MOUSE FIBROBLAST GROWTH FACTOR 21 (FGF-21) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE FGF-21 CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	MOUSE FGF-21 ELISA
Catalog No.	SK00145-08
Lot No.	
Formulation	96 T
Standard range	0.313-20 ng/ml
Sensitivity	0.156 ng/ml
Sample Volume	100 µl
Dilution	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma
Specificity	Mouse FGF-21
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2 °C - 8 °C

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

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INTRODUCTION

Mouse FGF-21 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure FGF-21 in serum and plasma. It contains recombinant FGF-21 and antibodies raised against this protein. It has been shown to accurately quantify recombinant FGF-21. Results obtained with naturally occurring FGF-21 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural FGF-21.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for FGF-21 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FGF-21 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for FGF-21 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link streptavidin 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 FGF-21 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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 the appropriate 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.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
FGF21 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against mouse FGF-21	**145-08-01**	**1 plate**
FGF-21 Standard – 20 ng/vial of recombinant mouse FGF-21 in a buffered protein base with preservatives; lyophilized	**145-08-02**	**1 vial**
Detection Antibody Concentrate – 120 µL / vial, 100-fold concentrated of biotinylated polyclonal antibody against mouse FGF-21 with preservatives; lyophilized	**145-08-03**	**1 vial**
Positive Control- one vial of recombinant mouse FGF-21, lyophilized	**145-08-04**	**1 vial**
Streptavidin-HRP Conjugate - 30 ul/vial, 400-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB06**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 12 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 12 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard SHOULD BE STORED at -20°C or -70°C for up to one month. Reconstituted Detection Antibody Concentrate (120 µl) SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 400-fold Concentrate 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

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.
Notice: Heparin can't be used as anticoagulant for FGF-21 assay.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

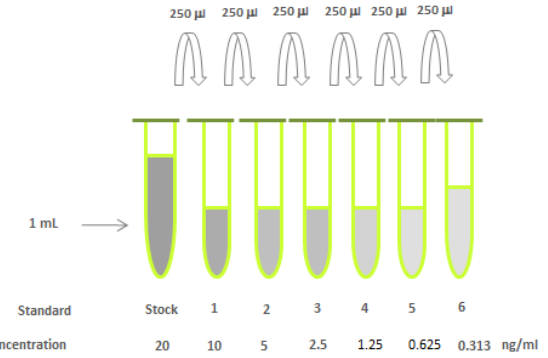
SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
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.
FGF-21 Standard - Refer to vial label for reconstitution volume. Reconstitute the mouse FGF-21 Standard with 1.0 ml of Dilution Buffer. This reconstitution produces a stock solution of 20 ng/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 20 ng/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	1000 µl	20 ng/ml
# 1	250 µl of stock	250 µl	10 ng/ml
# 2	250 µl of 1	250 µl	5 ng/ml
# 3	250 µl of 2	250 µl	2.5 ng/ml
# 4	250 µl of 3	250 µl	1.25 ng/ml
# 5	250 µl of 4	250 µl	0.625 ng/ml
# 6	250 µl of 5	250 µl	0.313 ng/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 120 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. 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.

Streptavidin-HRP Conjugate - Pipette 11.97 ml of **HRP Diluent Solution (DB01)** into a 15 ml centrifuge

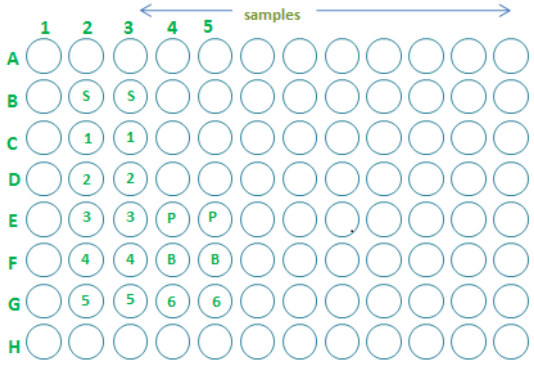
tube and transfer 30 µl of 400-fold concentrated stock solution to prepare working solution.

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

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, 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. Add 100 µL of Dilution Buffer to Blank well (F4, F5.
4. Add 100 µL of Standard (from B2, B3 to G2, G3), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 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. 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.
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, 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 standard 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.

CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant mouse FGF-21.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of mouse FGF-21 was 0.156 ng/ml.

TYPICAL DATA

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

FGF-21 STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.079)
0.313	0.045
0.625	0.074
1.25	0.121
2.5	0.191
5	0.356
10	0.625
20	1.351

- Lot No.:
- Positive Control: 1.5 – 4.0 ng/mL

SPECIFICITY

Mouse FGF-21 ELISA recognizes recombinant and natural mouse FGF-21. Our study data indicated that rat serum or EDTA plasma samples can be tested by this assay kit due to its samples dilution linear curves that were parallel to the standard curves.

Proteins	Cross-reactivity
Mouse FGF-21	100%
Human FGF-21	8%
Mouse FGF-23	0
Human FGF-19	0
Human FGF-17	0

REFERENCES

1: Mai K,et al. Relation between fibroblast growth factor-21, adiposity, metabolism, and weight reduction. Metabolism. 2010 Mar 31. [Epub ahead of print]

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3: Wang Y, Solt LA, Burris TP. Regulation of FGF21 expression and secretion by the retinoic acid receptor-related orphan receptor{alpha}. J Biol Chem. 2010 Mar 23. [Epub ahead of print]

4: Estall JL, et al. PGC-1alpha negatively regulates hepatic FGF21 expression by modulating the heme/Rev-Erb(alpha) axis. Proc Natl Acad Sci U S A. 2009 Dec 29;106(52):22510-5. Epub 2009 Dec 14.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇩
Add 100 µl of standard, samples, 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 Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate Solution to each well. Incubate 5-10 min on plate shaker. **Protect from light.**
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min