N-Terminal FGF-21 ELISA Assay Kit

Catalog Number:
N2131-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 3.0 30SEP2021

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INTENDED USE

The Eagle Biosciences N-Terminal FGF-21 ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of N-terminal FGF-21 levels in human serum or EDTA plasma. The Eagle Biosciences N-Terminal 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 non-truncated FGF021 is a potent cell membrane FGF receptor binder**. Whereas 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 samples.

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).

PRINCIPLE OF THE ASSAY

The Eagle Biosciences N-Terminal FGF-21 ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human N-terminal FGF-21 in serum and EDTA-plasma samples. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to difference 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 multi-epitopes of mid-regional and C-terminal human FGF-21.

Assay standards, controls and 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 N-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 N-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 N-terminal FGF-21 on the wall of the microtiter well is directly proportional to the amount of N-terminal 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 N-terminal FGF-21 in test samples is determined directly from this standard curve.

LIMITATIONS RELATED TO INTENDED USE

- Since there is no Gold Standard concentration available for human N-terminal 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 100 µL of human serum or EDTA plasma sample is required for human nFGF-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 or 13x100 glass or plastic tubes
- Disposable plastic 100 mL and 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 antibody to human N-terminal FGF-21
Format:	Ready to Use
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

2. **Human nFGF-21 Tracer Antibody (20x)**

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	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	10000 µL	500 µL
11	11000 µL	550 µL
12	12000 µL	600 µL

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:	12 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.

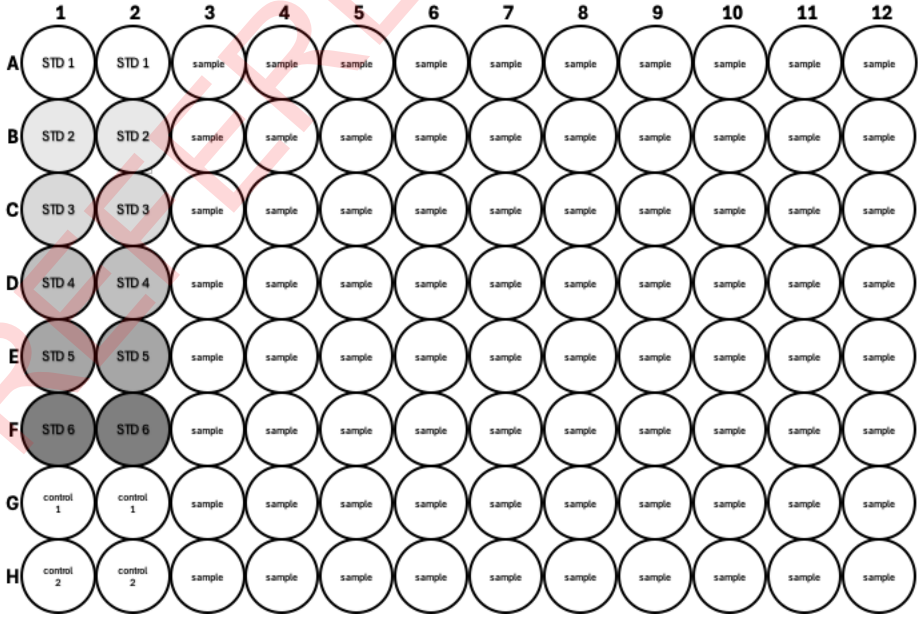
7. **Human FGF-21 Standards 1-6**

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 Opening: Stable until expiry if stored at -20°C
Preparation of Working Solution:	Add 0.5 mL of distilled or deionized water into each vial. 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 Opening: Stable until expiry if stored at -20°C
Preparation of Working Solution.	Add 0.5 mL of distilled or deionized water into each vial. 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 **100 µL of standards, controls and samples** into designated wells.
3. 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 **1 hour**.
4. 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 aspirate the contents. Alternatively, an automated microplate washer can be used.
5. Add **100 µL** of 1:21 diluted tracer antibody to each well.
6. 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 **1 hour.**
7. 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 aspirate the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of ELISA HRP Substrate into each of the wells
9. Cover the plate with one plate sealer and with aluminum foil to avoid exposure to light. Incubate plate at room temperature for **20 minutes**.
10. Remove the aluminum foil and plate sealer and add **100 µL** of ELISA Stop Solution into each wells. Mix gently
11. 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 STD 1 (0 pg/mL) from the average absorbance for all other readings to obtain the corrected value.
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.
4. The N-terminal FGF-21 concentrations for the controls and samples are read directly from the standard curve using their respective corrected absorbance.

QUALITY CONTROL

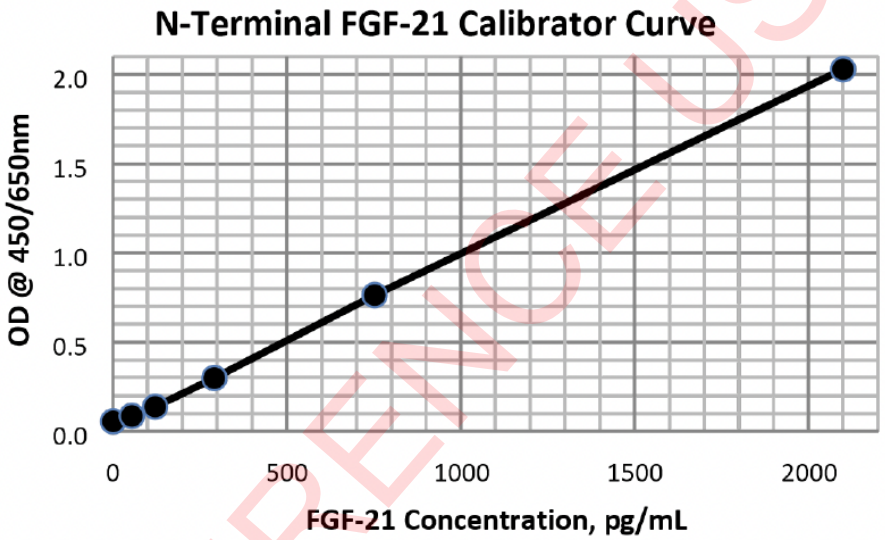
The test results are only valid if the test has been performed following the following instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated above 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 N-Terminal FGF-21 ELISA are represented. This curve should not be used in lieu of standard curve run with each assay.

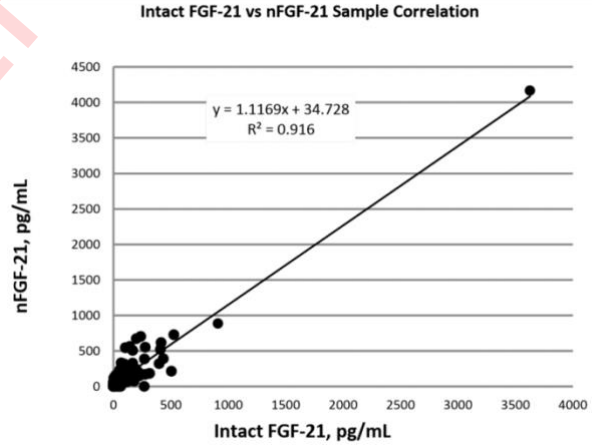
Well I.D	OD 450/650 nm Absorbance			Results (pg/mL)
	Readings	Average	Corrected	
STD 1: 0 pg/mL	0.055	0.055	0.000	

	0.054			
STD 2: 54 pg/mL	0.085 0.088	0.087	0.032	
STD 3: 121 pg/mL	0.135 0.138	0.137	0.082	
STD 4: 291 pg/mL	0.294 0.301	0.298	0.243	
STD 5: 752 pg/mL	0.757 0.770	0.764	0.709	
STD 6 : 2100 pg/mL	2.057 2.003	2.030	1.976	
Control 1	0.210 0.216	0.213	0.159	201.3
Control 2	0.526 0.516	0.521	0.467	512.1



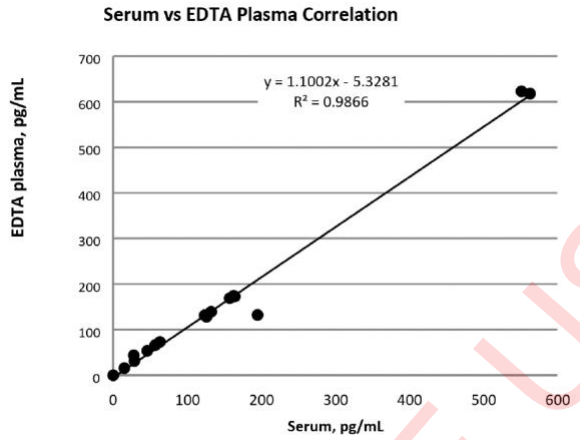
EXPECTED DATA

This human N-terminal FGF-21 ELISA was validated by testing the sample correlation against human intact FGF-21. Total of combined 138 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 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.



PERFORMANCE AND CHARACTERISTICS

Sensitivity

The sensitivity (lowest limit of detection) of this N-Terminal FGF-21 ELISA as determined by the corresponding OD value of 2-fold standard deviation about the mean on 16 duplicate determinations of zero standard is 4.7 pg/mL. The limit of quantitation in 95th percentile is 9.3 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

Intra-Assay Precision

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

Mean Human nFGF-21 Value (pg/mL)	CV%
225.49	1.9
528.89	1.1

Inter-Assay Precision

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

Mean Human nFGF-21 Value (pg/mL)	CV%
202.57	4.4
506.53	3.8

Linearity

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

	Expected	Observed	Recovery %
Level 6	-	2100	-
1:2	1050	1069.888	101.9%
1:4	525	504.475	96.1%
1:8	262.5	242.964	92.6%
Level 5	-	700	-
1:2	350	333.188	95.2%
1:4	175	160.554	91.7%
1:8	87.5	76.716	87.7%

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	-	46.769	-
+STD L3 (77.8)	62.2845	71.724	115.2%
+STD L4 (233.3)	140.0345	142.964	102.1%
+STD L5 (700)	373.3845	359.668	96.3%
Sample B	-	92.965	-
+STD L3 (77.8)	85.3825	80.456	94.2%
+STD L4 (233.3)	163.1325	147.121	90.2%
+STD L5 (700)	396.4825	359.861	90.8%

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.