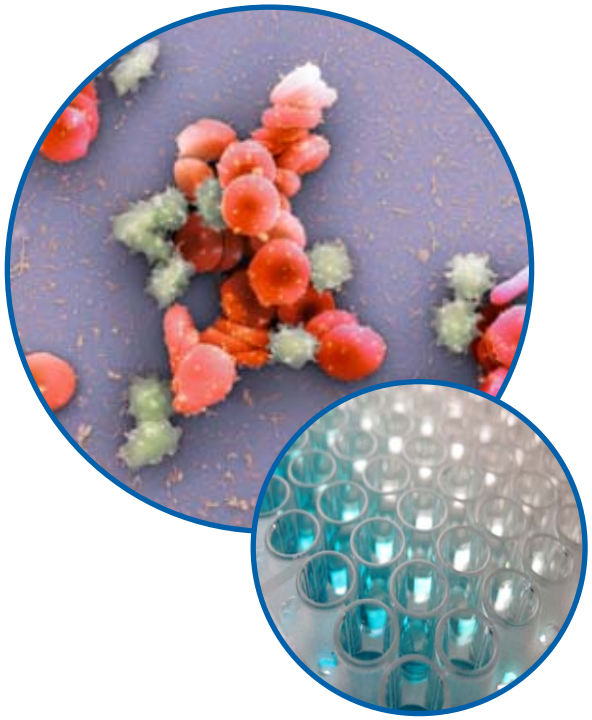


AdipoGen® LIFE SCIENCES

MANUAL

FGF-19 (human) ELISA Kit

[Fibroblast Growth Factor 19 (human) ELISA Kit]

For research use only. Not for diagnostic use

Version 1 (April-14-2014)

Cat. No. AG-45B-5002-KI01

Table of Contents

1. Intended Use 3

2. Introduction 3

3. General References 4

4. Assay Principle 5

5. Handling & Storage 5

6. Kit Components 5

7. Materials Required but *Not* Supplied 6

8. General ELISA Protocol 7

8.1. Preparation and Storage of Reagents 7

8.2. Sample Collection, Storage and Dilution 8

8.3. Assay Procedure (Checklist) 9

9. Calculation of Results 10

10. Typical Data 10

11. Performance Characteristics 11

12. Technical Hints and Limitations 12

13. Troubleshooting 13

14. Notes 14

1. Intended Use

The FGF-19 (human) ELISA Kit is to be used for the *in vitro* quantitative determination of human FGF-19 in cell culture supernatants, serum and plasma. This ELISA Kit is for research use only.

2. Introduction

Fibroblast growth factor 19 (FGF-19, also called FGF15 in rodents) is a member of a subfamily of fibroblast growth factors including FGF-21 and FGF-23 that govern nutrient metabolism (1). FGF-19 is expressed in the distal small intestine, where its synthesis is regulated by the nuclear bile acid receptor, FXR, after the postprandial uptake of bile acids. The primary source of endocrine FGF-19 is the ileum (2). FGF-19 mediates its effects through the cell surface proteins FGFR4 and β-Klotho and plays an important role in maintaining proper bile acid homeostasis (1, 3). Several pharmacologic studies in hyperglycaemic, obese animal models have shown that FGF-19 can improve metabolic rate and lower serum glucose and hepatic triglyceride and cholesterol levels (4, 5). Like insulin, FGF-19 functions as postprandial hormone to govern hepatic protein synthesis, glycogen synthesis and gluconeogenesis, but does not stimulate lipogenesis (6). Most recently it was shown that circulating FGF-19 is associated with remission of diabetes after Roux-en-Y gastric bypass surgery for obesity (7). FGF-19 and FGF-21 equally improved glucose parameters (8).

3. General References

(1) The FGF family: biology, pathophysiology and therapy: A. Beenken, et al.; *Nat. Rev. Drug Discov.* **8,** 235 (2009)

(2) Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis: T. Inagaki, et al.; *Cell Metab*. **4,** 217 (2005)

(3) Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man: T. Lundåsen, et al.; *J. Intern. Med.* **260,** 530 (2006)

(4) Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity: E. Tomlinson, et al.; *Endocrinology* **143,** 1741 (2002)

(5) Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes: L. Fu, et al.; *Endocrinology* **145,** 2594 (2004)

(6) FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis: S. Kir, et al.; *Science* **331,** 1621 (2011)

(7) A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass: G.S. Gerhard, et al.; *Diabetes Care* **36,** 1859 (2013)

(8) Dual actions of fibroblast growth factor 19 on lipid metabolism: X. Wu, et al.; *J. Lipid Res.* **54,** 325 (2013)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human FGF-19 in cell culture supernatants, serum and plasma. A polyclonal antibody specific for FGF-19 has been precoated onto the 96-well microtiter plate. Standards (STD) and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, FGF-19 is recognized by the addition of a biotinylated polyclonal antibody specific for human FGF-19 (DET). After removal of excess biotinylated antibody, streptavidin-peroxidase (STREP-HRP) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of human FGF-19 in the samples.

