

HUMAN SECRETED KLOTHO ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN SECRETED KLOTHO
CONCENTRATIONS IN SERUM AND EDTA
PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL
PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

| | |
|--|---|
| ELISA NAME | HUMAN SECRETED KLOTHO ELISA |
| Catalog No. | SK00708-06 |
| Lot No. | |
| Formulation | 96 T |
| Standard range | 625 - 40000 pg/mL |
| Sensitivity | 100 pg/mL |
| Sample Volume | 100 µL |
| Sample Type | Serum, EDTA Plasma |
| Dilution Factor | Optimal dilutions should be determined by each laboratory for each application |
| Specificity | Human secreted klotho |
| Calibration | Human secreted klotho recombinant |
| Intra-assay Precision | 4 - 6% |
| Inter-assay Precision | 8 - 12% |
| Storage | 2 – 8° C for 1 month. See page 2 for detail |
| This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol. | |

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DESCRIPTION

This Human Secreted Klotho ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human secreted klotho from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human secreted klotho and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural secreted klotho samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human secreted klotho. The capture antibody can bind to the human secreted klotho in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human secreted 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 secreted klotho bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL 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.

_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.

COMPONENTS PROVIDED

| DESCRIPTION | CODE | QUANTITY |
|---|------------------|-----------------|
| Klotho Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against secreted klotho. | 708-06-01 | 1 plate |
| Klotho Standard – refer to lot of recombinant human secreted klotho in a buffered protein base with preservative; lyophilized. | 708-06-02 | 1 vial |
| Detection Antibody Concentrate – refer to lot of biotinylated antibody against klotho with preservative; lyophilized. | 708-06-03 | 1 vial |
| Positive Control - one vial of recombinant human secreted klotho; lyophilized. | 708-06-04 | 1 vial |
| Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP. | SAHRP | 1 vial |
| Dilution Buffer – 40 mL of buffered protein based solution with preservative. | DB01 | 1 bottle |
| HRP Diluent Solution – 12 mL of buffered protein based solution with preservative. | DB68C | 1 bottle |
| Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative. | WB01 | 1 bottle |
| TMB Substrate Solution -11 mL of TMB substrate solution. | TMB01 | 1 bottle |
| Stop Solution - 11 mL of 0.5M HCl. | S-STOP | 1 bottle |
| Plate Sealer | EAPS | 1 piece |
| Plastic Pouch | P01 | 1 piece |

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent

Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2-8 °C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- 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.

PRECAUTION

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.

SAMPLE COLLECTION AND STORAGE

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.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

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.

Klotho Standard - Reconstitute the Klotho standard with refer to lot of Dilution Buffer. This reconstitution produces a stock solution of 40000 pg/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 (DB01) into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **40000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

| TUBE | STANDARD | DILUTION BUFFER | CONCENTRATION |
|-------|-----------------|-----------------|---------------|
| stock | Powder | Refer to lot | 40000 pg/ml |
| # 1 | 250 µl of stock | 250 µl | 20000 pg/ml |
| # 2 | 250 µl of 1 | 250 µl | 10000 pg/ml |
| # 3 | 250 µl of 2 | 250 µl | 5000 pg/ml |
| # 4 | 250 µl of 3 | 250 µl | 2500 pg/ml |
| # 5 | 250 µl of 4 | 250 µl | 1250 pg/ml |
| # 6 | 250 µl of 5 | 250 µl | 625 pg/ml |

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with refer to lot of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 ml of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of HRP Diluent Solution (DB68C) into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Protect from light.**

ELISA PROTOCOL

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. Add 100 µL of **Dilution Buffer (DB01)** to Blank wells.
3. 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 at room temperature.
4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 5 min.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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.

TYPICAL DATA

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

| STANDARD (PG/ML) | AVERAGE OD450NM (CORRECTED) |
|------------------|-----------------------------|
| Blank | 0 (refer to lot) |
| 625 | 0.033 |
| 1250 | 0.067 |
| 2500 | 0.129 |
| 5000 | 0.259 |
| 10000 | 0.499 |
| 20000 | 0.860 |
| 40000 | 1.656 |

SUMMARY OF ASSAY PROCEDURE

