Quantikine™ QuicKit™ ELISA

Human GDF-15 Immunoassay

Catalog Number QK957

For the quantitative determination of Growth 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 Differentiation Factor-15 (GDF-15), also known as macrophage inhibitory cytokine-1 (MIC-1), placental transforming growth factor-β, prostate-derived factor, and placental bone morphogenetic protein, is a divergent member of the transforming growth factor beta (TGF-β) superfamily (1-3). GDF-15 is initially synthesized as a 40 kDa inactive precursor protein. It is then proteolytically cleaved to release the active C-terminal fragment, which is assembled into a disulfide-linked homodimer of 28 kDa to become biologically active GDF-15 (1, 4). In humans, GDF-15 is predominantly expressed in the placenta, with low levels in the kidney, pancreas, and prostate. However, its expression can be rapidly induced by cytokines such as interleukin-1 and TGF-β (1-3).

GDF-15 has diverse biological functions. Early studies have shown that low serum GDF-15 levels correlate with miscarriages, indicating that it might be able to suppress inflammation in early pregnancy (5, 6). GDF-15 also plays an important role in tumorigenesis and metastasis. It has been observed that in many types of cancers, such as colorectal, breast, and prostate, the expression of GDF-15 is dramatically increased (7-9). Additionally, in cancer patients, serum levels of GDF-15 are elevated, which are of value in disease diagnosis and stratification (10-12). GDF-15 is strongly induced by the tumor suppressor gene p53 and other anti-tumorigenic agents, such as non-steroidal anti-inflammatory drugs and the peroxisome proliferator-activated receptor γ. These findings suggest that GDF-15 may be a downstream target of those signaling pathways that regulate cell cycle arrest and apoptosis (13-15). Through the modulation of neuronal pathways important in the regulation of appetite and energy homeostasis, GDF-15 mediates cancer-induced anorexia and weight loss (16).

GDF-15 has cardioprotective functions. In mouse models, induction of GDF-15 protects the heart from ischemia/reperfusion injury and its over-expression attenuates ventricular dilation and heart failure. Conversely, in GDF-15 gene-targeted mice, reduction of GDF-15 expression results in enhanced cardiac hypertrophic growth (17, 18). In humans, serum GDF-15 concentrations have been shown to be associated with the risk of acute coronary syndrome as well as its prognosis (19, 20). GDF-15 might exert its cardioprotective effects through activation of the PI3K-Akt signaling pathway and the Smad protein (21, 22).

GDF-15 is also involved in iron homeostasis. Tanno *et al.* have reported that in patients with β thalassemia, serum GDF-15 levels are elevated, which results in the suppression of the iron regulatory protein hepcidin. Significant induction of GDF-15 has also been observed in individuals with iron deficiency (23, 24).

The Quantikine™ QuicKit™ Human GDF-15 Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human GDF-15 levels in cell culture supernates, serum, plasma, and urine. It contains CHO cell-expressed recombinant human GDF-15 and antibodies raised against the recombinant protein. Results obtained using natural human GDF-15 showed linear curves that were parallel to the standard curves obtained using the QuicKit standards. These results indicate that this kit can be used to determine relative mass values for natural human GDF-15.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked polyclonal detection antibody specific for human GDF-15. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of GDF-15 bound. 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 the 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™ QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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
QuicKit™ Coated Microplate	899063	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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 GDF-15 Standard	899471	2 vials of recombinant human GDF-15 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human GDF-15 Capture Ab Concentrate	899469	Lyophilized tagged monoclonal antibody specific for human GDF-15.	May be stored for up to 1 month at 2-8 °C.*
Human GDF-15 Detection Ab Concentrate	899470	400 µL of a polyclonal antibody specific for human GDF-15 conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-19	895467	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-10	895266	21 mL 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
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human GDF-15 Controls (optional; R&D Systems®, Catalog # QC282)

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 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.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate samples may require dilution due to high endogenous levels.

Serum and plasma samples require a 2-fold dilution due to matrix effects. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-10.

Urine samples require a 10-fold dilution due to high endogenous levels. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-10.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human GDF-15 Capture Ab Concentrate - Refer to the vial label for reconstitution volume. Reconstitute the Human GDF-15 Capture Ab Concentrate with Assay Diluent RD1-19. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

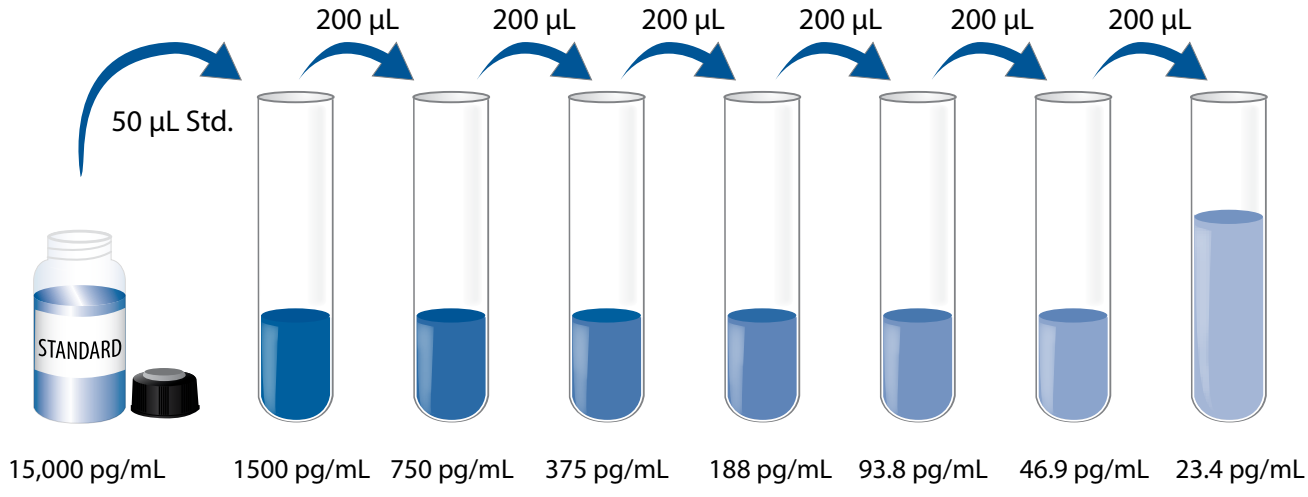
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-19. For a full plate, add 300 µL of reconstituted Human GDF-15 Capture Ab stock and 300 µL of Human GDF-15 Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-19 to get 6.0 mL of Human GDF-15 Antibody Cocktail.

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.

Human GDF-15 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human GDF-15 Standard with deionized or distilled water. This reconstitution produces a stock solution of 15,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-10 into the 1500 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 1500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-10 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, controls, and samples be assayed in duplicate.

1. Prepare all reagents, samples, and working standards 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 standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each 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.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. 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.
8. 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

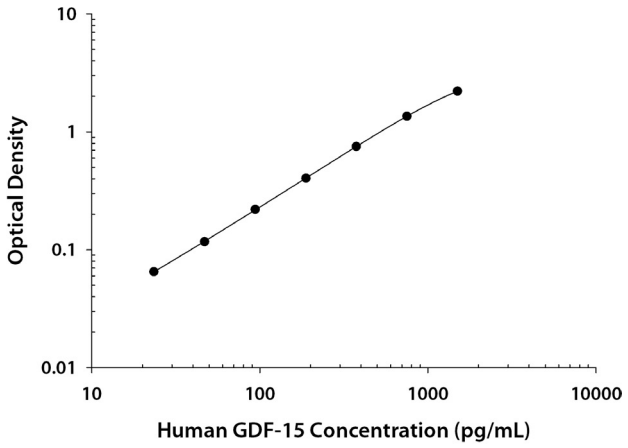
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human 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 is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.008 0.009	0.009	——
23.4	0.064 0.065	0.065	0.056
46.9	0.117 0.117	0.117	0.108
93.8	0.219 0.221	0.220	0.211
188	0.403 0.404	0.404	0.395
375	0.749 0.755	0.752	0.743
750	1.355 1.356	1.356	1.347
1500	2.194 2.226	2.210	2.201

PRECISION

Intra-Assay Precision (Precision within an assay)
Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	156	882	165	925
Standard deviation	6.12	58.8	9.49	77.6
CV (%)	3.9	6.7	5.7	8.4

RECOVERY

The recovery of human GDF-15 spiked to three levels in samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	92-113%
Serum (n=2)	77	66-84%
EDTA plasma (n=2)	74	66-83%
Heparin plasma (n=2)	77	66-93%
Urine (n=2)	104	94-116%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human GDF-15 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=3)*	Serum (n=2)*	EDTA plasma (n=2)*	Heparin plasma (n=2)*	Urine (n=2)*
1:2	Average % of Expected	98	107	106	107	93
	Range (%)	83-116	105-110	104-108	106-108	89-96
1:4	Average % of Expected	94	111	104	108	94
	Range (%)	85-107	108-114	98-110	99-118	86-102
1:8	Average % of Expected	103	112	102	103	90
	Range (%)	96-117	112-112	96-107	95-112	84-95
1:16	Average % of Expected	117	111	99	102	95
	Range (%)	110-122	107-115	87-111	91-112	88-103

*Samples were diluted prior to this assay.

SENSITIVITY

Sixteen assays were evaluated and the minimum detectable dose (MDD) of human GDF-15 ranged from 0.279-1.85 pg/mL. The mean MDD was 0.947 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 CHO cell-expressed recombinant human GDF-15 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human GDF-15 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=10)	556	204-1146	315
EDTA plasma (n=10)	511	249-1042	281
Heparin plasma (n=10)	539	243-1009	282
Urine (n=4)	4783	3243-8287	2353

Cell Culture Supernates:

HepG2 cells were cultured in MEM NEAA Earle's Salts supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until nearly confluent. The cell conditioned media was taken off and centrifuged to remove any cells or debris and stored at ≤ -20 °C. An aliquot of the cell culture supernate was removed, assayed for human GDF-15, and measured at 47,216 pg/mL.

