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R&D SYSTEMS

Quantikine® ELISA

Mouse/Rat GDF-15 Immunoassay

Catalog Number MGD150

For the quantitative determination of mouse and rat Growth and Differentiation Factor-15 (GDF-15) concentrations in cell culture supernates, 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

Growth and Differentiation Factor-15 (GDF-15), also known as macrophage inhibitory cytokine-1 (MIC-1), placental transforming growth factor-β, prostate-derived factor, nonsteroidal anti-inflammatory drug-activated gene, and placental bone morphogenetic protein, is a divergent member of the Transforming Growth Factor-β superfamily (1-4). GDF-15 is synthesized as a 40 kDa inactive precursor protein that is proteolytically cleaved to release the active C-terminal fragment, which is then secreted into the circulation as a bioactive disulfide-linked homodimer of 28 kDa.

Under normal conditions, GDF-15 is expressed at high levels in the placenta with lower expression levels in a variety of tissues including the kidney, liver, lung, pancreas, and prostate. It is also expressed in the epithelium of the central nervous system. Its expression by activated macrophages is induced by inflammatory cytokines including IL-1β and TGF-β (1).

GDF-15 has diverse biological functions with roles in inflammation, cancer, and metabolism, and it is associated with all-cause mortality and miscarriage (5-7). GDF-15 is upregulated under inflammatory conditions such as atherosclerosis or rheumatoid arthritis (8-10). Increased expression of GDF-15 is also observed in many cancers (6, 11-14). The metabolic effects of GDF-15 are associated with the modulation of neuronal pathways important in the regulation of appetite and energy homeostasis (15, 16). Increased levels of GDF-15 in the serum of individuals with advanced cancer or chronic disease are correlated with anorexia/cachexia in mice and humans (15, 17).

GDF-15 also exerts cardioprotective actions (18). In mouse models, induction of GDF-15 protects the heart from ischemia/reperfusion injury, and over-expression of GDF-15 attenuates ventricular dilation and heart failure. In humans, serum GDF-15 concentrations are associated with the risk of acute coronary syndrome as well as its prognosis (19, 20).

The Quantikine® Mouse/Rat GDF-15 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse and rat GDF-15 in cell culture supernates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant mouse GDF-15 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse or rat GDF-15 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 mouse and rat GDF-15.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat GDF-15 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any GDF-15 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat GDF-15 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 GDF-15 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, 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.
- 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/Rat GDF-15 Microplate	894754	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat GDF-15.	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/Rat GDF-15 Standard	894756	2 vials of recombinant mouse GDF-15 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/Rat GDF-15 Control	894757	2 vials of recombinant mouse GDF-15 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse/Rat GDF-15 Conjugate	894755	12 mL of a polyclonal antibody specific for mouse/rat GDF-15 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a 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	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.

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 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.

Cell 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 samples have 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

Urine samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse/Rat GDF-15 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

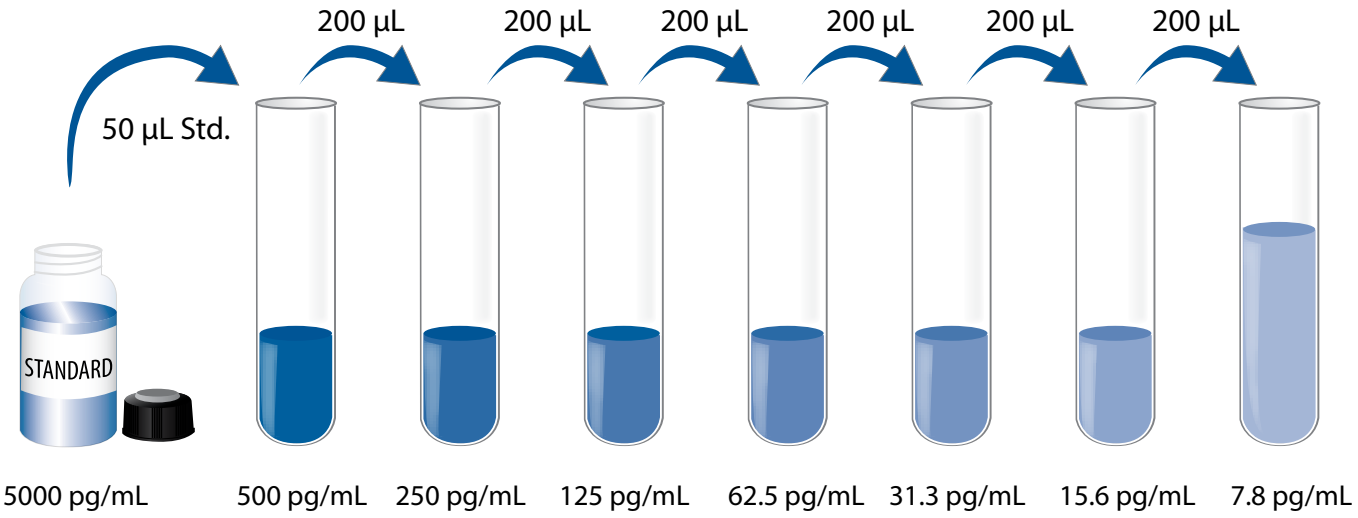
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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

