Version 25-02/25

User's Manual

Human Soluble Klotho ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This Human Soluble Klotho ELISA Kit contains the necessary components required for the quantitative measurement of natural human soluble Klotho from cell culture supernates and serum in a sandwich ELISA format.

This immunoassay contains human soluble Klotho and antibodies specifically raised against human soluble Klotho protein. Results from this immunoassay have shown to accurately quantify natural Soluble Klotho samples.

Principles of Testing

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Soluble Klotho. The capture antibody can bind to the human Soluble Klotho in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Soluble Klotho is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human Soluble Klotho bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

Reagents And Materials Provided

1. Soluble Klotho Microplate - 96 well polystyrene microplate coated with an antibody against human Soluble Klotho. 1 plate
2. Soluble Klotho Standard - 20 ng/vial of recombinant human Soluble Klotho in a buffered protein base with preservative; lyophilized. 1 vial
3. Detection Antibody Concentrate - 1.2 mL/vial, 10-fold concentrate of purified antibody biotinylated against human Soluble Klotho with preservative; lyophilized. 1 vial
4. Positive Control - one vial of human Soluble Klotho; lyophilized. 1 vial
5. Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP. 1 vial
6. Dilution Buffer- 60 mL of buffered protein based solution with preservative. 1 bottle
7. Wash Buffer - 50 mL of 10- fold concentrated buffered surfactant, with preservative. 1 bottle
8. TMB Substrate Solution - 11 mL of TMB substrate solution. 1 bottle
9. Stop Solution - 11 mL of 0.5M HCl. 1 bottle
10. Plate Sealer: 1
11. Plastic Pouch: 1

Materials Required But Not Supplied

 Microplate reader capable of absorbance measurement at 450 nm.

 Microplate shaker (250 - 300 rpm).

 Microplate washer or manifold dispenser.

 100 mL and 500 mL graduated cylinders.

 Multi-channel Pipette, Pipettes and pipette tips.

 Deionized or distilled water.

Storage

Unopened Kit: Store at 2 - 8°C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Detection Antibody concentrated solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution (protect from light) and other components may be stored at 2 - 8°C for up to 8 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

Specimen Collection And Preparation

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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes 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.

SAMPLE PREPARATION:

Serum samples may require 2 fold dilution. A suggested 2-fold dilution is 120 µL sample + 120 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

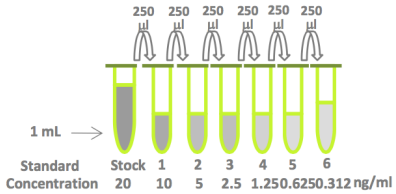
Reagent Preparation

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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Soluble Klotho Standard - Reconstitute the Soluble Klotho standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 20 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1-6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	20 ng/ml
# 1	250µl of stock	250µl	10 ng/ml
# 2	250µl of 1	250µl	5 ng/ml
# 3	250µl of 2	250µl	2.5 ng/ml
# 4	250µl of 3	250µl	1.25 ng/ml
# 5	250µl of 4	250µl	0.625 ng/ml
# 6	250µl of 5	250µl	0.3125 ng/ml



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 10.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Transfer 120 µL of 100- fold concentrated Streptavidin-HRP Conjugate stock solution to 11.88 mL of Dilution Buffer to prepare working solution. Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).

Assay Procedure

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

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 plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker (250-300rpm) at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin HRP Conjugate working solution to each well. Incubate for 1 hour on microplate shaker at room temperature. Protect from light.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 14-16 minutes on microplate shaker at room temperature. Protect from light.
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

Calculation

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x- axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

Typical Standard Curve

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.054)
0.313	0.039
0.625	0.072
1.25	0.147
2.5	0.289
5	0.592
10	1.031
20	1.602

Precision

Intra-assay Precision: 4-8%; Inter-assay Precision: 10-12%

Detection Range

0.313-20 ng/mL

Sensitivity

60 pg/mL

Specificity

PROTEINS CROSS-REACTIVITY

Human Soluble Klotho rec: 100%

Human FGF-23: 0

Human soluble klotho (extracellular domain) derived from E. Coli may not be detected by this elisa kit.

Precautions

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

Limitations

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.