (29-32). Plasma HMW Adiponectin levels increase in response to treatment with insulin-sensitizing thiazolidinediones (26, 33).

Adiponectin inhibits inflammation by antagonizing TNF-α induced vascular endothelial cell apoptosis and the upregulation of leukocyte adhesion proteins on the vascular endothelium (31, 34, 35). In macrophages, Adiponectin promotes polarization toward the M2 anti-inflammatory phenotype, inhibits TNF-α production, and interacts with C1q Rp to promote the clearance of Adiponectin-opsonized apoptotic cell debris (36-38). It protects against atherosclerosis by suppressing nitric oxide formation, the progression of macrophages into foam cells, and the migration of adventitial fibroblasts to the intima (39, 40). In nonmetabolic disorders such as rheumatoid arthritis and inflammatory bowel disease, however, Adiponectin levels are elevated and it can promote inflammation (41-44). Adiponectin also negatively regulates myelomonocytic progenitor cell growth (37).

The Quantikine® Rat Total Adiponectin/Acrp30 Immunoassay is a 2.5 hour solid phase ELISA designed to measure rat Adiponectin levels in cell culture supernates, tissue lysates, serum, plasma, and urine. It contains NS0-expressed recombinant rat Adiponectin and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant rat Adiponectin accurately. Results obtained using natural rat Adiponectin showed dose-response 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 rat Adiponectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat Adiponectin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat Adiponectin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of Adiponectin bound in the initial step. The sample values are then read off the standard curve.

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.
- 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.

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
Rat Adiponectin Microplate	894268	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat Adiponectin.	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.*
Rat Adiponectin Standard	894270	Recombinant rat Adiponectin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	May be stored for up to 1 month at ≤ -20 °C.*
Rat Adiponectin Control	894271	Recombinant rat Adiponectin in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Rat Adiponectin Conjugate	894269	12 mL of a polyclonal antibody specific for rat Adiponectin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 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	895174	23 mL of diluted hydrochloric 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Prior to assay, tissues must be lysed according to the directions in the Sample Values section.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 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 20 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.*

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

SAMPLE PREPARATION

Cell culture supernate samples may require dilution.

Tissue lysate, 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 RD5-26 (diluted 1:4)*. Complete the 1000-fold dilution by adding 15 µL of the diluted sample to 285 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Rat Adiponectin Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

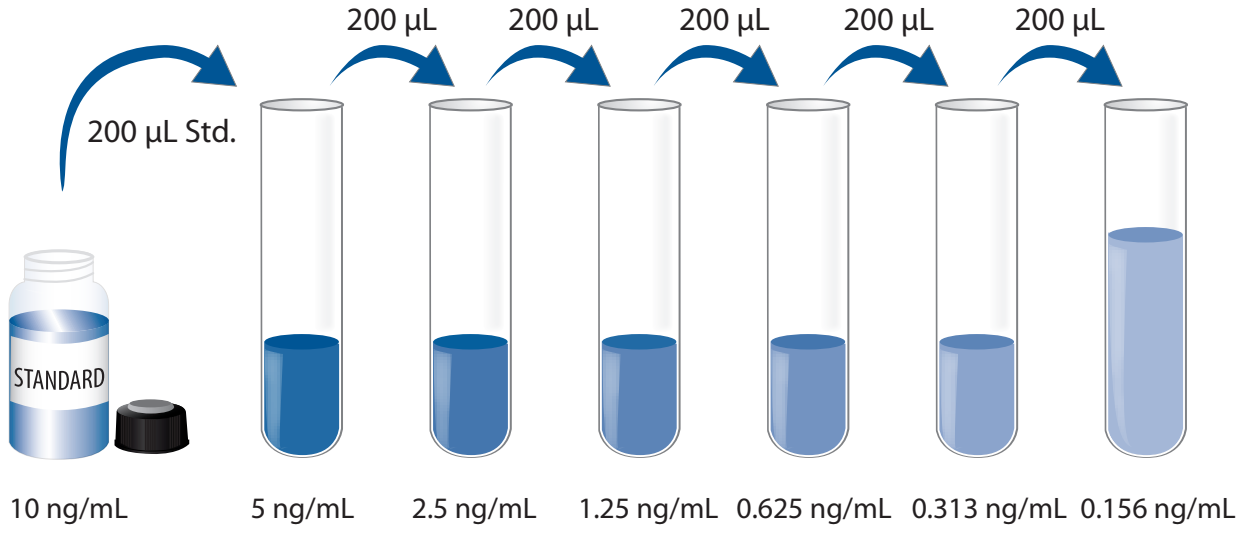
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Rat Adiponectin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat Adiponectin Standard with Calibrator Diluent RD5-26 (diluted 1:4). This reconstitution produces a stock solution of 10 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Rat Adiponectin Standard (10 ng/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) 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, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, control, 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 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1 hour 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 four times for a total of five 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 100 µL of Rat Adiponectin Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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