Mouse/Rat GDF-15 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat GDF-15 Standard with deionized or distilled water. Do not substitute other diluents. This reconstitution produces a stock solution of 5000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 500 pg/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 500 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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 standards, control, and samples 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 RD1W 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/Rat GDF-15 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.

CALCULATION OF RESULTS

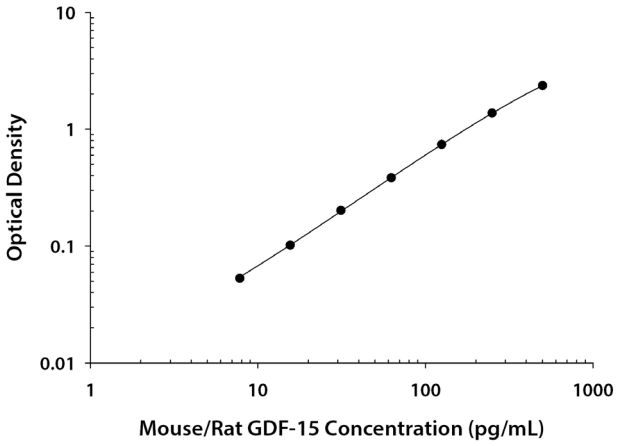
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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/rat GDF-15 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 ia provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.024	0.024	—
7.8	0.076 0.078	0.077	0.053
15.6	0.124 0.127	0.126	0.102
31.3	0.220 0.231	0.226	0.202
62.5	0.408 0.408	0.408	0.384
125	0.757 0.769	0.763	0.739
250	1.376 1.413	1.395	1.371
500	2.362 2.416	2.389	2.365

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)	25.6	60.0	190	26.7	63.1	196
Standard deviation	0.690	1.42	5.49	1.62	2.84	10.7
CV (%)	2.7	2.4	2.9	6.1	4.5	5.5

RECOVERY

The recovery of mouse/rat GDF-15 spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture media (n=4)	102	91-110%
Serum (n=4)	91	82-101%
EDTA plasma (n=4)	95	81-109%
Heparin plasma (n=4)	95	87-104%
Urine* (n=4)	97	88-106%

*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 mouse/rat GDF-15 in each matrix were diluted with calibrator diluent and assayed.

Mouse Samples		Cell culture supernates* (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine* (n=4)
1:2	Average % of Expected	99	102	104	104	99
	Range (%)	95-104	99-104	101-108	103-106	97-101
1:4	Average % of Expected	96	106	105	106	99
	Range (%)	92-99	106-107	99-111	105-108	97-100
1:8	Average % of Expected	96	112	110	110	98
	Range (%)	91-100	107-114	103-118	106-116	95-100
1:16	Average % of Expected	95	117	111	113	97
	Range (%)	89-97	114-121	108-119	108-118	89-102

*Samples were diluted prior to assay.

