

**DEPARTMENT OF PEDIATRICS
ALL INDIA INSTITUTE OF MEDICAL SCIENCES**

27 January 2017

Ref. : Pediatrics/Genetic/Proprietary/Human Fibroblast Growth Factor-21 Elisa kit/2016-17


SUBJECT: Purchase of Human Fibroblast Growth Factor-21 Elisa kit for Genetic Unit, Department of Pediatrics, AIIMS, New Delhi- 29, on proprietary basis- Inviting comments thereon.

The Genetic Unit, Department of Pediatrics has been procuring the above cited item (i.e. **Human Fibroblast Growth Factor-21 Elisa kit (Cat. No. KT879)**) from M/s Epitope Diagnostics Inc., USA on proprietary basis. The proposal has been submitted by M/s Immunoconcept India Pvt. Ltd., F-242A, Street No. 7, Pandav Nagar, Mayur Vihar Phase-I, Delhi- 110091. The proprietary certification and authorization certifications are attached & uploaded on website.

The above document are being uploaded for open information to submit objections, comments, if any, from any manufacturer regarding proprietary nature of the item within 15 days from the date of issue/uploading of the notification giving reference **Pediatrics/Genetic/Proprietary/Human Fibroblast Growth Factor-21 Elisa kit/2016-17**,

The comments should be sent to Dr. Madhulika Kabra, Professor, Genetic Unit, Department of Pediatrics, Old O.T. Block, (behind C.N.Center) AIIMS, New Delhi, on or before **15 February 2017 upto 2:00 p.m.**, failing which it will be presumed that any other vendor is having no comment to offer and case will be decided on merits.

Yours Sincerely,


Dr. Madhulika Kabra
Professor &
Faculty in-charge Genetic Unit

Encl.

1. PAC Certificate
2. Authorization certificate



DL No.: E(0441)15/W/20-B 21-I

IMMUNOCONCEPT INDIA PVT. LTD.Quotation

To,	Ref	VT/ICPL/DRMK/030117
Dr. Madhulika Kabra,	Date	03/01/2017
Division Of Genetics,	Phone	011- 22753711, 64662566,
(Department Of Pediatrics)	Fax	011-22753710
Ansari Nagar	Email	sales@immunoconceptindia.com
AIIMS		harendra@immunoconceptindia.com
New Delhi-110029	Web	www.immunoconceptindia.com
Email;		

Subject: Quotation of FGF 21 ELISA KIT

S. No	Product	Company	Pack Size/Kit	Price/Kit (INR)
01.	Human Fibroblast Growth Factor-21(FGF-21) Elisa Kit Cat No.KT879	Epitope Diagnostics Inc, USA	96 Wells	

TERMS & CONDITIONS:

- VAT@5% Charged extra.
- Supply will be made within 4-5 weeks after receiving your written confirmation order
- Price is valid for 3 Months from the date of quotation issued.
- Payment in Advance.
- If you need any further information, please feel free to contact us.

Thanking You

For Immunoconcept India Pvt. Ltd

Vaishali
VAISHALI TYAGI
(Authorized Signatory)



F-242A, Street No. 7, Pandav Nagar, Mayur Vihar Phase-I, Delhi-110091
E-Mail : sales@immunoconceptindia.com, Web.: www.immunoconceptindia.com
Tel : +91-11-22753711, 43063564, TeleFax : +91-11-22753710
CTN No. : 117400001 2015PTC270472

Date: 2017-01-04

To whom so ever it may concern

It is certified our product given below,

1. Human Fibroblast Growth Factor 21 Elisa Kit Cat#. KT 879 Pack size 1x96 Well/kit

Is unique and propriety item worldwide with aforesaid reference numbers.

It is true and evident with the best of our knowledge.

Thanking You



Stefanie Lenart
Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, USA
Tel: +1-858-693-7877
Fax: +1-858-693-7678



6 JAN 2017

EPITOPE DIAGNOSTICS, INC. IS A US FDA REGISTERED AND STATE OF CALIFORNIA DEPARTMENT OF HEALTH SERVICES FOOD AND DRUG BRANCH LICENSED MEDICAL DEVICE MANUFACTURER



FDA

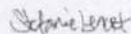
DEVICE MANUFACTURING LICENSE NO: 65362 ISO13485:2003 CERTIFIED

LETTER OF AUTHORIZATION

To whom it may concern:

We, Epitope Diagnostics, Inc., herewith confirm that we have appointed **Immunoconcept India Pvt.Ltd.**, (F-242, Street No.7, Pandav Nagar, Mayur Vihar Phase-1, Delhi-11009 India) as our exclusive authorized distributor in India, to sell your complete range of products in the territorial of India.

This authorization is valid until December 2017



Stefanie Lenart
Epitope Diagnostics, Inc.
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ATTESTED


NOTARY PUBLIC DELHI
GOVT. OF NCT OF DELHI

EPITOPE DIAGNOSTICS, INC. IS A US FDA REGISTERED AND STATE OF CALIFORNIA DEPARTMENT OF HEALTH SERVICES FOOD AND DRUG BRANCH LICENSED MEDICAL DEVICE MANUFACTURER



FDA

DEVICE MANUFACTURING LICENSE NO: 65362 ISO13485:2003 CERTIFIED

15.6 JAN. 2017



TIN No. : 07630410872
DL No.: E(0441)15/W/20-B 21-B

IMMUNOCONCEPT INDIA PVT. LTD.

Date 05.01.2017

RATE - CERTIFICATE (To whom so ever it may concern)

It is certify that the rate quoted in this tender/quotation/Performa Invoice by Immunoconcept India Pvt. Ltd are the best lowest and we have not quoted anywhere(Government/Semi-Government and private in India) lower than quoted in this tender with the best of our knowledge If found so you are free to take action deemed fit against us. In this concern, if you want any clarification; please feel free to contact us.

Thanking You

Vaishali Tyagi
Immunoconcept India Pvt. Ltd.
(Authorized Signatory)



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CIN No. : U74999DL2015PTC279473



Epitope Diagnostics, Inc.

EDI™ Human Intact FGF-21 ELISA Kit

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



KT 879

EU:



12x8



2-8°C

INTENDED USE

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.

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 related to 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).

ASSAY PRINCIPLE

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.

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.

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

1. Anti-Human FGF-21 Antibody Coated Microplate (Cat. No. 30619)

One microplate with 12 x 8 well-breakable strips (96 wells total) coated with antibody to human 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 FGF-21 Tracer Antibody (Cat. No. 30620)

One vial contains 0.4 mL concentrated HRP-labeled anti-human FGF-21 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 contains 8 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 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)

Chisholm



One bottle containing 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 Standards (Cat. No. 30621 – 30626)

Six vials each contain a different concentration 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 standard.** The standards are ready to use. 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. 30627 – 30628)

Two vials each contain a different concentration 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.** 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.

SAFETY PRECAUTIONS

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.

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 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.

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 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.

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 standards (Cat. 30621-30626) and controls (Cat. 30627-30628) 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 (Cat. 30620) by 1:21 fold dilution of the conjugation antibody with the FGF-21 Tracer Antibody Diluent (Cat. 30600). Following is a table that outlines the relationship of strips used and antibody mix prepared.

Strip no.	FGF-21 Tracer Antibody Diluent	FGF-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 µL	225 µL
10	5000 µL	250 µL
11	5500 µL	275 µL
12	6000 µL	300 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. 30619) in a holder to run human intact FGF-21 standards, controls and unknown samples in duplicate.

(2) Test Configuration

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

- (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 μ L of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (7) Add 100 μ L of ELISA HRP Substrate (Cat. 10020) 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 μ L of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
- (10) Read the absorbance at 450/650 nm within 10 minutes in a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all standards, 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 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.

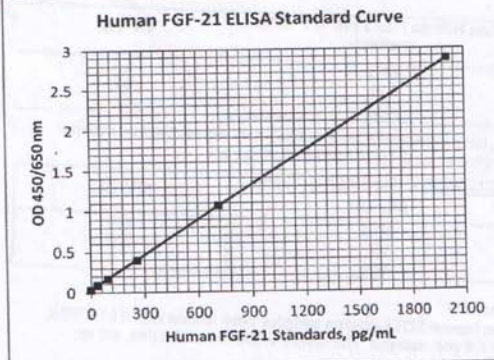
The human intact FGF-21 concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance.

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	
0 pg/mL	0.037 0.036	0.037	0.000	
32.5 pg/mL	0.087 0.086	0.087	0.050	
91 pg/mL	0.172 0.169	0.170	0.133	
255 pg/mL	0.398 0.399	0.399	0.302	
714 pg/mL	1.067 1.069	1.068	1.031	
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



EXPECTED VALUES

Thirty two normal adult plasma samples were measured with this human intact FGF-21 ELISA. 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 or serum samples.

LIMITATION OF THE PROCEDURE

1. 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.
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 inactivate 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. We recommend that all assays include the laboratory's own FGF-21 controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity (LoD)

The sensitivity (lowest limit of detection) of this human intact FGF-21 ELISA as determined by the corresponding OD value of 2 fold standard deviation above the mean on 20 duplicate determination of zero standard is 1.7 pg/mL.

High Dose "hook" effect

This assay has showed that it did not have any high dose "hook" effect up to 20,000 pg/mL.

Precision

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

Mean Human Intact FGF-21 Value (pg/mL)	CV (%)
63.2	5.7
171	4.2
480	5.4

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

Mean Human Intact FGF-21 Value (pg/mL)	CV (%)
69.8	6.9
181	3.0
486	1.9

Linearity

Two human EDTA-plasma samples were diluted with 0.01M PBS, pH 7.4 and assayed. The results in the value of pg/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	286	-	-
	1:2	138	143	96
	1:4	75	72	104
	1:8	37.9	36	105
	1:16	19.5	18	108
2	Neat	61.8	-	-
	1:2	32.1	30.9	104
	1:4	15.9	15.5	103
	1:8	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:

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	45.9 (serum)	91	64.9	68.5	95
		255	150	151	100
		714	388	380	102
2	40.4 (plasma)	91	71.2	65.7	108
		255	148	148	100
		714	406	377	108

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

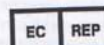
- Yie J, et al. FGF21 N- and C-termini play different roles in receptor interaction and activation. *FEBS Lett.* 2009 Jan 5;583:19-24.
- Micanovic R, et al. Different roles of N- and C-termini in the functional activity of FGF21. *J Cell Physiol.* 2009 May;219(2):227-34.
- Yusuke Murata, et al. FGF21 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 92121, USA



MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
Authorized Representative In Europe	

Human Intact FGF-21 ELISA: Condensed Assay Protocol

- 50 µL Calibrators, controls and test samples
- 50 µL Tracer Antibody

Incubate @ RT for 2 hrs
on ELISA plate shaker
wash 5 x

- 100 µL TMB Substrate

Incubate @ RT for 20 min
static

- 100 µL Stop Solution
Read absorbance at 450/650 nm