Epitope Diagnostics, Inc.

EDI™ Human cFGF-21 ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human C-terminal FGF-21 Level in EDTA-Plasma or Serum

REF **KTR 874** Σ 96

For Research Use Only. Not for Use in Diagnostic Procedures.

INTENDED USE

This "sandwich" ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of **human C-terminal FGF-21** level in EDTA-plasma or serum. It measures both the intact FGF-21 and the C-terminal FGF-21 fragments that must not be C-terminally truncated while the N-terminal end of the FGF-21 may be truncated. The test is useful in clinical study related to diabetes and obesity, and is for research use only.

SUMMARY OF PHYSIOLOGY

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. **The C-terminal non-truncated FGF-21 is a potent cell membrane β-Klotho binder**. Whereas, a C-terminal truncated FGF-21 (1-170) 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).

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human C-terminal 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 FGF-21. One of the antibodies specifically binds to the C-terminal human FGF-21 (175-181) and the other is to the multi-epitopes of mid-regional and N-terminal human FGF-21.

Assay calibrators, controls and patient samples are added directly to wells of a microplate that is coated with an anti-human FGF-21 (175-181) specific antibody. After the first incubation period, a horseradish peroxidase-conjugated anti-human FGF-21 polyclonal antibody is added to each well. After the second incubation period, the antibody on the wall of microtiter well captures human C-terminal FGF-21 in the sample and further forms "sandwich" with the tracer antibody. Unbound proteins in each microtiter well are washed away. An immunocomplex of "anti-FGF-21 antibody --- human C-terminal 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 C-terminal FGF-21 on the wall of the microtiter well is directly proportional to the amount of C-terminal FGF-21 in the sample. A calibrator curve is generated by plotting the absorbance versus the respective human C-terminal FGF-21 concentration for each calibrator on point-to-point or 4 parameter curve fit. The concentration of human C-terminal FGF-21 in test samples is determined directly from this calibrator curve.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. **Anti-Human FGF-21 Antibody Coated Microplate (Cat. No. 31098)**
   One microplate with 12 x 8 well-breakable strips (96 wells total) coated with antibody to human C-terminal FGF-21. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **Human cFGF-21 Tracer Antibody (Cat. No. 31099)**
   One vial containing **0.6 mL** concentrated HRP-labeled anti-human FGF-21 polyclonal antibody in a stabilized protein matrix. This reagent must be diluted with FGF-21 Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **FGF-21 Tracer Antibody Diluent (Cat. No. 30600)**
   One vial containing **12 mL** ready-to-use buffer. It should be only used for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **ELISA Wash Concentrate (Cat. No. 10010)**
   One bottle contains **30 mL** of 30-fold concentrate. Before use the contents must be diluted with 870 mL of demineralized water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

5. **ELISA HRP Substrate (Cat. No. 10020)**

One bottle contains **12 mL** of tetramethylbenzidine (TMB) with stabilized hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. ELISA Stop Solution (Cat. No. 10030)
One bottle contains **12 mL** of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

7. Human FGF-21 Calibrators (Cat. No. 31111 – 31116)
Six vials each contain different concentrations of human FGF-21 in a lyophilized bovine serum-based matrix with a non-azide, non-mercury preservative. **Refer to vials for exact concentration for each calibrator.** These reagents should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

8. Human FGF-21 Controls (Cat. No. 31117 – 31118)
Two vials each contain different concentrations of human FGF-21 in a lyophilized bovine serum- based matrix with a non-azide, non-mercury preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and is for research use only. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are 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.

MATERIALS REQUIRED BUT NOT PROVIDED

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.

SPECIMEN COLLECTION

Only 100 µL of human serum or EDTA plasma sample is required for human cFGF-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 samples should be separated from the cells right after collection or at least within one hour of blood collection and should be transferred to a clean test tube right after centrifugation. Serum and EDTA plasma samples can be stored at 2-8ºC for no more than 72 hours, otherwise must be stored at -20ºC. Avoid more than three freeze-thaw cycles of specimen.

SPECIMEN SHIPMENT

Collected EDTA-plasma or serum samples should be shipped to designated laboratory in frozen condition with dry ice. If frozen condition is not available, samples should be shipped with blue ice in an insulated container for a maximum of 48 hours.

ASSAY PROCEDURE

1. 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 (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
(3) Reconstitute kit calibrators (Cat. 31111-31116) and controls (Cat. 31117-31118) by adding 1.0 mL distilled water into each vial. Gently mix and dissolve the entire particle before use. The reconstituted calibrators and controls should be stored at -20ºC right after use.
(4) Prepare working human FGF-21 tracer antibody (Cat. 31090) by 1:21 fold dilution of the conjugation antibody with the cFGF-21 Tracer Antibody Diluent (Cat. 30600). Following is a table that outlines the relationship of strips used and antibody mix prepared.