5. Handling & Storage

- Reagents must be stored at 2-8°C when not in use.
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

• 1 vial human FGF-19 Standard (lyophilized)	(2000 pg)	(STD)
• 1 vial Detection Antibody (100x)	(120 µl)	(DET)
• 1 vial HRP Labeled Streptavidin (200x)	(60 µl)	(STREP-HRP)
• 2 bottles Wash Buffer 10X	(2 x 25 ml)	(Wash Buffer 10X)
• 1 bottle Assay Buffer 5X	(20 ml)	(Assay Buffer 5X)
• 1 bottle TMB Substrate Solution	(12 ml)	(TMB)
• 1 bottle Stop Solution	(12 ml)	(STOP)
• 1 plate coated with FGF-19 Antibody	(12 x 8-well strips)	
• 2 plate Covers (plastic film)		
• 2 silica Gel Minibags		

7. Materials Required but *Not* Supplied

- Microtiterplate reader at 450nm
- Calibrated precision pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. General ELISA Protocol

8.1. Preparation and Storage of Reagents

NOTE: *Prepare just the appropriate amount of the buffers necessary for the assay.*

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml distilled or deionized water) to obtain Wash Buffer 1X. The 1x Wash buffer may be stored at 2-8°C for up to one month.

- **Assay Buffer 5X** has to be diluted with deionized water 1:5 before use (e.g. 10 ml Assay Buffer 5X + 40 ml distilled or deionized water) to obtain Assay Buffer 1X. The 1x Assay buffer may be stored at 2-8°C for up to one month.

- **Detection Antibody (DET) 100X** (after a spin-down) has to be diluted to 1:100 in Assay Buffer 1X (20 µl DET + 1.98 ml Assay Buffer 1X). 100µl of the 1x Detection antibody is required per well.

  NOTE: *Prepare only as much 1x Detection antibody as needed. The diluted Detection Antibody is not stable and cannot be stored!*

- **HRP Labeled Streptavidin (STREP-HRP) 200X** (after a spin-down) has to be diluted to 1:200 with Assay Buffer 1X (50 µl in 9.95 ml of Assay Buffer 1X). 100µl of the 1x STREP-HRP is required per well.

  NOTE: *Prepare only as much 1x STREP-HRP as needed. The diluted STREP-HRP is not stable and cannot be stored!*

- **Human FGF-19 Standard (STD)** has to be reconstituted with 1ml of Assay Buffer 1X.

  - This reconstitution produces a stock solution of 2000 pg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes **at RT.** Mix well prior to making dilutions.

  NOTE: *The reconstituted standard is aliquoted and stored at -20°C!*

  - Dilute the standard protein concentrate (STD) in Assay Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in Assay Buffer 1X is recommended.

  - Suggested standard points are:

    2000, 1000, 500, 250, 125, 62.5, 31.2, and 0 pg/ml.

-

To obtain	**Add**	Into
2000 pg/ml	2000 pg/ml stock	-
1000 pg/ml	250 µl of FGF-19 (2000 pg/ml)	250 µl of Assay buffer 1X
500 pg/ml	250 µl of FGF-19 (1000 pg/ml)	250 µl of Assay buffer 1X
250 pg/ml	250 µl of FGF-19 (500 pg/ml)	250 µl of Assay buffer 1X
125 pg/ml	250 µl of FGF-19 (250 pg/ml)	250 µl of Assay buffer 1X
62.5 pg/ml	250 µl of FGF-19 (125 pg/ml)	250 µl of Assay buffer 1X
31.2 pg/ml	250 µl of FGF-19 (62.5 pg/ml)	250 µl of Assay buffer 1X
0 pg/ml	250 µl of Assay buffer 1X	Empty tube

-
- ***NOTE:*** *Do not store the diluted standard solutions.*

8.2. Sample Collection, Storage and Dilution

Cell Culture Supernatants, serum and plasma have to be diluted in Assay Buffer 1X. Starting dilution of 1/2 is recommended.

