Quantikine® HS ELISA

Human FGF basic Immunoassay

Catalog Number HSFB00D
SSFB00D
PHSFB00D

For the quantitative determination of human Fibroblast Growth Factor basic (FGF basic) concentrations in 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

FGF basic, also called FGF-2 (fibroblast growth factor-2) or previously, HBGF-2 (heparin-binding growth factor-2), is the most intensively studied of the mitogenic proteins of the FGF family (1-7). Family members share 35-60% amino acid (aa) sequence identity, but only FGF acidic and basic lack signal peptides and are secreted by an alternate pathway. The 18 kDa FGF basic isoform can be found in both the cytoplasm and the nucleus and is also the form that is secreted (8-10). Storage pools are likely within the cell or on the cell surface heparan sulfate proteoglycans (HSPG) (2). Transcription from alternate start sites produces 21-23 kDa forms found only in the nucleus (8, 9). High and low molecular weight human FGF basic isoforms target the expression of different genes (9, 10). The 18 kDa human FGF basic sequence shares 97% and 99% aa sequence identity with mouse/rat and bovine/ovine FGF basic, respectively (6, 7). Expression of FGF basic is nearly ubiquitous; however, disruption of the mouse FGF basic gene gives relatively mild cardiovascular, skeletal, and neuronal phenotypes, suggesting compensation by other FGF family members (11-15). Transgenic over-expression of FGF basic mainly influences development and mineralization of bone (4, 16, 17).

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). 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). It 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-2, which may correlate with tumor vascularity (2, 5).

The Quantikine® HS Human FGF basic Immunoassay kit is a 5.5 hour solid phase ELISA designed to measure human FGF basic levels in serum, plasma, and urine. It contains *E. coli*-expressed recombinant human FGF basic and antibodies raised against the recombinant factor. It has been shown to quantitate recombinant human FGF basic accurately. Results obtained using natural human FGF basic showed linear curves that were parallel to the standard curves obtained using the Quantikine® HS kit standards. These results indicate that this kit can be used to determine relative mass values for natural human FGF basic.

PRINCIPLE OF THE ASSAY

DUE TO THE HIGH SENSITIVITY OF THIS KIT, PLEASE NOTE THE FOLLOWING PRECAUTIONS.

- **Alkaline phosphatase is detectable in saliva.** Take precautionary measures (*e.g. wear a mask and gloves*) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).
- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize nonspecific binding of the conjugate.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human FGF basic has been pre-coated onto a microplate. Standards 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 human 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. After an incubation period, an amplifier solution is added to the wells and color develops in proportion to the amount of FGF basic 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.
- Although this kit has been designed to eliminate matrix problems, there may exist some samples that give falsely elevated values when assayed neat. If samples generate values that are suspiciously high or higher than the highest standard, dilute the samples in 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

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing 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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Neither the addition of the Substrate Solution nor the Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the plate in the same order as the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # HSFB00D	CATALOG # SSFB00D	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human FGF basic HS Microplate	893336	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human FGF basic.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human FGF basic HS Conjugate	893634	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human FGF basic, conjugated to alkaline phosphatase with preservatives.	Store for up to 1 month at 2-8 °C.*
Human FGF basic HS Standard	893337	1 vial	6 vials	Recombinant human FGF basic in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-97	895929	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD6-52	895438	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895188	1 vial	6 vials	100 mL/vial of a 10-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Substrate	895884	1 vial	6 vials	Lyophilized NADPH with stabilizers.	**Store in an upright position** for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Substrate Diluent	895885	1 vial	6 vials	7 mL/vial of a buffered solution with stabilizers.	
Amplifier	895886	1 vial	6 vials	Lyophilized amplifier enzymes with stabilizers.	
Amplifier Diluent	895887	1 vial	6 vials	7 mL/vial of a buffered solution containing INT-violet with stabilizer.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

HSFB00D contains sufficient materials to run an ELISA on one 96 well plate.
SSFB00D (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PHSFB00D). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm, preferably with dual wavelength correction set at 650 nm or 690 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated platewasher.
- 1000 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.
- Human FGF basic HS Controls (optional; R&D Systems®, Catalog # QC41).

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Alkaline phosphatase is detectable in saliva. Take precautionary measures (*e.g. wear a mask and gloves*) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).

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.

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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes 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 citrate 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: *Heparin plasma is not recommended for use in this assay.*
Grossly hemolyzed samples are not suitable 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. Assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Alkaline phosphatase is detectable in saliva.* *Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).*

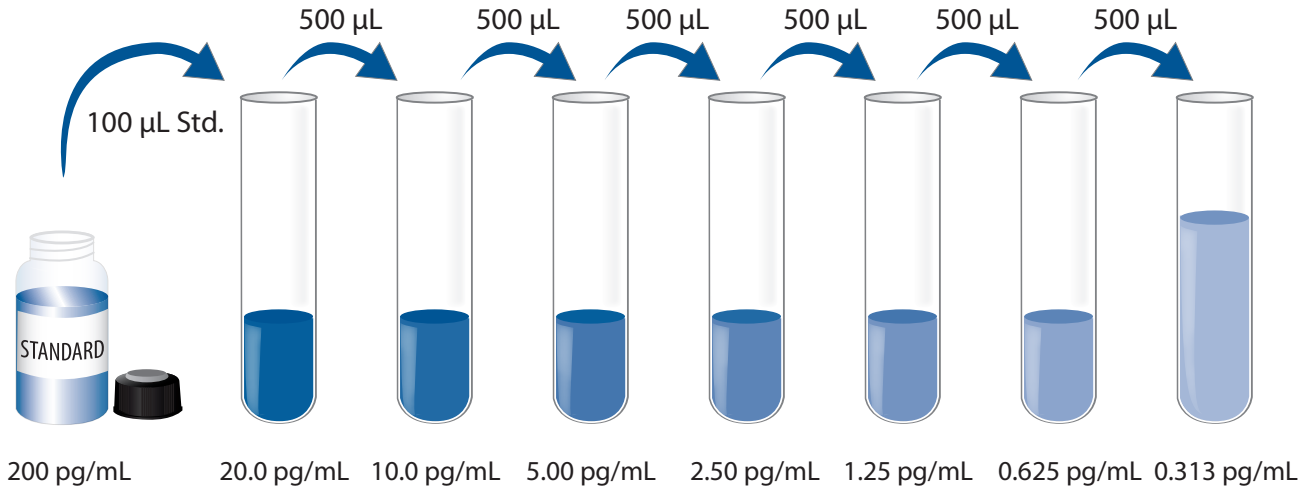
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to 900 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Reconstitute the lyophilized Substrate in 6.0 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier in 6.0 mL of Amplifier Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Human FGF basic HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the FGF basic HS Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polyproylene tubes. Pipette 900 µL of Calibrator Diluent RD6-52 into the 20.0 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 20.0 pg/mL standard serves as the high standard. Calibrator Diluent RD6-52 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, samples, and controls be assayed in duplicate.

Note: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

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 100 µL Assay Diluent RD1-97 to each well.
4. Add 100 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 as a record of standards and samples assayed.
5. Wash
   a. Remove liquid from the wells by aspirating or inverting the plate and decanting the contents.
   b. Remove excess liquid by grasping the plate firmly and smartly rapping the inverted plate on a clean paper towel at least 5 times.
   c. Fill each well with 400 µL of Wash Buffer using a squirt bottle, manifold dispenser, or autowasher.
   d. Remove liquid from the wells by aspirating or inverting the plate and decanting the contents.
   e. Repeat steps b, c, and d 5 times for a total of 6 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.
6. Add 200 µL of Human FGF basic HS Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 50 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker. **Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature on the shaker.
   Note: *Addition of Amplifier Solution initiates color development.*
10. Add 50 µL of Stop Solution to each well. Addition of Stop Solution does not affect color in the wells.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

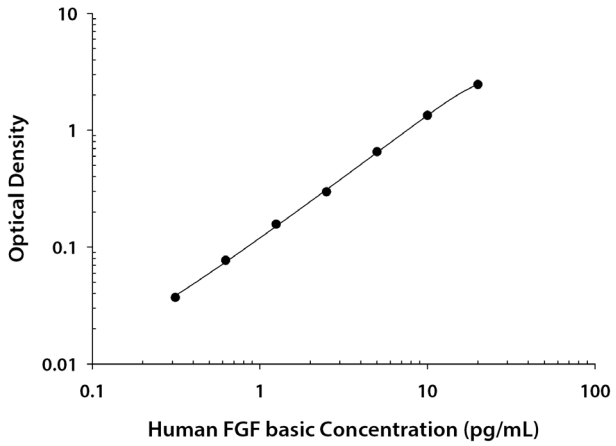
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human 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	0.033 0.035	0.034	—
0.313	0.069 0.073	0.071	0.037
0.625	0.107 0.114	0.111	0.077
1.25	0.188 0.193	0.191	0.157
2.50	0.318 0.345	0.331	0.297
5.00	0.684 0.689	0.687	0.653
10.0	1.367 1.378	1.373	1.339
20.0	2.473 2.518	2.495	2.461

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 forty 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	40	40	40
Mean (pg/mL)	1.31	5.32	11.4	1.51	5.71	11.1
Standard deviation	0.10	0.23	0.40	0.12	0.27	0.91
CV (%)	7.6	4.3	3.5	7.9	4.7	8.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum (n=4)	100	88-116%
EDTA Plasma (n=4)	99	85-109%
Citrate Plasma (n=4)	97	83-118%
Urine (n=4)	109	100-120%

LINEARITY

To assess linearity of the assay, the following samples were spiked with high concentrations of human FGF basic, diluted with calibrator diluent, and assayed.

		Serum (n=4)	EDTA plasma (n=4)	Citrate plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	106	105	113	99
	Range (%)	100-110	98-112	112-115	93-104
1:4	Average % of Expected	105	107	112	97
	Range (%)	100-114	103-111	107-115	94-99
1:8	Average % of Expected	98	108	112	88
	Range (%)	83-116	102-115	104-117	86-93
1:16	Average % of Expected	103	111	107	88
	Range (%)	92-112	104-117	99-115	84-92

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human FGF basic ranged from 0.01-0.07 pg/mL. The mean MDD was 0.03 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 human FGF basic produced at R&D Systems®.

SAMPLE VALUES

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

Sample	Mean of Detectable (pg/mL)	Detectable	Range (pg/mL)
Serum (n=30)	0.776	75%	ND-2.42
EDTA plasma (n=30)	5.05	97%	ND-16.5
Citrate plasma (n=15)	3.78	93%	ND-9.12
Urine (n=15)	2.75	40%	ND-6.80

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human FGF basic.

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

Recombinant human:

β-ECGF
FGF-3
FGF-4
FGF-5
FGF-6
FGF-8a
FGF-8e
FGF-8f
FGF-9
FGF-10
FGF-12
FGF-16
FGF-17
FGF-18
FGF-19
FGF-20
FGF-21
FGF-22
FGF-23
FGF acidic
FGF-BP
FGF R1α (IIIb)
FGF R1α (IIIc)
FGF R1β (IIIb)
FGF R1β (IIIc)
FGF R2α (IIIb)
FGF R2α (IIIc)
FGF R2β (IIIb)
FGF R2β (IIIb)
FGF R2β (IIIc)
FGF R3 (IIIb)
FGF R3 (IIIc)
FGF R4
KGF/FGF-7
Klotho
Klotho β
Pentraxin 3/TSG-14

Recombinant mouse:

FGF-4
FGF-6
FGF-8b
FGF-8c
FGF-9
FGF-10
FGF-23
FGF acidic
FGF R2β (IIIb)
FGF R2β (IIIc)
FGF R3 (IIIc)
KGF/FGF-7
Klotho
Klotho β
Pentraxin 3/TSG-14

Recombinant rat:

FGF-BP

Cross-reactivity was observed with the following:

Factor	% Cross-reactivity
Natural bovine FGF basic	26
Recombinant bovine FGF basic	38
Recombinant mouse FGF basic	30
Recombinant rat FGF basic	79

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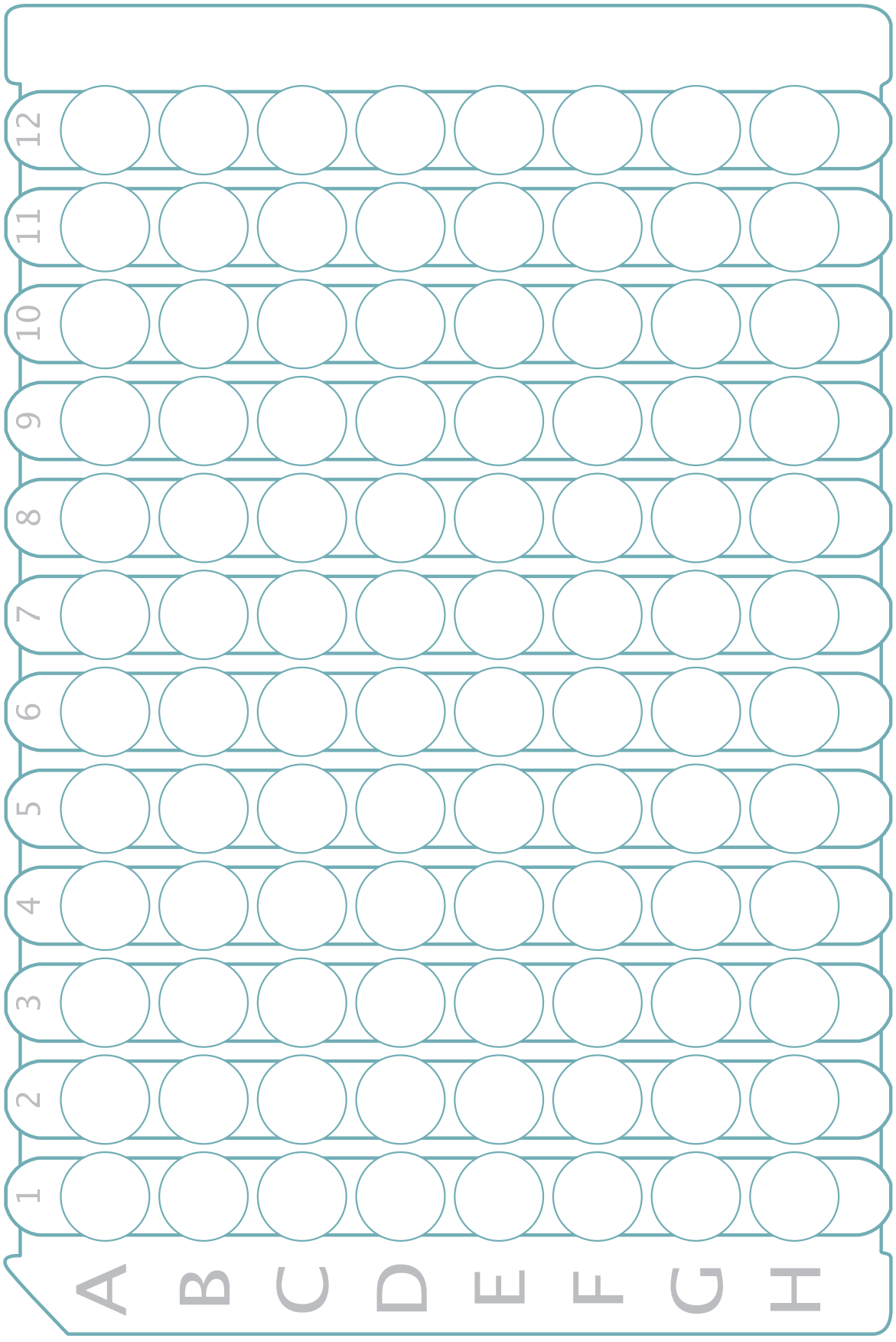
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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