



EAGLE
BIOSCIENCES

Intact FGF-21 ELISA Assay Kit

Catalog Number:

F2131-K01 (1 x 96 wells)

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

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EAGLE BIOSCIENCES, INC.

20A Northwest Blvd., Suite 112, Nashua, NH 03063

Phone: 617-419-2019 Fax: 617-419-1110

WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences Intact FGF-21 ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Intact FGF-21 levels in human serum or EDTA plasma. The Eagle Biosciences Intact FGF-21 ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

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

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. The 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. The assay which determines the fragment of the FGF-21 might overestimate the biological activity of the protein in a test sample. Circulating FGF-21 is a biomarker and its levels are increased in patients with non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, mellitus, gestational diabetes and obesity, Cushing's syndrome, patients with lipodystrophy induced by HIV-1 and patients with chronic renal disease or end-stage renal disease (ESRD).

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Intact FGF-21 ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human intact FGF-21 in serum and EDTA-plasma samples. 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 N-terminal human FGF-21 (1-7) and the other is 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. 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 the microtiter well captures human 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 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 intact FGF-21 on the wall of the microtiter well is directly proportional to the amount of intact FGF-21 in the sample. A calibrator 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 calibrator curve.

LIMITATIONS RELATED TO INTENDED USE

- Since there is no Gold Standard concentration available for human intact FGF-21 measurement, the values of assay Standards were established by correlation to a highly purified FGF-21 standard.

- For sample values reading greater than the highest Standard, it is recommended to re-assay samples with dilution.
- Bacterial or fungal contamination of plasma specimens or reagents, or cross-contamination between reagents may cause erroneous results.
- Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
 - Do not pipette by mouth.
 - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
 - Wear protective clothing and disposable gloves.
 - Wash hands thoroughly after performing the test.
 - Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A calibrator curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.5 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage

Only 50 μ L of human serum or EDTA plasma sample 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 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 2-8°C for no more than 72 hours, otherwise must be stored at -20°C. Avoid more than three freeze-thaw cycles of specimen.

Specimen Pre-Treatment

No pretreatment required once serum and EDTA plasma is collected.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 25, 50, 100 and 1000 μL
- Repeating dispenser suitable for delivering 100 μL
- Disposable pipette tips suitable for above volume dispensing
- Disposable 12x75 mm glass or plastic tubes
- Disposable plastic 1000 mL bottle with caps
- Aluminum foil
- Deionized or distilled water.
- 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.
- Orbital shaker capable of big radius of 170 rpm, or small radius of 400 rpm.

REAGENTS PROVIDED

1. MICROPLATE

Contents: One microplate with 12x8 break-apart well strips coated with anti-human FGF-21 antibody
 Format: Ready to Use
 Storage: 2-8°C
 Stability: Stable until the expiry date printed on the label.

2. Human FGF-21 Tracer Antibody (21x)

Contents: One bottle containing concentrated HRP-labeled anti-human FGF-21 polyclonal antibody in a stabilized protein matrix.
 Format: Concentrated; Requires Preparation
 Volume: 0.6 mL/bottle
 Storage: 2-8°C
 Stability: Concentrated: Stable until the expiry date printed on the label. After preparation: Discard after use.
 Preparation of Working Solution: **Dilute 1:21** in Tracer Antibody Diluent according to table below. Prepare working solution for number of strips needed to test. Discard any that is left over

Strip No.	nFGF-21 Tracer Antibody Diluent	nFGF-21 Tracer Antibody
1	500 μL	25 μL
2	1000 μL	50 μL
3	1500 μL	75 μL
4	2000 μL	100 μL
5	2500 μL	125 μL
6	3000 μL	150 μL
7	3500 μL	175 μL
8	4000 μL	200 μL

9	4500 μ L	225 μ L
10	5000 μ L	250 μ L
11	5500 μ L	275 μ L
12	6000 μ L	300 μ L

3. FGF-21 Tracer Antibody Diluent

Contents: One vial containing a ready-to-use buffer for use with the Tracer Antibody dilution.
 Format: Ready to Use
 Volume: 8 mL
 Storage: 2-8°C
 Stability: Stable until the expiry date printed on the label

4. ELISA Wash Concentrate (30x)

Contents: One bottle of concentrated wash buffer containing a surfactant in phosphate buffered saline with non-azide, non-mercury preservative.
 Format: Concentrated; Requires Preparation
 Volume: 30 mL/bottle
 Storage: 2-8°C
 Stability: Unopened: Stable until the expiry date printed on the label.
 After Preparation: Stable at RT until expiry on the label.
 Preparation of Working Solution: **1:30 Dilution.** To prepare to run a full plate, use all 30 mL of wash concentrate with 870 mL of deionized or distilled water.