8.3. Assay Procedure (Checklist)

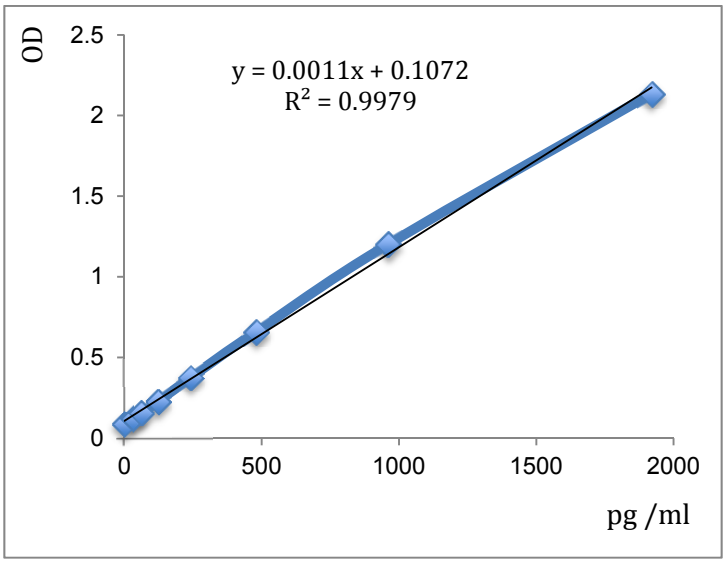
☐	1. Determine the number of 8-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the bag with 2 silica gel minibags and stored at 4°C. **NOTE:** *Remaining 8-well strips coated with* FGF-19 *antibody when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.*
☐	2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted serum, plasma or cell culture supernatant samples in duplicate to the wells **(see 8.1. Preparation and Storage of Reagents and 8.2 Preparation of Samples).**
☐	3. Cover the plate with plastic film and incubate for **1 h at room temperature.**
☐	4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	5. Add 100 µl to each well of the 1x Detection Antibody **(DET) (see 8.1 Preparation and Storage of Reagents)**.
☐	6. Cover the plate with plastic film and incubate for **1 h at room temperature.**
☐	7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	8. Add 100 µl to each well of the diluted HRP Labeled Streptavidin **(STREP-HRP) (see 8.1. Preparation and Storage of Reagents)**.
☐	9. Cover the plate with plastic film and incubate for **20 min at room temperature**.
☐	10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	11. Add 100 µl to each well of TMB substrate solution **(TMB)**.
☐	12. Allow the color reaction to develop **at room temperature in the dark for 15 minutes**. Do not cover the plate.
☐	13. Stop the reaction by adding 100 µl of Stop Solution (**STOP**). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (**STOP**) is added.
	! CAUTION: CORROSIVE SOLUTION !
☐	14. Measure the OD at 450 nm in an ELISA reader.

9. Calculation of Results

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 pg/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding FGF-19 concentration (pg/ml) on the vertical axis (see **10.** TYPICAL DATA).
- Calculate the FGF-19 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate the concentration of human FGF-19 in the sample.

10. Typical Data

The following data are obtained using the different concentrations of standard as described in this protocol:



Human FGF-19 (pg/ml)	Absorbance (450nm)
0	0.088
31.2	0.121
62.5	0.155
125	0.225
250	0.374
500	0.660
1000	1.201
2000	2.136

Figure: Standard curve

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of human FGF-19 that can be detected by this assay is 10 pg/ml. **NOTE:** *The Limit of detection was measured by adding three standard deviations to the mean value of 50 zero standard.*

B. Assay range: 31.2 pg/ml – 2000 pg/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant human FGF-19. It does not cross-react with human FGF-21.

D. Intra-Inter assay precision:

Intra-assay Precision (Precision within an assay)	C.V	<4.5%
Inter-assay Precision (Precision between assays)	C.V	<5.6%

E. Recovery:

The recovery of the assay was determined by adding various amounts FGF-19 to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 97%.

F. Expected values:

Human FGF-19 levels range in plasma or serum from 10pg /ml to >1000pg /ml.

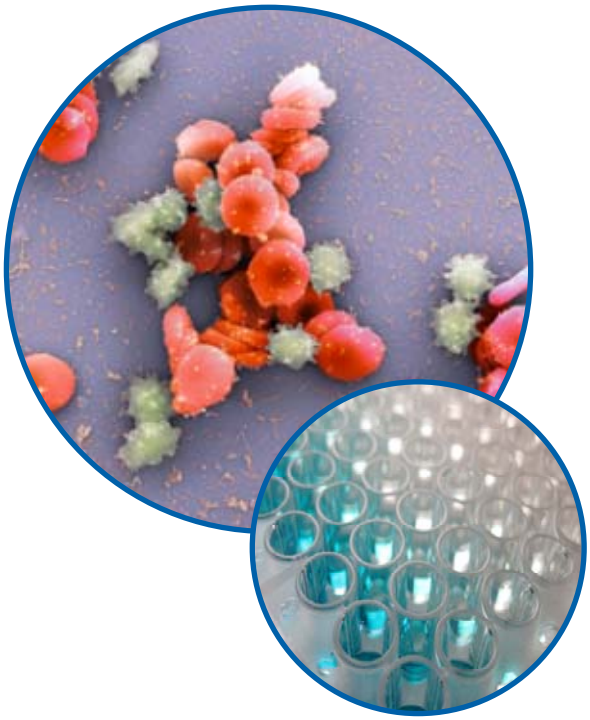
12. Technical Hints and Limitations

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Solution protected from light.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

13. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of STREP-HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

14. Notes



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