Quantikine® ELISA

Human FABP4 Immunoassay

Catalog Number DFBP40

For the quantitative determination of human Fatty Acid Binding Protein 4 (FABP4) concentrations in cell culture supernates, cell lysates, 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

Fatty Acid Binding Protein 4 (FABP4), also known as Adipocyte Fatty Acid Binding Protein (A-FABP) and Adipocyte Protein 2 (aP2), is a 15 kDa member of the FABP family of cytoplasmic lipid chaperones (1). It shares 92% and 89% amino acid sequence identity with the mouse and rat orthologs, respectively. FABPs are a group of small, cytosolic proteins that are highly expressed in tissues that exhibit high rates of fatty acid (FA) uptake and metabolism. These proteins reversibly bind hydrophobic ligands and regulate numerous processes including the uptake, intracellular trafficking, storage, and metabolism of FAs, the maintenance of cellular membrane FA levels, and regulation of lipid-mediated gene expression (2). FABPs are composed of a β-barrel that is formed from ten anti-parallel β-strands and contains a lipid-binding pocket (1-3).

FABP4 is the predominant FABP protein found in adipocytes and is often used as a marker for adipocyte differentiation (1-6). It is also expressed in macrophages, dendritic cells, and endothelial cells (2, 3, 6, 7). FABP4 expression is regulated by multiple factors including FAs, PPARγ agonists, and Insulin, and its levels increase with lipolytic stimulation (5, 7, 8). It is a key mediator of FA intracellular transport and metabolism in adipose tissue. It has the highest binding affinity for saturated long-chain FAs, but can also bind to unsaturated FAs, arachidonic acid, and retinoic acid (1-3, 9-11). FABP4 interacts with FAs in donor membranes via collisional interaction and transfers them to various cellular compartments including the lipid droplet, endoplasmic reticulum, and mitochondria (1, 3, 7, 12). FABP4 also shuttles specific molecules into the nucleus to the nuclear receptor PPARγ and facilitates ligand binding, thereby regulating transcriptional activity of PPARγ (1-3, 8, 13). FABP4 is believed to control lipolysis. It can bind to activated Hormone-Sensitive Lipase and increase its hydrolytic activity, and targeted disruption of FABP4 in mice reduces basal and hormone-stimulated lipolysis (1, 2, 7, 14). FABP4 is also believed to facilitate inflammatory responses. It has been shown to potentiate activation of the JNK/AP-1 and IKK/NFκB signaling pathways and increase production of multiple pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and CCL2/MCP-1 (7, 15, 16). FABP4 expression in adipocytes and macrophages is associated with the development of Insulin resistance, hypertriacylglycerolaemia, and atherosclerosis (1, 2). Furthermore, FABP4 has been found to be overexpressed in multiple cancer types including ovarian, bladder, glioblastoma, and oral, and is believed to play a role in tumor progression (8, 17-20).

FABP4 has also been characterized as a circulating adipokine. Serum levels of FABP4 are enhanced in several pathologies related to adipose tissue dysfunction and inflammation including obesity and metabolic syndrome, type 2 diabetes, HIV-associated lipodystrophy, polycystic ovary syndrome, and nonalcoholic fatty liver disease (1, 2, 6, 8, 12, 21-25). Elevated circulating FABP4 levels are also associated with carotid atherosclerosis and plaque instability, various types of cardiovascular disease, and with poor clinical outcomes in patients with acute ischaemic stroke (1, 2, 8, 12, 26-30). Moreover, serum FABP4 levels increase with the development of type 2 diabetes, metabolic syndrome, and atherogenic dyslipidaemia in the general population (1, 2, 31-34).

The Quantikine Human FABP4 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human FABP4 in cell culture supernates, cell lysates, serum, and plasma. It contains *E. coli*-expressed recombinant human FABP4 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human FABP4 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 naturally occurring human FABP4.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human FABP4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FABP4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human FABP4 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 FABP4 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.

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 FABP4 Microplate	894776	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human FABP4.	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 FABP4 Standard	894778	2 vials of recombinant human FABP4 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 FABP4 Conjugate	894777	21 mL of a polyclonal antibody specific for human FABP4 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-89	895881	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-68	896030	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
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 and samples.
- Human FABP4 Controls (optional; R&D Systems, Catalog # QC186).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 1 (R&D Systems, Catalog # 890713)
- PBS

PRECAUTIONS

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.

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.

Cell Lysates - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

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.
Samples containing excessively high levels of protein are not suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5-68.

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples.

1. Wash cells three times in cold PBS.
2. Resuspend cells at 1 x 10^7 cells/mL in Cell Lysis Buffer 1.
3. Incubate with gentle agitation for up to 60 minutes at room temperature.
4. Centrifuge at 8000 x g for 10 minutes to remove cell debris.
5. Assay immediately or aliquot the lysis supernates and store at ≤ -70 °C until ready for use.

REAGENT PREPARATION

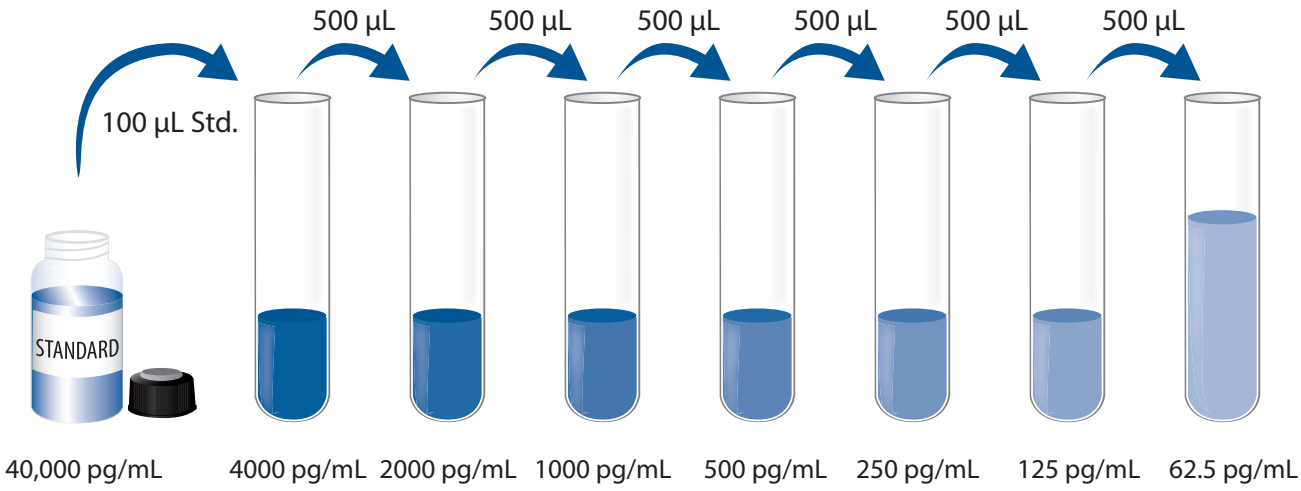
Bring all reagents to room temperature before use.

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 FABP4 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FABP4 Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/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 RD5-68 into the 4000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-68 serves as the zero standard (0 pg/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 RD1-89 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 FABP4 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

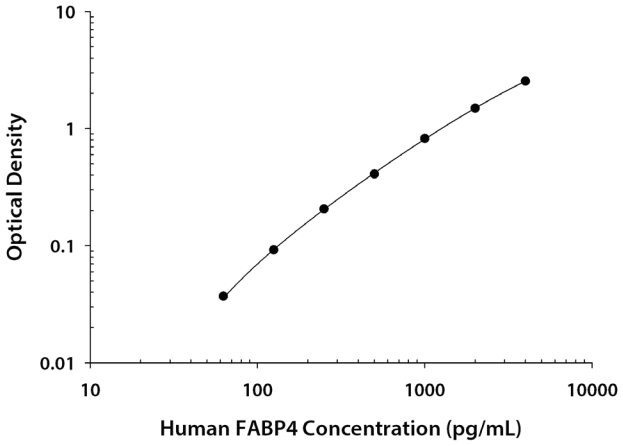
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 FABP4 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.