Note: *Rat samples were evaluated and no significant difference in linearity or recovery was observed from the data above.*

SENSITIVITY

Twenty-eight assays were evaluated and the minimum detectable dose (MDD) of mouse/rat GDF-15 ranged from 0.254-2.20 pg/mL. The mean MDD was 0.686 pg/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 *E. coli*-expressed recombinant mouse GDF-15 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse/rat GDF-15 in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	77.0	47.4-97.1	19.5
EDTA plasma (n=5)	241	54.4-874	354
Heparin plasma (n=5)	110	82.4-169	34.7
Urine (n=10)	2376	632-6255	1547

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	27.5	10.5-49.4	14.3
Heparin plasma (n=5)	42.7	11.1-145	57.3
Urine (n=10)	4972	1922-11,550	2747

Rat Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
EDTA plasma (n=5)	31.5	80	ND-68.8

ND=Non-detectable

Cell Culture Supernates:

C2C12 mouse myoblast cells were cultured in DMEM supplemented with 10% fetal bovine serum to 80% confluency. Cell were washed with PBS and cultured in DMEM supplemented with 5% equine serum for 6 days with media changes every other day. When the cells were 50% differentiated, they were unstimulated or stimulated with 1000 µM H_2O_2 for 1 day. Aliquots of the cell culture supernates were removed, assayed for mouse/rat GDF-15, and measured 4630 pg/mL and 7450 pg/mL, respectively.

Hepa 1-6 mouse hepatoma cells (1 x 10^6 cells/mL) were cultured for 6 days in DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for mouse/rat GDF-15, and measured 6255 pg/mL.

H4-II-E-C3 rat hepatoma cells (2 x 10^6 cells/mL) were cultured for 3 days in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for mouse/rat GDF-15, and measured 514 pg/mL.

SPECIFICITY

This assay recognizes natural mouse and rat GDF-15, recombinant mouse mature GDF-15 and precursor GDF-15. It does not detect monomeric recombinant mouse GDF-15.

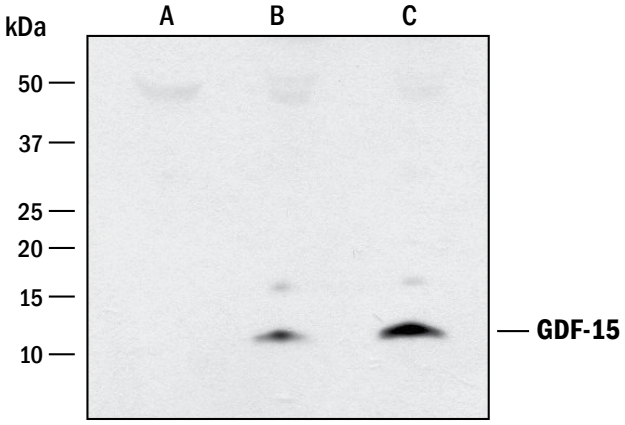
The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat GDF-15 control were assayed for interference. No significant cross-reactivity or interference was observed.

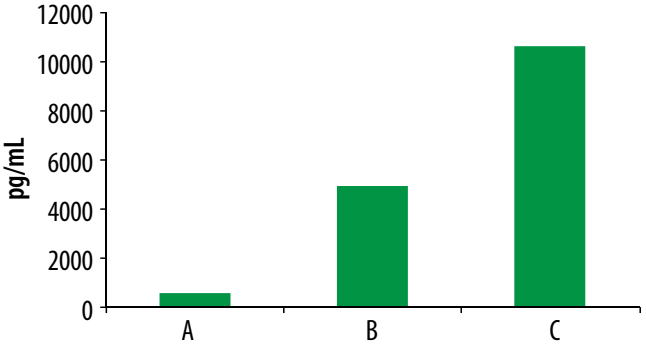
Recombinant mouse:

GDF-1
GDF-3
GDF-5
GDF-6
GDF-7
GDF-8
GDF-9
GDF-15 (monomer, aa 189-303)

Recombinant human:

GDF-11
GDF-15





Conditioned media samples from C2C12 mouse myoblast cells were analyzed by Western Blot and Quantikine® ELISA. **(A)** The undifferentiated sample was from cells cultured in DMEM supplemented with 10% fetal bovine serum. **(B)** The differentiated sample was from cells cultured in DMEM supplemented with 5% equine serum for 6 days. **(C)** Other differentiated media was cultured in DMEM and equine serum and then treated with 1000 µM H_2O_2 for 48 hours. 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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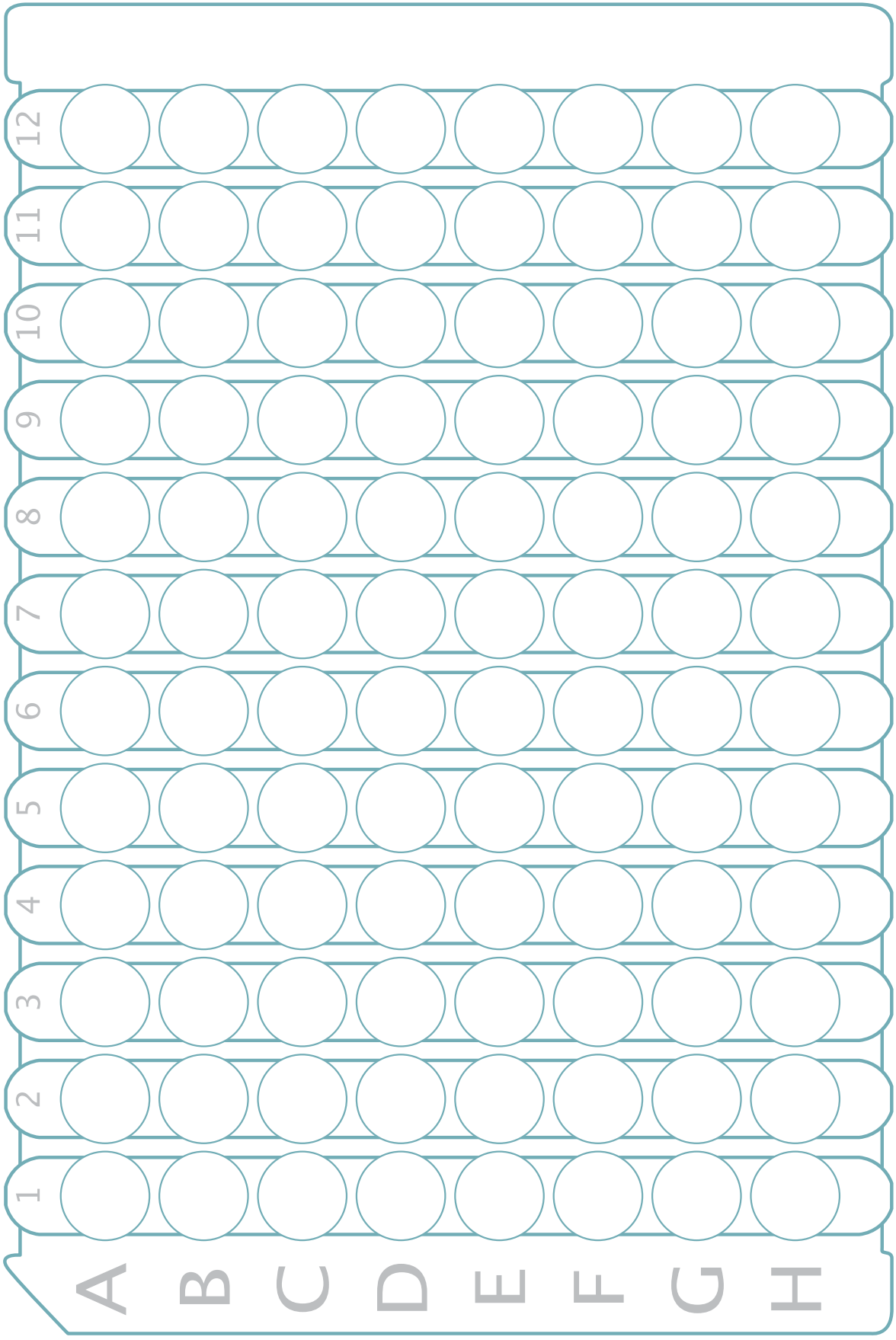
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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