

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

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



ALWAYS REFER TO LOT SPECIFIC  
PROTOCOL PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ AND CHECK ALL ITEMS OF EACH KIT  
BEFORE USING THIS PRODUCT.

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

**PRODUCT INFORMATION:**  
**THIS KIT IS FOR ONE TIME USE ONLY.**

ELISA NAME	HUMAN FGF-21 ELISA KIT
Catalog No.	SK00145-01
Lot No.	20114777
Formulation	96 T
Standard range	31.25- 2000 pg/ml
Sensitivity	5 ~ 7 pg/ml
Sample require	100 µl
Dilution Factor	<i>Optimal dilutions should be determined by each laboratory for each application</i>
Sample Type	Serum, EDTA Plasma
Specificity	Human FGF-21
Calibration	Human FGF-21 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8°C for 6 months, see page 2-3 for detail
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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**DESCRIPTION**

This Human FGF-21 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human FGF-21 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human FGF-21 and monoclonal antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural FGF-21 samples.

**ASSAY OVERVIEW**

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human FGF-21. The capture antibody can bind to the human FGF-21 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human FGF-21 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human FGF-21 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
<b>FGF-21 Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against FGF-21.	<b>145-01-01</b>	<b>1 plate</b>
<b>FGF-21 Standard</b> – 24000 pg/vial of recombinant Human FGF-21 in a buffered protein base with preservatives; lyophilized.	<b>145-01-02</b>	<b>1 vial</b>
<b>Detection Antibody</b> – 1.8 mL / vial, 10-fold concentrated of a purified IgG biotinylated against FGF-21 with preservatives; lyophilized.	<b>145-01-03</b>	<b>1 vial</b>
<b>Positive Control</b> – one vial of recombinant FGF-21 , lyophilized	<b>145-01-04</b>	<b>1 vial</b>
<b>Streptavidin HRP Conjugate</b> -120 µl/vial, 100-fold concentrated solution of Streptavidin HRP conjugate	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> - 45 mL/vial of buffered protein based solution with preservatives	<b>DB10</b>	<b>1 Bottle</b>
<b>HRP Diluent Solution</b> - 12 mL/vial of buffered protein based solution with preservatives	<b>DB08B</b>	<b>1 Bottle</b>
<b>Wash Buffer 20X</b> -25 ml/vial, 20-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 Bottle</b>
<b>TMB Substrate Solution</b> -11 ml / vial of TMB substrate solution	<b>TMB01</b>	<b>1 Bottle</b>
<b>Stop Solution</b> - 11 ml /vial of 0.25M HCL	<b>S-STOP</b>	<b>1 Bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 Piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 Piece</b>

**STORAGE**

**Unopened Kit:** Store at 2 – 8°C for up to 6 months. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20°C or -70°C. **Streptavidin HRP Conjugate** and **TMB Substrate Solution** should be stored only at 2 – 8 °C. Do not use kit past expiration date.

**ADDITIONAL MATERIALS REQUIRED**

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 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**

**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.

**Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

**SAMPLE PREPARATION**

Human EDTA plasma or serum may not require dilution.

Optimal dilutions should be determined by each laboratory for each application.

**Use polypropylene test tubes.**

**REAGENT PREPARATION**

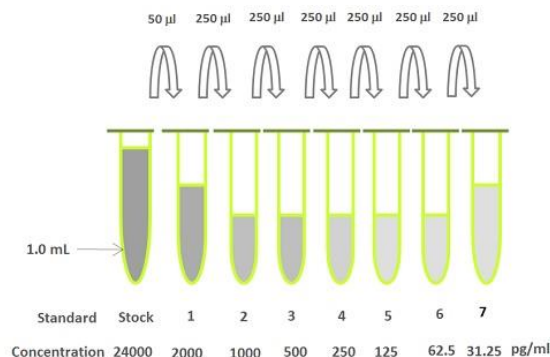
**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute **25 mL** of **Wash Buffer Concentrate (20-fold)**

into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

**FGF-21 Standard** - Reconstitute the FGF-21 Standard with 1.0 mL of **Dilution Buffer (DB10)**. This reconstitution produces a stock solution of 24000 pg/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 #2 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 Dilution Buffer serves as the zero standard (0 pg/mL). The optional set 4000pg/mL as the highest standard and use 4-fold serial dilution as standard. Store the stock solution at -70 °C for a few days

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	24000 pg/ml
optional	100 µl of stock	500µl	4000 pg/mL
# 1	50µl of stock	550µl	2000 pg/ml
# 2	250µl of 2	250µl	1000 pg/ml
# 3	250µl of 3	250µl	500 pg/ml
# 4	250µl of 4	250µl	250 pg/ml
# 5	250µl of 5	250µl	125 pg/ml
# 6	250µl of 6	250µl	62.5 pg/ml
# 7	250µl of 7	250µl	31.25 pg/ml



**Positive Control**- Reconstitute the Positive Control with 1 mL of **Dilution Buffer (DB10)**. Discard the positive control after use. It is for one time use only.

