HUMAN FIBROBLAST GROWTH FACTOR 21 (FGF21) ELISA SET

FOR THE QUANTITATIVE DETERMINATION OF HUMAN FGF21 CONCENTRATIONS IN SERUM AND PLASMA

PRODUCT INFORMATION:

ELISA NAME	HUMAN FGF21 ELISA SET
Catalog No.	SK00145-10
Lot No.	
Formulation	960 T
Standard range	31.25 – 2000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µl
Sample Type	Serum and Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human FGF21
Clibration	Human FGF21 Recombinant
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	2 °C - 8 °C

READ ENTIRE PROTOCOL BEFORE BEGINNING EXPERIMENT.

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

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

INTRODUCTION

Human FGF21 ELISA set contains basic components required for the development of sandwich ELISA to measure human FGF21 in serum and plasma. It contains recombinant human FGF21 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human FGF21. Each kit contains sufficient material to run ELISAs on approximately ten 96-well plates, provided that the following conditions are met:

- The assay is run as summarized in the ELISA Protocol.
- The recommended buffers, diluents, substrates and solutions are used.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for FGF21 will be coated onto a microplate. Standards and samples are pipetted into the wells and any FGF21 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for FGF21 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, Streptavidin HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of FGF21 bound in the initial step. The color development is stopped and the intensity of the color is measured.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Capture Antibody – 1.05 mL/vial of 100-fold concentrated purified monoclonal antibody against human FGF21 in PBS; lyophilized.	145-10-01	1 vial
FGF21 Standard – 40 ng/vial of human FGF21 in a buffered protein base with preservative; lyophilized.	145-10-02	1 vial
Detection Antibody – 1.05 mL/vial of 100-fold concentrated purified biotinylated antibody against FGF21 with preservative; lyophilized.	145-10-03	1 vial
Streptavidin HRP Conjugate - 1100 µL/vial of 100-fold concentrated solution of Streptavidin HRP conjugate.	SAHRP	1 vial

STORAGE

Unopened Capture Antibody, Standard and Detection Antibody: Store at -20° C or -70° C for up to 6 months. Do not use past expiration date.
Opened / Reconstituted Reagents: Aliquot the reconstituted standard (stock) solution and store at -20° C or -70° C for up to 2 months. Diluted standard working solution should be prepared and used immediately.
SAHRP Conjugate 100-fold concentrated solution (protect from light) may be stored at 2 – 8 °C for up to 6 months. **DO NOT FREEZE SAHRP**.

ADDITIONAL MATERIALS REQUIRED

- PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2PO4, 1.5 mM KH2PO4, pH 7.4, 0.2 µm filtered).
- Microplate (P001)
- Wash Buffer (0.05% Tween-20 in PBS, pH 7.4)
- Coating Buffer (Code: CS01)
- Blocking Buffer (BSA based. Code: BS-01)
- Concentrated Dilution Buffer (Code: BD01), Antibody Diluent Solution (Code: BD18) and HRP Diluent Solution (Code: BD06) **Note:** BD01 is for standard dilution. BD18 is for Detection Antibody dilution. BD06 is for HRP dilution
- Substrate Solution (TMB)
- Stop Solution (0.5M HCl)
- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- **Note: Wash Buffer, Blocking Buffer, Diluent Solutions (BD01, BD06 and BD18), Coating Buffer (CS01), Substrate Solution and Stop Solution, etc. are available for purchase online at www.aviscerabioscience.com**

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Capture Antibody - Reconstitute the Capture Antibody with 1.05 mL of **PBS** to produce a 100-fold concentrated stock solution. Pipette 9.9 mL of **1x Coating Buffer** into a 15 mL centrifuge tube and transfer 100 µL of 100-fold concentrated stock solution to prepare working solution (for one plate). **Note:** Capture Antibody should be diluted without any carrier proteins.

FGF21 Standard - Reconstitute the FGF21 Standard with 1.0 mL of **1x Dilution Buffer** (**BD01**). This reconstitution produces a stock solution of 40 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to aliquot. Aliquot and store at -70° C for up to 2 months. A seven point standard curve using 2-fold serial dilutions in 1x Dilution Buffer is suggested. A high standard of 2000 pg/mL is recommended.

Detection Antibody - Reconstitute the Detection Antibody with 1.05 mL of **Antibody Diluent Solution** (**BD18**) to produce a 100-fold concentrated stock solution. Pipette 9.9 mL of the 1x Dilution Buffer into a 15 mL centrifuge tube and transfer 100 µL of 100-fold concentrated stock solution to prepare working solution (for one plate).

Streptavidin HRP Conjugate - Pipette 9.9 mL of **HRP Diluent Solution** (**BD06**) into a 15 mL centrifuge tube and transfer 100 µL of 100-fold concentrated stock solution to prepare working solution (for one plate). **Note:** 1x working solution of Streptavidin-HRP conjugate should be used immediately **(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 (on day performing assay) as directed in the previous sections.
2. Coat a 96-well microplate with 100 µL per well of Capture Antibody Working Solution. Seal with plate sealer and incubate overnight at 2-8 °C.
3. Aspirate each well and wash, repeating the process two times for a total of three 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.
4. Add 150 µL per well of Blocking Buffer to each well. Seal with plate sealer and incubate for 5 hours at room temperature or overnight at 2-8 °C.
5. Aspirate each well and let plate dry completely before beginning assay.
6. Add 100 µL of standard dilutions and samples per well. Seal with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
7. 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.
8. 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.
9. Repeat the aspiration/wash as in step 7.
10. Add 100 µL of Streptavidin HRP Conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
11. Repeat the aspiration/wash as in step 7.
12. Add 100 µL of Substrate Solution to each well. Incubate for 20-30 minutes on microplate shaker at room temperature. **Protect from light.**
13. 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.
14. 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 DATA

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

MPO (ng/mL)	Absorbance 450nm (Corrected)
Blank	0 (0.071)
31.25	0.020
62.5	0.048
125	0.080
250	0.158
500	0.319
1000	0.603
2000	1.181

- **Lot:**

SPECIFICITY

PROTEIN	CROSS-REACTIVITY
Human FGF-21	100%
Mouse FGF-21	0
Human FGF-19	0
Human FGF-23, C-Terminal	0
Human FGF-23, N-Terminal	0
Human FGF-23	0
Human FGF-17	0
Human FGF-10	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS (SAMPLES AND STANDARDS ON DAY PERFORMING THE ASSAY)

↓

Add 100 µL of Capture Antibody working solution to each well. Incubate overnight at 2-8 °C.

↓

Aspirate and wash 3 times.

↓

Add 150 µL of Blocking Buffer to each well. Incubate 5 hours at RT or overnight at 2-8 °C.

↓

Aspirate and let each well in plate dry completely.

↓

Add 100 µL of standards and samples to the wells. Incubate for 2 hours on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µL Detection Antibody working solution to each well. Incubate for 2 hours on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µL SAHRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

↓

Add 100 µl Substrate Solution to each well. Incubate 20-30 min on the plate shaker at RT. **Protect from light.**

↓

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