PRODUCT INFORMATION & MANUAL

Human Klotho Valukine™ ELISA

VAL147

For the quantitative determination of natural and recombinant human Klotho concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version202104.1

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I. BACKGROUND

Klotho, also called Klotho-alpha, is the founding member of the Klotho family within the glycosidase-1 superfamily (1, 2). Klotho is expressed in areas concerned with calcium regulation, predominantly in the kidney distal convoluted tubules, but also in the brain choroid plexus (which produces cerebrospinal fluid) and the parathyroid (1). The 1012 amino acid (aa) type I transmembrane protein contains a 32 aa signal sequence, a 948 aa extracellular domain (ECD) containing two extracellular glycosidase-like domains, a 21 aa transmembrane domain and an 11 aa intracellular domain. Within the ECD, human Klotho shares 87%, 90%, 90% and 86% aa identity with mouse, rat, bovine and equine Klotho, respectively. Although a truncated 549 aa isoform predicts a soluble 70 kDa form, the form found in plasma and cerebrospinal fluid is a 130 kDa form produced by proteolytic cleavage of the glycosylated 135 kDa full-length Klotho (3, 4). A prominent intracellular 120 kDa form of Klotho is localized to endoplasmic reticulum and Golgi membranes (4).

The phenotype of Klotho - deficient mice resembles premature aging, including arteriosclerosis, osteoporosis, skin atrophy, infertility, emphysema and premature death (2). Conversely, excess Klotho extends lifespan (5). Klotho acts as a cofactor for interaction of FGF23 with FGF R1 (6). This interaction negatively regulates 1 alpha -hydroxylase, the rate - limiting enzyme in the synthesis of 1,25(OH)2D3 (vitamin D) (7). Klotho - deficient mice show severe hyperphosphatemia and ectopic calcification of soft tissues due to excess vitamin D (2, 7). Both Klotho and Klotho-beta are cofactors for FGF19 binding (8). Klotho also shows glucuronidase activity which activates the renal ion channel TRPV5 to reabsorb urinary calcium (9). Klotho has been reported to downregulate insulin or IGF-1 signaling in adipocytes, to bind and antagonize Wnt molecules, and to facilitate release of parathyroid hormone (10-12).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Klotho has been pre-coated onto a microplate. Standards, samples and a biotinylated detection antibody specific for human Klotho are pipetted into the wells and any Klotho present is bound by the immobilized antibody. After washing away any unbound substances, streptavidin-HRP are pipetted into the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Klotho bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate and human serum.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	207.5	625.5	2266.9	199.4	609.3	2214.8
Standard Deviation	6.7	10.4	62.3	11.0	20.1	77.0
CV%	3.2	1.7	2.7	5.5	3.3	3.5

B. RECOVERY

The recovery of human Klotho spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 117.3-122.4% with an average of 120.3%.

The recovery of human Klotho spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 90.3-111.4% with an average of 100.0%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human Klotho is typically less than 5.2 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified *E. Coli*-expressed recombinant human Klotho produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Klotho and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	100.3	92.8-105.3
1:4	101.8	94.2-113.0
1:8	105.4	96.4-122.0
1:16	105.8	92.0-120.6

F. SAMPLE VALUES

Serum - Four human serum samples were evaluated for the presence of human Klotho in this assay. Two samples measured less than the lowest human Klotho standard, 71.6 pg/mL, and two samples measured 31.7 pg/mL and 104.6 pg/mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

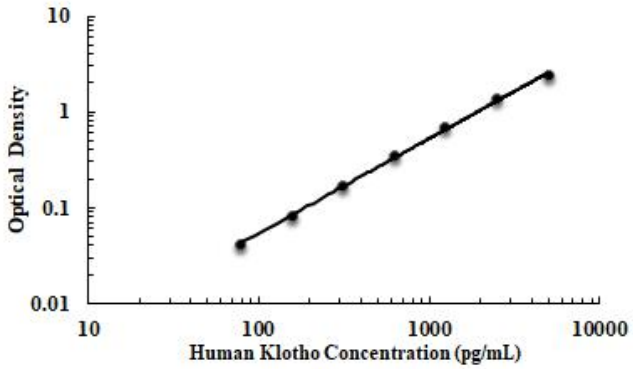
Recombinant human	Recombinant Mouse
β -Klotho	Klotho
FGF-19	
FGF-23	
FGF R1α (IIIb)/Fc Chimera	
FGF R1α (IIIc)/Fc Chimera	
FGF R1β (IIIb)/Fc Chimera	
FGF R1β (IIIc)/Fc Chimera	

