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

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

PURCHASE INFORMATION:

ELISA NAME	**MOUSE/RAT FGF21 ELISA**
Catalog No.	SK00145-03
Lot No.	
Formulation	96 T
Standard range	6 - 20,000 pg/mL
Dynamic range	32 - 4000 pg/mL
Sensitivity	19 - 30 pg/mL
Sample Volume	50 µL
Dilution Factor	***Optimal dilutions should be determined by each laboratory for each application.***
Sample Type	Serum, EDTA plasma
Specificity	Mouse, Rat
Calibration	Mouse FGF-21 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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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 antibody specific against mouse FGF21, which is pre-coated onto the microplates. Following a wash to remove any unbound 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
FGF21 Microplate - 96 well microplate pre-coated with an antibody against mouse FGF21.	**145-03-01**	**1 plate**
FGF21 Standard – 20 ng/vial of recombinant mouse FGF21 in a buffered protein base with preservative; lyophilized.	**145-03-02**	**1 vial**
Biotin Solution Concentrate – 600 µL/vial, 10-fold concentrate of biotinylated mouse FGF21 with preservative; lyophilized.	**145-03-03**	**1 vial**
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 preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative. Ready to use.	**DB18**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB06**	**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 8 months. For longer storage, unopened Standard, Positive

Control and Biotin Solution Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Biotin concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. Reconstituted Biotin concentrated solution CANNOT BE STORED at 2 – 8 °C. Streptavidin-HRP Conjugate 100-fold concentrated solution(protect from light) and other components may be stored at 2 – 8 °C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. 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.
- 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

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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

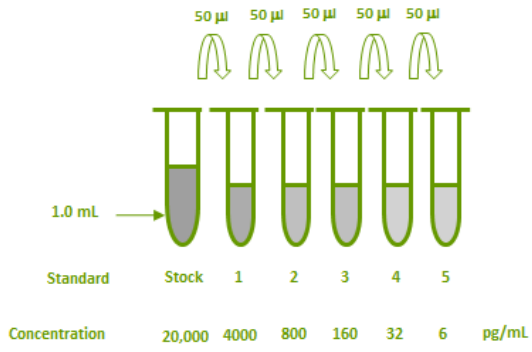
REAGENT PREPARATION

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.

Mouse FGF21 Standard - Refer to vial label for reconstitution volume. Reconstitute the **Mouse FGF21** standard with 1 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 #5. 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	1 mL	20,000 pg/mL
# 1	50 µL of stock	200 µL	4000 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
# 5	50 µL of 4	200 µL	6 pg/mL



Positive Control - Reconstitute the **Positive Control** with 1 mL of Dilution Buffer. **Note**: Positive Control could be reused within a few days if stored at -20 °C or -70 °C.

Biotin Solution Concentrate – Reconstitute the **Biotin Solution Concentrate** with 600 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer the 600 µL concentrated stock solution to 5.4 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 **HRP Diluent Solution (DB06)** 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 **(protect from light)**.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standard solutions, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Leave wells G4 and G5 as Blank. **DO NOT ADD ANY STANDARD SOLUTIONS OR BIOTIN WORKING SOLUTION INTO BLANK WELLS.**
4. Set B4 and B5 as total binding (TB). Add 50 µL of **Dilution Buffer** per well.
5. Add 50 µL per well of **standard solutions** from #5 to stock solution (reverse order of serial dilution) to the appropriate wells (B2, B3 to G2, G3). Add 50 µL per well of **Positive Control** into wells F4 and F5. Add 50 µL per well of **samples** into other wells. 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 50 µL per well of **1x Biotin working solution** into total binding, standards, positive control and sample wells. Cover with plate sealer and incubate at room temperature for 2 hours. **Note: DO NOT ADD Biotin working 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 60 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 on microplate shaker for 3-10 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 solutions, positive control 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.

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)
Blank	0 (0.060)
Total Binding	1.360
6	1.125
32	1.036
160	0.850
800	0.479
4000	0.254
20000	0.112

- Lot No.:
- Positive Control:

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Mouse FGF21	100%
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 indicates that rat serum and EDTA plasma samples were competitively bound to the antibody that was used in this kit formulation. Its dynamic dilution curves were parallel to the standard curves obtained using the ELISA standard, which means rat serum and EDTA plasma samples cross-react with mouse FGF21 ELISA kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50 µl of standard, samples, positive control to the well, except blanks. Incubate 2 hours on the plate shaker at RT.
↓
DO NOT ASPIRATE AND WASH PLATE. Add 50 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 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 **3-10** minutes on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 minutes.

REFERENCES

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