5. ELISA HRP Substrate

Contents: One bottle containing tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
 Format: Ready to Use
 Volume: 12 mL/bottle
 Storage: 2-8°C
 Stability: Stable until the expiry date printed on the label.

6. ELISA Stop Solution

Contents: One bottle containing 0.5 M sulfuric acid
 Format: Ready to Use
 Volume: 12 mL/bottle
 Storage: 2-8°C
 Stability: Stable until the expiry date printed on the label.

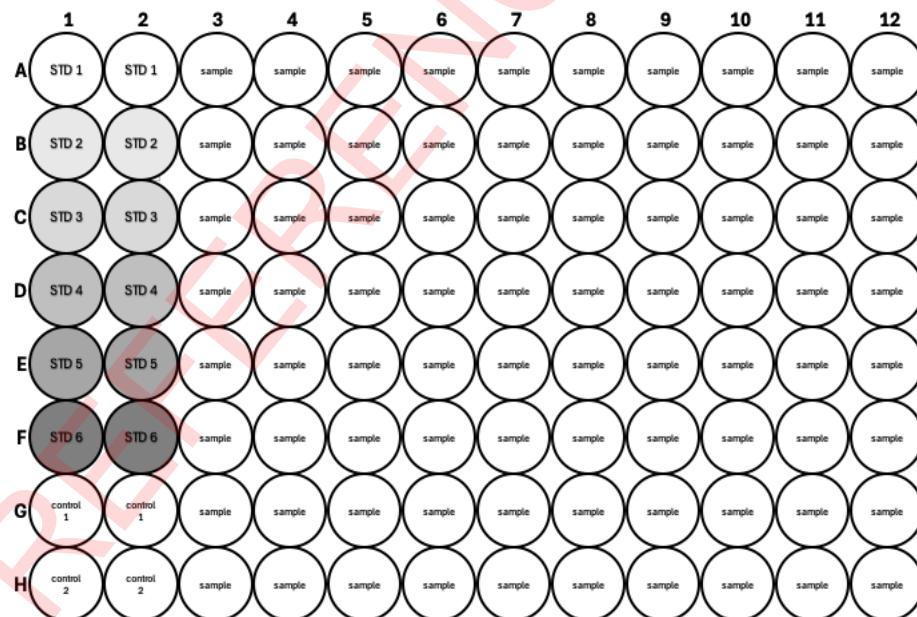
7. Human FGF-21 Standards 1-6

Contents:	Six vials each containing different concentrations of human FGF-21 in a bovine serum-based matrix with a non-azide, non-mercury preservative.
Format:	Lyophilized, needs reconstitution
Storage:	2-8°C, -20°C after reconstitution
Stability	Stable until the expiry date printed on the label. After
Preparation of Working Solution:	Opening: Stable until expiry if stored at -20°C Add 0.5 mL of distilled or deionized water into each vial. Let sit for 10 minutes and gently mix and dissolve the entire particle before use.

8. Human FGF-21 Controls

Contents:	Two vials each containing different concentrations of human FGF-21 in a bovine serum-based matrix with a non-azide, non-mercury preservative.
Format:	Lyophilized, needs reconstitution
Storage:	2-8°C, -20°C after reconstitution
Stability:	Stable until the expiry date printed on the label. After
Preparation of Working Solution.	Opening: Stable until expiry if stored at -20°C Add 0.5 mL of distilled or deionized water into each vial. Let sit for 10 minutes and gently mix and dissolve the entire particle before use.

RECOMMENDED ASSAY LAYOUT*



*Layout subject to change based on standard and control quantities

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Place a sufficient number of antibody coated microwell strips in a holder to run Standards, controls and unknown samples in duplicate.
2. Add **50 μ L of Standards, controls and samples** into designated wells.
3. Add **50 μ L** of 1:21 diluted tracer antibody working solution to each well and mix gently by tapping the plate.
4. Cover the plate with one plate sealer and incubate plate with orbital shaker (170 rpm-big radius, or 400 rpm-small radius) at room temperature for **120 minutes**.
5. Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 μ L of working wash solution into each well and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Add **100 μ L** of ELISA HRP Substrate into each of the wells and mix gently by tapping the plate
7. Cover the plate with one plate sealer and with aluminum foil to avoid exposure to light. Incubate plate at room temperature for **20 minutes**.
8. Remove the aluminum foil and plate sealer and add **100 μ L** of ELISA Stop Solution into each wells. Mix gently
9. Read the absorbance at **450/650 nm** within 10 minutes in a microplate reader using point-to-point curve fitting.