A sample containing 12.5 ng/mL of recombinant rat Klotho reads as 750 pg/mL (6.0% cross-reactivity)

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



pg/mL	OD	Average	Corrected
0	0.025 0.026	0.026	-
78.1	0.065 0.068	0.067	0.041
156.3	0.107 0.109	0.108	0.083
312.5	0.193 0.199	0.196	0.171
625	0.372 0.374	0.373	0.348
1250	0.698 0.706	0.702	0.677
2500	1.374 1.382	1.378	1.353
5000	2.349 2.436	2.393	2.367

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Klotho Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a Rat antibody against Human Klotho.	1 plate
Human Klotho Standard	Recombinant human Klotho in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human Klotho Detection Antibody	Biotinylated Klotho antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (2×)	Concentrated buffered diluent used to dilute standard and samples.	1 vial
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
Color Reagent A	Stabilized hydrogen peroxide.	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine).	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="5">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent (2×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.
- 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

B. SAMPLE PREPARATION

Cell Culture Supernates samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×).

Serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

Detection Antibody- Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Reagent Diluent (1×) to the working concentration of 2 µg/mL. Prepare at least 15 minutes prior to use.

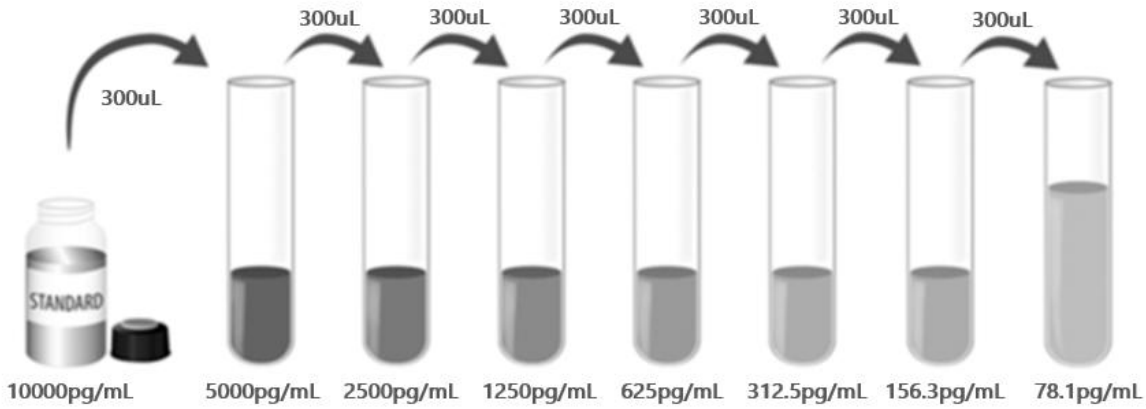
Streptavidin-HRP B (1×) **-** Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Human Klotho Standard - Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 10000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*if you have any question, please seek help from our Technical Support.

Pipette 300 µL of the appropriate Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. The Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

VII.ASSAY PROCEDURE

Note: *Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of standard, or prepared sample per well. Add 50 µL of the Detection Antibody diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm.** A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 20 minutes at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm.** Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm.** Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections

in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

10. CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Klotho concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