**Detection Antibody** - Reconstitute the Detection Antibody Concentrate with 1.8 mL of **Dilution Buffer (DB10)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly Pipette 9.45mL of Antibody Diluent Solution into the 15 mL centrifuge

tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. For partial strip test, freshly prepare the 900 µL per strip of working solution. Store the stock solution at -20 °C for a few days.

**Streptavidin-HRP Conjugate** – For 96 wells test, freshly Transfer 110 µL of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 10.89 mL of **HRP Diluent Solution (DB08B)** to prepare working solution (*protect from light*). *The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 20-30 min. For the partial strip test, freshly prepare 900 µL per strip of working solution. Always store the stock solution (100-fold concentrated) at 2 ~ 8 °C for 10 months.*

## ELISA PROTOCOL

**Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples 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 per well of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard solution from #9 to 1 (reverse order of serial dilution), sample, or positive control per well. Cover with the plate Sealer. Incubate for 2 hours on microplate 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 90 minutes on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 45 minutes on microplate 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 10-12 minutes on microplate 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 microplate reader set to 450 nm within 3 min.

## CALCULATION OF RESULTS

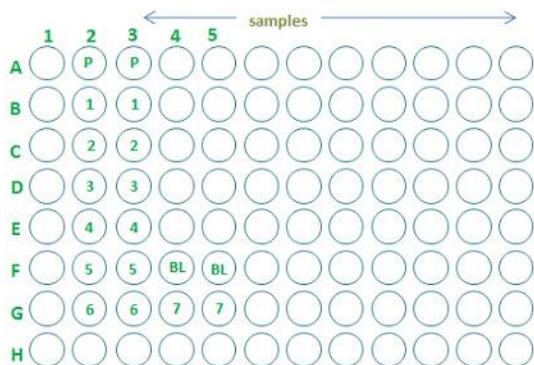
Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-parameter curve fit.

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

## 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
Mouse FGF 18	0
Human Secreted Klotho	0

Well Position



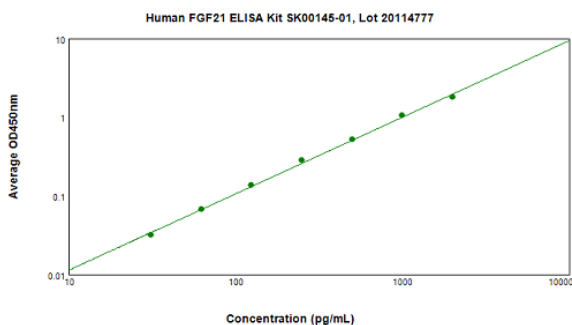
**TYPICAL DATA**

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

STANDARD (PG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.113)
31.25	0.032
62.5	0.067
125	0.137
250	0.282
500	0.519
1000	1.045
2000	1.801
4000 (optional)	2.599

- Lot No.:20114777
- Positive Control: 500 - 2000 pg/ml (log-log)

Standard curve by log-log fit



**SUMMARY OF ASSAY PROCEDURE**

<b>PREPARE REAGENTS, SAMPLES AND STANDARDS</b>
↓
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 90 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate solution to each well. Incubate 45 minutes on the plate shaker at RT. <b>Protect from light.</b>
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-12 minutes on the plate shaker at RT. <b>Protect from light.</b>
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.

Sample Test

The research samples were diluted by Dilution Buffer (DB10). Its linearity and recovery was assayed by SK00145-01.

Sample	Dilution Factor	Assayed (pg/mL)	Final (pg/mL)	Recovery (%)
Human EDTA Plasma	1 X	459.935	459.935	100
Human EDTA Plasma	2 X	231.670	463.340	101
Human EDTA Plasma	4 X	119.150	476.600	104

Manufacture Date: 25 July, 2023.

Expire Date: 30 May, 2024.

Lot No.: 20114777