CALCULATIONS

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 for all other readings to obtain the corrected value.
3. The calibrator curve is generated by the absorbance of all Standards. Appropriate computer assisted data reduction programs may also be used for the calculation of results.
4. The N-terminal FGF-21 concentrations for the controls and samples are read directly from the calibrator curve using their respective corrected absorbance.

QUALITY CONTROL

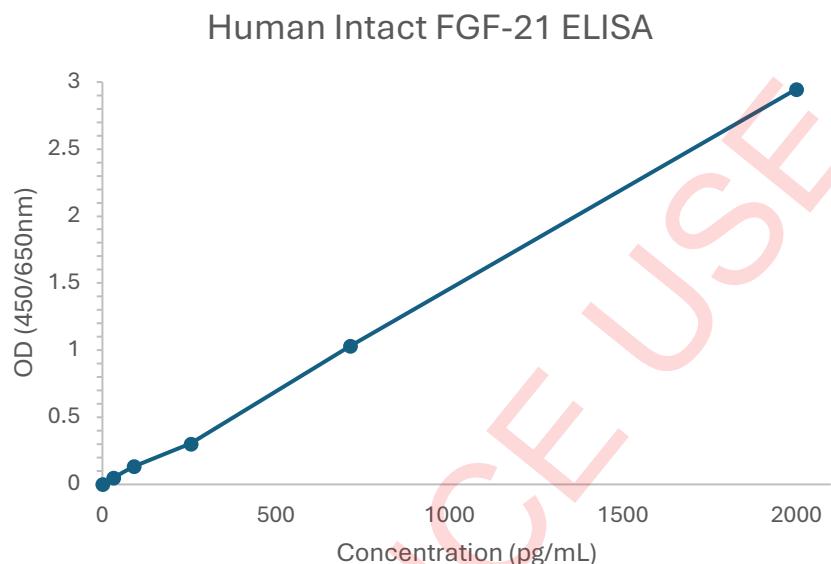
The test results are only valid if the test has been performed following the described procedure. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated above and/or labeled. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation, the following technical issues should be reviewed. Expiration dates of prepared reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

EXAMPLE DATA AND STANDARD CURVE

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

Well I.D	OD 450/650 nm Absorbance			Results (pg/mL)
	Readings	Average	Corrected	
STD 1: 0 pg/mL	0.037 0.036	0.037	0.000	
STD 2: 32.5 pg/mL	0.087 0.086	0.087	0.050	
STD 3: 91.0 pg/mL	0.172 0.169	0.170	0.133	
STD 4: 255 pg/mL	0.398 0.399	0.399	0.302	
STD 5: 714 pg/mL	1.067	1.068	1.031	

	1.069			
STD 6 : 2000 pg/mL	2.835 2.903	2.869	2.946	
Control 1	0.126 0.129	0.127	0.371	60.83
Control 2	0.736 0.721	0.729	1.200	481.29



PERFORMANCE AND CHARACTERISTICS

Sensitivity

The sensitivity (lowest limit of detection) of this FGF-21 ELISA as determined by the corresponding OD value of 2-fold standard deviation about the mean on 20 duplicate determinations of zero standard is found to be 1.7 pg/mL

Intra-Assay Precision

The intra-assay precision is validated by measuring three EDTA-plasma donor samples in a single assay with 16 replicate determinations.

Mean FGF-21 Value (pg/mL)	CV%
63.2	5.7
171.0	4.2
480.0	5.4

Inter-Assay Precision

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

Mean FGF-21 Value (pg/mL)	CV%

69.8	6.9
181.0	3.0
486.0	1.9

Linearity

Linearity was validated using two human EDTA-plasma samples diluted with standard matrix or standard level 1 at pH 7.4 and were assayed

	Expected	Observed	Recovery %
Sample A	-	286.0	-
50%	143.0	138.0	96%
25%	72.0	75.0	104%
12.5%	36.0	37.9	105%
6.25%	18.0	19.5	108%
Sample B	-	61.8	-
50%	30.9	32.1	104%
25%	15.5	15.9	103%
12.5%	7.7	7.2	94%

Spike Recovery

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

	Expected	Observed	Recovery %
Sample A (serum)	-	45.9	-
+91 pg/mL	68.5	64.9	95%
+255 pg/mL	151.0	150.0	100%
+714 pg/mL	380.0	388.0	102%
Sample B (EDTA Plasma)	-	40.4	-
+91 pg/mL	65.7	71.2	108%
+255 pg/mL	148.0	148.0	100%
+714 pg/mL	377.0	406.0	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
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.

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.

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