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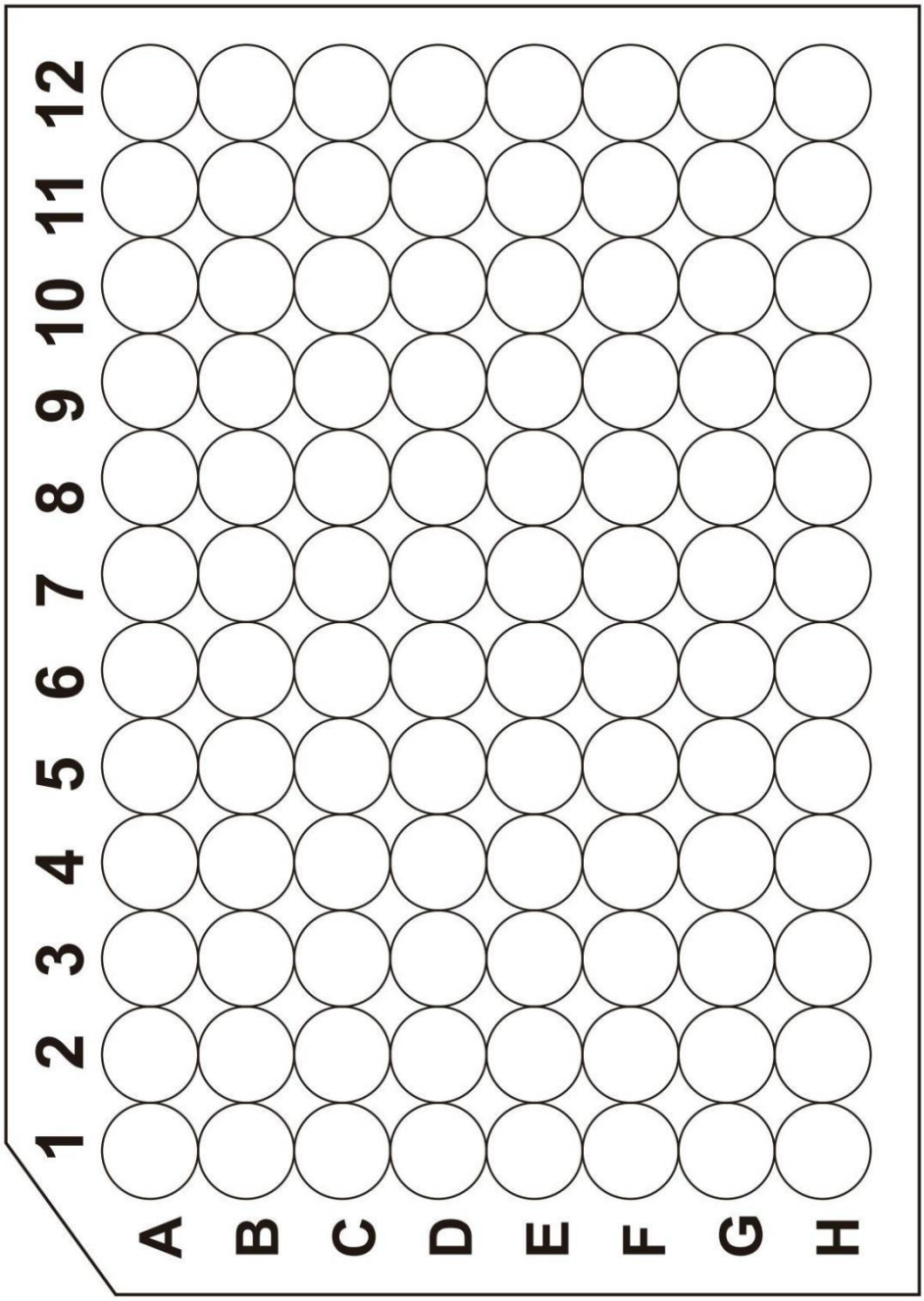
2. Kuro-o, M. et al. (1997) Nature 390:45.
3. Shiraki-Iida, T. et al. (1998) FEBS Lett. 424:6.
4. Imura, A. et al. (2004) FEBS Lett. 565:143.
5. Kurosu, H. et al. (2005) Science 309:1829.
6. Kurosu, H. et al. (2006) J. Biol. Chem. 281:6120.
7. Tsujikawa, H. et al. (2003) Mol. Endocrinol. 17:2393.
8. Wu, X. et al. (2007) J. Biol. Chem. 282:29069.
9. Chang, Q. et al. (2005) Science 310:490.
10. Yamamoto, M. et al. (2005) J. Biol. Chem. 280:38029.
11. Liu, H. et al. (2007) Science 317:803.
12. Imura, A. et al. (2007) Science 316:1615.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Klotho Valukine™ ELISA 试剂盒

目录号：VAL147

适用于定量检测天然和重组人 Klotho 的浓度

科研专用，不可用于临床诊断

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有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

目录

I. 背景

Klotho也叫Klotho-alpha，是糖苷酶-1超家族中Klotho家族的创始成员 (1, 2)。Klotho在与钙调节有关的区域表达，主要在肾远端曲小管，也在脑脉络丛(产生脑脊液)和甲状旁腺中表达 (1)。它是1012个氨基酸(aa)的I型跨膜蛋白，包含一个32 aa的信号序列，一个948 aa的胞外结构域(ECD)，ECD包括两个胞外糖苷酶样结构域，一个21 aa的跨膜域和一个11 aa的胞内域。在ECD区域内，人Klotho与小鼠、大鼠、牛和马分别具有87%、90%、90%和86%的氨基酸同源性。虽然有一个截短形式的549 aa被预测为可溶性70 kDa亚型，但在血浆和脑脊液中发现的形式是由135 kDa糖基化的全长Klotho蛋白水解断裂而产生的130 kDa亚型(3, 4)。Klotho在细胞内主要以120kDa的亚型存在于内质网和高尔基体膜上(4)。

