EAGLE BIOSCIENCES

Human Intact Fibroblast Growth Factor (FGF-21) ELISA

Catalog Number:

F2131-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 8.0 (13 NOV 23)

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INTENDED USE

The Eagle Biosciences Human Intact Fibroblast Growth Factor 21 (FGF-21) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative measurement of human Intact Fibroblast Growth Factor 21 (FGF-21) levels in serum or EDTA plasma. This assay doesn't detect human FGF-21 fragments. The Eagle Biosciences Human Intact Fibroblast Growth Factor 21 (FGF-21) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

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 is increased in patient with nonalcoholic fatty liver disease (NAFLD), type 2 diabetes, gestational diabetes and obesity. An increase of circulating FGF-21 is also found in patient with Cushing's syndrome, patient with lipodystrophy induced by HIV-1 and patient with chronic renal disease or end-stage renal disease (ESRD).

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Human Intact Fibroblast Growth Factor 21 (FGF-21) ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human intact FGF-21 in serum and/or 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 is specifically binds to the N-terminal human FGF-21 (1-7) and the other is specifically to the C-terminal human FGF-21 (175-181). Assay calibrators, controls and patient samples are added directly to wells of 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 an unbound protein in each microtiter well is 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 calibration curve is generated by plotting the absorbance versus the respective human intact FGF-21 concentration for each calibrator on point-to-point or 4 parameter curve fit. The concentration of human intact FGF-21 in test samples is determined directly from this calibration curve.

REAGENTS: Preparation and Storage

This Intact Fibroblast Growth Factor 21 (FGF-21) ELISA Assay 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 the expiration date.

Allow all reagents to come to room temperature prior to use. Regents from different kit lot numbers should not be combined or interchanged.

1. Anti-Human FGF-21 Antibody Coated Microplate

Microplate coated with human FGF-21 antibody

Qty: 1 x 96 well microplate
Storage: 2-8°C
Preparation: Ready to Use

2. Human FGF-21 Tracer Antibody

HRP-labeled anti-hFGF-21 antibody in a stabilized protein matrix

Qty: 1 x 0.4 mL
Storage: 2-8°C
Preparation: 21X Concentrate, must be diluted with FGF-21 Tracer Antibody Diluent before use

3. FGF-21 Tracer Antibody Diluent

Ready-to-use buffer. It should only be used for tracer antibody dilution according to the assay procedure

Qty: 1 x 8 mL
Storage: 2-8°C
Preparation: Ready to Use

4. ELISA Wash Concentrate

Wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative

Qty: 1 x 30 mL
Storage: 2-25°C
Preparation: 30X Concentrate, must be diluted with 870 mL of demineralized water, mix well before use

5. ELISA HRP Substrate

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 12 mL
Storage: 2-8°C
Preparation: Ready to Use

6. ELISA Stop Solution

0.5M sulfuric acid

Qty: 1 x 12 mL
Storage: 2-25°C
Preparation: Ready to Use

7. Human FGF-21 Standards

Human FGF-21 in BSA based matrix with a non-azide, non-mercury preservative. Refer to each vial for exact concentration

Qty: 6 x Vials
Storage: 2-8°C, <-20°C for long term storage. Do not exceed 3 freeze-thaw cycles
Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

8. Human FGF-21 Controls

Human FGF-21 in BSA based matrix with a non-azide, non-mercury preservative. Refer to each vial for exact concentration.

Qty: 2 x Vials
Storage: 2-8°C, <-20°C for long term storage. Do not exceed 3 freeze-thaw cycles
Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

SAFETY PRECAUTIONS

The Human Intact Fibroblast Growth Factor 21 (FGF-21) Assay Kit reagents are for research use only. Source material which contains reagents of bovine serum albumin 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 hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. Keep out of reach skin, eyes and/or clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µL, 100 µL, and 1000 µL
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or glass or plastic tubes.
- Disposable plastic 100 mL and 1000 mL bottle with caps.
- Aluminum foil

- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm/650nm.
- Deionized or distilled water
- Calibrated timer

SPECIMEN 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 Vaccutainer. 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 – 20ºC if the assay is not to be performed within 48 hours. Avoid more than three times freeze-thaw cycles of specimen. Collect 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 at room temperature in an insulated container for a maximum of 48 hours. Samples must **not** be shipped refrigerated, such as with blue ice pack. Serum sample can also be used for FGF-21 measurement. Serum sample collection should perform as suggested by manufacturer of the sample collection tubes.

REAGENT PREPARATION

1. Prior to use allow all reagents to come to room temperature (20-25°C). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

ASSAY PROCEDURE

1. Place a sufficient number of microwell strips/wells in a holder to run calibrators, controls and 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	SAMPLE 4
H	CAL 4	C 2	SAMPLE 4

3. Prepare antibody working solution with the human FGF-21 tracer antibody by 1:21 fold dilution of the conjugation antibody with the FGF-21 Tracer Antibody Diluent. For each strip, 25 µL human FGF-21 Tracer Antibody and 500 µL of FGF-21 Tracer Antibody Diluent is used (This antibody working solution should be freshly prepared before testing).

4. Add **50 µL** of calibrators, controls and samples in duplicate into the designated microwells.
5. Add **50 µL** of antibody working solution to each well. Mix gently by tapping the plate
6. Cover the plate with one plate sealer and aluminum foil. Incubate plate at **room temperature (20-25ºC)** with orbital shaking **170 rpm (bigger radius) or 400 rpm (smaller radius)** for **120 minutes**.
7. Remove plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of ELISA HRP Substrate into each of the wells. Mix gently by tapping the plate.
9. Cover the plate with one plate sealer and aluminum foil. Incubate plate at **room temperature (20-25ºC)** for **20 minutes.**
10. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix by gently tapping the plate.
11. Read the absorbance at **450/650 nm** within **10 minutes** in a microplate reader

PROCEDURAL NOTES

1. It is recommended that all 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 away from direct light in the original container and should be stored in a dark area avoiding unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Exposure of plates to humidity drastically reduces the shelf life.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation time(s) and/or temperature(s) other than those specified in the package insert may affect the results.
7. Avoid air bubbles in the microwell as it could result in lower binding efficiency and higher CV% of a duplicate reading
8. All reagents should be mix thoroughly prior 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 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration 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 intact FGF-21 concentrations for the controls and patient samples are read directly from the calibration curve using their respective corrected absorbance values.

LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration available for human Intact FGF-21 measuremnt, the values of assay calibrators were established by correlation to a highly purified FGF-21 calibrator.
2. For sample values reading greater than the highest calibrator, it is recommended to re-assay samples with dilution.
3. Bacterial of fungal 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 intact FGF-21 levels. EagleBio recommends laboratories to include their own FGF-21 controls in addition to those provided with kit.

EXPECTED VALUES

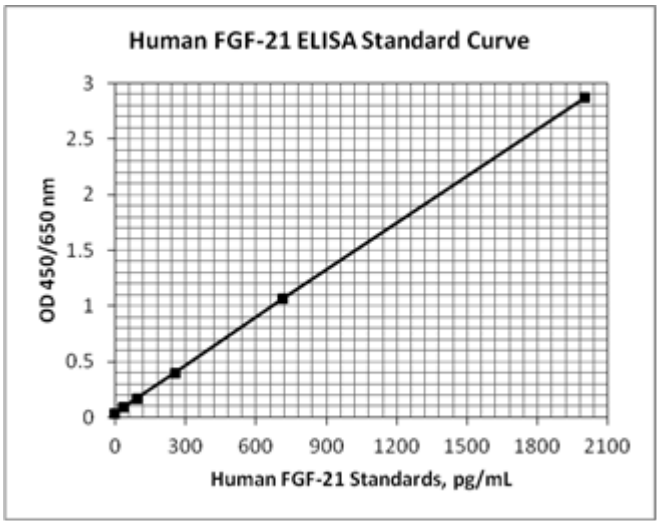
Thirty two normal adult plasma samples were measured using proposed test kit. The normal range was found to be < 200 pg/mL. It is strongly recommended that each laboratory should establish its own normal range based on normal donor serum or EDTA-plasma samples.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting calibrator curve from Human Intact Fibroblast Growth Factor 21 (FGF-21) ELISA Assay Kit are represented.

Well I.D.	OD 450/650 nm Absorbance			Results pg/mL
	Readings	Average	Corrected	
Calibrator Level 1: 0.0 pg/mL	0.037	0.037	0.000	
	0.036			
Calibrator Level 2: 32.5 pg/mL	0.087	0.087	0.50	
	0.086			
Calibrator Level 3: 91.0 pg/mL	0.172	0.170	0.133	
	0.169			
Calibrator Level 4: 255.0 pg/mL	0.398	0.399	0.302	
	0.399			
Calibrator Level 5: 714.0 pg/mL	1.067	1.068	1.031	
	1.069			
Calibrator Level 6: 2000.0 pg/mL	2.835	2.869	2.946	
	2.903			
Control 1	0.126	0.127	0.371	60.83
	0.129			
Control 2	0.736	0.729	1.200	481.29
	0.721			

Note: This curve should not be used in lieu of calibrator curve run with each assay



PERFORMANCE CHARACTERISTICS

Limit of Detection

The limit of detection (sensitivity) of human intact FGF-21 ELISA was determined by the corresponding OD value of 2 fold standard deviation above the mean on 20 duplicate determination of zero calibrator, and was found to be 1.7 pg/mL.

Precision

The intra-assay precision was validated by measuring three donor EDTA-plasma samples in a single assay with 16-replicate determinations. The results are summarized below.

Sample	Mean Value (pg/mL)	CV (%)
1	63.2	5.7
2	171	4.2
3	480	5.4

The inter-assay reproducibility was validated by measuring three control samples in duplicate in 12 individual assays. The results are summarized below.

Sample	Mean Value (pg/mL)	CV (%)
1	69.8	6.9
2	181	3.0
3	486	1.9

Linearity

Two human EDTA-plasma samples were diluted with standard matrix or calibrator level 1 at pH 7.4 and were assayed. The results are as follows:

Sample	Observed Value (pg/mL)	Expected Value (pg/mL)	Recovery %
Sample A	45.9	-	-
50%	138.0	143.0	96%
25.0%	75.0	72.0	104%
12.5%	37.9	36.0	105%
6.25%	19.5	18	108%
Sample B	61.8	-	-
50%	32.1	30.9	104%
25.0%	15.9	15.5	103%
12.5%	7.2	7.7	94%

Spike Recovery

Two patient samples were spiked with various amounts of human intact FGF-21 (1 vol. + 1 vol. mixture) and assayed. The results in the value of ng/mL are as follows:

Sample	Observed Value (pg/mL)	Expected Value (pg/mL)	Recovery (%)
Serum Sample A	45.9	-	-
+91 pg/mL	64.9	68.5	95
+255 pg/mL	150	151	100
+714 pg/mL	388	380	102
Serum Sample B	40.4	-	-
+91 pg/mL	71.2	65.7	108%
+255 pg/mL	148.0	148.0	100%
+714 pg/mL	406.0	377.0	108%

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.
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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

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.