Datasheet for ABIN1305174

FGF21 ELISA Kit

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Overview

Quantity:	96 tests
Target:	FGF21
Binding Specificity:	intact
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.7-2000 pg/mL
Minimum Detection Limit:	1.7 pg/mL
Application:	ELISA

Product Details

Purpose:	This sandwich ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human intact FGF-21 level in EDTA-plasma or serum. This assay does not detect human FGF-21 fragments. Indications for use:The test is useful as an aid in diagnosis of primary muscle-manifesting respiratory chain deficiencies, nonalcoholic fatty liver disease and other conditions related to type 2 diabetes, gestational diabetes and obesity.
Brand:	ED™
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. Anti-Human FGF-21 Antibody Coated Microplate Two vials each contain a different concentration of human FGF-21 in a lyophilized bovine

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	serum-based matrix with a non-azide, non-mercury preservative. Refer to vials for exact concentration range for each control. The controls are ready to use. Both controls should be stored at 2-8 °C and are stable until the expiration date on the kit box.
Material not included:	1. Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, and 1000 µL etc. 2. Repeating dispenser suitable for delivering 100 µL. 3. Disposable pipette tips suitable for above volume dispensing. 4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes. 5. Disposable plastic 100 mL and 1000 mL bottle with caps. 6. Aluminum foil. 7. Deionized or distilled water. 8. Plastic microtiter well cover or polyethylene film. 9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system. 10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Target Details

Target:	FGF21
Alternative Name:	FGF-21 (FGF21 Products)
Background:	Fibroblast Growth Factor 21 (FGF-21) belongs to the FGF-19 subfamily, which includes FGF-19, FGF-21 and FGF-23. The FGF-19 family members are potent endocrine hormones in the regulation of a diverse physiological homeostasis. The intact FGF-21 is a small protein comprising 181 amino acids. Administration of recombinant FGF-21 lowered plasma glucose and insulin levels, reduced hepatic and circulating triglycerides and cholesterol levels, and improved insulin sensitivity, energy expenditure, hepatic steatosis and obesity in a range of insulin-resistant animal models. The physiological functions of FGF-21 are relied on the intact molecular structure and amino acid sequence in its N-terminal and C-terminal region. An N-terminal truncated FGF-21 (7-181) is a potent inhibitor that competitively inhibits the biological activity of intact FGF-21 (1-181). Therefore, it is important to measure the circulation intact FGF-21 level in the assessment of the physiological and pathophysiological condition. An assay that determines the fragment of the FGF-21 might overestimate the biological activity of the protein in test sample.Circulation FGF-21 is a biomarker and its levels are increased in patients with nonalcoholic fatty liver disease (NAFLD), type 2 diabetes, gestational diabetes and obesity. An increase of circulating FGF-21 is also found in patients with Cushing's syndrome, patients with lipodystrophy induced by HIV-1 and patients with chronic renal disease or end-stage renal disease (ESRD).

Target Details

Pathways:	RTK Signaling

Application Details

Comment:	Intact FGF-21 ELISA Kit
Sample Volume:	50 µL
Assay Time:	4 h
Plate:	Pre-coated
Protocol:	This ELISA is designed, developed and produced for the quantitative measurement of human intact FGF-21 in serum and EDTA-plasma sample. The assay utilizes the two-site sandwich technique with two selected antibodies that bind to different epitopes of human intact FGF-21. One of the antibodies specifically binds to the N-terminal human FGF-21 (1-7) and the other is specific to the C-terminal human FGF-21 (175-181).Assay standards, controls and patient samples are added directly to wells of a microplate that is coated with an anti-human FGF-21 (1-7) specific antibody. Simultaneously, a horseradish peroxidase-conjugated anti-human FGF-21 (175-181) specific antibody is added to each well. After the first incubation period, the antibody on the wall of microtiter well captures human FGF-21 in the sample and unbound proteins in each microtiter well are washed away. A sandwich of anti-FGF-21 antibody --- human intact FGF-21 --- HRP conjugated tracer antibody is formed. The unbound tracer antibody is removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to human intact FGF-21 on the wall of the microtiter well is directly proportional to the amount of intact FGF-21 in the sample. A standard curve is generated by plotting the absorbance versus the respective human intact FGF-21 concentration for each standard on point-to-point or 4 parameter curve fit. The concentration of human intact FGF-21 in test samples is determined directly from this standard curve.
Reagent Preparation:	(1) Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged. (2) ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details. (3) Reconstitute kit standards and controls by adding 0.5 mL distilled water into each vial. Gently mix and dissolve the entire particle before use. The reconstituted standards and controls should be stored at -20 °C right after use.

	(4) Prepare working human FGF-21 tracer antibody by 1:21 fold dilution of the conjugation antibody with the FGF-21 Tracer Antibody Diluent . Following is a table that outlines the relationship of strips used and antibody mix prepared.
Sample Collection:	Only 50 µL of human EDTA-plasma is required for human FGF-21 measurement in singlet. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected with lavender-top Vacutainer. Separate the plasma from cells by centrifugation (850 ? 1500xg for 10 minutes). The plasma should be separated from the cells right after collection or at least within one hour of blood collection. The plasma should be transferred to a clean test tube right after centrifugation. Plasma samples should be stored at ? 20 °C if the assay is not to be performed within 48 hours. Avoid more than three freeze-thaw cycles of specimen. Serum sample can also be used for FGF-21 measurement. Serum sample collection should be performed as suggested by the manufacturer of the sample collection tubes.
Assay Procedure:	(1) Place a sufficient number of antibody-coated microwell strips(Cat. 30619(in a holder to run human intact FGF-21 standards, controls and unknown samples in duplicate. (2) Test Configuration (3) Add 50 µL of standards, controls and patient plasma/serum samples into the designated microwell. (4) Add 50 µL of 1:21 diluted tracer antibody to each well (5) Cover the plate with one plate sealer and incubate plate with orbital shaking 170 rpm at room temperature for 2 hours. (6) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (7) Add 100 µL of ELISA HRP Substrate into each of the wells. (8) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light. Incubate plate at room temperature for 20 minutes. (9) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (10) Read the absorbance at 450/650 nm within 10 minutes in a microplate reader.
Calculation of Results:	1. Calculate the average absorbance for each pair of duplicate test results. 2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance. 3. The standard curve is generated by the absorbance of all standards. Appropriate computer assisted data reduction programs may also be used for the calculation of results. The human intact FGF-21 concentrations for the controls and patient samples are read directly from the

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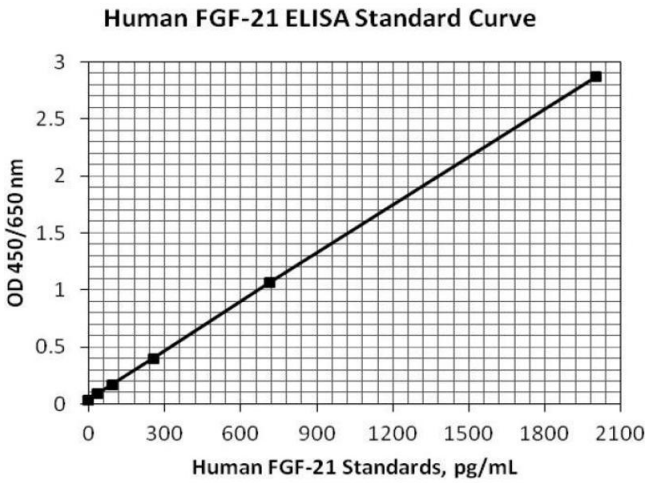
	standard curve using their respective corrected absorbance.
Assay Precision:	The intra-assay precision is validated by measuring three donor EDTA-plasma samples in a single assay with 16 replicate determinations. The inter-assay precision is validated by measuring three control samples in duplicate in 12 individual assays.
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Human Intact Fibroblast Growth Factor 21 (FGF-21) Assay Kit reagents must be used in a professional laboratory environment and is for in vitro diagnostic use. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from donor health animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.
Storage:	4 °C

Images



ELISA

Image 1.