Quantikine® ELISA

Human FGF-21 Immunoassay

Catalog Number DF2100

For the quantitative determination of human Fibroblast Growth Factor 21 (FGF-21) concentrations in cell culture supernates, 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.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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; all three are induced by a nuclear receptor heterodimer that includes RXR (retinoid X receptor), and all three bind FGF receptors (FGF R) indirectly through co-receptors of the klotho family (5-9). FGF-21 binds to β-Klotho 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). Mature human FGF-21 shares 81% aa sequence identity with mouse and rat FGF-21.

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, FGF-21 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 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 winter (22). In diet-induced obese mice and mouse models of diabetes such as db/db and ob/ob, administration or transgenic overexpression 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® Human FGF-21 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human FGF-21 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human FGF-21 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human FGF-21 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-21.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human FGF-21 has been pre-coated onto a microplate. Standards 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 human 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 and color develops in proportion to the amount of FGF-21 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.
- If samples generate values higher than the highest standard, dilute the samples with 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.
- 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-21 Microplate	893425	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human FGF-21 Conjugate	893426	21 mL of a polyclonal antibody specific for human FGF-21 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human FGF-21 Standard	893427	Recombinant human FGF-21 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1S	895137	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-10	895468	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:2 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	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.
- **Polypropylene** test tubes for dilution of standards.
- Human FGF-21 Controls (optional; R&D Systems®, Catalog # QC24).

PRECAUTIONS

Calibrator Diluent RD6-10 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.

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 heparin or EDTA 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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

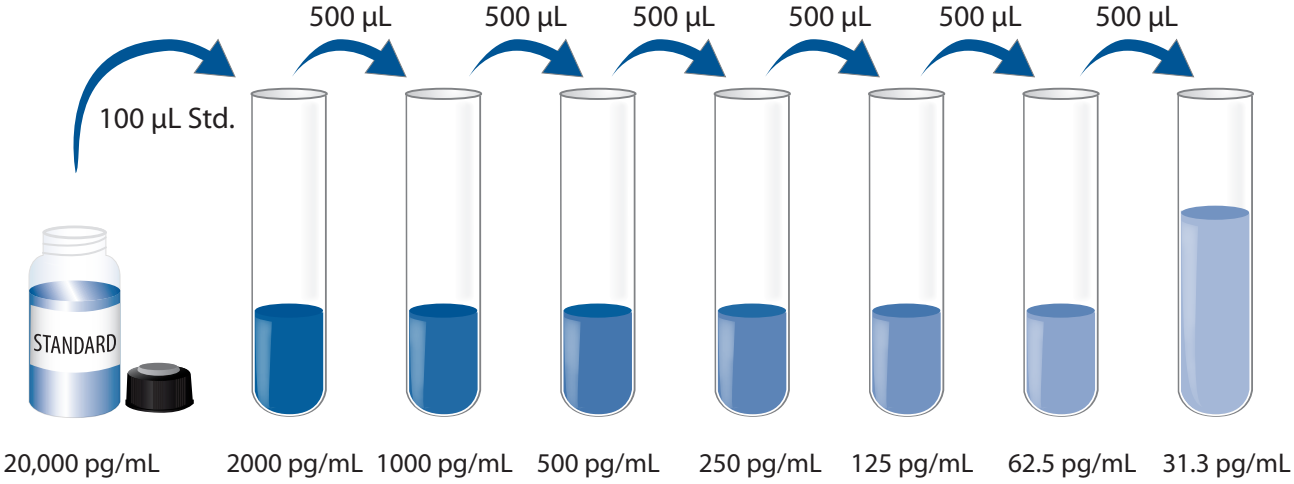
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6-10 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD6-10 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD6-10 (diluted 1:2).

Human FGF-21 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FGF-21 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 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6-10 (diluted 1:2) into the 2000 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 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD6-10 (diluted 1:2) 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 100 µL of Assay Diluent RD1S 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 200 µL of Human 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **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 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

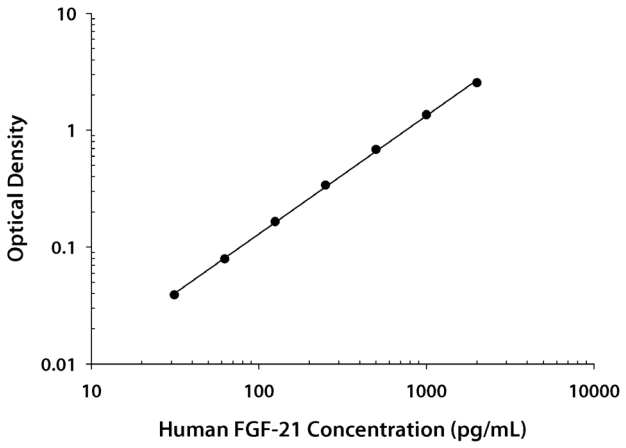
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the human FGF-21 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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	0.034 0.034	0.034	—
31.3	0.072 0.074	0.073	0.039
62.5	0.111 0.114	0.113	0.079
125	0.198 0.200	0.199	0.165
250	0.361 0.384	0.373	0.339
500	0.708 0.728	0.718	0.684
1000	1.368 1.409	1.389	1.355
2000	2.549 2.594	2.572	2.538

