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

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN FGF-21 ELISA
Catalog No.	SK00145-01
Lot No.	
Formulation	96 T
Standard range	15.6-2000 pg/ml
Sensitivity	5~7 pg/ml
Sample require	100 µl
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Serum, EDTA Plasma
Specificity	Human FGF-21
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	4 °C

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

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INTRODUCTION

Human FGF-21 Immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human FGF-21 in cell culture supernates, serum and plasma. It contains recombinant Human FGF-21 and antibodies raised against this protein. It has been shown to accurately quantify recombinant Human 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 Human FGF-21.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An 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 antibody specific for FGF-21 is added to the wells. Following a wash to remove any unbound antibody reagent, A 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 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
FGF-21 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified IgG against FGF-21.	**145-01-01**	**1 plate**
FGF-21 Standard – 1000 pg/vial of recombinant Human FGF-21 in a buffered protein base with preservatives; lyophilized.	**145-01-02**	**2 vials**
Detection Antibody– 1.05 mL / vial, 10-fold concentrated of a purified IgG biotinylated against FGF-21 with preservatives; lyophilized.	**145-01-03**	**1 vial**
Positive Control – one vial of recombinant FGF-21 , lyophilized	**145-01-04**	**1 vial**
Streptavidin HRP Conjugate -60 µl/vial, 200-fold concentrated solution of Streptavidin HRP conjugate	**SAHRP**	**1 vial**
Dilution Buffer- 60mL/vial of buffered protein based solution with preservatives	**DB06**	**1 Bottle**
Antibody Diluent Solution- 12 mL/vial of buffered protein based solution with preservatives	**DB12**	**1 Bottle**
HRP Diluent Solution- 12 mL/vial of buffered protein based solution with preservatives	**DB01**	**1 Bottle**
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 Bottle**
TMB Substrate Solution-11 ml / vial of TMB substrate solution	**TMB01**	**1 Bottle**
Stop Solution- 11 ml /vial 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 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrated should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard , Antibody Solution SHOULD BE STORED at -20 °C or – 70°C for up to one month. Streptavidin HRP Conjugate 200-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 with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 12 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.

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 is 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.

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, heparin, or citrate 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) (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.

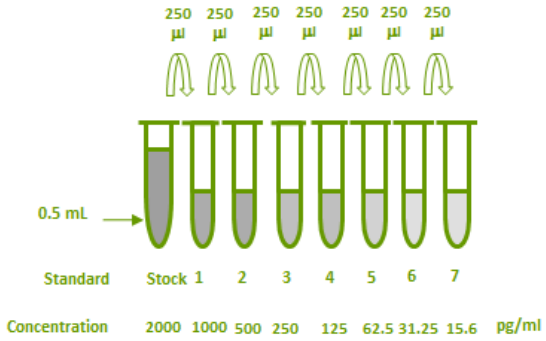
REAGENT PREPARATION

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 **FGF-21** Standard with 0.5 ml of **Dilution Buffer**. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5ml	2000 pg/ml
# 1	250µl of stock	250µl	1000 pg/ml
# 2	250µl of 1	250µl	500 pg/ml
# 3	250µl of 2	250µl	250 pg/ml
# 4	250µl of 3	250µl	125 pg/ml
# 5	250µl of 4	250µl	62.5 pg/ml
# 6	250µl of 5	250µl	31.25 pg/ml
# 7	250µl of 6	250µl	15.6 pg/ml

Detection Antibody- Reconstitute the **Detection Antibody concentrated** with 1.05 mL of **Antibody Diluent Solution** to produce a 10-fold concentrated stock solution. Pipette 9.45mL of the appropriate

Antibody Diluent Solution into the 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.



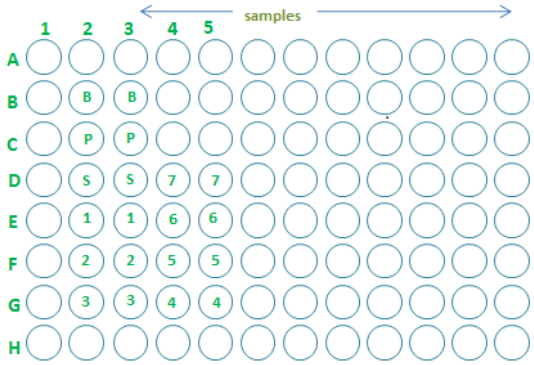
Streptavidin HRP Conjugate - Transfer 60 µl of 200-fold concentrated **Streptavidin HRP conjugate** stock solution to 11.94 mL of **HRP Diluent Solution** to prepare working solution. *Note: 1 x working solution of Streptavidin HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the **Positive Control** with 0.5 mL of **Dilution Buffer**. *Positive Control should be prepared and used immediately*. Reconstituted Positive Control CAN NOT BE REUSED.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended That Blank, standards, positive control and samples be assayed in duplicate.

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 with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard solution from #7 to S (reverse order of serial dilution) (from D4, D5 to G4, G5, G2, G3 to D2, D3), sample, or positive control per well (C2, C3). Cover with the 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 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 60 minutes 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 10 ~20 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 FGF-21 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 recombinant Human FGF-21.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of FGF-21 Was 5~7 pg/mL.

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

TYPICAL DATA

These standard curve data* are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0(0.052)
15.6	0.014
31.25	0.021
62.5	0.048
125	0.077
250	0.163
500	0.410
1000	0.734
2000	1.547

- **Lot No.:**
- **Positive Control: 106-200 pg/ml**

LINEARITY

To assess the linearity of the assay, pooled serum samples were diluted with Dilution Buffer DB06 and assayed.

SERUM SAMPLES	RECOVERY (%)
1 x	100
2 x	96.5
4 x	91.5

To assess the linearity of the assay, pooled EDTA plasma samples were diluted with Dilution Buffer DB06 and assayed.

PLASMA SAMPLES	RECOVERY (%)
1 x	100
2x	96.2

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇩
Add 100µl 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 solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate Solution to each well. Incubate 10~ 20 min on the bench top. **Protect from light.**
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min