Code No. 27997

Human FGF21 Assay Kit - IBL

INTRODUCTION

FGF19 subfamily composed of FGF19, FGF21 and FGF23 binds the receptors, α/βKlotho and it is characterized as a factor group which has hormonal functions. The novel system consisted of α/βKlotho and FGF19 subfamily regulates integrally metabolism of minerals, lipids, energy and amino acids. This novel regulating system involves important homeostatic mechanism for biological body such as metabolism of bile acid and cholesterol in the liver (FGF19), energy and glycolipids metabolism (FGF21) and phosphate, calcium and vitamin D metabolism in kidney (FGF23) in fasting state.

FGF21 is secreted in the liver and it is reported that it has a lipolytic action in fatty tissues. FGF21 binds a receptor consisted of FGFR and βKlotho.
FGF21 reacts in emptiness and it accelerates lipid metabolism in liver, Triacylglycerol (TG) is used as lipid acid and Ketone bodies are produced by the metabolism. As a result, it is used as an energy source. In this process, FGF21 is released and acts as a hormone-like factor.

In relation to the concentration of FGF21 in blood, it is measured and reported that the correlation with many diseases like gestational diabetes mellitus, mitochondrial dysfunction in HIV infection, friedreich ataxia, coronary artery disease, carotid atherosclerosis, non-alcoholic fatty liver disease, rheumatoid arthritis, diabetic nephropathy, type2 diabetes, dyslipidemia and, obesity and metabolic syndrome.

This kit can assay Human blood FGF21 density.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human FGF21.

MEASUREMENT RANGE

31.3 – 2,000 pg/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

This IBL's assay kit is capable for the quantitative determination Human FGF21 in serum, EDTA plasma and cell culture supernatant. Guideline of dilution for serum and EDTA plasma samples of normal human is around 2-8 fold.

KIT COMPONENT

1	Precoated plate	: Anti-Human FGF21(Rf21) Rat IgG MoAb	96Well x 1
2	Labeled antibody Conc. (30X)	HRP conjugated Anti- Human FGF21(Rf4) Rat IgG MoAb Fab'	0.4mL x 1
3	Standard	: Recombinant Human FGF21	0.5mL x 2
4	EIA buffer*		30mL x 1
5	Solution for Labeled antibody*		12mL x 1
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution*		12mL x 1
8	Wash buffer Conc.*		50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

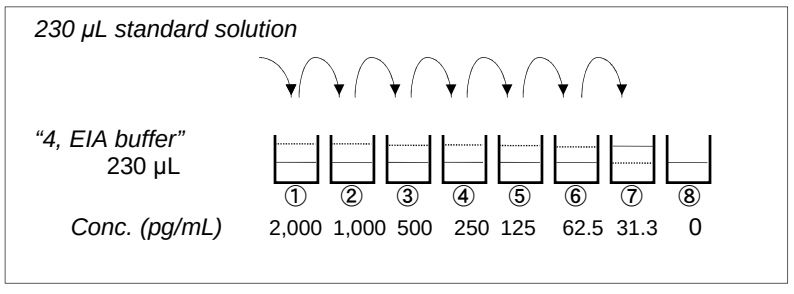
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
<u>This operation should be done just before applying labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 4,000pg/mL Human FGF21 standard.
4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	2,000 pg/mL
Tube-2	1,000 pg/mL
Tube-3	500 pg/mL
Tube-4	250 pg/mL
Tube-5	125 pg/mL
Tube-6	62.5 pg/mL
Tube-7	31.3 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 2,000 pg/mL and 31.3 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.



5) Dilution of test sample
Test samples should be diluted with "4, EIA buffer" suitably.
Guideline of dilution for serum and EDTA plasma samples of normal human is around 2-8 fold.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation O/N at 4 °C with plate lid				
4 times (wash buffer more than 350 µL)				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL)				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate over night at 4°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid.*
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid.*
8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
10) Add 100 µL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the solution. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

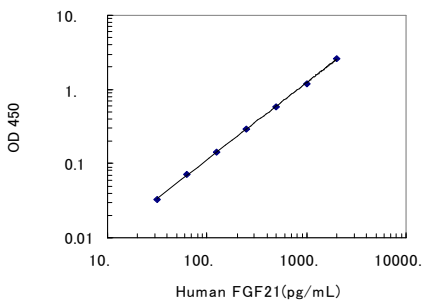
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", suitably.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer in this kit for washing the precoated plate.
Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.
8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