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.018	0.015	—
62.5	0.051 0.053	0.052	0.037
125	0.102 0.112	0.107	0.092
250	0.220 0.222	0.221	0.206
500	0.424 0.424	0.424	0.409
1000	0.824 0.850	0.837	0.822
2000	1.476 1.531	1.504	1.489
4000	2.544 2.567	2.556	2.541

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 (pg/mL)	349	1155	2595	407	1236	2619
Standard deviation	20.2	39.7	117	51.6	156	291
CV (%)	5.8	3.4	4.5	12.7	12.6	11.1

RECOVERY

The recovery of human FABP4 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	108	100-118%
Cell lysis buffer (n=2)	108	92-117%
Serum* (n=4)	99	82-113%
EDTA plasma* (n=4)	98	88-113%
Heparin plasma* (n=4)	95	83-103%

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

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human FABP4 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Cell lysates (n=3)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	107	109	99	101	102
	Range (%)	102-113	100-116	94-106	95-105	94-110
1:4	Average % of Expected	104	105	103	102	106
	Range (%)	91-113	94-110	99-109	98-110	98-115
1:8	Average % of Expected	103	105	104	105	108
	Range (%)	84-121	93-114	95-119	98-117	99-120
1:16	Average % of Expected	102	107	104	112	105
	Range (%)	91-116	98-119	94-121	103-120	95-118

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

SENSITIVITY

Twenty-six assays were evaluated and the minimum detectable dose (MDD) of human FABP4 ranged from 2.70-14.2 pg/mL. The mean MDD was 6.55 pg/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 human FABP4 manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human FABP4 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=36)	16,213	4080-36,520	9047
EDTA plasma (n=36)	16,491	3949-34,328	8802
Heparin plasma (n=36)	15,400	4256-38,880	8761

Cell Culture Supernates/Cell Lysates:
Human mesenchymal stem cells (MSCs) were differentiated to adipocytes *in vitro* for 14 days using StemXVivo® Osteogenic/Adipogenic Base Media (R&D Systems, Catalog # CCM007) and StemXVivo Adipogenic Supplement (R&D Systems, Catalog # CCM011). Aliquots of the cell culture supernates were removed and assayed for human FABP4. Cells were lysed and assayed for human FABP4. Results were normalized to total protein concentration.

Cell Line	Cell Culture Supernate Value (pg/mL)	Cell Lysate Value (pg/mg)
Undifferentiated MSCs	ND	N/A
Differentiated adipocytes	55,192	6,366,738

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human FABP4.

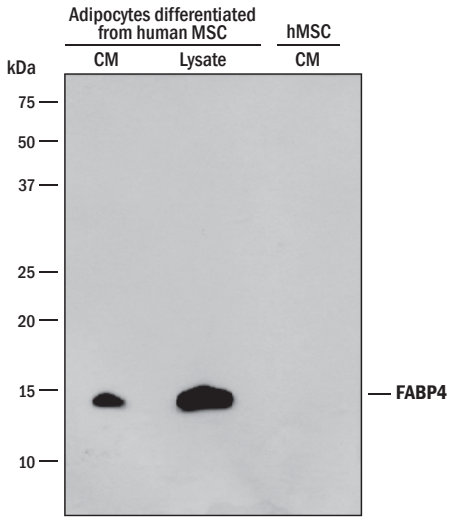
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human FABP4 control were assayed for interference. No significant cross-reactivity or interference was observed.

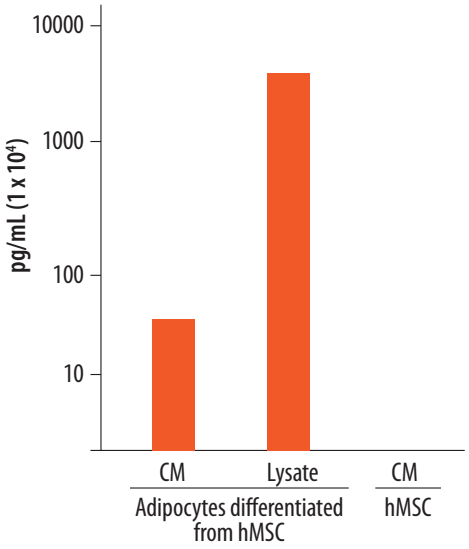
Recombinant human:

FABP1
FABP2
FABP3
FABP5
FABP7
FABP8
FABP9
HSL
PPARγ
PPARγ1
PPARγ2

Recombinant mouse:

FABP4





Conditioned media and lysate samples from human MSC-differentiated adipocytes were analyzed by Western blot and Quantikine ELISA. For the Western blot, diluted 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 shows a direct correlation with the ELISA value for these samples.