Klotho缺陷小鼠的表型类似于早衰，包括动脉硬化、骨质疏松、皮肤萎缩、不孕不育、肺气肿和早亡(2)。相反，过量的Klotho可延长寿命(5)。Klotho是FGF23与FGF R1相互作用的辅助因子(6)。这种相互作用负调控1α -羟化酶，这是合成维生素D (1,25 (OH)2D3)的限速酶(7)。Klotho缺陷小鼠由于维生素D过量而出现严重高磷血症和软组织异位钙化(2, 7)。Klotho和Klotho-beta都是FGF19结合的辅助因子(8)。Klotho还显示了葡萄糖苷酸酶的活性，它可以激活肾脏离子通道TRPV5来重新吸收尿钙(9)。也有报道称Klotho可以下调脂肪细胞中的胰岛素或IGF-1信号，结合和拮抗Wnt分子，促进甲状旁腺激素的释放(10-12)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Klotho抗体包被于微孔板上，样品和标准品中的人Klotho会与固定在板上的抗体结合，接着加入生物素化的抗人Klotho检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本和人血清样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释剂（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的储存等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	207.5	625.5	2266.9	199.4	609.3	2214.8
标准差	6.7	10.4	62.3	11.0	20.1	77.0
CV%	3.2	1.7	2.7	5.5	3.3	3.5

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人Klotho，测定其回收率。回收率范围在117.3-122.4%，平均回收率在120.3%。

在人血清样本中掺入检测范围内不同水平的人Klotho，测定其回收率。回收率范围在90.3-111.4%，平均回收率在100.0%。

C. 灵敏度

人Klotho的最低可测剂量（MDD）一般小于5.2pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此ELISA试剂盒经由R&D Systems生产的大肠杆菌表达的高纯度重组人Klotho蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人Klotho，然后用标准品稀释剂（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值(%)	范围 (%)
1:2	100.3	92.8-105.3
1:4	101.8	94.2-113.0
1:8	105.4	96.4-122.0
1:16	105.8	92.0-120.6

F. 样本预值

血清样本 - 使用本试剂盒检测了4份人血清样本中Klotho的水平，其中2份样本的检测值均低于人Klotho最低标准品71.6pg/mL，2份样本的检测值分别为31.7pg/mL和104.6 pg/mL。

G. 特异性

将以下因子配置成50ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

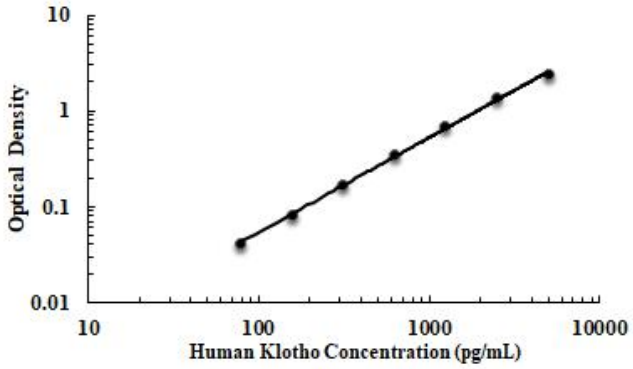
Recombinant human	Recombinant Mouse
β-Klotho	Klotho
FGF-19	
FGF-23	
FGF R1α (IIIb)/Fc Chimera	
FGF R1α (IIIc)/Fc Chimera	
FGF R1β (IIIb)/Fc Chimera	
FGF R1β (IIIc)/Fc Chimera	

含有12.5 ng/mL重组大鼠Klotho 的样品测量值为750pg/mL（6.0%交叉反应）。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



pg/mL	OD	Average	Corrected
0	0.025 0.026	0.026	-
78.1	0.065 0.068	0.067	0.041
156.3	0.107 0.109	0.108	0.083
312.5	0.193 0.199	0.196	0.171
625	0.372 0.374	0.373	0.348
1250	0.698 0.706	0.702	0.677
2500	1.374 1.382	1.378	1.353
5000	2.349 2.436	2.393	2.367

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Klotho Microplate	包被大鼠抗人 Klotho 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human Klotho Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human Klotho Detection antibody	生物素化的 Klotho 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）	1 瓶
Calibrator Diluent (2×)	浓缩的标准品稀释剂（2×）	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
Color Reagent A	显色液 A	1 瓶
Color Reagent B	显色液 B	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="9">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP B</td><td rowspan="5">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释剂（2×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×标准品稀释剂</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封：2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450nm检测波长的吸收值及540nm或570nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 振荡器（速度可调至500±50 rpm）
- 500mL量筒

