

Code No. 27998

**Human soluble  $\alpha$ -Klotho Assay Kit - IBL****INTRODUCTION**

" $\alpha$ -klotho" was identified as an extremely down-regulated gene in the genetically-modified mouse of which phenotype is very similar to various symptoms of human aging. Then, the sequences of  $\alpha$ -klotho genes have been identified in various species including humans based on the one of mouse.  $\alpha$ -Klotho protein is a 130 kDa, one-transmembrane protein and its expression is confirmed in the kidneys and the parathyroid glands.

In recent years, it has become clear that  $\alpha$ -Klotho is an important molecule within a living organism regulating the metabolism of mineral such as calcium and phosphorus. Therefore, it is considered that, in  $\alpha$ -klotho mouse, early aging-like symptoms are induced by impaired mineral homeostasis caused by decreased expression of  $\alpha$ -Klotho. Meanwhile, it is reported that the long N-terminal extracellular domain which comprises the major portion of sequence of  $\alpha$ -Klotho protein is released free into blood by shedding. However, there are many unclear points about functions and changes in concentration of free (soluble)  $\alpha$ -Klotho protein, so it has been required to develop the measurement system of  $\alpha$ -Klotho. This ELISA kit can measure Human soluble  $\alpha$ -Klotho protein in human blood.

**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 soluble  $\alpha$ -Klotho.

**MEASUREMENT RANGE**

93.75 – 6,000 pg/mL

**INTENDED USE**

This IBL's assay kit is capable for the quantitative determination human soluble  $\alpha$ -Klotho in serum, plasma and urine.

**KIT COMPONENT**

1	Precoated plate : Anti-Human Klotho (67G3) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human Klotho (91F1) Mouse IgG MoAb Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant human soluble $\alpha$ -Klotho	0.5mL x 2
4	EIA buffer :	30mL x 2
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 <sub>2</sub> SO <sub>4</sub>	12mL x 1
8	Wash buffer Conc. : (40X) 0.05% Tween20 in phosphate buffer	50mL x 1

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

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

**2. Preparation**

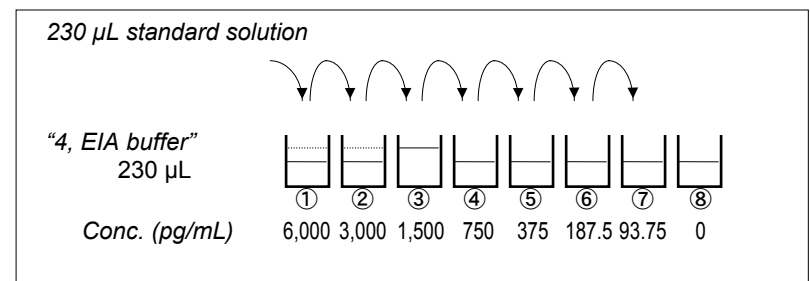
- 1) Preparation of wash buffer  
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This 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  $\mu$ L. (Dilute 30  $\mu$ L of "2, Labeled antibody Conc." with 870  $\mu$ L of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100  $\mu$ L in each well.)  
This operation should be done just before the application of 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 12,000 pg/mL human soluble  $\alpha$ -Klotho standard.
- 4) Dilution of Standard  
Prepare 8 tubes for dilution of "3, Standard". Put 230  $\mu$ L each of "4, EIA buffer" into the tube.  
Specify the following concentration of each tube."

Tube-1	6,000 pg/mL
Tube-2	3,000 pg/mL
Tube-3	1,500 pg/mL
Tube-4	750 pg/mL
Tube-5	375 pg/mL
Tube-6	187.5 pg/mL
Tube-7	93.75 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230  $\mu$ L of Standard solution into tube-1 and mix it gently. Then, put 230  $\mu$ L of tube-1 mixture into tube-2. Dilute two times standard solution in series to set

up 7 points of diluted standard between 6,000 pg/mL and 93.75 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.

**5) Dilution of test sample**

Test samples need to be diluted with "4, EIA buffer" accordingly. The recommended dilution for serum, plasma or urine samples is 2 - 4 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 $\mu$ L	Diluted standard (Tube 1-7) 100 $\mu$ L	EIA buffer (Tube-8) 100 $\mu$ L	EIA buffer 100 $\mu$ L
Incubation for 60 minutes at room temperature with plate lid				
Washing 7 times				
Labeled Antibody	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L	-
Incubation for 30 minutes at room temperature with plate lid				
Washing 9 times				
Chromogen	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L	100 $\mu$ 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  $\mu$ L each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100  $\mu$ 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 for 60 minutes at room temperature after covering it with plate lid.
- 4) Wash each well of the precoated plate vigorously with wash buffer using the washing bottle. Then, fill each well with wash buffer and leave the precoated plate laid for 15-30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.  
*In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.*
- 5) Pipette 100  $\mu$ 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 room temperature after covering it with plate lid.
- 7) Wash the precoated plate 9 times in the same manner as 4).
- 8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100  $\mu$ L from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of "6, Chromogen".
- 10) Pipette 100  $\mu$ L of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid 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 liquid. 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. Especially, urine samples are less stable than serum or plasma samples against temperature and freeze/thawing. Therefore, freeze urine samples rapidly after collection and avoid refreezing.
- 2) Test samples need to be diluted with "4, EIA buffer", as the need arises.
- 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 contained 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.

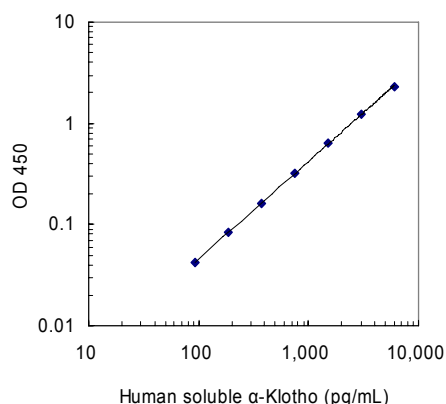
- 7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
- 8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

**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

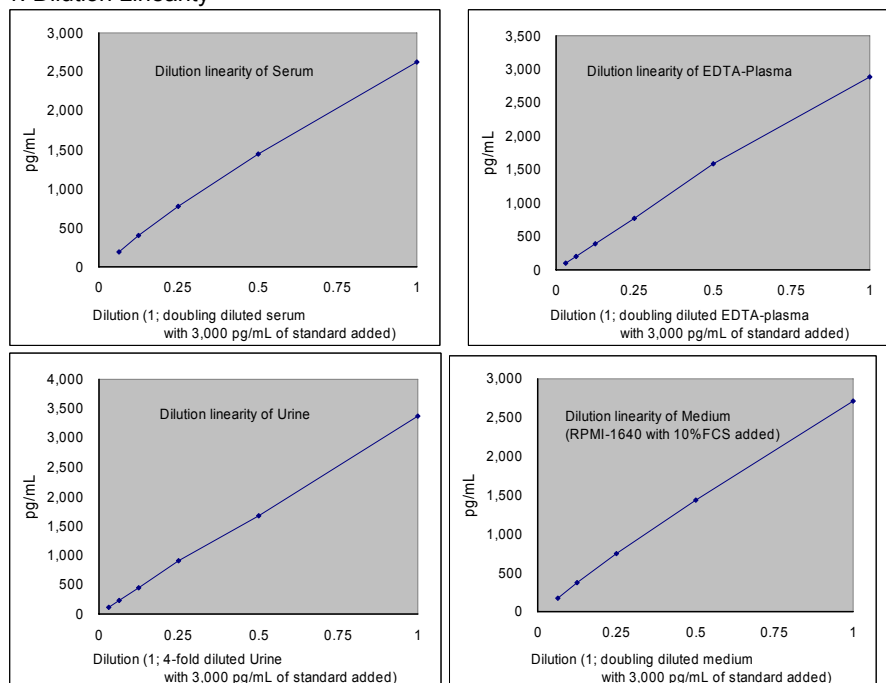
Conc. (pg/mL)	Absorbance (450nm)
6,000	2.307
3,000	1.245
1,500	0.635
750	0.324
375	0.166
187.5	0.087
93.75	0.046
0 (Test Sample Blank)	0.004



\* 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. Dilution Linearity**



**2. Added Recovery Assay**

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
Human Serum (x4)	1764.53	1494.92	84.7
	1014.53	924.85	91.2
	639.53	602.29	94.2
	452.03	419.16	92.7
Human Plasma (EDTA) (x4)	1770.47	1677.46	94.7
	1020.47	984.78	96.5
	645.47	629.21	97.5
	457.97	435.74	95.1
Human Urine (x2)	1500.00	1349.55	90.0
	750.00	713.85	95.2
	375.00	355.67	94.8
	187.50	181.27	96.7
10%FCS added RPMI-1640 (x2)	1500.00	1349.55	90.0
	750.00	713.85	95.2
	375.00	355.67	94.8
	187.50	181.27	96.7

**3. Intra - Assay**

Measurement Value (pg/mL)	SD value	CV value (%)	n
2968.78	92.26	3.1	24
757.34	20.65	2.7	24
186.64	6.62	3.5	24

**4. Inter - Assay**

Measurement Value (pg/mL)	SD value	CV value (%)	n
2903.01	85.44	2.9	5
706.32	45.72	6.5	5
165.47	18.82	11.4	5

**5. Specificity**

Compound	Cross Reactivity
Human $\alpha$ -Klotho	100 %
Human osteopontin	<0.1 %
Human VEGF (165)	<0.1 %
Human PDGF	<0.1 %

**6. Sensitivity**

6.15 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.", 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. Yamazaki Y, Imura A, Urakawa I, Shimada T, Murakami J, Aono Y, Hasegawa H, Yamashita T, Nakatani K, Saito Y, Okamoto N, Kurumatani N, Namba N, Kitaoka T, Ozono K, Sakai T, Hataya H, Ichikawa S, Imel EA, Econs MJ, Nabeshima Y. Establishment of sandwich ELISA for soluble alpha-Klotho measurement: Age-dependent change of soluble alpha-Klotho levels in healthy subjects. Biochem Biophys Res Commun. 2010 Jul 30;398(3):513-8.

Version 1.3

Made in Japan.