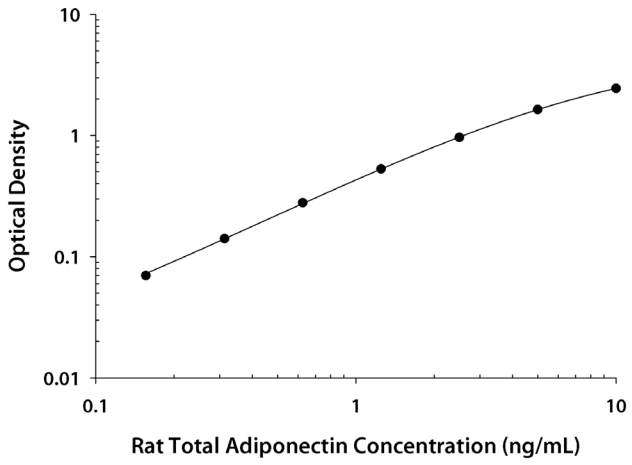
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 rat Adiponectin 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	0.009 0.009	0.009	—
0.156	0.078 0.080	0.079	0.070
0.313	0.148 0.152	0.150	0.141
0.625	0.287 0.288	0.288	0.279
1.25	0.535 0.543	0.539	0.530
2.5	0.965 0.983	0.974	0.965
5	1.632 1.665	1.649	1.640
10	2.434 2.473	2.454	2.445

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)	0.587	1.14	3.42	0.595	1.19	3.48
Standard deviation	0.021	0.025	0.112	0.056	0.083	0.272
CV (%)	3.6	2.2	3.3	9.4	7.0	7.8

RECOVERY

The recovery of rat Adiponectin spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=6)	109	100-116%
Urine (n=4)	106	91-113%

LINEARITY

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

		Cell culture supernates* (n=4)	Tissue lysates* (n=2)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine (n=4)
1:2	Average % of Expected	95	98	94	97	97	100
	Range (%)	90-98	97-100	92-97	96-98	93-99	96-103
1:4	Average % of Expected	96	98	93	94	96	100
	Range (%)	89-104	96-100	89-96	93-97	89-103	96-102
1:8	Average % of Expected	92	99	92	96	96	100
	Range (%)	87-99	97-102	90-95	95-97	90-102	94-104
1:16	Average % of Expected	92	96	94	97	97	98
	Range (%)	88-96	95-97	90-96	96-99	93-105	92-104

*Samples were diluted prior to assay.

SENSITIVITY

Sixty-four assays were evaluated and the minimum detectable dose (MDD) of rat Adiponectin ranged from 0.002-0.023 ng/mL. The mean MDD was 0.004 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-derived recombinant rat Adiponectin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of rat Adiponectin in this assay.

Sample Type	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum (n=18)	4.49	3.02-6.78	1.16
EDTA plasma (n=10)	3.02	2.09-4.19	0.712
Heparin plasma (n=10)	3.02	1.14-6.91	1.76

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Urine (n=18)	0.942	0.262-3.21	0.901

Cell Culture Supernates - Organs from rats were removed, rinsed in PBS, and kept on ice. Organs were homogenized with a tissue homogenizer and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of rat Adiponectin.

Tissue Type	(ng/mL)
Heart	25.3
Liver	10.5

Tissue Lysates - Abdominal fat from rats was rinsed with PBS, cut into 1-2 mm pieces, and then frozen at -70 °C. Cells were thawed and then frozen again for a total of two freeze-thaw cycles. Debris was then removed by centrifugation. An aliquot of the supernate was removed, assayed for rat Adiponectin, and measured 3120 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat Adiponectin. This assay recognizes LMW, MMW, and HMW recombinant rat Adiponectin.