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- Color Reagent B可能引起皮肤、眼睛和呼吸道刺激，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃冰箱内，避免反复冻融。样本可能需要用标准品稀释剂（1×）稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 x g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。

B. 样本准备工作

细胞上清样本需要用标准品稀释剂（1×）2倍稀释后进行检测，例如：100μL样本+100μL标准品稀释剂（1×）。

血清样本需要用标准品稀释剂（1×）2倍稀释后进行检测，例如：100μL样本+100μL标准品稀释剂（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温

洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20mL浓缩洗涤液用蒸馏水或去离子水稀释配置成500mL工作浓度的洗涤液。未用完的放回4 ℃。

标准品稀释剂（1×）：使用蒸馏水或去离子水稀释配置成标准品稀释剂（1×）。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配置成试剂稀释液（1×）。

检测抗体：参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶。再用检测试剂稀释液（1×）稀释至工作浓度2μg/mL，至少在使用前15分钟准备。

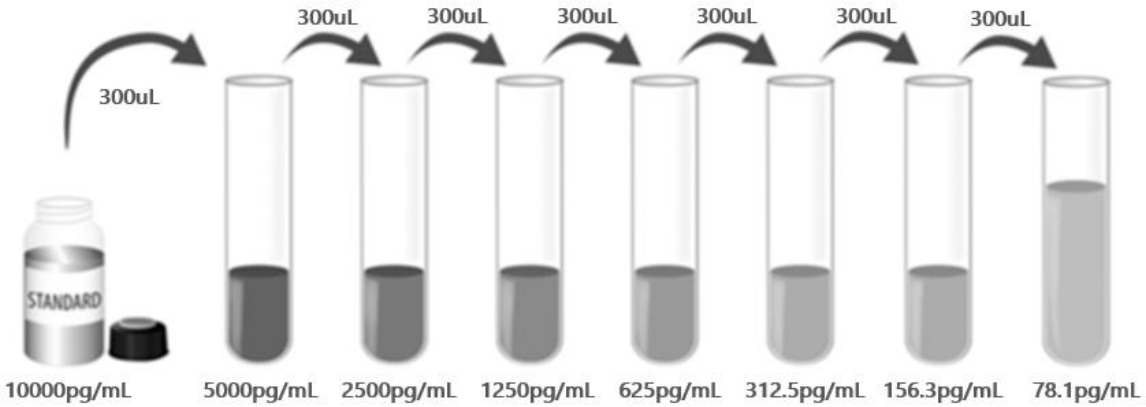
链霉亲和素- HRP B：用试剂稀释液（1×）将链霉亲和素- HRP B（40×）稀释至工作浓度链霉亲和素- HRP B（1×）。

显色剂：按试验所需用量（100 μL/孔）将显色液A和显色液B等体积混合，避光保存，现用现配，须在15分钟内使用。

人Klotho 标准品：冻干标准品的重溶体积请参考瓶身标签，得到浓度为 10000 pg/mL 标准品母液。轻轻震摇至少 15 分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入 300 μL标准品稀释剂（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。5000 pg/mL管作标准曲线最高点，标准品稀释剂（1×）可用作标准品零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板胶纸可保证结果的准确性；
- 混合后的显色底物在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同显色底物上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII.操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔100μL。然后每个微孔内加入50μL配置好的检测抗体，用封板膜封住反应孔，**室温500±50rpm水平振荡孵育2小时。**说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；（样本需要稀释，详情参见样本制备部分。）
4. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 μL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
5. 在每个微孔内加入100 μL稀释好的链霉亲和素- HRP B 工作液。用封板膜封住反应孔，**室温500±50rpm水平振荡孵育20分钟，注意避光；**
6. 重复第4步洗板操作 ；
7. 在每个微孔内加入100 μL显色剂，**室温500±50rpm水平振荡孵育30分钟，注意避光；**
8. 在每个微孔内加入50 μL终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色；
9. 加入终止液后30分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果没有使用双波长校正，结果准确度可能会受影响；
10. **计算结果：**将每个标准品和样品的校正吸光度值（OD450-OD540/OD570），复孔读数取平均值，然后减去平均零标准品OD值。使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。通过样本的OD值，可从标准曲线上得到样本中人Klotho的浓度。如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置