HUMAN FIBROBLAST GROWTH FACTOR 23 (FGF-23) ELISA KIT

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN FGF-23 ELISA**
Catalog No.	SK00147-09
Lot No.	
Formulation	96 T
Standard Range	31.25 - 2000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL per well
Sample Type	Serum, EDTA Plasma
Specificity	Human FGF-23
Calibration	Human FGF-23 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
This kit contains sufficient materials to run 40 samples duplicated provided that assay is run according to protocol.	

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.

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DESCRIPTION

This Human FGF-23 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human FGF-23 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human FGF-23 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural FGF-23 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human FGF-23. The capture antibody can bind to the human FGF-23 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 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 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with IgG against human FGF-23.	**147-09-01**	**1 plate**
FGF-23 Standard – 2000 pg/vial of FGF-23 in a buffered protein base with preservative; lyophilized.	**147-09-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of biotinylated IgG against FGF-23 with preservative; lyophilized.	**147-09-03**	**1 vial**
Positive Control - one vial of FGF-23; lyophilized.	**147-09-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL of 100-fold concentrated Streptavidin-HRP Conjugate.	**SAHRP**	**1 vial**
Dilution Buffer - 6 mL of 10-fold concentrated buffered protein based solution with preservative.	**DB101**	**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 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.

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

Human serum and plasma samples may require a 2-fold dilution. A suggested 10 -fold dilution is 25 µl sample + 225 µl Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**

Use polypropylene test tubes.

REAGENT PREPARATION

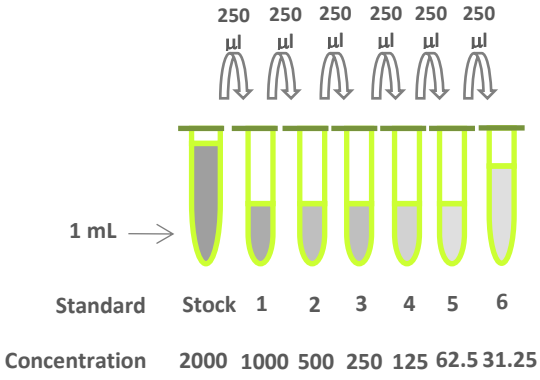
Bring all reagents to room temperature before use.

Dilution Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 6 mL of 10-fold concentrated Dilution Buffer into deionized water 54 mL to prepare 60 mL of 1x Dilution Buffer.

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 Standard - Reconstitute the FGF-23 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 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 into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 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	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml



Positive Control – Reconstitute the Positive Control with 1.0 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 10.8 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 – Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-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 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 20-25 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 15 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 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 (0.117)
31.25	0.022
62.5	0.045
125	0.084
250	0.179
500	0.424
1000	0.853
2000	1.768

Lot No.:

Positive Control: 100 ~ 400 pg/mL

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human FGF-23	100
Human FGF-21	0
Human FGF-19	0
Human FGF-15	0

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 20-25 min on the plate shaker at RT. **Protect from light.**

Add 100 µl of Stop Solution to each well. Read 450nm within 15 min.