Strip no.	cFGF-21 Tracer Antibody Diluent	cFGF-21 Tracer Antibody
1	1000 µL	50 µL
2	2000 µL	100 µL
3	3000 µL	150 µL
4	4000 µL	200 µL
5	5000 µL	250 µL
6	6000 µL	300 µL
7	7000 µL	350 µL
8	8000 µL	400 µL
9	9000 µL	450 µL
10	1000 µL	500 µL
11	1100 µL	550 µL
12	1200 µL	600 µL

Note: this antibody mixture must be freshly prepared right before testing.

2. Assay Procedure

(1) Place a sufficient number of antibody coated microwell strips (Cat. 31098) in a holder to run human cFGF-21 calibrators, controls and unknown samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	CAL 1	CAL 5	SAMPLE 1
B	CAL 1	CAL 5	SAMPLE 1
C	CAL 2	CAL 6	SAMPLE 2
D	CAL 2	CAL 6	SAMPLE 2
E	CAL 3	C 1	SAMPLE 3
F	CAL 3	C 1	SAMPLE 3
G	CAL 4	C 2	
H	CAL 4	C 2	

(3) Add **100 µL** of calibrators, controls and patient plasma/serum samples into the designated microwell.
(4) Cover the plate with one plate sealer and incubate plate with orbital shaking 170 rpm (big radius) or 400 rpm (smaller radius) at room temperature for **1 hour**.
(5) 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.
(6) Add **100 µL** of 1:21 diluted tracer antibody to each well

(7) Cover the plate with one plate sealer and incubate plate with orbital shaking 170 rpm (big radius) or 400 rpm (smaller radius) at room temperature for **1 hour**.
(8) 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.
(9) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(10) 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.**
(11) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(12) Read the absorbance at 450/650 nm within 10 minutes in a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

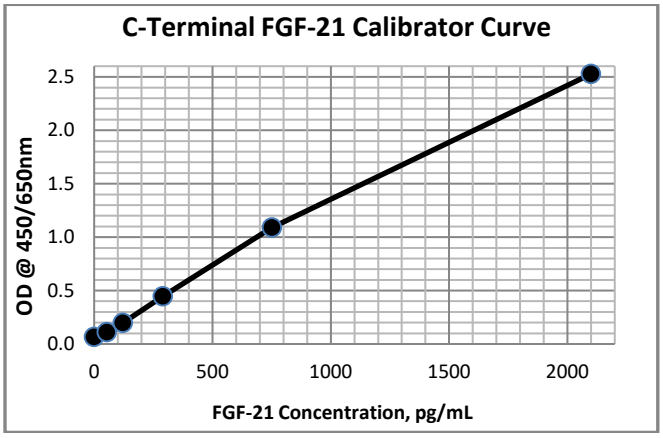
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the CAL 1 (0 pg/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibrator curve is generated by the absorbance of all calibrators. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The human C-terminal FGF-21 concentrations for the controls and patient samples are read directly from the calibrator curve using their respective corrected absorbance.

EXAMPLE DATA AND CALIBRATOR CURVE

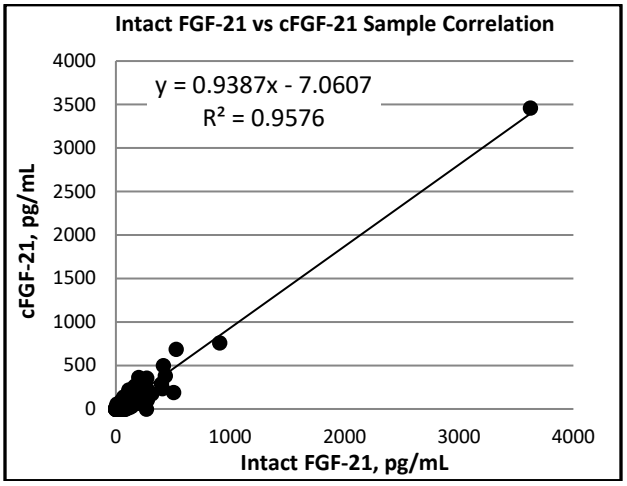
A typical absorbance data and the resulting calibrator curve from human cFGF-21 ELISA are represented. **This curve should not be used in lieu of calibrator curve run with each assay.**

Well I.D.	OD 450/650 nm Absorbance			Results (pg/mL)
	Readings	Average	Corrected	
CAL-1: 0 pg/mL	0.066 0.066	0.066	0.000	
CAL-2: 54.0 pg/mL	0.112 0.112	0.112	0.046	
CAL-3: 121 pg/mL	0.197 0.197	0.197	0.131	
CAL-4: 291 pg/mL	0.449 0.446	0.448	0.382	
CAL-5: 752 pg/mL	1.089 1.094	1.092	1.026	
CAL-6: 2100 pg/mL	2.546 2.508	2.527	2.461	
Control 1	0.307 0.302	0.305	0.239	194
Control 2	0.748 0.739	0.744	0.678	503.1



