Quantikine® ELISA

Mouse/Rat FGF basic/FGF2/bFGF Immunoassay

Catalog Number MFB00

For the quantitative determination of mouse and rat Fibroblast Growth Factor basic (FGF basic) concentrations in cell culture supernates, tissue 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

FGF basic, also called bFGF, renamed FGF-2 (fibroblast growth factor-2) and previously named HBGF-2 (heparin-binding growth factor-2), is one of the 22 mitogenic proteins of the FGF family (1-7). Family members share 35-60% amino acid (aa) sequence identity, but some FGFs such as FGF basic lack signal peptides (2, 3). FGF basic is secreted by an alternate pathway requiring phosphorylation by the Tec kinase and interaction with specific membrane lipids (8, 9). The 18 kDa FGF basic isoform can be found in both the cytoplasm and the nucleus, and is also the form that is secreted (10-12). Storage pools are likely within the cell, or on the cell surface heparan sulfate proteoglycans (HSPG) (2). Transcription from alternate start sites produces higher molecular weight forms (mainly 21-23 kDa) found in the nucleus (11-14). High and low molecular weight FGF basic isoforms target the expression of different genes (10, 11, 14, 15). The 18 kDa mouse and rat FGF basic sequences share 99% amino acid sequence identity with each other, and 97% with human and bovine FGF basic (6, 7). Expression of FGF basic is nearly ubiquitous (6, 7, 13). However, expression is inducible, and certain signals can alter the proportion of isoforms produced (13, 16). For example, estrogen selectively stimulates production of high molecular weight nuclear forms (16).

Four FGF tyrosine kinase receptors (FGF R) and their splice variants show differential binding of FGFs (1). FGF basic preferentially binds FGF R1c and 2c, for which it has picomolar affinity (1, 2). FGF basic also has a number of other binding partners that fine-tune FGF basic activities, according to their locations and quantities. These include heparin, integrin αvβ3, soluble FGF R1, FGF-binding protein, free gangliosides, thrombospondin, pentraxin 3, fibrinogen, α2-macroglobulin, platelet-derived growth factor, and platelet factor-4, all of which bind with nanomolar affinity (2). These molecules may act as co-receptors or adhesion partners on cells, decoys, or reservoirs in the extracellular matrix and scavengers or chaperones as free proteins (2). Binding of FGF basic to cell surface HSPG is particularly critical, and is required for binding, dimerization, and activation of FGF R (1, 2). Cell surface and matrix binding promotes paracrine and autocrine signaling, with very little soluble FGF basic released to the circulation under normal circumstances (2, 5, 17).

FGF basic modulates such normal processes as angiogenesis, wound healing, tissue repair, learning and memory, and embryonic development and differentiation of heart, bone, and brain (2-4, 10, 14-26). Secreted FGF basic is particularly important in angiogenesis, directly due to its enhancing effects on endothelial growth and migration, and indirectly due to cooperation with the angiogenic molecule, VEGF (2). FGF basic is upregulated in response to inflammation via mediators such as TNF-α, IL-1β, IL-2, PDGF, and nitric oxide (2). Many human tumors express FGF basic, and its expression may correlate with tumor vascularity (2, 5, 17). Disruption of the mouse FGF basic gene gives relatively mild cardiovascular, skeletal, and neuronal phenotypes, possibly due to compensation by other FGF family members (14, 20-26). Transgenic over-expression of FGF basic mainly influences development and mineralization of bone (4, 27, 28).

The Quantikine® Mouse/Rat FGF basic/FGF2/bFGF Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse and rat FGF basic in cell culture supernates, tissue lysates, serum, and plasma. It contains *E. coli*-expressed recombinant rat FGF basic and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural FGF basic 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 FGF basic.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat FGF basic has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any FGF basic present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse/rat FGF basic 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 FGF basic 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.
- For best results, pipette reagents and samples into the center of each well.
- 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 FGF basic Microplate	894654	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat FGF basic.	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 FGF basic Standard	894656	2 vials of recombinant rat FGF basic 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 FGF basic Control	894657	2 vials of recombinant rat FGF basic 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 FGF basic Conjugate	894655	12 mL of a monoclonal antibody specific for mouse/rat FGF basic 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 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
- Test tubes for dilution of standards and samples

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347)
- PBS

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.

Note: *Animal serum used in the preparation of cell culture media may contain high levels of FGF basic. For best results, do not use animal serum for growth of cell cultures when assaying for FGF basic production. If animal serum is used as a supplement in the media, precautions should be taken to prepare the appropriate control and run the control in the immunoassay to determine the baseline concentration of FGF basic.*

Tissue Lysates - Tissues must be lysed prior to assay as described in the Sample Values section.

Mouse 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.

Rat 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.

Mouse Plasma - Collect plasma using EDTA 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.

Rat Plasma - Collect plasma using EDTA 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: *Heparin plasma is not suitable for use in this assay.*
Citrate plasma has not been validated for use in this assay.

SAMPLE PREPARATION

Tissue lysate samples require a 20-fold dilution. A suggested 20-fold dilution is 15 µL of lysate + 285 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

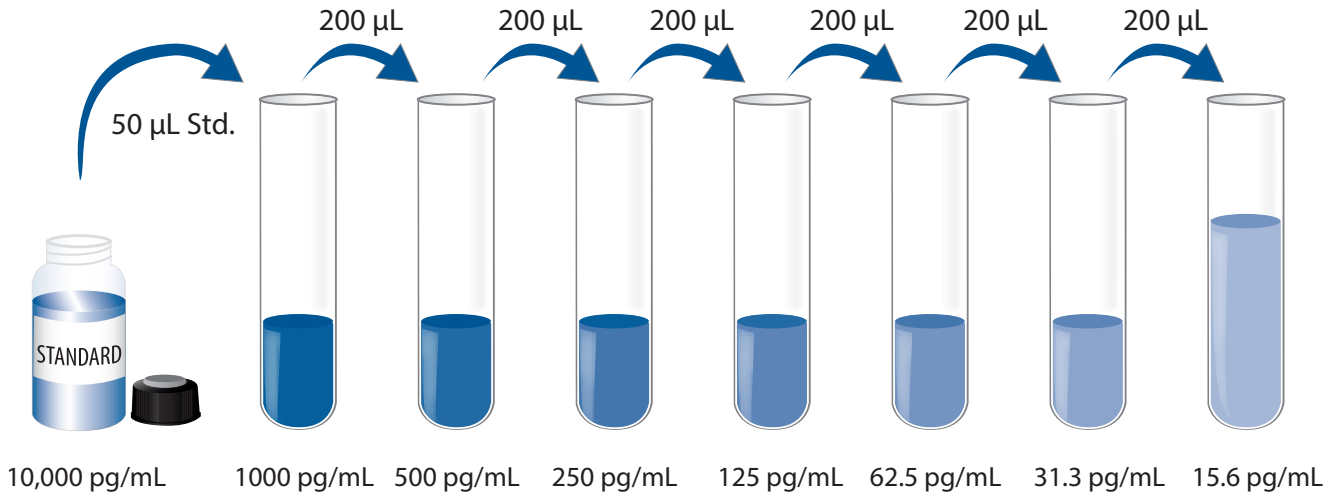
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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 FGF basic Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat FGF basic Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard 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 1000 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 1000 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, standards, control, and samples as directed by 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. 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 FGF basic 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 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

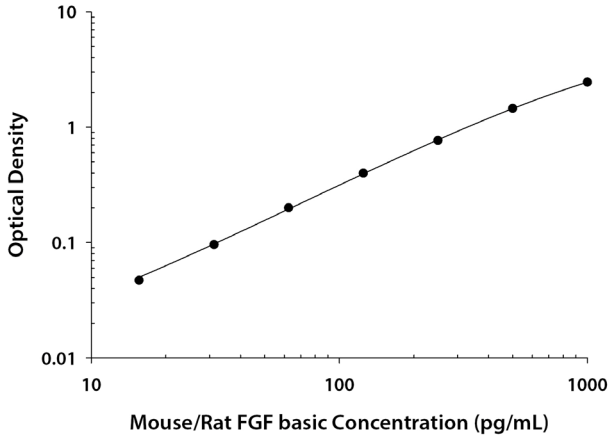
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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/rat FGF basic 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	0.010 0.011	0.011	—
15.6	0.057 0.059	0.058	0.047
31.3	0.107 0.107	0.107	0.096
62.5	0.210 0.212	0.211	0.200
125	0.410 0.410	0.410	0.399
250	0.773 0.780	0.777	0.766
500	1.456 1.472	1.464	1.453
1000	2.411 2.511	2.461	2.450

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 kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	49.5	122	394	45.0	104	382
Standard deviation	1.17	2.7	12.0	3.80	5.03	20.5
CV (%)	2.4	2.2	3.1	8.4	4.8	5.4

RECOVERY

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

Mouse Samples	Average % Recovery	Range
Cell culture samples (n=3)	101	91-110%
Tissue lysates* (n=4)	97	80-106%
Serum (n=4)	95	84-110%
EDTA plasma (n=4)	97	84-110%

*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 FGF basic were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

Mouse Samples		Cell culture supernates (n=4)	Tissue lysates* (n=4)	Serum (n=4)	EDTA plasma (n=4)
1:2	Average % of Expected	104	107	103	97
	Range (%)	97-111	105-110	97-106	90-107
1:4	Average % of Expected	103	106	107	99
	Range (%)	84-114	96-116	95-114	88-109
1:8	Average % of Expected	100	104	110	103
	Range (%)	93-107	92-111	99-116	88-115
1:16	Average % of Expected	102	96	106	106
	Range (%)	97-107	82-111	100-115	91-118

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

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

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of FGF basic ranged from 0.447-3.68 pg/mL. The mean MDD was 1.42 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 rat FGF basic (Accession # P13109 aa11-154) produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of FGF basic in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=11)	201	32.5-585	207
EDTA plasma (n=7)	45.3	23.4-113	31.2

Note: *Ten rat serum and five rat EDTA plasma samples were evaluated for the presence of FGF basic and no detectable levels were observed.*

Cell Culture Supernates - Tissues from individual mice or rats were removed and rinsed in PBS and kept on ice. The tissue was homogenized using a tissue homogenizer and seeded into media containing 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. Aliquots of the cell culture supernates were removed and assayed for levels of FGF basic.

Species	Brain (pg/mL)	Spleen (pg/mL)
Mouse	136	907
Rat	1556	877

Tissue Lysates - Organs from mice were rinsed with PBS, chopped into 1-2 mm pieces, and homogenized with a tissue homogenizer. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. An aliquot of each tissue lysate was removed and assayed for levels of natural FGF basic.

Tissue	(pg/mL)
Mouse brain	3440
Mouse spleen	18,620

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat FGF basic.

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

Recombinant mouse:

FGF-4
FGF-6
FGF-8b
FGF-8c
FGF-9
FGF-10
FGF-15
FGF-17
FGF-21
FGF-23
FGF acidic
FGF R2α (IIIb)
FGF R2α (IIIc)
FGF R2β (IIIb)
FGF R2β (IIIc)
FGF R3α (IIIb)
FGF R3α (IIIc)
FGF R4
FGF R5
GM-CSF
KGF/FGF-7
Klotho
Klotho β
Syndecan-2
TSG-14

Recombinant rat:

FGF-10
FGF-BP

Recombinant human:

FGF R1
FGF R1α (IIIb)
FGF R1α (IIIc)
FGF R1β (IIIb)
FGF R1β (IIIc)

Bovine FGF basic cross-reacts 100% in this assay.

Recombinant human FGF basic is detectable in this assay. For optimal measurement of human FGF basic, use the Quantikine® Human FGF basic/FGF2/bFGF ELISA Kit, Catalog # DFB50 or Quantikine® High Sensitivity FGF basic/FGF2/bFGF ELISA Kit, Catalog # HSFB00D.

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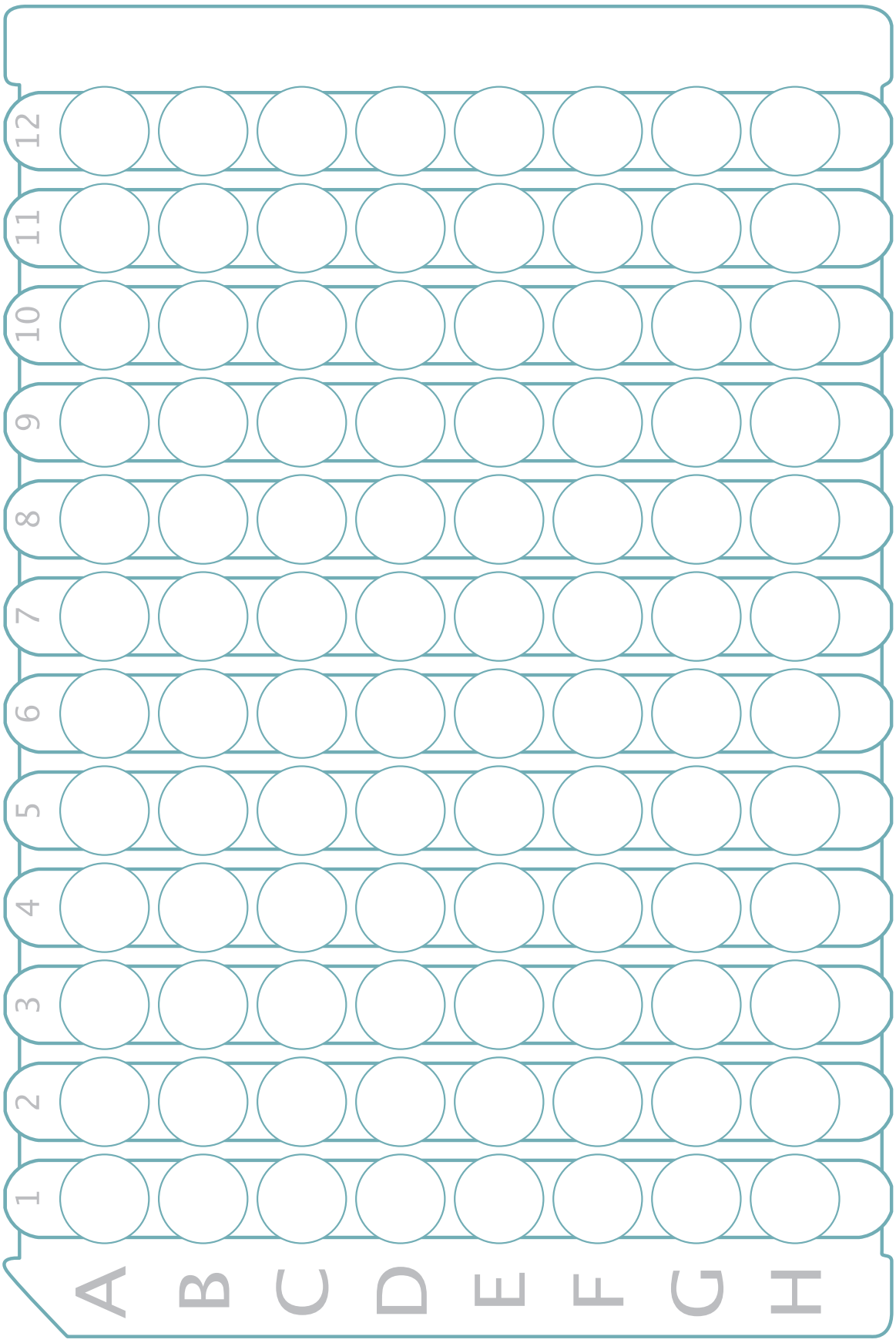
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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