Quantikine® ELISA

Mouse Serum Amyloid A Immunoassay

Catalog Number MSAA00

For the quantitative determination of mouse Serum Amyloid A (SAA) concentrations in cell culture supernates, tissue 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

Serum Amyloid A1 (SAA1), like the closely related SAA2, is a multifunctional acute phase protein that is dramatically upregulated in the liver in response to pro-inflammatory cytokines (1, 2). Elevated levels of circulating SAA1 are associated with acute inflammation as well as chronic inflammatory diseases such as cancer, atherosclerosis, and metabolic syndrome (3-7). In contrast, SAA3 is induced by inflammatory stimulation in intestinal epithelium and adipocytes (8, 9) while SAA4 is constitutively expressed (10). Mature mouse SAA1 shares 72%, 72%, and 67% amino acid (aa) sequence identity with human, rabbit and equine SAA1, respectively (11). It shares 91%, 70%, and 54% aa sequence identity with mouse SAA2, SAA3, and SAA4, respectively. Circulating levels of SAA1 can increase by as much as 1000-fold during inflammation. It is secreted as a 12 kDa nonglycosylated protein and circulates as a component of the HDL complex (4). During inflammation, SAA displaces ApoA1 as the major apolipoprotein in HDL, weakening the role of HDL as a reverse (lipid clearing) cholesterol transporter (4). HDL particles containing SAA1 can interact with heparan sulfate proteoglycans, leading to extraction of SAA1 from HDL and the formation of amyloid aggregates during systemic amyloidosis (12, 13). SAA1 additionally induces cholesterol efflux from adipocytes and smooth muscle cells (14, 15). It serves as a ligand for FPRL1, TLR2, TLR4, RAGE, and Integrin α2bβ3 on multiple cell types including monocytes, macrophages, neutrophils, smooth muscle cells, vascular endothelial cells, rheumatoid synoviocytes, and platelets. These interactions induce cell chemotaxis as well as the generation of pro-inflammatory cytokines and chemokines (16-25). SAA is cleared from the circulation by CD36/SR-B3 and SR-B1 mediated uptake (26, 27). SAA can bind the surface of Gram negative bacteria and function as an opsonin to aid clearance by macrophages (28, 29). It also functions as a retinol and retinoic acid binding protein (30).

The Quantikine® Mouse Serum Amyloid A Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse SAA in cell culture supernates, tissue culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse SAA1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse SAA. Results obtained using natural mouse SAA showed dose response curves that were parallel to the standard curves obtained using the Quantikine® mouse kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse SAA.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse SAA has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse SAA present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse SAA 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 mouse SAA 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
Mouse SAA Microplate	898285	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse SAA.	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.*
Mouse SAA Standard	898287	2 vials of recombinant mouse SAA in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse SAA Control	898288	2 vials of recombinant mouse SAA in a buffered protein base with preservatives, lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse SAA Conjugate	898286	12 mL of a polyclonal antibody specific for mouse SAA conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1N	895488	12 mL of a buffered protein solution 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.
- Test tubes for dilution of standards and samples.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.

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. Please 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.

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

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 2000 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.*

SAMPLE PREPARATION

Serum and plasma samples require a 200-fold dilution prior to assay due to high endogenous levels. A suggested 200-fold dilution can be achieved by adding 10 µL of sample to 90 µL of Calibrator Diluent RD5-26 (diluted 1:4)*. Complete the 200-fold dilution by adding 10 µL of the diluted sample to 190 µL Calibrator Diluent RD5-26 (diluted 1:4).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse SAA 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.

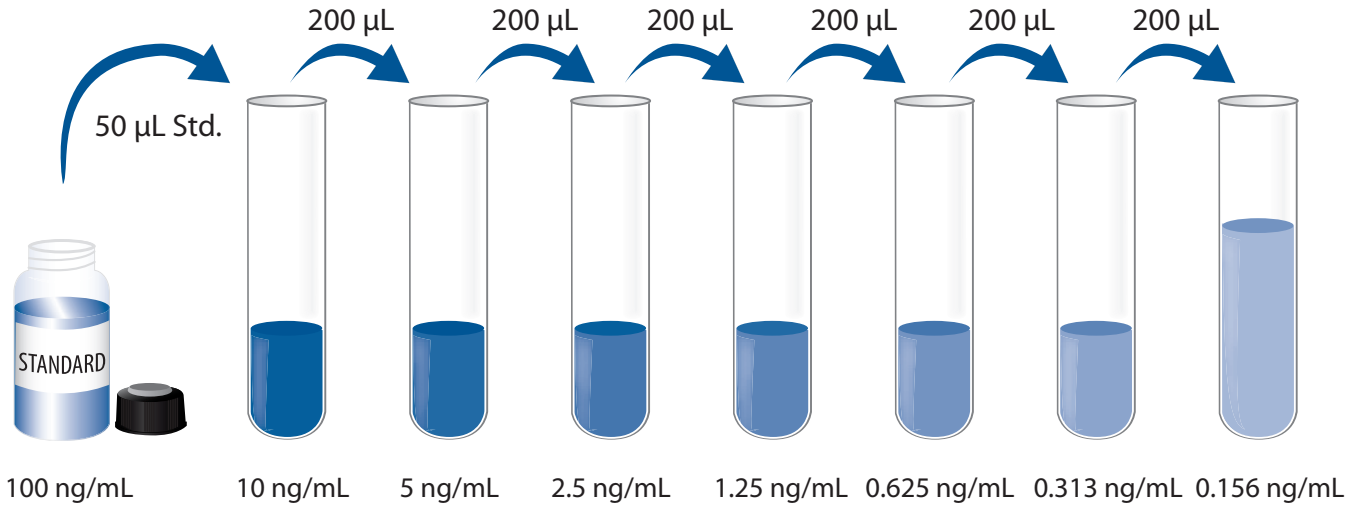
Calibrator Diluent RD5-26 (diluted 1:4) - Add 10 mL of Calibrator Diluent RD5-26 Concentrate to 30 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-26 (diluted 1:4).

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.

Mouse SAA Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse SAA Standard with deionized or distilled water. Do not substitute other diluents. This reconstitution produces a stock solution of 100 ng/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Note: *Do not use rocker.*

Pipette 450 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the 10 ng/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The 10 ng/mL standard 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 samples, control, and standards be assayed in duplicate.

1. Prepare all reagents, standard dilutions, 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 RD1N 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 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 100 µL of Mouse SAA 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 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 the Sample Preparation section.

CALCULATION OF RESULTS

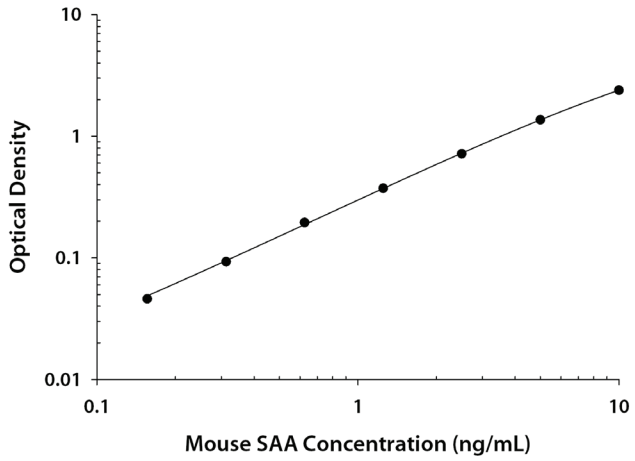
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse SAA 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 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.



(ng/mL)	O.D.	Average	Corrected
0	0.006 0.006	0.006	—
0.156	0.052 0.052	0.052	0.046
0.313	0.099 0.099	0.099	0.093
0.625	0.197 0.204	0.201	0.195
1.25	0.375 0.383	0.379	0.373
2.5	0.718 0.730	0.724	0.718
5	1.353 1.388	1.371	1.365
10	2.369 2.416	2.393	2.387

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)	0.413	1.35	2.35	0.459	1.35	2.51
Standard deviation	0.020	0.073	0.110	0.036	0.082	0.178
CV (%)	4.8	5.4	4.7	7.8	6.1	7.1

RECOVERY

The recovery of mouse SAA spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Culture media (n=4)	93	84-106%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse SAA were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	96	98	97	97
	Range (%)	88-102	93-101	92-102	95-99
1:4	Average % of Expected	93	97	97	96
	Range (%)	86-99	91-101	93-103	93-99
1:8	Average % of Expected	92	99	99	98
	Range (%)	89-97	91-106	93-106	92-101
1:16	Average % of Expected	99	96	93	95
	Range (%)	88-105	91-101	89-96	94-98

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Thirty assays were evaluated and the minimum detectable dose (MDD) of mouse SAA ranged from 0.003-0.022 ng/mL. The mean MDD was 0.006 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 a highly purified *E. coli*-expressed recombinant mouse SAA1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse SAA in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=10)	581	176-1699	512
EDTA plasma (n=5)	391	165-851	285
Heparin plasma (n=5)	1984	248-4718	1991

Cell Culture Supernates - AML12 mouse liver hepatocyte cells were cultured in DMEM/Kaighn's F12 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 5 µg/mL of recombinant human Insulin, and 5 µg/mL of recombinant human Apo-Transferrin. Cells were grown unstimulated or stimulated with 10 ng/mL of recombinant mouse IL-6, 10 ng/mL of recombinant mouse IL-1β, and 10 ng/mL of recombinant mouse TNF-α for 3 days. Aliquots of the cell culture supernates were removed and assayed for mouse SAA.

Condition	(ng/mL)
Unstimulated	ND
Stimulated	7.93

ND=Non-detectable

Tissue Culture Supernates - Livers from mice were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. Cells were 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 24 hours. An aliquot of the tissue culture supernate was removed, assayed for mouse SAA, and measured 1.80 ng/mL.

SPECIFICITY

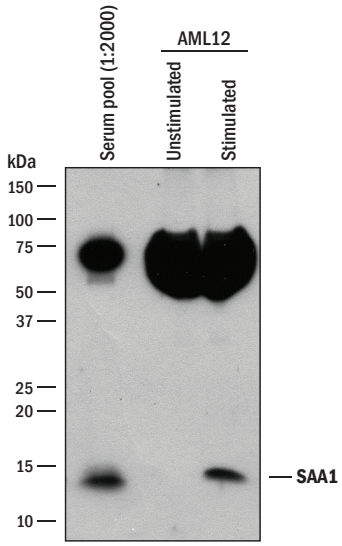
This assay recognizes natural and recombinant mouse SAA.

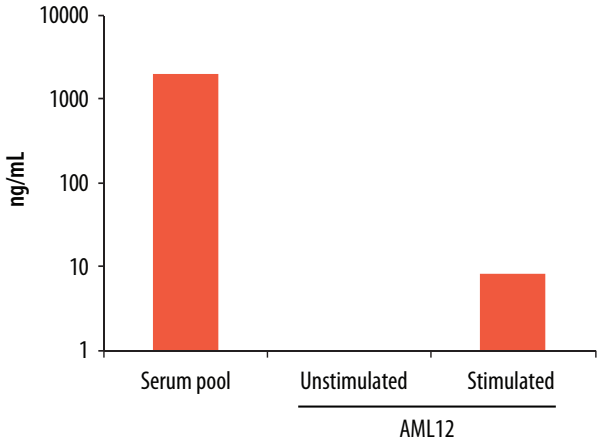
The factors listed below were prepared at 100 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range mouse SAA control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
SAA3

Recombinant human:
SAA1
SAA2
SAA4

Recombinant mouse Serum Amyloid A2 cross-reacts approximately 85.3% in this assay.





Normal mouse serum pool and cell culture media were analyzed by Western blot and Quantikine ELISA. For the Western blot, a diluted serum sample and undiluted AML12 samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody used in this kit. The Western blot band intensity shows a direct correlation with the ELISA sample values.

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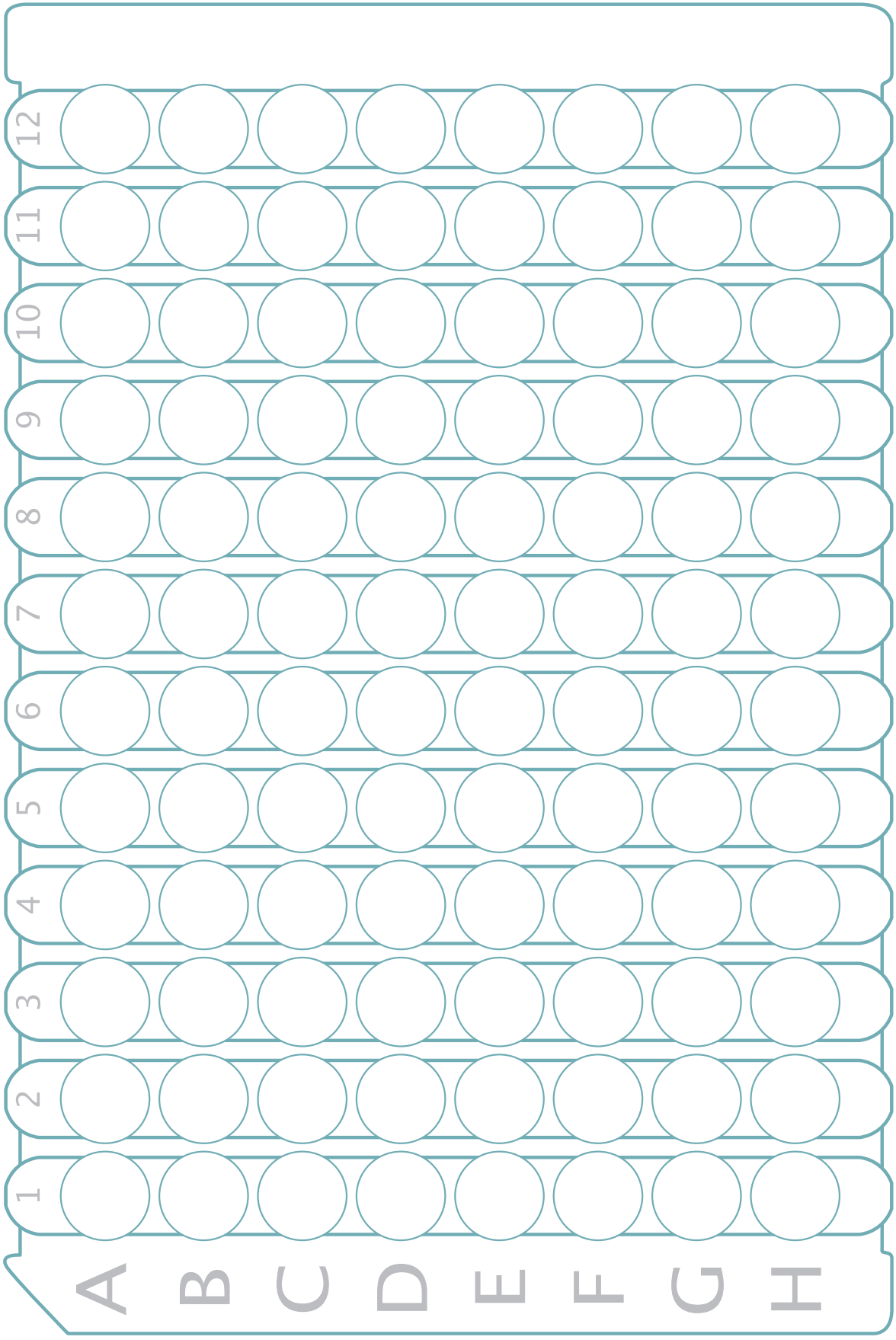
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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