Quantikine® ELISA

Human FGF acidic Immunoassay

Catalog Number DFA00B

For the quantitative determination of human Fibroblast Growth Factor acidic (FGF acidic) 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

Fibroblast Growth Factor acidic (FGF acidic), also known as FGF-1, endothelial cell growth factor (ECGF), and heparin-binding growth factor-1 (HBGF-1), is a 17-18 kDa non-glycosylated polypeptide that is secreted by a variety of cell types (1-4). The molecule is synthesized as a 155 amino acid (aa) protein that has no distinctive signal peptide sequence. As such, this precludes its secretion via classical ER/Golgi pathways. It appears, however, that there is a unique secretory pathway that involves heat shock proteins, phosphatidyl serine, synaptotagmin-1 and annexin (5, 6). The outcome of FGF acidic secretion is an extracellular disulfide-linked homodimer whose covalent bonds can be broken by reducing agents to release potentially bioactive FGF acidic. Unlike FGF-2, no 5' alternate start sites are known to exist for FGF acidic. A 60 aa splice variant of FGF acidic has been reported that consists of the first 57 amino acids of the full-length molecule plus three additional C-terminal amino acids. Although its function is unknown, experimentally it can bind FGF receptors but will not activate them (7). In full-length form, FGF acidic has a nuclear localization sequence comprising aa residues 21-27 (8). Human FGF acidic exhibits 95% aa identity to mouse and rat FGF acidic, and 92% aa identity to bovine FGF acidic (9-11). Cells known to express FGF acidic include breast epithelium (12), neurons (motor and sensory) (13), skeletal and smooth muscle cells (14, 15), renal proximal tubule cells (16), endothelial cells (17), macrophages (17), keratinocytes (18) and fibroblasts (18).

There are five receptors for the FGF family (FGF R1-R5) (19, 20). The first four receptors are all Ig-superfamily (IgSF) type I transmembrane tyrosine kinase receptors, while FGF R5 is an IgSF member without a tyrosine kinase domain. Notably, FGF acidic is reported to bind to FGF receptors 1 through 4 but not to FGF R5 (19). FGF acidic can activate a tyrosine kinase-mediated signaling pathway upon binding and/or utilize a ligand-receptor internalization event to reach intracellular organelles and the nucleus (21, 22). Receptor activation is associated with a concurrent FGF-heparin interaction. Heparin binding to FGF acidic can be either receptor inhibiting or activating. When activating, heparin seems to serve as a centerpiece linking two FGF/FGF R complexes. This approximation of two FGF R molecules initiates receptor dimerization and signal transduction (20, 23-25). FGF acidic will also bind cell-surface heparin alone, with internalization but no cell activation (26).

FGF acidic is best known for its mitogenic activity on endothelial cells (27, 28). It is suggested that both membrane receptor activation and internalization is necessary for a full mitogenic response (22). Other cells shown to proliferate in response to FGF acidic include smooth muscle cells (28), hepatocytes (29), mammary epithelium (30) and fibroblasts (31). FGF acidic also plays a number of roles in development and regeneration. It is proposed to inhibit neuronal differentiation via the Notch-Delta pathway (32), induce the differentiation of prolactin-secreting cells in the anterior pituitary (33) and promote the regeneration of peripheral nerve axons following injury (34). In addition, FGF acidic serves as a costimulatory molecule during select $CD4^+$ T cell proliferation, prompting an increase in IL-2 secretion (35).

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human FGF acidic has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FGF acidic present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human FGF acidic 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 FGF acidic 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.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate 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.
- 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 FGF acidic Microplate	890659	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human FGF acidic.	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 FGF acidic Conjugate	892520	21 mL of a polyclonal antibody specific for human FGF acidic conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human FGF acidic Standard	892521	Recombinant human FGF acidic in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-77	895545	17 mL of a buffered solution with blue dye and preservatives. *May contain crystals. Warm to room temperature to dissolve.*	
Calibrator Diluent RD5-5	895485	21 mL of a buffered protein base with preservatives. *For cell culture supernate/ urine samples.*	
Calibrator Diluent RD6X	895152	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
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/vial 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.
- **Polypropylene** test tubes for dilution of standards.
- Human FGF acidic Controls (optional; R&D Systems®, Catalog # QC22).

PRECAUTIONS

Calibrator Diluent RD6X contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 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.

Note: *Do not assay supernates from cell culture media supplemented with animal brain extracts.*

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.*
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, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

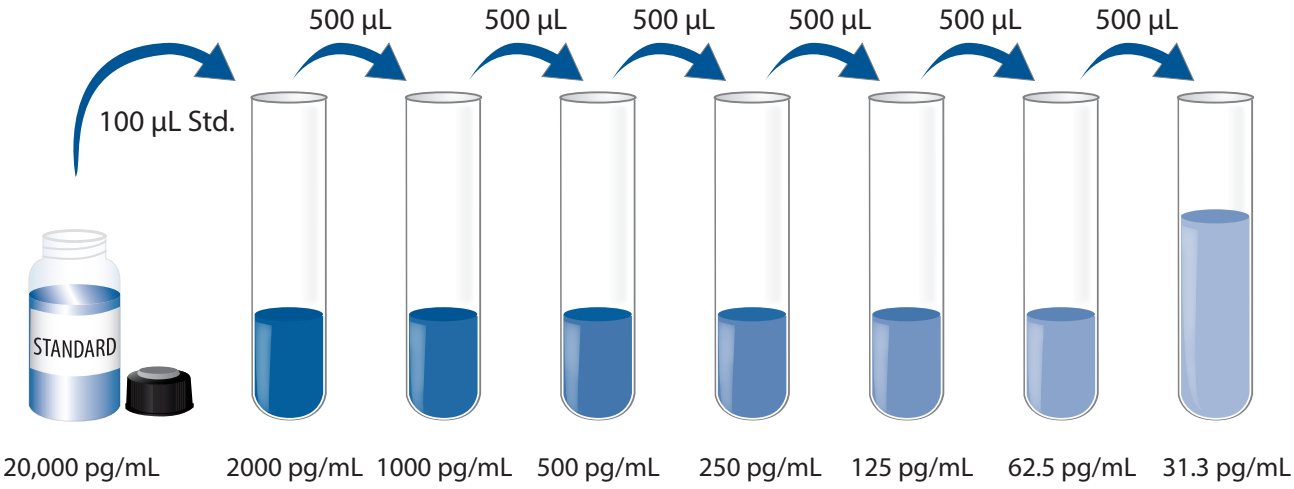
Bring all reagents to room temperature before use.

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 FGF acidic Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FGF acidic Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-5 (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6X (*for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate calibrator diluent 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.

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 150 µL of Assay Diluent RD1-77 to each well. *Assay Diluent RD1-77 may contain crystals, mix gently until the crystals have completely dissolved.*
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 200 µL of Human FGF acidic 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 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 if 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.

CALCULATION OF RESULTS

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

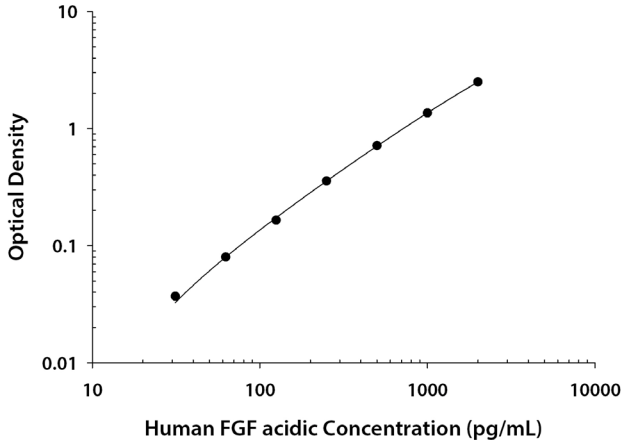
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human FGF acidic 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

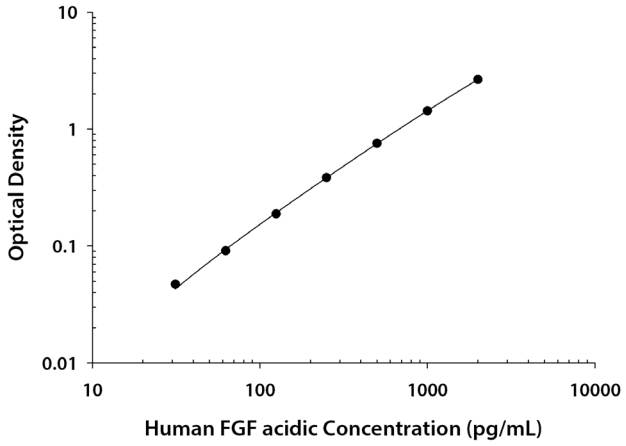
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.047 0.049	0.048	—
31.3	0.084 0.085	0.085	0.037
62.5	0.127 0.128	0.128	0.080
125	0.209 0.217	0.213	0.165
250	0.398 0.412	0.405	0.357
500	0.746 0.780	0.763	0.715
1000	1.396 1.415	1.406	1.358
2000	2.506 2.585	2.546	2.498

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.059 0.059	0.059	—
31.3	0.101 0.110	0.106	0.047
62.5	0.144 0.156	0.150	0.091
125	0.238 0.256	0.247	0.188
250	0.427 0.459	0.443	0.384
500	0.789 0.836	0.813	0.754
1000	1.449 1.528	1.489	1.430
2000	2.637 2.774	2.706	2.647

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.

CELL CULTURE SUPERNATE/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	208	485	766	204	477	761
Standard deviation	7.6	21.8	22.9	15.0	29.3	48.7
CV (%)	3.7	4.5	3.0	7.4	6.1	6.4

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	179	441	749	178	433	713
Standard deviation	8.3	31.8	17.6	15.0	36.3	61.0
CV (%)	4.6	7.2	2.3	8.4	8.4	8.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	105	93-113%
Serum (n=4)	98	89-108%
EDTA plasma (n=4)	102	93-109%
Heparin plasma (n=4)	97	92-110%
Urine (n=4)	99	89-108%

SENSITIVITY

Sixty-four assays were evaluated and the minimum detectable dose (MDD) of human FGF acidic ranged from 1.19-13.9 pg/mL. The mean MDD was 5.68 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.

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human FGF acidic were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	102	102	100	97	98
	Range (%)	98-106	98-107	98-102	94-101	86-103
1:4	Average % of Expected	109	98	93	91	100
	Range (%)	104-112	90-107	88-99	87-96	96-108
1:8	Average % of Expected	108	99	96	94	104
	Range (%)	103-112	93-112	86-109	88-97	99-114
1:16	Average % of Expected	107	103	101	98	103
	Range (%)	102-111	98-112	97-104	92-102	96-111

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human FGF acidic produced at R&D Systems®.

SAMPLE VALUES

Serum - Sixty-one serum samples from apparently healthy volunteers were evaluated for the presence of human FGF acidic in this assay. No medical histories were available for the donors used in this study. Two samples measured 54 pg/mL and 109 pg/mL. The remainder measured less than the lowest standard, 31.3 pg/mL.

Plasma - Thirty-six EDTA and thirty-six heparin plasma samples from apparently healthy volunteers were evaluated for the presence of human FGF acidic in this assay. No medical histories were available for the donors used in this study. One EDTA plasma sample measured 117 pg/mL. One heparin plasma sample measured 117 pg/mL. The remainder measured less than the lowest standard, 31.3 pg/mL.

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

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=28)	56.7	46	ND-96

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the supernate were removed on days 1 and 5 and assayed for human FGF acidic. All samples measured less than the lowest standard, 31.3 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human FGF acidic.

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 FGF acidic standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
FGF basic
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-17
FGF-18
FGF R1α (IIIb)
FGF R1α (IIIc)
FGF R1β (IIIb)
FGF R1β (IIIc)
FGF R2α (IIIb)
FGF R2α (IIIc)
FGF R2β (IIIb)
FGF R2β (IIIc)
FGF R3α (IIIc)
FGF R4
Flt-3 Ligand
Flt-4
G-CSF
GM-CSF
HB-EGF
HGF
HRG-α
IGF-I
IGF-II
KGF/FGF-7
M-CSF
MSP
MSP β chain
β-NGF
PDGF-AA
PDGF-AB
PDGF-BB
PlGF
$VEGF_{121}$
$VEGF_{165}$
VEGF/PlGF
VEGF-D

Recombinant mouse:

FGF-8b
FGF-8c
FGF R2β (IIIb)
FGF R2β (IIIc)
FGF R3α (IIIb)
Flt-3 Ligand
G-CSF
GM-CSF
M-CSF
PlGF-2
$VEGF_{120}$
$VEGF_{164}$

Recombinant rat:

GM-CSF
β-NGF
PDGF-BB

Recombinant porcine:

GM-CSF

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF

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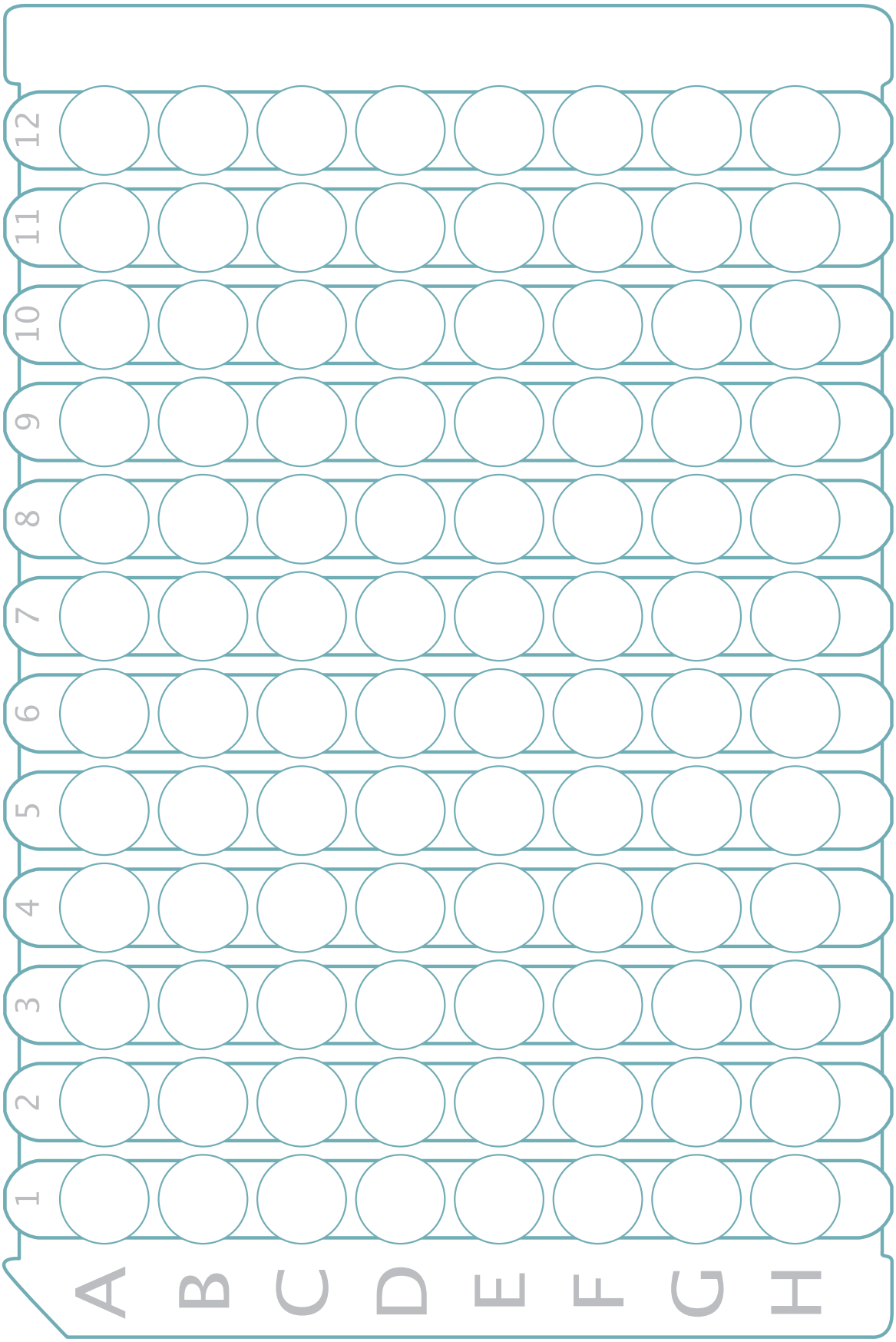
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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