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)	204	672	1324	245	742	1411
Standard deviation	8.0	19.8	46.2	26.6	47.2	73.9
CV (%)	3.9	2.9	3.5	10.9	6.4	5.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	93-108%
Serum (n=4)	105	87-119%
Heparin plasma (n=4)	98	83-111%
EDTA plasma (n=4)	103	92-112%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human FGF-21 were serially diluted with the 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)
1:2	Average % of Expected	105	101	101	102
	Range (%)	103-107	96-108	99-103	97-105
1:4	Average % of Expected	105	98	102	104
	Range (%)	102-107	96-103	98-104	99-110
1:8	Average % of Expected	101	98	100	101
	Range (%)	99-103	94-102	98-101	97-104
1:16	Average % of Expected	95	90	93	101
	Range (%)	91-104	86-94	90-97	99-105

SENSITIVITY

Fifty-five assays were evaluated and the minimum detectable dose (MDD) of human FGF-21 ranged from 1.61-8.69 pg/mL. The mean MDD was 4.67 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-21 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human FGF-21 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)
Serum (n=35)	172	97	ND-914
EDTA plasma (n=35)	201	97	ND-1155
Heparin plasma (n=35)	186	97	ND-1012

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood leukocytes (PBLs) were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for human FGF-21. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human FGF-21.

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-21 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
EGF (pro)
EGF R
EG-VEGF
FGF-3
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-12
FGF-16
FGF-17
FGF-18
FGF-19
FGF-20
FGF-22
FGF-23
FGF acidic
FGF basic
FGF R1α
FGF R2α
FGF R2β
FGF R3
FGF R4
Flt-3
Flt-3/Flk-2 Ligand
Flt-4
G-CSF
G-CSF R
GM-CSF
HB-EGF
HGF
HGF Activator
HGF R
HRG-α
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
IGF-I
IGF-I R
IGF-II
IGF-II R
IGFBP-rp1
KGF/FGF-7
Klotho
β-Klotho
M-CSF
M-CSF R
MSP
MSP β
MSP R
NGF R
β-NGF
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PDGF-CC
PDGF-DD
PDGF Rα
PDGF Rβ
PlGF
PPARα
VEGF121
VEGF162
VEGF165
VEGF-B167
VEGF-C (Cys156Ser)
VEGF-C (wild type)
VEGF-D
VEGF/PlGF
VEGF R1
VEGF R2
VEGF R3

Recombinant mouse:

FGF-8b
FGF-8c
FGF-23
FGF basic
FGF R2β
FGF R3
Klotho

Recombinant rat:

FGF basic
FGF-BP

Recombinant canine:

KGF/FGF-7

Natural Proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF

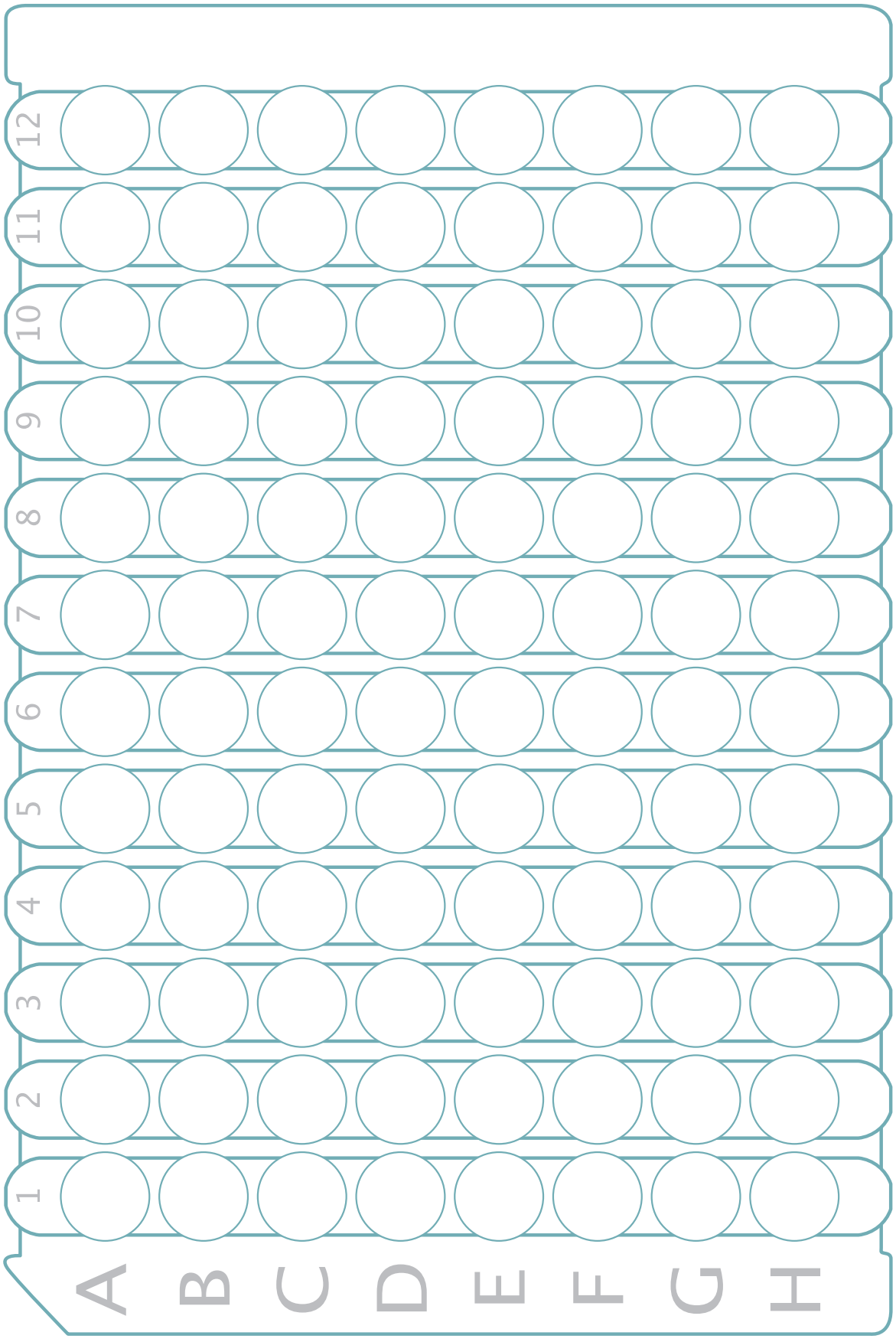
Recombinant mouse FGF-21 cross-reacts approximately 21% in this assay.

REFERENCES

1. Itoh, N. and D.M. Ornitz (2004) Trends Genet. **20**:563.
2. Ryden, M. (2009) Cell. Mol. Life Sci. **66**:2067.
3. Goetz, R. *et al.* (2007) Mol. Cell. Biol. **27**:3417.
4. Nishimura, T. *et al.* (2000) Biochim. Biophys. Acta **1492**:203.
5. Moore, D.D. (2007) Science **316**:1436.
6. Kharitonenkov, A. *et al.* (2005) J. Clin. Invest. **115**:1627.
7. Yie, J. *et al.* (2009) FEBS Lett. **583**:19.
8. Micanovic, R. *et al.* (2009) J. Cell Physiol. **219**:227.
9. Kurosu, H. and M. Kuro-o (2009) Mol. Cell. Endocrinol. **299**:72.
10. Ogawa, Y. *et al.* (2007) Proc. Natl. Acad. Sci. USA **104**:7432.
11. Kurosu, H. *et al.* (2007) J. Biol. Chem. **282**:26687.
12. Suzuki, M. *et al.* (2008) Mol. Endocrinol. **22**:1006.
13. Mai, K. *et al.* (2009) Diabetes **58**:1532.
14. Lundasen, T. *et al.* (2007) Biochem. Biophys. Res. Commun. **360**:437.
15. Badman, M.K. *et al.* (2007) Cell Metab. **5**:426.
16. Inagaki, T. *et al.* (2007) Cell Metab. **5**:415.
17. Zhang, X. *et al.* (2008) Diabetes **57**:1246.
18. Wang, H. *et al.* (2008) Mol. Cell. Biol. **28**:188.
19. Moyers, J.S. *et al.* (2007) J. Cell. Physiol. **210**:1.
20. Oishi, K. *et al.* (2008) FEBS Lett. **582**:3639.
21. Hsuchou, H. *et al.* (2007) Peptides **28**:2382.
22. Ishida, N. (2009) PPAR Res. **2009**:412949.
23. Xu, J. *et al.* (2009) Diabetes **58**:250.
24. Berglund, E.D. *et al.* (2009) Endocrinology **150**:4084.
25. Chen, W.W. *et al.* (2008) Exp. Clin. Endocrinol. Diabetes **116**:65.
26. Li, L. *et al.* (2008) Diabetes Res. Clin. Pract. **82**:209.
27. Coskun, T. *et al.* (2008) Endocrinology **149**:6018.
28. Galman, C. *et al.* (2008) Cell Metab. **8**:169.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES