Human Klotho ELISA Kit

Enzyme Immunoassay for the quantification of Human Klotho in serum, plasma, cell culture supernatants

Catalog number: ARG81264

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

This gene encodes a type-I membrane protein that is related to beta-glucosidases. Reduced production of this protein has been observed in patients with chronic renal failure (CRF), and this may be one of the factors underlying the degenerative processes (e.g., arteriosclerosis, osteoporosis, and skin atrophy) seen in CRF. Also, mutations within this protein have been associated with ageing and bone loss. [provided by RefSeq, Jul 2008]

May have weak glycosidase activity towards glucuronylated steroids. However, it lacks essential active site Glu residues at positions 239 and 872, suggesting it may be inactive as a glycosidase in vivo. May be involved in the regulation of calcium and phosphorus homeostasis by inhibiting the synthesis of active vitamin D (By similarity). Essential factor for the specific interaction between FGF23 and FGFR1 (By similarity).

The Klotho peptide generated by cleavage of the membrane-bound isoform may be an anti-aging circulating hormone which would extend life span by inhibiting insulin/IGF1 signaling. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Klotho has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Klotho present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Klotho is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-

enzyme reagent, a substrate solution (TMB) 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 by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Klotho in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 5 ng/vial	4°C
Standard/Sample diluent	1 X 16 ml	4°C
Antibody conjugate concentrate	2 vial (60 µl)	4°C
Antibody diluent buffer	16 ml	4°C
HRP-Streptavidin concentrate	2 vial (60 µl)	4°C (Protect from light)
HRP-Streptavidin diluent buffer	16 ml	4°C
20X Wash buffer	25 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)

- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

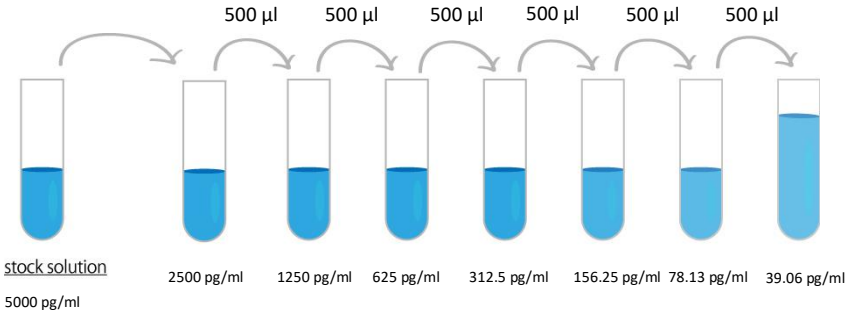
minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge (1000 x g) for 15 minutes at 2-8 °C within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Antibody conjugate: Dilute 100X antibody conjugate concentrate into 1X antibody diluent buffer with Antibody diluent buffer to yield 1X detection antibody solution.
- 1X HRP-Streptavidin Solution: Dilute 100X HRP-Streptavidin concentrate solution into 1X HRP-Streptavidin diluent buffer with HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.
- Sample: If the initial assay found samples contain Klotho higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
  (It is recommended to do pre-test to determine the suitable dilution factor).
- Standards: Reconstitute the standard with 1 ml Standard/Sample diluent to yield a stock concentration of 5000 pg/ml. Allow the stock standard to

sit for at least 15 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml, 78.13 pg/ml, 39.06 pg/ml.



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (Standard/Sample diluent) into wells. Incubate for 1.5 h at 37 °C.
3. Aspirate each well and wash, repeating the process three times for a total four washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of

liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

4. Add 100 µl 1X Antibody conjugate into each well. Cover wells and incubate for 1 hour at 37 °C.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well. Cover wells and incubate for 30 minutes at 37 °C.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well. Incubate for 10-20 minutes at 37°C in dark.
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing
10. Read the OD with a microplate reader at 450nm immediately. (optional: read at 610-650 nm as the reference wave length)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the

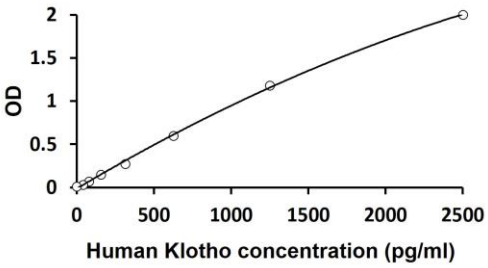
corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human Klotho ranged from 39- 2500 pg/ml. The mean MDD was 40 pg/ml.

Specificity

This assay recognizes natural and recombinant Human Klotho. No significant cross-reactivity or interference with the factors below was observed:

50 ng/ ml of recombinant proteins:

Human: IL1 alpha, IL2, IL4, IL6, IL8, IL10, TNF alpha, IFN gamma

Mouse: IL2, IL6

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.