

Code No. 27996

Human FGF19 Assay Kit - IBL**INTRODUCTION**

FGF19 subfamily composed of FGF19, FGF21 and FGF23 binds the receptors, α/β Klotho and it is characterized as a factor group which has hormonal functions. The novel system consisted of α/β Klotho 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.

FGF19 is secreted from intestine and transported to liver through bloodstream. It suppresses synthesis of bile acid in liver and regulates the function of liver that preserves sugar as a glycogen. FGF19 binds a receptor consisted of FGFR4 and β Klotho. FGF19 is studied in relation to digestive disorders such as diarrhea and constipation which might be related to FGF19 hormonal abnormality and metabolic disorders such as diabetes.

The concentration of FGF19 in blood is measured and reported its involvement with many disease such as polycystic ovary syndrome history, bile acid diarrhea, coronary artery disease, pediatric nonalcoholic fatty liver disease, impaired fasting glucose, quiescent Crohn's colitis, hepatocellular carcinoma, type 2 diabetic patients with metabolic syndrome and anorexia nervosa.

This kit can assay Human blood FGF19 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 FGF19.

MEASUREMENT RANGE

23.4 ~ 1,500 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 FGF19 in EDTA plasma and cell culture supernatant. Guideline of dilution for EDTA plasma samples of normal human is around 4-16fold.

KIT COMPONENT

1	Precoated plate	: Anti-Human FGF19(12B1)Mouse IgG MoAb	96Well x 1
2	Labeled antibody Conc.	:	
	(30X) HRP conjugated Anti- Human FGF19(16A1)Mouse IgG Fab'		0.4mL x 1
3	Standard	: Recombinant Human FGF19	0.5mL x 2
4	EIA buffer	: 1% BSA, 0.05% Tween20 in PBS	30mL x 1
5	Solution for Labeled antibody	: 1% BSA, 0.05% Tween20 in PBS	12mL x 1
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution	: 1N H ₂ SO ₄	12mL x 1
8	Wash buffer Conc.	: (40X) Phosphate buffer	50mL x 1

OPERATION MANUAL**1. Materials needed but not supplied**

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

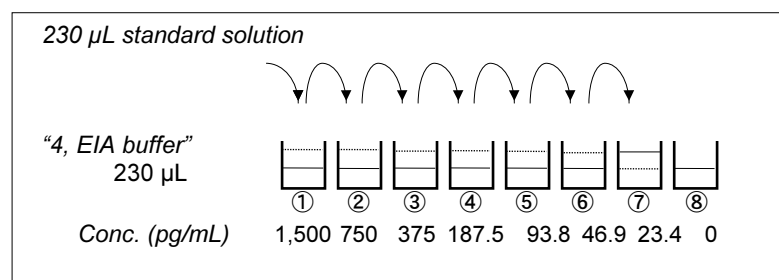
2. Preparation

- 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 μ L. (Dilute 30 μ L of "2, Labeled antibody Conc." with 870 μ L of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μ L in each well.)
This operation should be done just before applying labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard
Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 3,000pg/mL Human FGF19 standard.
- 4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 μ L each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	1,500 pg/mL
Tube-2	750 pg/mL
Tube-3	375 pg/mL
Tube-4	187.5 pg/mL
Tube-5	93.8 pg/mL
Tube-6	46.9 pg/mL
Tube-7	23.4 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 μ L of Standard solution into tube-1 and mix it gently. Then, put 230 μ L of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 1,500 pg/mL and 23.4 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/plasma samples of normal mouse/rat is around 4-16 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 μ L	Diluted standard (Tube 1-7) 100 μ L	EIA buffer (Tube-8) 100 μ L	EIA buffer 100 μ L
Incubation O/N at 4 °C with plate lid				
Washing 4 times				
Labeled Antibody	100 μ L	100 μ L	100 μ L	-
Incubation for 30 minutes at 4°C with plate lid				
Washing 5 times				
Chromogen	100 μ L	100 μ L	100 μ L	100 μ L
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μ L	100 μ L	100 μ L	100 μ L
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 μ L each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μ L 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 each well of the precoated plate 4 times with wash buffer using a washing bottle or a plate washer in following way.
After shaking off (or aspiration of) the solution in wells, fill each well with wash buffer and shake off the wash buffer completely from the precoated plate. This procedure must be repeated 4 times. Then, drain the precoated plate completely on paper towel.
Please refer to 5) and 6) in SPECIAL ATTENTION below, and be careful not to miss a well.
- 5) Pipette 100 μ L 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 precoated plate 5 times in the same manner as 4).
In case of using a plate washer, we recommend manually washing in the manner mentioned above at least the last one time.
- 8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 μ L 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 μ L 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.

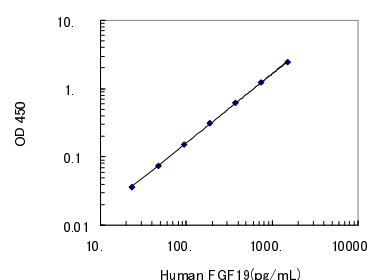
- 6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
- 7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.
- 8) Measurement should be done within 30 minutes after 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)
1,500	2.436
750	1.267
375	0.649
187.5	0.332
93.8	0.175
46.9	0.095
23.4	0.059
0 (Test Sample Blank)	0.023



* 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.

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

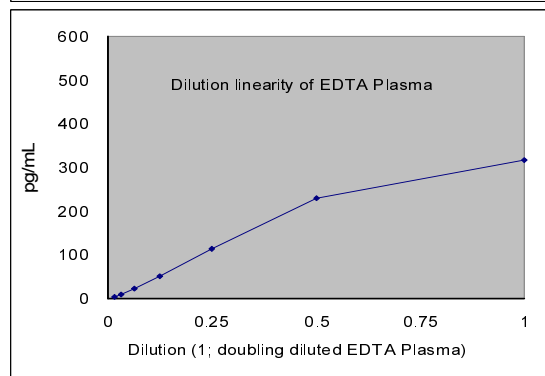
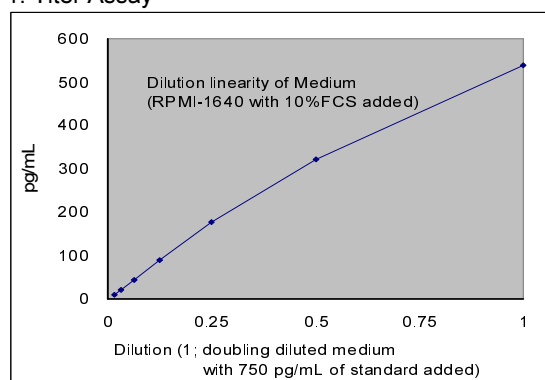
Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

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

PERFORMANCE CHARACTERISTICS

1. Titer Assay



2. Intra - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
721.8	18.5	2.6	5
177.8	4.9	2.8	5
55.7	4.0	7.2	5

3. Inter - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
713.1	16.8	2.4	3
177.5	6.3	3.5	3
56.7	4.2	7.5	3

4. Specificity

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

5. Sensitivity

7.2 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.)

Version 1.

Made in Japan.

IBL FGF related products

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