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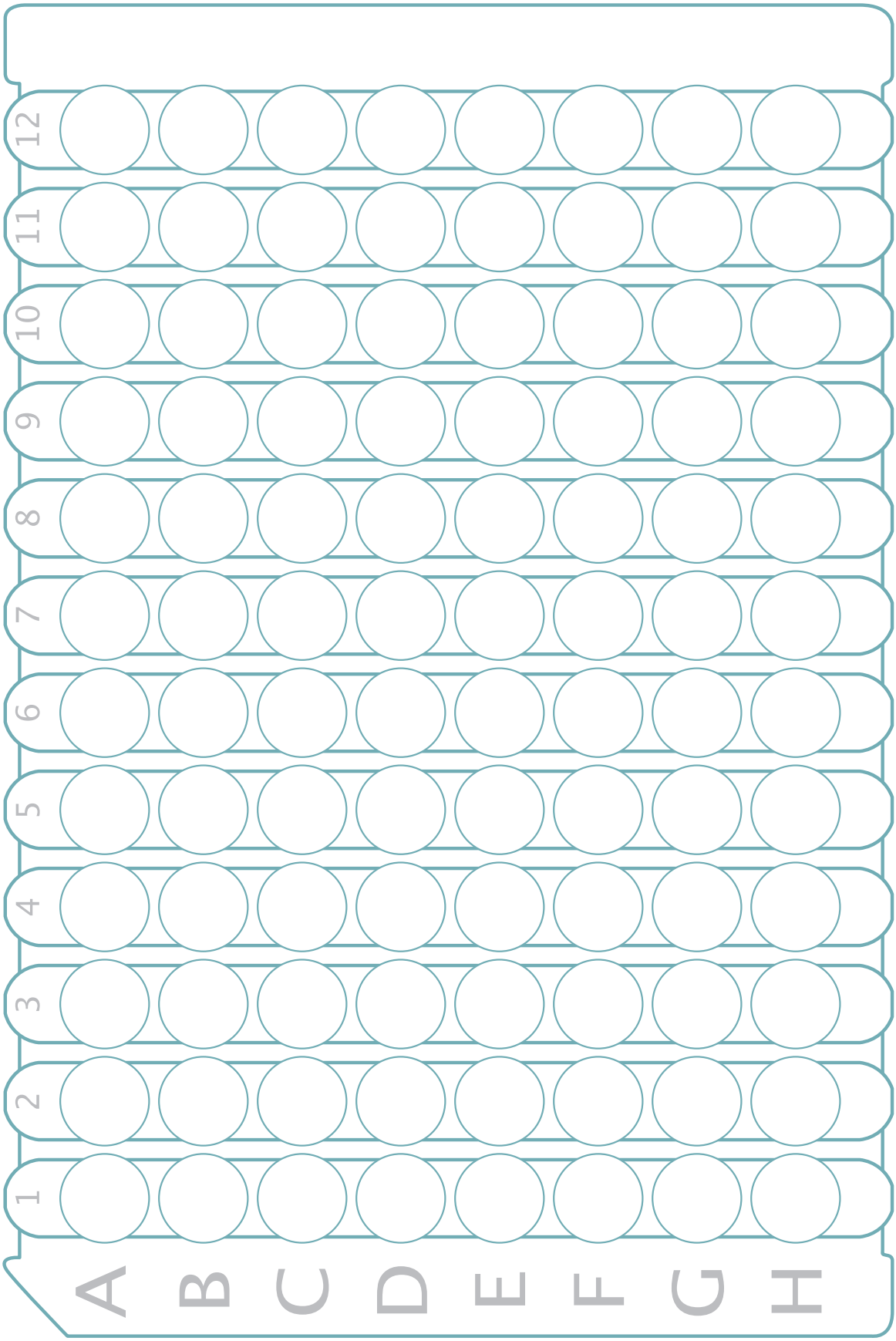
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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