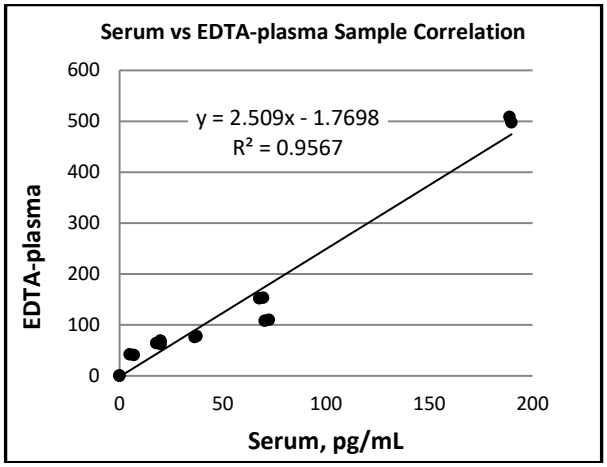
EXPECTED VALUES

This human C-terminal FGF-21 ELISA was validated by testing the sample correlation against human intact FGF-21 (KT879). Total of combined138 serum/plasma samples were measured.



The normal range was found to be less than 200 pg/mL. It is strongly recommended that each laboratory should establish its own normal range based on normal donor EDTA-plasma and serum samples.

Total of 16 EDTA plasma and 16 serum samples were measured side-by-side with this ELISA kit. It was found that EDTA-plasma samples give a higher value than serum samples. It is recommended to use serum for this kit.



LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration available for human C-terminal FGF-21 measurement, the values of assay calibrators were established by correlation to a highly purified FGF-21 standard.
2. For sample values reading greater than the highest standard, it is recommended to re-assay samples with dilution.
3. Bacterial or fungal contamination of plasma specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known human N-terminal FGF-21 levels. We recommend that all assays include the laboratory's own C-terminal FGF-21 controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity (lowest limit of detection) of this human cFGF-21 ELISA as determined by the corresponding OD value of 2-fold standard deviation above the mean on 20 duplicate determination of zero calibrator is 3.7 pg/mL. The Limit of Quantitation in 95th percentile is 7.2 pg/mL

High Dose "hook" effect

This assay has showed that it did not have any high dose "hook" effect up to 236, 900 pg/mL.

Precision

The intra-assay precision is validated by measuring two samples in a single assay with 16 replicate determinations.

Mean Human cFGF-21 Value (pg/mL)	CV (%)
229.4	4.4
538.8	2.2

The inter-assay precision is validated by measuring three control samples in duplicate in 12 individual assays.

Mean Human cFGF-21 Value (pg/mL)	CV (%)
205.4	7.7
513.3	3.2

Linearity

Linearity was validated using Level 5 and Level 6, diluted with Calibrator Matrix and assayed.

	Expected	Observed	% Recovery
Level 6	-	2100	-
1:2	1050	1120.648	106.7%
1:4	525	487.366	92.8%
1:8	262.5	238.659	90.9%
Level 5	-	700	-
1:2	350	330.07	94.3%
1:4	175	159.266	91.0%
1:8	87.5	71.957	82.2%

Spike Recovery

Two serum samples were spiked, in equal volume, with various amounts of human nFGF-21 and assayed. The results in the value of pg/mL are as follows:

	Expected	Observed	% Recovery
Sample A	-	34.831	-
+level 3: 77.8	56.3155	62.439	110.9%
+Level 4: 233.3	134.0655	133.281	99.4%
+Level 5: 700	367.4155	338.587	92.2%
Sample B	-	67.696	-
+level 3: 77.8	72.748	67.542	92.8%
+Level 4: 233.3	150.498	132.871	88.3%
+Level 5: 700	383.848	337.826	88.0%

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. Yie J, et al. FGF21 N- and C-termini play different roles in receptor interaction and activation. FEBS Lett. 2009 Jan 5;583:19-24.
2. Micanovic R, et al. Different roles of N- and C- termini in the functional activity of FGF21. J Cell Physiol. 2009 May;219(2):227-34.
3. Yusuke Murata, et al. FGF21 as an Endocrine Regulator in Lipid Metabolism: From Molecular Evolution to Physiology and Pathophysiology. Journal of Nutrition and Metabolism, Vol 2011, Article ID 981315, 8 pages

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

This product is developed and manufactured by
Epitope Diagnostics, Inc.
San Diego, CA 92126, USA

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
Read instructions before use	Use by
	Lot No.

Human cFGF-21 ELISA: Condensed Assay Protocol

1. **100 µl Calibrators, controls and test samples**

   ↓ *Incubate @ RT for 1 hr on ELISA plate shaker*
   wash 5 x

2. **100 µl Tracer Antibody**

   ↓ *Incubate @ RT for 1 hr on ELISA plate shaker*
   wash 5 x

3. **100 µl TMB Substrate**

   ↓ *Incubate @ RT for 20 min static*

4. **100 µl Stop Solution at 450/650 nm** **Read absorbance**