HIGH SENSITIVITY HUMAN FIBROBLAST GROWTH FACTOR 23 C-TERMINAL PEPTIDE (FGF-23 CT) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN FGF-23 C-TERMINAL PEPTIDE CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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	**HIGH SENSITIVITY HUMAN FGF-23 C-TERMINAL PEPTIDE ELISA KIT**
Catalog No.	SK00147-18
Lot No.	
Formulation	96 T
Standard Range	3.9 - 1000 pg/mL
Sensitivity	3 pg/mL
Sample Volume	100 µL per well
Sample Type	Serum, EDTA Plasma
Specificity	Human FGF-23 C-Terminal Peptide (180-251) at 100% and Intact mature FGF-23 (25-251) at 3%
Calibration	Human FGF-23 C-Terminal Peptide Recombinant
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C for 1 month. See page 2 ~3 for detail
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
2348 WALSH AVE., SUITE C
SANTA CLARA, CA 95051
USA
TEL: (408) 982 0300
Email: Sales@AvisceraBioscience.com
Website: www.AvisceraBioscience.com
Website: www.AvisceraBioscience.net

DESCRIPTION

This High Sensitivity Human FGF-23 C-Terminal Peptide ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human FGF-23 C-Terminal Peptide from serum and plasma in a sandwich ELISA format. The 2000 pg/ml of intact human FGF-23 (25-251) recombinant protein has been measured by this elisa kit at 62.5 pg/mL. The cross-reactivity of human intact FGF23 recombinant with this ELISA kit was 3%.

This immunoassay contains recombinant human FGF-23 C-Terminal Peptide and a monoclonal antibody raised against human FGF23 CT recombinant as a the capture antibody. Results from this immunoassay have shown to accurately quantify recombinant and natural FGF-23 C-Terminal Peptide in samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human FGF-23 C-terminal Peptide. The capture antibody can bind to the human FGF-23 CT in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human FGF-23 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 is added to the wells and color develops in direct proportion to the amount of human FGF-23 C Terminal Peptide immunoreactivity bound in the standard dilutions 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.
_Each laboratory must determine the optimal dilution factors for 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
FGF-23 CT Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against human FGF-23 CT.	**147-18-01**	**1 plate**
FGF-23 CT Standard – refer to lot of human FGF-23 C-terminal peptide in a buffered protein base with preservative; lyophilized.	**147-18-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial of 10-fold concentrate of biotinylated IgG against human FGF-23 with preservative; lyophilized.	**147-18-03**	**1 vial**
Positive Control - one vial of FGF-23 CT; lyophilized.	**147-16-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL of 100-fold concentrated Streptavidin-HRP Conjugate.	**SAHRP**	**1 vial**
Dilution Buffer Concentrate - 45 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB118C**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08B**	**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**
Plastic Pouch	**P01**	**1**

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, Antibody Diluent Solution 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 (350 – 400 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.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order Code: 00700-01-25, 25 TIU) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

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.

FGF-23 CT Standard - Reconstitute the Human FGF-23 CT standard with refer to lot of Dilution Buffer. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a 4-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 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	xxxx
# 1	Refer to lot	Refer to lot	4000 pg/ml
# 2	150 µl of 1	450 µl	1000 pg/ml
# 3	150 µl of 2	450 µl	250 pg/ml
# 4	150 µl of 3	450 µl	62.5 pg/ml
# 5	150 µl of 4	450 µl	15.6 pg/ml
# 6	150 µl of 5	450 µl	3.9 pg/ml

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

Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB118C)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB118C)** 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 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**. The working solution of Streptavidin HRP Conjugate should be used within a 1 hour.

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 per well of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions, 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 working solution to each well. Cover with plate sealer. 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 TMB Substrate Solution to each well. Incubate for 15-20 minutes 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 within 3 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

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 or 4-parameter 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 is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (0.065)
3.9	0.031
15.6	0.121
31.25	0.242
62.5	0.510
250	1.183
1000	2.796
4000	3.488

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human FGF-23 C-Terminal Peptide (180-251)	100
Human FGF-23 Intact mature	3
Human FGF-23 N-Terminal Peptide (25-179)	0
Human Secreted Klotho	0
Human FGF-21	0
Human FGF-19	0
Human FGF-15	0

The 2000 pg/ml of intact human FGF23 recombinant in Dilution Buffer DB01 was detected by this ELISA Kit SK00147-18 at 62.5 pg/mL. The cross-reactivity of intact human FGF23 recombinant with HS FGF23CT (Human) ELISA Kit SK00147-18 is at 3%.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Streptavidin-HRP working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl of TMB Substrate Solution to each well. Incubate 15-20 minutes on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl of Stop Solution to each well. Read 450nm within 3 min.