HUMAN FGF-19 ELISA KIT

PURCHASE INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

FOR THE QUANTITATIVE DETERMINATION OF HUMAN FGF-19 CONCENTRATIONS IN SERUM AND EDTA PLASMA

ELISA NAME	**HUMAN FGF-19 ELISA**
Catalog No.	SK00148-01
Lot No.	
Formulation	96 T
Standard range	39-2500 pg/ml
Sensitivity	15 pg/ml
Sample Volume	100 μl
Sample Type	Serum, EDTA plasma
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Specificity	Human FGF-19
Calibration	Human FGF-19 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	Refer to lot specific

This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.

THE STANDARD CURVE IS PROVIDED FOR DEMONSTRATION ONLY.
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.

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
         Info@AvisceraBioscience.com
www.AvisceraBioscience.com

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DESCRIPTION

This Human FGF-19 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human FGF-19 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human FGF-19 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural FGF-19 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human FGF-19. The capture antibody can bind to the human FGF-19 in the standard and samples. After washing the plate of any unbound substances, a polyclonal antibody against human FGF-19 is added to the wells. After another washing of the plate, Anti IgG-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-19 bound in the standard solutions 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 with a pretest.

_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-19 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against FGF-19.	148-01-01	1 plate
FGF-19 Standard – lot specific of recombinant human FGF-19 in a buffered protein base with preservative; lyophilized.	148-01-02	1 vial
Detection Antibody Concentrate – lot specific concentrate of antibody against FGF-19 with preservative; lyophilized.	148-01-03	1 vial
Positive Control – one vial of recombinant human FGF-19; lyophilized.	148-01-04	1 vial
Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 50 mL of buffered protein based solution with preservative.	DB08A	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 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 1 months. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate and Dilution Buffer should be stored at -20°C.

Do not use kit past expiration date.

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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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C or -70°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 or 70°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. Aliquot and store samples at -20°C ~ -70°C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order code: 00740-01-25, 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. A pretest is suggested to determine the dilution factor.
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-19 Standard - Reconstitute the FGF-19 standard with lot specific Dilution Buffer (**DB08A**). Pipette 250 μl of Dilution Buffer into tubes #2 to #7. Use the stock solution to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The **2500 pg/ml** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/ml).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	Lot specific	
# 1	Lot specific	Lot specific	2500 pg/ml
# 2	250 μl of 1	250 μl	1250 pg/ml
# 3	250 μl of 2	250 μl	625 pg/ml
# 4	250 μl of 3	250 μl	312.5 pg/ml
# 5	250 μl of 4	250 μl	156 pg/ml
# 6	250 μl of 5	250 μl	78 pg/ml
# 7	250 μl of 6	250 μl	39 pg/ml

Positive Control - Reconstitute the Positive Control with lot specific of Dilution Buffer (**DB08A**) to prepare Positive Control working solution.

Detection Antibody Concentrate - Reconstitute each Detection Antibody Concentrate vial with refer to lot of Dilution Buffer (**DB08A**) to produce a 10-fold concentrated stock solution. Pipette 9.45 ml of Dilution Buffer (**DB08A**) into a 15 ml centrifuge tube and transfer 1.05 ml of 10-fold concentrated stock solution to prepare working solution.

Anti Rabbit IgG-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer (**DB08A**) 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

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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 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 micro-plate 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 micro-plate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Anti IGG-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on micro-plate 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 using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the FGF-19 concentrations versus the log of the O.D. and the best fit line can be determined by regression

analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (lot specific)
39	0.042
78	0.092
156	0.157
312.5	0.302
625	0.567
1250	1.066
2500	2.057

SPECIFICITY

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

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SUMMARY OF ASSAY PROCEDURE

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