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

Recombinant rat:
C-Reactive Protein/CRP*
Fas Ligand
Leptin
TNF-α

Recombinant mouse:
C1qL2
C1q R1/CD93
gAdiponectin/Acrp30*

Recombinant human:
Adiponectin/Acrp30
gAdiponectin/Acrp30
HMW Adiponectin/Acrp30
LMW Adiponectin/Acrp30
MMW Adiponectin/Acrp30
Cadherin-13
Calreticulin
Complement Component C1qA

*Prepared at 5 µg/mL in calibrator diluent or a mid-range control.

Recombinant mouse Adiponectin/Acrp30 cross-reacts approximately 0.03%, and recombinant mouse HMW Adiponectin/Acrp30 cross-reacts approximately 0.09% in this assay.

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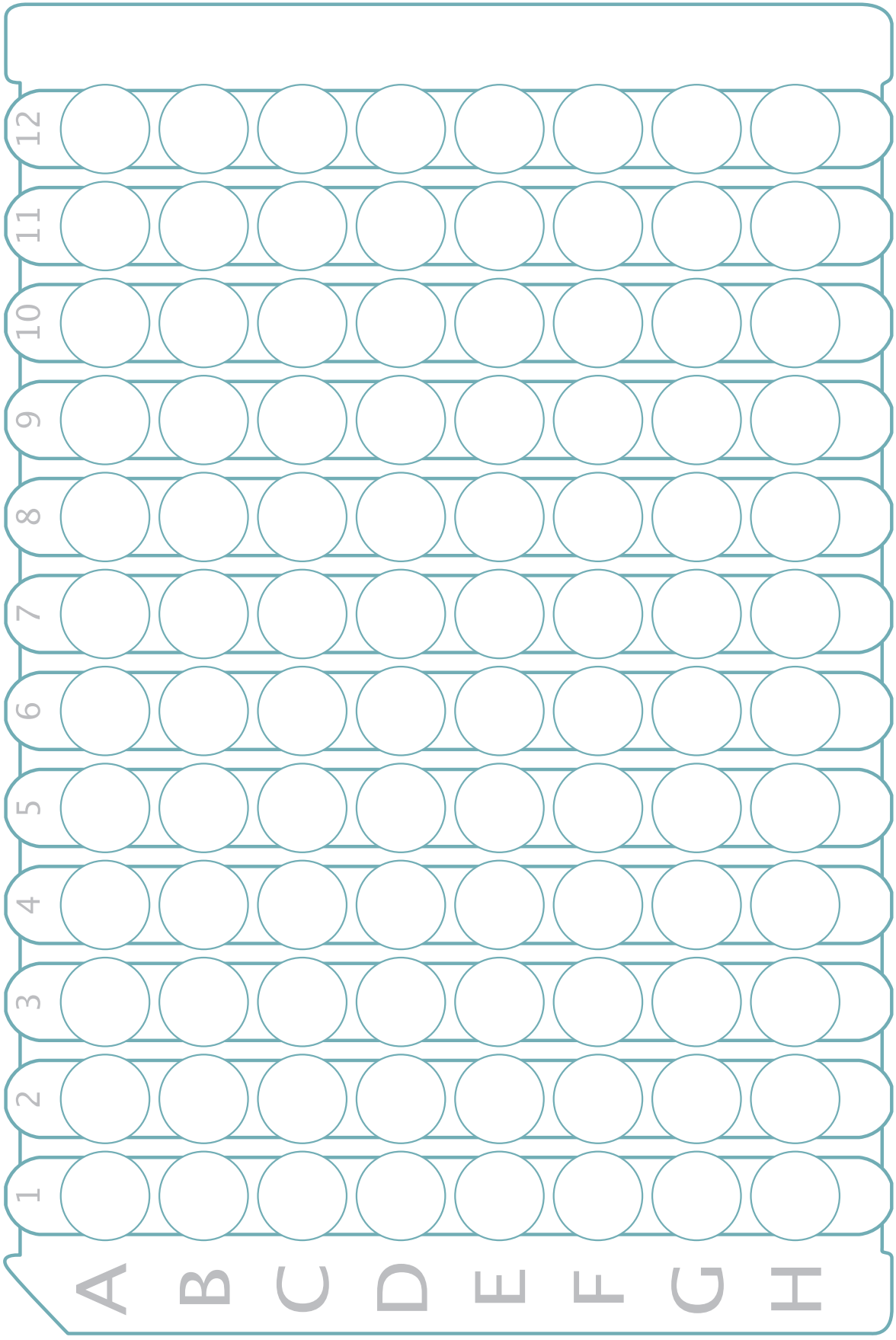
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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