ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human FGF-21 ELISA Kit

(Catalog Number: 31180)

For the quantitative determination of human FGF-21 concentrations in serum, plasma or cell culture supernate samples

IMD (Hong Kong)

Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)

Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

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INTRODUCTION

Fibroblast growth factor 21 (FGF-21) is a novel protein that has been implicated in the regulation of lipid and glucose metabolism under fasting and ketotic conditions[1,2]. In murine models, FGF-21 is predominantly expressed in liver, but it is also expressed in adipose tissue and pancreatic β-cells[3,4]. FGF-21 stimulates glucose uptake in adipocytes. It also protects animals from diet-induced obesity when overexpressed in transgenic mice and lowers blood glucose and triglyceride levels when administered to diabetic rodents[5]. When administered daily for 6 weeks to diabetic rhesus monkeys, FGF-21 caused a dramatic decline in fasting plasma glucose, fructosamine, triglycerides, insulin, and glucagon[6]. Furthermore, elevated plasma FGF-21 concentrations in humans appear to be related to the presence of hepatic and peripheral insulin resistance[7].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with affinity purified polyclonal antibody against human FGF-21. Standards and samples are pipetted into the wells and any human FGF-21 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-labelled polyclonal antibody against human FGF-21 is added to the wells. After wash step to remove any unbound reagents, streptavidin-horseradish peroxidase conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution 3,3′,5,5′-Tetramethylbenzidine (TMB) is added and color develops in proportion to the amount of human FGF-21 bound initially. Color reaction is stopped by 2M H_2SO_4 and the optical density of the wells are determined using a microtiter plate reader at 450nm. Since the increases in absorbance are directly proportional to the amount of captured human FGF-21, the unknown sample concentration can be calculated from the standard curve included in each assay.

INTENDED USE

This Human FGF-21 ELISA kit is designed for quantification of human FGF-21 in serum, plasma and cell culture supernate samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a polyclonal antibody against human FGF-21, sealed
2. 10×Wash buffer, 50 mL
3. 5×Assay buffer, 20 mL
4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against human FGF-21, 0.12 mL
5. Human FGF-21 standard, 1920 pg of recombinant human FGF-21, lyophilized
6. 200×STP-HRP solution, 0.06 mL
7. Substrate solution, 12 mL, ready for use
8. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human FGF-21 microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 mL) with 80 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 mL) with 450 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

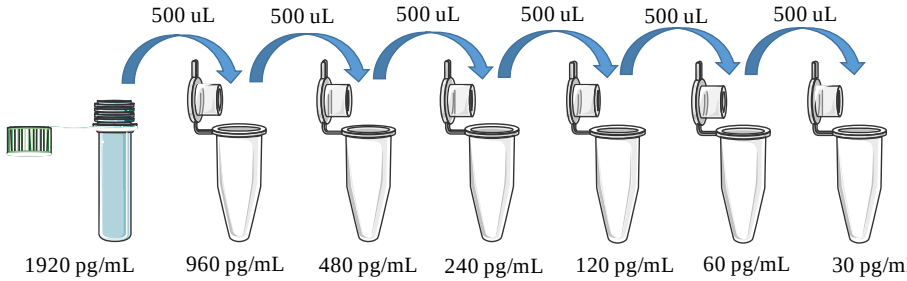
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µL of the 1×STP-HRP solution is required per well. Prepare only as much 1×STPHRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human FGF-21 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 1920 pg/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of 1×Assay buffer to 960, 480, 240, 120, 60, 30 pg/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 pg/mL). The reconstituted standard stock should be aliquoted and stored at -80ºC for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **2-fold** dilution in the 1×Assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µL of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human FGF-21 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.

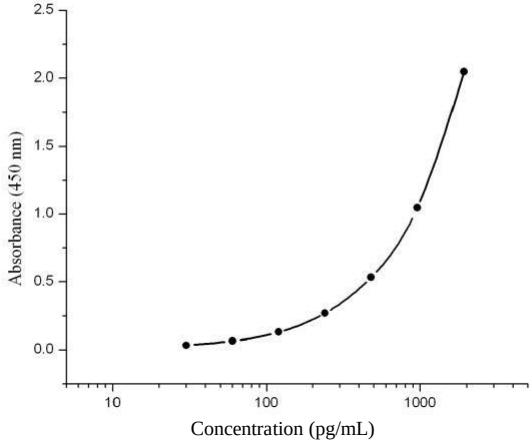
3. Determine human FGF-21 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Human FGF-21 (pg/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.094	0
30	0.127	0.033
60	0.158	0.064
120	0.224	0.13
240	0.362	0.268
480	0.626	0.532
960	1.141	1.047
1920	2.14	2.046

Human FGF-21 standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of human FGF-21 that can be detected by this assay is 30 pg/mL.

B. Specificity

Cross reactivity of recombinant proteins

Analyte	Cross Reactivity
Mouse FGF-21	No
Human FABP4	No
Human LCN2	No
Human Adiponectin	No
Human ANGPL4	No

C. Precision

Intra-assay Precision (Precision within an assay)

Two samples of known concentration were tested 12 times on one plate.

Sample	Mean (pg/mL)	SD (pg/mL)	CV (%)
1	93.3	3.72	4.0
2	547	27.6	5.0

Inter-assay Precision (Precision between assays)

Two samples of known concentration were tested in 10 separate assays.

Sample	Mean (pg/mL)	SD (pg/mL)	CV (%)
1	273.5	27.9	10.2
2	335	11.8	3.5

D. Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human FGF-21 were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

Sample 1

Dilution	Measured (pg/mL)	Expected (pg/mL)	Recovery (%)
Neat(non-diluted)	448	448	100
1:2	245	224	109
1:4	123	112	109
1:8	63.6	56	113

Sample 2

Dilution	Measured (pg/mL)	Expected (pg/mL)	Recovery (%)
Neat(non-diluted)	318	318	100
1:2	170	159	106
1:4	84.3	79.5	106

1:8	43.1	39.75	108

REFERENCES

1. Kharitonenkov A, et al. (2005) *J Clin Invest;* 115*:* 1627– 1635.
2. Badman MK, Pissios P, et al. (2007) *Cell Metab;* 5*:* 426– 437.
3. Nishimura T, et al. (2000) *Biochim Biophys Acta*; 1492*:* 203– 206.
4. Kurosu H, et al. (2007) *J Biol Chem;* 282*:* 26687– 26695.
5. Kharitonenkov A, et al. (2005) *J. Clin. Invest;* 115: 1627–35.
6. Kharitonenkov, et al. (2007) *Endocrinology*; 148:774-81.
7. Chavez AO, et al. (2009) *Diabetes Care*; 32:1542-6.

SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation