MOUSE/RAT FIBROBLAST GROWTH FACTOR 21 (FGF21) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE OR RAT FGF21 CONCENTRATIONS IN SERUM AND EDTA PLASMA

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

PURCHASE INFORMATION:

ELISA NAME	MOUSE/RAT FGF21 ELISA
Catalog No.	SK00145-03
Lot No.	
Formulation	96 T
Standard range	32 - 20,000 pg/mL
Dynamic range	200 - 4000 pg/mL
Sensitivity	19 - 30 pg/mL
Sample Volume	50 µL
Dilution Factor	2~4 **(Optimal dilutions should be determined by each laboratory for each application.)**
Sample Type	Serum, EDTA plasma
Specificity	Mouse, Rat
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8°C

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

INTRODUCTION

Mouse FGF21 ELISA employs the quantitatively competitive enzyme immunoassay technique in which mouse FGF21 present in samples compete with a fixed amount of biotinylated mouse FGF21 for sites on purified rabbit IgG specific against mouse FGF21. During the incubation period, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplates. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution is added. The intensity of the color measured is in inverse proportion to the amount of mouse FGF21 bound in the initial step. The sample values are then read off the standard curve.

Mouse FGF21 ELISA has been shown to accurately quantify the recombinant and natural mouse FGF21. Results obtained using natural mouse FGF21 showed dose response curves that were parallel to the standard curves obtained using the kit standards.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_Some vials contain small quantities of material, therefore centrifuge before use.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product be handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate - 96 well microplate pre-coated with Goat anti Rabbit IgG	**RM01**	**1 plate**
FGF21 Standard – 10 ng/vial of recombinant mouse FGF21 in a buffered protein base with preservatives; lyophilized.	**145-03-01**	**2 vials**
Mouse FGF21 Antibody - 200µL/vial, 10-fold Concentrate of Rabbit Anti Mouse FGF21 Purified IgG in buffered protein base with preservatives; lyophilized	**145-03-02**	**2 vials**
Biotin Solution Concentrate - 200µL/vial, 10-fold Concentrate of Mouse FGF21 biotinylated with preservatives; lyophilized.	**145-03-03**	**2 vials**
Positive Control – one vial of recombinant mouse FGF21, lyophilized (optional)	**145-03-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives. Ready to use.	**DB06**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservatives.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 mL of contains 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Solution Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Biotin Solution and Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Reconstituted Biotin Solution CAN NOT BE STORED at 2 - 8°C. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 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.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles. **Note:** FGF-21 is detectable in fetal bovine serum. Animal free cell culture supernatants only.
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 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.
Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation.

SAMPLE PREPARATION

Serum and plasma samples may need a 2~4-fold dilution. A suggested 2-fold dilution is 60 µL sample + 60 µL Dilution Buffer. A suggested 4-fold dilution is 30 µL sample + 90 µL Dilution Buffer.
Use polypropylene test tubes.

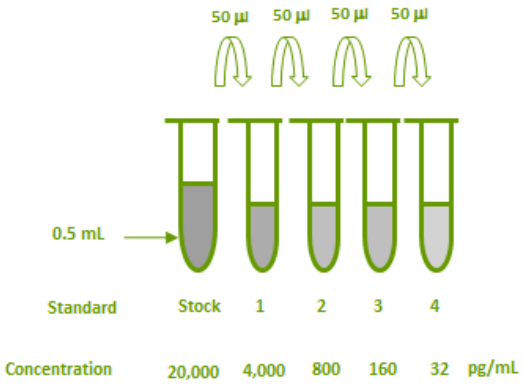
REAGENT PREPARATION

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 Wash Buffer.

Mouse FGF 21 Standard - Refer to vial label for reconstitution volume. Reconstitute the **Mouse FGF21** standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20,000 pg/mL standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5 mL	20,000 pg/mL
# 1	50 µL of stock	200 µL	4,000 pg/mL
# 2	50 µL of 1	200 µL	800 pg/mL
# 3	50 µL of 2	200 µL	160 pg/mL
# 4	50 µL of 3	200 µL	32 pg/mL



Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note**: Positive Control should be prepared and used immediately.

Mouse FGF21 Antibody - Reconstitute the **Mouse FGF21 Antibody Concentrate** with 200 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer the 200 µL concentrated stock solution to 1.8 mL of Dilution Buffer in a 15 mL centrifuge tube to prepare **1X Antibody working solution.**

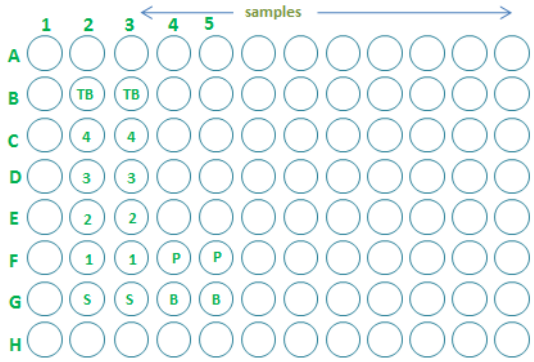
Biotin Solution Concentrate – Reconstitute the **Biotin Solution Concentrate** with 200 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer the 200 µL concentrated stock solution to 1.8 mL of Dilution Buffer in a 15 mL centrifuge tube to prepare **1X Biotin working solution.**

Streptavidin-HRP Conjugate - Transfer 120 µL of 100-fold concentrated **Streptavidin-HRP Conjugate** stock solution to 11.88 mL of Dilution Buffer in a 15 mL centrifuge tube to prepare Streptavidin-HRP working solution. **Note**: 1X working solution of Streptavidin-HRP Conjugate should be used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards and positive control be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Leave well G4 and G5 as Blank. **DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.**
4. Set B2 and B3 as total binding (TB). Add 50 µL of **Dilution Buffer** per well.
5. Add 50 µL per well of **standard solution** from #4 to stock solution (reverse order of serial dilution) to the appropriate wells (C2, C3 to G2, G3). Add 50 µL per well of **Positive Control** into wells F4, F5. Add 50 µL per well of **samples** into other wells. Add 25 µL of **1X Antibody working solution** into each well, except for blanks. Cover with plate sealer and incubate on microplate shaker (250-300rpm) at room temperature for 2 hours. **NOTE: DO NOT ASPIRATE AND WASH PLATE**. **PROCEED IMMEDIATELY TO THE NEXT STEP.**
6. Add 25 µL per well of **1X Biotin working solution** into total binding, standard, PC and sample wells. Cover plate with plate sealer and incubate at room temperature for 2 hours. **Note: DO NOT ADD Biotin Solution to Blank wells.**
7. Aspirate wells and wash 4 times with 300 µl of **1X Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to every well, including the blank wells. Incubate on microplate shaker for 45 minutes at room temperature. **Protect from light.**
9. Aspirate and wash as step 7.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 10-15 minutes at room temperature. **Protect from light**.
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. It is recommended to add the stop solution when the total binding or the lowest standard has developed a blue color.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, PC, and samples and subtract the average blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant mouse FGF21.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of mouse FGF21 was 19-30 pg/mL

TYPICAL DATA

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

MOUSE FGF21 STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Total Binding	0.814
32	0.684
160	0.648
800	0.465
4000	0.214
20000	0.065
Blank	0 (0.108)

- Lot No.:
- Positive Control: 400 - 1000 pg/mL

LINEARITY

To assess the linearity of the assay, pooled research mouse plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
2 X	1248.379	2496.758	100
4 X	656.689	2626.756	105.2

SPECIFICITY

This assay recognizes both natural and recombinant mouse FGF21. The factors listed below were prepared at 200 ng/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEINS	CROSS-REACTIVITY
Mouse FGF21	100%
Human FGF21	78
Human FGF19	0
Mouse FGF23	0
Mouse Leptin	0
Mouse gAdiponectin	0
Rat Visfatin	0
Mouse FABP-4	0
Human Chemerin	0
Mouse gCTRP9	0
Rat RBP-4	0
Mouse Vaspin	0

Mouse FGF21 ELISA kit recognizes recombinant and endogenous mouse FGF21. The data also indicated that rat serum samples were competitively bound to antibody that was used in this kit formulation condition. Its dynamic dilution curves were parallel to the standard curves obtained using the ELISA standard. That means rat serum samples cross-react with mouse FGF21 ELISA kit.

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
↓
Add 50µl of standard, samples, positive control to each well, except blanks. Add 25 µl of 1X Antibody working solution to each well, except blanks. Incubate 2 hours on the plate shaker at RT.
↓
DO NOT ASPIRATE AND WASH PLATE. Add 25 µl 1X Biotin working solution to each well, except blanks. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to all wells. Incubate 45 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-15 minutes on the bench top. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 minutes

REFERENCES

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