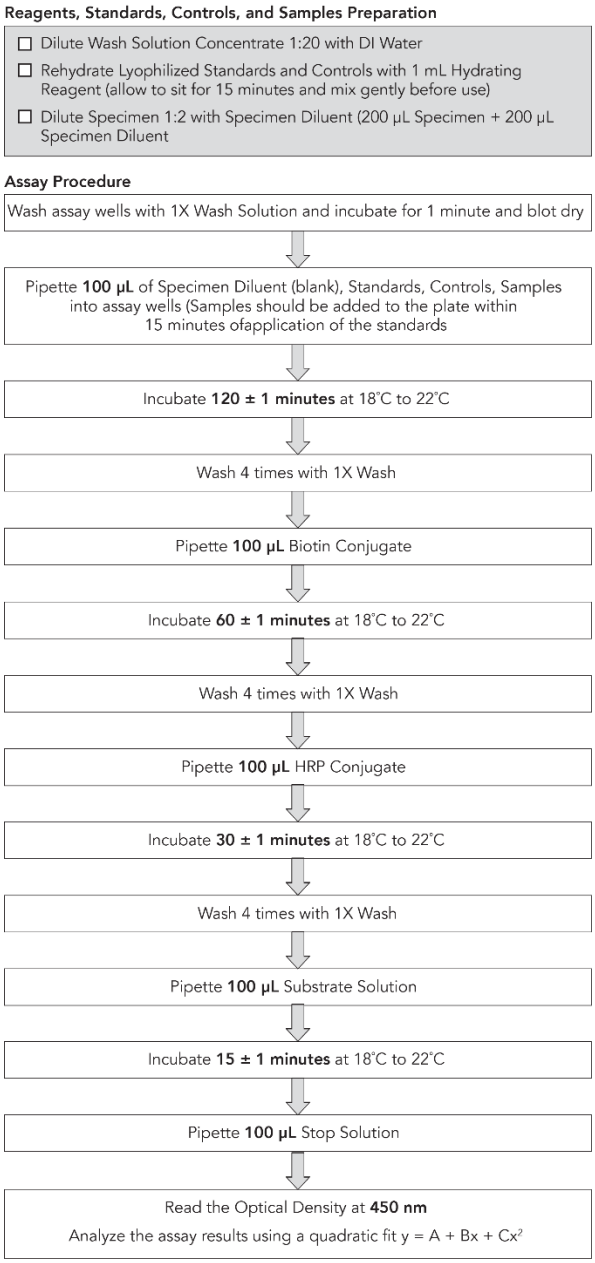
QUIDEL

MicroVue™ Bone Klotho EIA

An enzyme immunoassay to quantitatively measure human α-soluble Klotho protein in plasma and serum

For **Research Use Only**. Not for use in diagnostic procedures.

A symbols glossary can be found at quidel.com/glossary.



SUMMARY AND EXPLANATION

Klotho, named after one of the Fates in Greek mythology, is a type-I transmembrane protein that was discovered in mice with an association to aging.[1] Though a single gene, it has three major isoforms: the transmembrane form, a shed soluble form, and a truncated soluble form.[2] The extracellular portion of the protein consists of two glycosyl hydrolase domains referred to as KL1 and KL2. Upon cleavage at the base of the extracellular domain the protein becomes the shed soluble form and is an active form that functions as a hormone. Importantly, Klotho is a receptor/co-receptor of the phosphate regulation protein FGF-23.[3] While Klotho/FGF-23 homeostasis has primarily been focused on phosphate and vitamin D regulation, continuing research is associating Klotho and FGF-23 with functionality that impacts the heart,[4] bones,[5] blood vessels,[6] and parathyroid gland.[7]

PRINCIPLE OF THE PROCEDURE

The MicroVue Klotho EIA is a sandwich enzyme immunoassay in a microtiter plate format utilizing a capture monoclonal anti-Klotho antibody specific to the KL2 region coated on the plate, a detection mouse monoclonal anti-Klotho antibody specific to the KL1 region, a goat anti-mouse secondary antibody conjugated to horseradish peroxidase, and an enzymatic substrate to quantify Klotho.

REAGENTS AND MATERIALS PROVIDED

A | F Klotho Standards **Parts 142000 – 1420500** **1.0 mL each**
(Lyophilized) Each contains a known concentration of Klotho diluted in PBS, protein stabilizers, 0.05% Tween-20

L Klotho Low Control **Part 1420700** **1.0 mL**
(Lyophilized) Each contains a known concentration of Klotho diluted in PBS, protein stabilizers, 0.05% Tween-20

H Klotho High Control **Part 1420600** **1.0 mL**
(Lyophilized) Each contains a known concentration of Klotho diluted in PBS, protein stabilizers, 0.05% Tween-20

❶ **Microassay Plate** **Part 1419600** **12 x 8 wells**
Eight-well strips coated with a murine monoclonal antibody specific for human Klotho in a resealable foil pouch

❷ **Stop Solution** **Part A9947** **12 mL**
Contains 1N (4%) Hydrochloric Acid

❸ **20X Wash Solution Concentrate** **Part A9957** **1 x 50 mL**
Contains phosphate buffered saline (PBS), 1.0% Tween-20 and 0.035% ProClin 300

❹ **Specimen Diluent** **Part 1422000** **50 mL**
Contains PBS, and protein stabilizers),

❺ **TMB Substrate** **Part 5059** **12 mL**
Ready to use. Contains 3,3',5,5'-tetramethylbenzidene (TMB) and Hydrogen Peroxide (H_2O_2)

❻ **Klotho Biotin Conjugate** **Part 1419800** **12 mL**
Contains biotin-conjugated monoclonal anti-human Klotho antibody suspended in stabilizing buffer with preservative.

❼ **Klotho HRP Conjugate** **Part 1419900** **12 mL**
Contains horseradish peroxidase-conjugated monoclonal anti-human Klotho antibody suspended in HRP stabilizing buffer with preservative

❽ **Hydrating Reagent** **Part A3675** **25 mL**
Contains 0.035% ProClin 300

Tween® 20 is a registered trademark of ICI Americas Inc.
ProClin® is a registered trademark of Rohm and Haas Company.

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes to deliver 25 µL to 300 µL
- Labware suitable for liquid measurement of 7 mL to 300 mL
- Tubes for dilution of samples
- Container for wash buffer dilution
- Deionized or distilled water
- Plate reader capable of reading at 450 nm
- 4-parameter calibration curve fitting software

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures (U.S. only).
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- The Stop Solution (1N HCl is considered corrosive and can cause irritation. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
- The Substrate is light sensitive. Avoid prolonged exposure to bright or direct light. Store reagents in the dark when not in use.
- Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of reagents.
- For accurate measurement of samples, add samples and standards precisely. Pipette carefully using only calibrated equipment.
- Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN COLLECTION AND STORAGE*).
- Avoid microbial or cross-contamination of specimens or reagents.
- Test each sample in duplicate.
- Do not use a microassay well for more than one test.
- Using incubation times and temperatures other than those indicated in the *ASSAY PROCEDURE* section may give erroneous results.
- Do not allow microassay wells to dry once the assay has begun.
- When [adding or] removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
- Heat inactivated, hyperlipemic, or contaminated specimens may give erroneous results.
- To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
- This assay may be performed with any validated washing method.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

Store the unopened kit at 2°C to 8°C. After the kit is opened, the 20X Wash Solution Concentrate may be stored at 2°C to 25°C.

SPECIMEN COLLECTION AND PREPARATION

Handle and dispose of all specimens using Universal Precautions. The proper collection and storage of specimens is essential. Serum or EDTA plasma specimens should be collected aseptically using standard techniques. The specimens should be tested immediately, stored at 4°C, or on ice for no longer than four (4) hours before being assayed. If the specimen cannot be tested within four (4) hours under the guidelines detailed above, the specimen should be frozen at –70°C, or below. Thaw frozen (≤–70°C) specimens rapidly in a 37°C water bath until just thawed. Do not leave specimens at 37°C. Frozen specimens should be tested as soon as possible after thawing. Repeated freezing and thawing is not recommended. If samples are to be re-frozen for further analysis, we suggest freezing multiple aliquots of the specimen to prevent repeated freeze/thaw cycles.

Prior to use allow all reagents to come to room temperature and mix by gentle swirling and inversion.

Reagents from different kit lot numbers should not be combined or interchanged. Store the kit at 2°C to 8°C upon receipt.

For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

REAGENT PREPARATION

Equilibrate reagents to 18°C to 25°C prior to use.

Coated Strips

Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table in *ASSAY PROCEDURE* section). Ensure that the pouch containing any unused strips is completely resealed.

Wash Buffer

Prepare the Wash Solution for washing the micro-assay wells by diluting 50 mL of the 20X Wash Solution Concentrate up to a final volume of one (1) liter with distilled or deionized water. Mix thoroughly before use. The Wash Solution is stable for 30 days when stored in a clean container at 2°C to 8°C. If cloudiness occurs, discard the reagent.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See REAGENT PREPARATION before proceeding.

Sample Dilution/Incubation

1. Dilute serum and/or plasma samples 1:2 with Sample Diluent (e.g. 150 µL serum + 150 µL Sample Diluent).
2. Place desired number of Coated Strips in Stripwell Frame just prior to use. Label strips to prevent mix-up in case of accidental removal from Stripwell Frame.

Washing Step

3. Prepare required amount of 1x Wash Buffer by diluting Wash Buffer concentrate 1:20 with deionized water. Store at 18°C to 28°C. Use 1X Wash Buffer within 30 days of preparation.
4. Add at least 300 µL of 1X Wash Buffer to each well and incubate for 1 minute. Manually invert/empty strips. Vigorously blot the strips dry on paper.

Sample Addition

5. Pipette 100 µL of specimen Diluent (blank), standards, controls, and samples into assay wells. Samples should be added to the plate within 15 minutes of application of standards.
6. Incubate samples for 120± 1 minute at 18°C to 22°C.

Washing Step

7. Manually invert/empty strips. Add at least 300 µL of 1X Wash Buffer to each well and manually invert/empty strips. Repeat three more times for a total of four washes. Vigorously blot the strips dry on paper towels after the last wash.

Biotin Conjugate Incubation

8. Add 100 µL of Biotin Conjugate to each well.
9. Incubate 60 ± 1 minutes at 18°C to 22°C.

Washing Step

10. Manually invert/empty strips. Add at least 300 µL of 1X Wash Buffer to each well and manually invert/empty strips. Repeat three more times for a total of four washes. Vigorously blot the strips dry on paper towels after the last wash.

HRP Conjugate

11. Pipette 100 µL of HRP Conjugate into each well.
12. Incubate for 30 ± 1 minute at of 18°C to 22°C

Washing Step

13. Manually invert/empty strips. Add at least 300 µL of 1X Wash Buffer to each well and manually invert/empty strips. Repeat three more times for a total of four washes. Vigorously blot the strips dry on paper towels after the last wash.

Substrate Incubation

14. Add 100 µL of TMB Substrate Solution to each well.
15. Incubate for 15±1minute minutes at 18°C to 22°C.

Stop/Read

16. Add 100 µL of Stop Solution to each well to stop the reaction.
17. Read the optical density at 450 nm. Assure that no large bubbles are present in wells and that the bottoms of the strips are clean. Read strips within 15 minutes of Stop Solution addition.
18. Use quantitation software with a quadratic fit (see below) to analyze the MicroVue Klotho EIA results.

Equation: $y = A+Bx+Cx^2$

19. Determine concentration of samples and Controls from the standard curve.
    Dilute samples greater than 36 ng/mL in Sample Diluent and retest. Control values should be within the range specified in the Certificate of Analysis supplied with the kit.

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation.

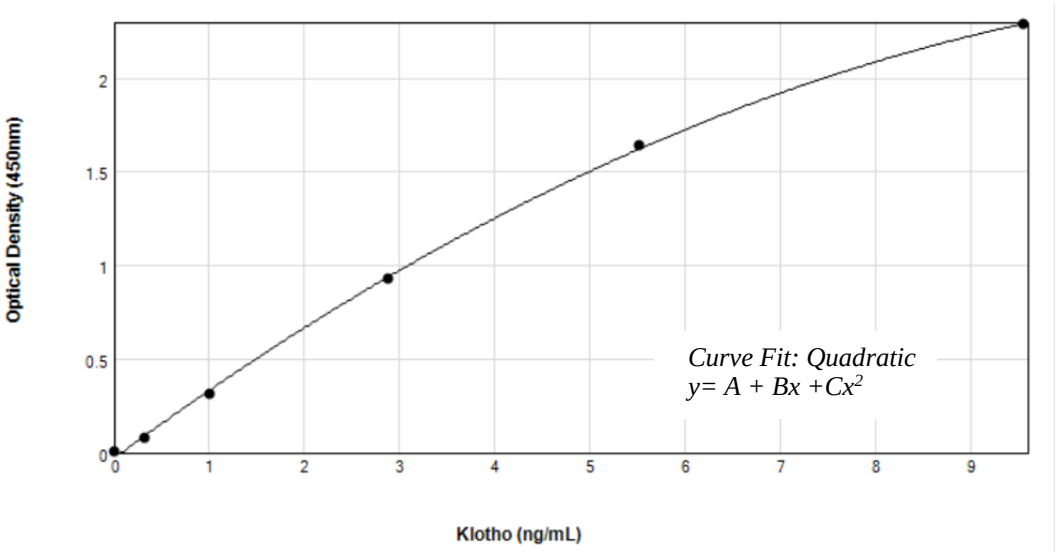
Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the samples should be repeated.

Quidel recommends that all assays include the laboratory's own human Klotho controls in addition to those provided with this kit.

INTERPRETATION OF RESULTS

The Standard curve is generated using the blank-subtracted A_{450} values for each Klotho Standard (on the y axis) and the assigned concentration for each Klotho Standard (on the x axis). Most plate reading software and computers are capable of performing these calculations. Sample results must be corrected for the dilution made. If the sample was diluted 1:2, multiply the ng/mL value by 2 for the final result of Klotho in ng/mL. An example of a typical standard curve is shown in figure 1.

Figure 1. Representative Standard Curve



PERFORMANCE OF THE TEST

Limits of Detection

LOD: The limit of detection (LOD) for the Klotho EIA is 0.04 ng/mL

LLOQ: The lower limit of quantitation (LLOQ) for the Klotho EIA is 0.13 ng/mL

ULOQ: The upper limit of quantitation (ULOQ) for the Klotho EIA is 18.00 ng/mL

Precision

Within-run and between-run precision was determined by assaying 18 replicates of two (2) plasma samples and two (2) serum samples in 10 different runs

Sample	Klotho (ng/mL)	Within-run[1] C.V. (%)	Between-run[2] C.V. (%)
EDTA Plasma	1.27	4.6	18.7
	8.41	4.3	9.9
Serum	0.93	3.7	10.7
	13.15	5.3	10.5
[1]n = 18 replicates		[2]n = 10 runs	

Linearity

Linearity was performed by diluting high-titer with low-titer of each sample type to generate nine levels of analyte-containing samples and comparing observed values with expected values. Typical results are provided below.

Sample		**Observed Klotho (ng/mL)**	**Expected Klotho (ng/mL)**	**% Recovery**
Serum	Low-titer	0.968	0.968	100
	2	2.413	2.497	97
	3	4.014	4.025	100
	4	5.896	5.554	106
	5	7.598	7.082	107
	6	8.980	8.611	104
	7	10.764	10.139	106
	8	12.409	11.668	106
	High-titer	13.196	13.196	100
Plasma	Low-titer	1.220	1.220	100
	2	3.280	3.335	98
	3	5.610	5.450	103
	4	7.900	7.565	104
	5	9.950	9.680	103
	6	11.650	11.795	99
	7	13.540	13.910	97
	8	15.280	16.025	95
	High-titer	18.140	18.140	100

Recovery – Spike Recovery

Spike recovery was determined by spiking serum and plasma samples with known concentrations of Klotho and comparing observed values with expected values. Typical results are provided below.

Sample Type	Sample	Observed Conc. (ng/mL)	Expected Results* (ng/mL)	% Recovery**
Serum	Spiked	15.07	14.87	101.3
	Un-Spiked	9.95	n/a	n/a
	Spike Amount	4.92	n/a	n/a
Plasma	Spiked	7.24	6.41	113.0
	Un-Spiked	1.26	n/a	n/a
	Spike Amount	5.15	n/a	n/a

* Spiked Sample Expected Results (ng/mL) = (Un-spiked sample observed + spike amount observed)
** % Recovery = Observed Results/Expected Results x 100%

Dilution Recovery

Dilution recovery was performed by serially diluting samples prior to testing and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Expected Klotho (ng/mL)	Measured Klotho (ng/mL)	% Recovery
Serum	2.0 (neat)	21.40	21.40	100%
	3.0	13.62	14.27	95%
	4.5	8.92	9.51	94%
	6.8	5.74	6.34	91%
	10.1	3.98	4.23	94%
	15.2	2.78	2.82	99%
	22.8	1.92	1.88	102%
	34.2	1.40	1.25	112%
Plasma	2.0 (neat)	17.60	17.60	100%
	3.0	11.34	11.73	97%
	4.5	7.54	7.82	96%
	6.8	5.18	5.21	99%
	10.1	3.66	3.48	105%
	15.2	2.50	2.32	108%
	22.8	1.72	1.55	111%
	34.2	1.24	1.03	120%

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

REFERENCES

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REF 8050 – MicroVue Klotho EIA Kit

RUO

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PI8050000EN01 (09/20)

GLOSSARY

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Consult e-labeling instructions for use
RUO For Research use only	 Contains sufficient for 96 determinations
CONT Contents/Contains	CONTROL Control