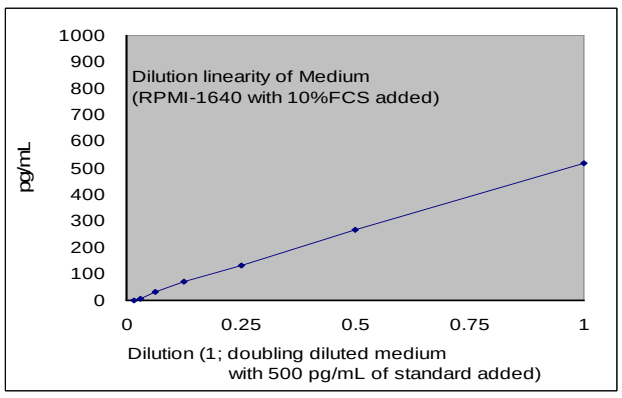
Conc. (pg/mL)	Absorbance (450nm)
2,000	2.642
1,000	1.238
500	0.619
250	0.324
125	0.174
62.5	0.101
31.3	0.062
0 (Test Sample Blank)	0.029

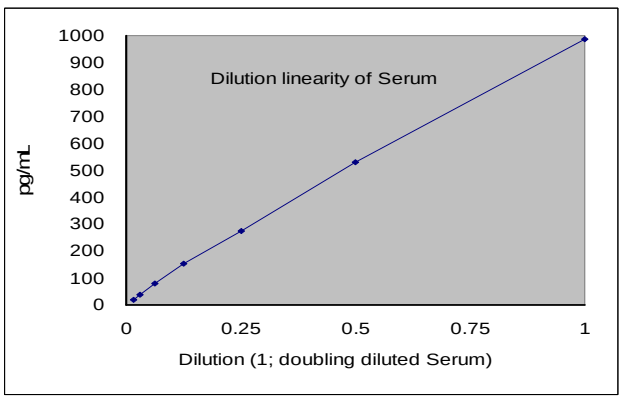


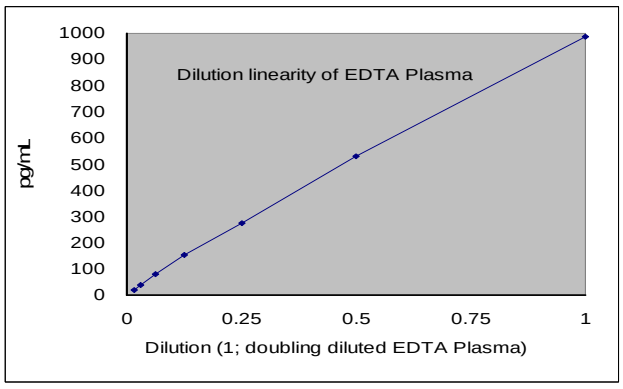
＊ The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay (Samples with standard added are used.)







2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measured Value (pg/mL)	%
Human Serum x2	1464.4	1487.7	101.6
	1214.4	1259.3	103.7
	1089.4	1125.1	103.3
Human Plasma (EDTA) x2	1447.3	1400.6	96.8
	1197.3	1220.9	102.0
	1072.3	1095.8	102.2
Medium (with10%FCS) x2	500	531.5	106.3
	250	294.4	117.8
	125	155.3	124.2

3. Intra - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
1010.3	27.1	2.7	24
238.6	7.1	3.0	24
78.0	4.4	5.6	24

4. Inter - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
1001.5	29.4	2.9	5
242.8	10.1	4.1	5
78.1	5.3	6.8	5

5. Specificity

Substance	Cross-Reactivity
Human FGF21	100%
Human FGF19	<0.1%

6. Sensitivity

29.4 pg/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”, “4, EIA buffer” or “8, Wash buffer Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

REFERENCE

1. Maeda R, Imura A, Nabeshima Y. Complex regulation and diverse functions of alpha-klotho. Contrib Nephrol. 2013;180:25-46.

2. Tomiyama K, Maeda R, Urakawa I, Yamazaki Y, Tanaka T, Ito S, Nabeshima Y, Tomita T, Odori S, Hosoda K, Nakao K, Imura A, Nabeshima Y. Relevant use of Klotho in FGF19 subfamily signaling system in vivo. Proc Natl Acad Sci USA. 2010 Jan 26;107(4):1666-71

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Made in Japan.

IBL FGF related products

Code	Product	Volume
27997	**Human FGF21 Assay Kit-IBL**	**96Well**
27996	Human FGF19 Assay Kit-IBL	96Well
27998	Human soluble α-Klotho Assay Kit - IBL	96Well