JAR cells were cultured in DMEM NEAA Earle's Salts supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until nearly confluent. The cell conditioned media was taken off and centrifuged to remove any cells or debris and stored at ≤ -20 °C. An aliquot of the cell culture supernate was removed, assayed for human GDF-15, and measured at 29,417 pg/mL.

JEG-3 cells were cultured in MEM NEAA Earle's Salts supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until nearly confluent. The cell conditioned media was taken off and centrifuged to remove any cells or debris and stored at ≤ -20 °C. An aliquot of the cell culture supernate was removed, assayed for human GDF-15, and measured at 5651 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human 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 low level recombinant human GDF-15 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

GDF-5
GDF-7
GDF-9
GDF-11
TGF-β RII/Fc
TFG-β2

Recombinant mouse:

GDF-5
GDF-6
GDF-7
GDF-8

Recombinant human GFRAL does not cross-react, but does interfere at concentrations > 0.5 ng/mL.

Recombinant mouse GDF-15 does not cross-react, but does interfere at concentrations > 2.0 ng/mL.

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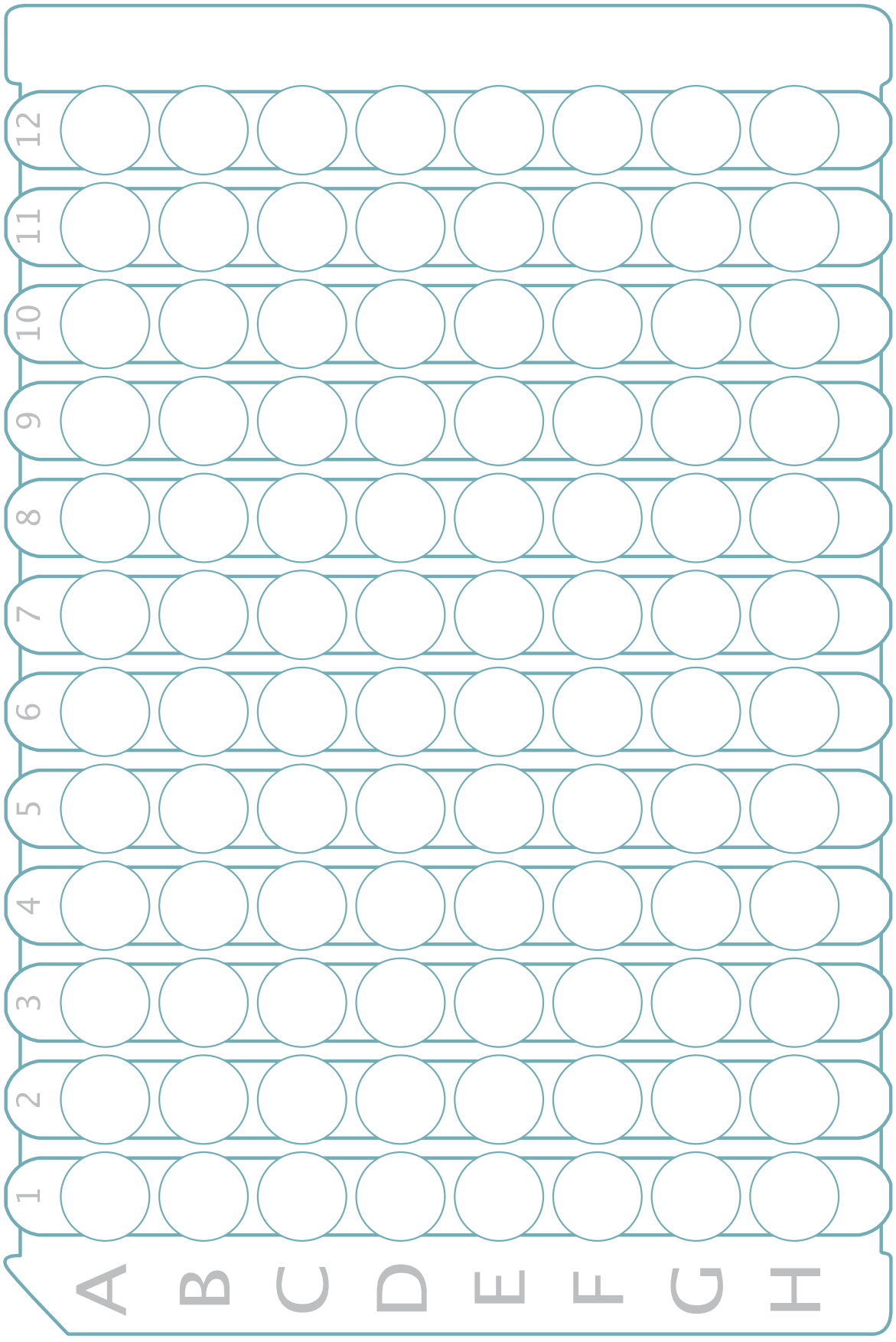
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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