FGF21 (Human) ELISA Kit

Catalog Number KA1849

96 assays

Version: 06

Intended for research use only

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Introduction

Background

Fibroblast growth factor 21 (FGF21) is a member of endocrine FGF subfamily, along with FGF19 and FGF23. The secreted human FGF21 is expressed in liver, pancreas, and white adipose tissue. It contains 209 amino acids in the precursor and 181 amino acids in the mature protein with a molecular mass of about 20 kDa, and has 75% homology with mouse FGF-21 (1). FGF21 signals through cell-surface tyrosine kinase FGF receptors complexed with a cofactor β-Klotho (2). FGF21 is a novel metabolic regulator involved in glucose metabolism, lipolysis, and ketogenesis and triglyceride clearance, growth hormone signaling, and metabolic diseases (3). In rodent models of diabetes, it stimulates glucose uptake in adipocytes, protects animals from diet-induced obesity, and lowers blood glucose and triglyceride (4). Serum FGF21 levels are increased in patients with metabolic diseases including nonalcoholic fatty liver disease, type 2 diabetes, gestational diabetes, obesity, Cushing's syndrome, HIV-1-induced lipodystrophy, and chronic kidney hemodialysis (5-7). Conversely, circulating FGF21 concentrations were reduced in subjects with anorexia nervosa (8). FGF21 is a biomarker for metabolic diseases and a candidate for the treatment of insulin resistance.

Principle of the Assay

The FGF21 (Human) ELISA Kit is designed for detection of human FGF21 in plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures FGF21 in less than 5 hours. A polyclonal antibody specific for FGF21 has been pre-coated onto a 96-well microplate with removable strips. FGF21 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for FGF21, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Human FBG21 Microplate: A 96-well polystyrene microplate coated with a polyclonal antibody against FGF21.	96 (8x12) wells
Sealing Tapes: Pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human FGF21 Standard: Human FGF21 in a buffered protein base (lyophilized).	2 ng
Biotinylated Human FGF21 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human FGF21.	140 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate.	80 µL
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ Prepare all reagents (working diluents buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and biotinylated antibody vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- ✓ Standard Curve: Reconstitute the 2 ng of Human FGF21 Standard with 1 mL of MIX Diluent to generate a stock solution of 2 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (2 ng/mL) 1:2 with MIX Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625, and 0.0313 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and use within 30 days.

Standard Point	Dilution	[FGF21] (ng/mL)
P1	Standard (2 ng/mL)	2.000
P2	1 part P1 + 1 part MIX Diluent	1.000
P3	1 part P2 + 1 part MIX Diluent	0.500
P4	1 part P3 + 1 part MIX Diluent	0.250
P5	1 part P4 + 1 part MIX Diluent	0.125
P6	1 part P5 + 1 part MIX Diluent	0.063
P7	1 part P6 + 1 part MIX Diluent	0.031
P8	MIX Diluent	0.000

- ✓ Biotinylated Human FGF21 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Sample Preparation

- ✓ Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:2 with MIX Diluent and assay. Store the remaining samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)

- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. Dilute samples 1:2 into MIX Diluent and assay. Store serum at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.

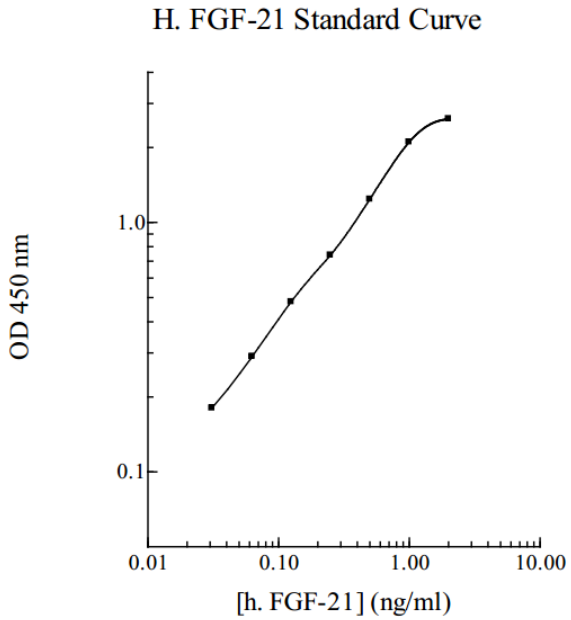
Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Human FGF21 Standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last sample addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
5. Add 50 µL of Biotinylated Human FGF21 Antibody to each well and incubate for 2 hours.
6. Wash the microplate as described above.
7. Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.



The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Performance Characteristics

- ✓ The minimum detectable level of FGF21 is typically 0.03 ng/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.7 % and 7.2% respectively.

- ✓ Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
No dilution	107%	103%
1:2	100%	99%
1:4	96%	97%

✓ Recovery

Standard Added Value	0.06 – 1.0 ng/mL
Recovery %	85-110%
Average Recovery %	97%

✓ Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	<20%
Mouse	<20%
Rat	<50%
Swine	<50%
Rabbit	None

Resources

References

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Plate Layout

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