Quantikine® ELISA

Mouse/Rat FGF-21 Immunoassay

Catalog Number MF2100

For the quantitative determination of mouse or rat Fibroblast Growth Factor 21 (FGF-21) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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 21 (FGF-21) is a member of the FGF gene family, which contains 22 mammalian members. Based on its structure, it is further classified as a member of the FGF-19 subfamily, which also includes FGF-19 and FGF-23 (1-4). FGF family members contain a 120 amino acid (aa) core FGF domain that exhibits a β-trefoil structure. FGF-19 subfamily members, unlike other FGFs, lack one strand of the β-trefoil and bind poorly to extracellular matrix molecules such as heparin (3). They are consequently more diffusible than other FGFs and are considered endocrine rather than paracrine (1-4). All three subfamily members impact some aspect of metabolism, are induced by a nuclear receptor heterodimer that includes RXR (retinoid X receptor), and bind FGF receptors (FGF R) indirectly through co-receptors of the klotho family (5-9). FGF-21 binds to Klotho β via its C-terminal sequence. This binding, along with amino acids at the N-terminus, is required for signaling through FGF R (7, 8). FGF-21 is selective for FGF R1 isoform 1c, with varying reports of using isoforms 2c or 3c (10-12). Presence of the required klotho and FGF R family members determines tissue specificity of FGF-19 subfamily members, and thus concentrates FGF-21 activity within adipose tissue (3, 9-11).

FGF-21 is produced by hepatocytes in response to free fatty acid (FFA) stimulation of a PPARα/RXR dimeric complex (4, 13-15). This situation occurs during starvation, diabetic ketosis, or following the ingestion of a high-fat/low-carbohydrate or ketogenic diet (5, 14-16). Upon FGF-21 secretion, white adipose tissue is induced to release FFAs from triglyceride stores. Once FFAs reach the hepatocytes, they are oxidized and reduced to acetyl-CoA (16). The acetyl-CoA is recombined into 4-carbon ketone bodies (acetoacetate and β-hydroxybutyrate), released, and transported to peripheral tissues for energy generation (5, 15, 16).

FGF-21 production is also induced upon differentiation of human or mouse fibroblasts to adipocytes (17, 18). In adipose tissue, it induces glucose uptake by signaling in synergy with PPARγ to increase production of the glucose transporter, GLUT1 (10, 12, 19). FGF-21 production follows a circadian pattern in mice (20). It diffuses across the blood-brain barrier, and in mice, this may facilitate induction of a state of torpor, or decreased activity, in response to increased FGF-21 (16, 21). These characteristics appear to induce a hibernation-like state during fasting and short days in the winter season (22). In diet-induced obese mice and mouse models of diabetes such as db/db and ob/ob, administration or transgenic over-expression of FGF-21 restores circulating glucose and triglyceride values to near normal and increases insulin sensitivity (5, 6, 14, 23, 24). In some of these states and in human obesity and type II diabetes, FGF-21 is already elevated prior to treatment, suggesting that resistance to FGF-21 is possible (17, 25, 26). Although FGF-21 administration corrects obesity in mice, it is unclear whether the same benefit would be seen in humans (2, 3, 17, 26-28).

The Quantikine Mouse/Rat FGF-21 immunoassay is a 4.5 hour solid-phase ELISA designed to measure FGF-21 in mouse or rat cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse FGF-21 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse FGF-21. Results obtained using natural mouse or rat FGF-21 showed dose response curves that were parallel to the standard curves obtained using the Quantikine mouse kit standards. These results indicate that this kit can be used to determine relative mass values for natural FGF-21.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat FGF-21 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any FGF-21 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat FGF-21 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-21 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, further dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard 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 FGF-21 Microplate	893722	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat FGF-21.	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-21 Standard	893724	2 vials of recombinant mouse FGF-21 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard any unused reconstituted Standard and Control after use. Use a new Standard and Control for each assay.
Mouse/Rat FGF-21 Control	893725	2 vials of recombinant mouse FGF-21 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-21 Conjugate	893723	12 mL of a polyclonal antibody specific for mouse/rat FGF-21 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-41	895514	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5Y	895201	21 mL of a buffered protein solution with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6Z	895466	21 mL of diluted 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	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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.

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. Please 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. 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 has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

Mouse and rat serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD6Z.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

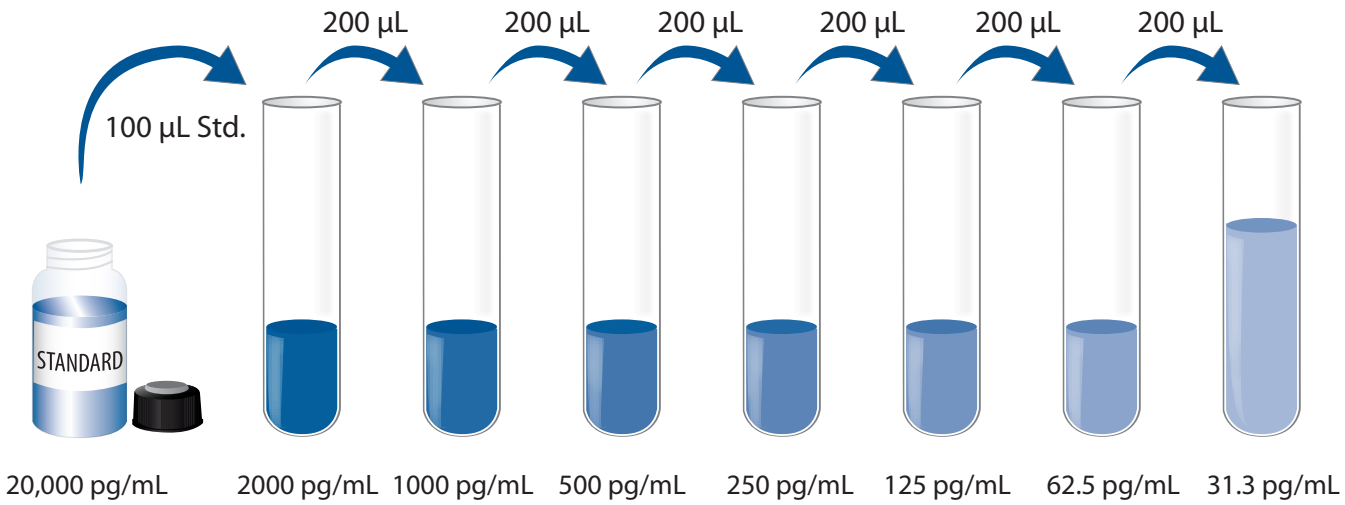
Mouse/Rat FGF-21 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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.

Mouse/Rat FGF-21 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat FGF-21 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5Y (*for cell culture supernate samples*) or Calibrator Diluent RD6Z (*for serum/plasma samples*) into the 2000 pg/mL tube and 200 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but 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, 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 RD1-41 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Gently tap the plate to ensure thorough mixing. 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 four times for a total of five 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-21 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. **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.).

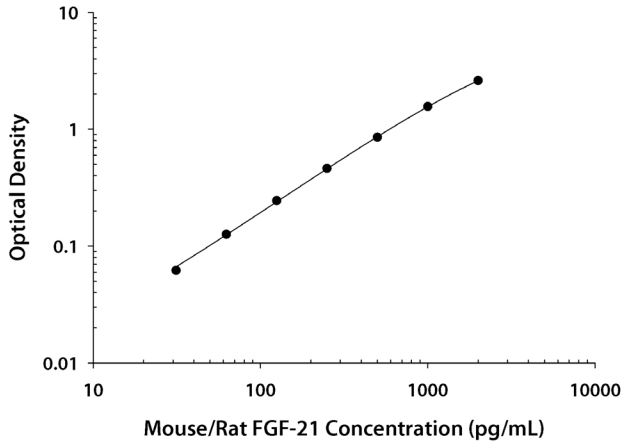
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat FGF-21 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

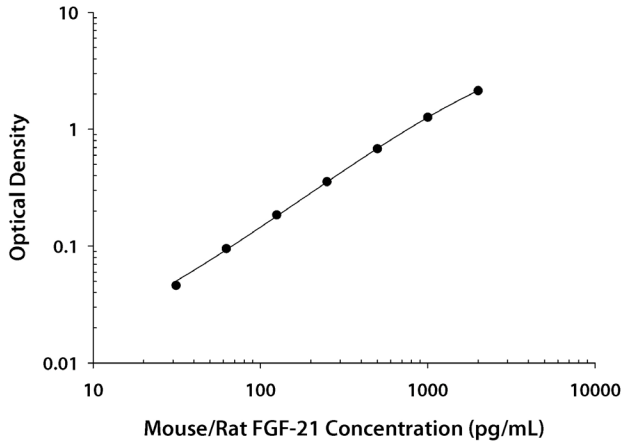
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.046 0.046	0.046	—
31.3	0.106 0.110	0.108	0.062
62.5	0.171 0.172	0.172	0.126
125	0.291 0.291	0.291	0.245
250	0.499 0.517	0.508	0.462
500	0.899 0.901	0.900	0.854
1000	1.592 1.623	1.608	1.562
2000	2.644 2.655	2.650	2.604

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.041 0.041	0.041	—
31.3	0.086 0.087	0.087	0.046
62.5	0.136 0.136	0.136	0.095
125	0.226 0.226	0.226	0.185
250	0.395 0.397	0.396	0.355
500	0.711 0.729	0.720	0.679
1000	1.287 1.326	1.307	1.266
2000	2.170 2.184	2.177	2.136

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 ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	84.4	226	754	94.5	231	745
Standard deviation	5.24	9.60	19.7	6.61	10.8	31.0
CV (%)	6.2	4.2	2.6	7.0	4.7	4.2

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)	118	317	1032	120	302	977
Standard deviation	7.35	13.5	25.2	8.91	18.0	65.9
CV (%)	6.2	4.3	2.4	7.4	6.0	6.7

RECOVERY

The recovery of mouse/rat FGF-21 spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture samples (n=6)	96	88-101%
Serum* (n=4)	95	83-103%
EDTA plasma* (n=4)	97	86-104%
Heparin plasma* (n=4)	93	83-101%

Rat Samples	Average % Recovery	Range
Cell culture samples (n=2)	91	87-95%
Serum* (n=4)	100	93-108%
EDTA plasma* (n=4)	101	93-119%
Heparin plasma* (n=4)	97	94-104%

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

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse/rat FGF-21 in each matrix were diluted with the appropriate Calibrator Diluent and assayed.

Mouse		Cell culture supernates (n=6)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	101	100	102
	Range (%)	96-103	99-103	97-101	99-104
1:4	Average % of Expected	104	108	104	107
	Range (%)	97-106	103-114	101-105	99-111
1:8	Average % of Expected	106	109	105	111
	Range (%)	99-109	104-114	105-106	103-115
1:16	Average % of Expected	106	107	103	110
	Range (%)	98-113	104-112	99-105	101-118

Rat		Cell culture supernates (n=6)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	103	102	102	103
	Range (%)	102-104	100-103	99-108	101-106
1:4	Average % of Expected	110	106	104	105
	Range (%)	109-112	102-108	100-108	102-109
1:8	Average % of Expected	115	108	106	110
	Range (%)	112-118	106-110	103-109	105-117
1:16	Average % of Expected	114	107	105	109
	Range (%)	113-115	101-113	102-109	102-119

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

SENSITIVITY

One hundred sixty-two assays were evaluated and the minimum detectable dose (MDD) of mouse/rat FGF-21 ranged from 0.93-13.4 pg/mL. The mean MDD was 3.81 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density 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 FGF-21 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Mouse and rat samples were evaluated for the presence of FGF-21 in this assay.

	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum (n=20)	887	115-3212	874
Mouse heparin plasma (n=20)	877	283-2280	622
Mouse EDTA plasma (n=20)	878	219-1650	385
Rat EDTA plasma (n=20)	741	88-1876	522

	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Rat serum (n=20)	402	90	ND-2222
Rat heparin plasma (n=20)	970	95	ND-3428

ND=Non-detectable

Cell Culture Supernates - L-929 mouse fibroblast cells (0.5 x 10^5 cells/mL) were cultured in MEM (NEAA) supplemented with 10% equine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were either unstimulated or stimulated for 3 days with 1 µg/mL LPS or 10 µg/mL PHA and 10 ng/mL PMA. Aliquots of the cell culture supernates were assayed for levels of mouse/rat FGF-21.

Condition	Observed Levels (pg/mL)
Unstimulated	387
LPS	300
PHA and PMA	185

SPECIFICITY

This assay recognizes natural and recombinant FGF-21.

The factors listed below were prepared at 50 ng/mL in the appropriate Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat FGF-21 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

FGF-6
FGF-7
FGF-8b
FGF-8c
FGF-10
FGF-15
FGF-23
FGF acidic
FGF basic
FGF-R2α (IIIb)
FGF-R2α (IIIc)
FGF-R2β (IIIb)
FGF-R2β (IIIc)
FGF-R3 (IIIb)
FGF-R3 (IIIc)
FGF-R4
FGF-R5
FGF-R5β

Recombinant human:

FGF-19
FGF-23
FGF-R1
FGF-R1α (IIIb)
FGF-R1α (IIIc)
FGF-R1β (IIIb)
FGF-R1β (IIIc)

Recombinant mouse Klotho β shows 0.3% cross-reactivity in this assay.

Recombinant human FGF-21 shows 1.4% cross-reactivity in this assay.

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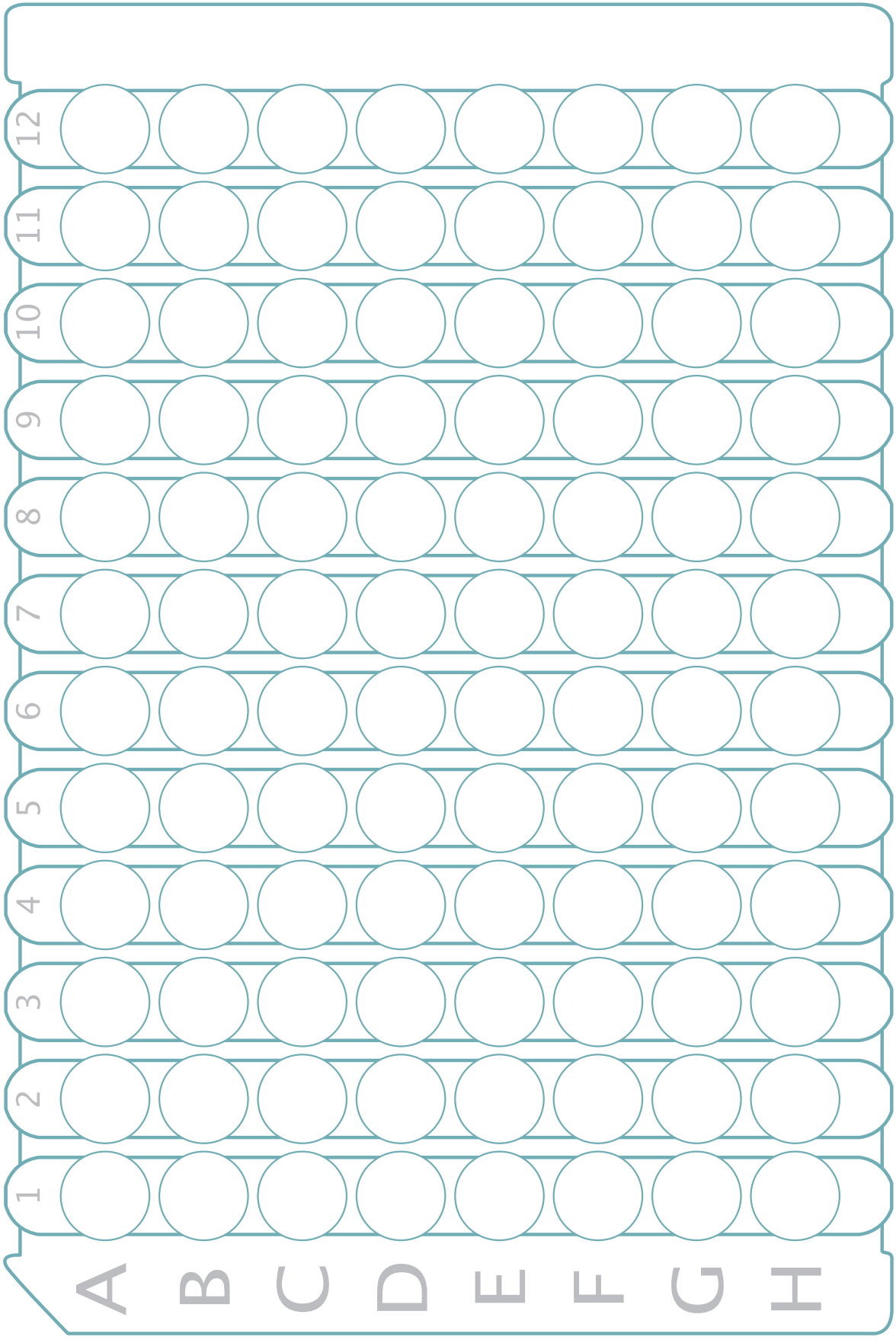
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES