Quantikine® ELISA

Human FGF-19 Immunoassay

Catalog Number DF1900

For the quantitative determination of human Fibroblast Growth Factor 19 (FGF-19) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The Fibroblast Growth Factor (FGF) family consists of at least 22 highly conserved proteins and are found in many species ranging from *C. elegans* and *Drosophila* to humans (1). FGFs are heparin-binding growth factors with a core 120 amino acid (aa) FGF domain that allows for a common tertiary structure. In general, FGFs are expressed during embryonic development and in restricted adult tissues. They act on cells of mesodermal and neuroectodermal origin to regulate diverse physiologic functions including angiogenesis, cell growth, pattern formation, metabolic regulation, cell migration, neurotrophic effects, and tissue repair (2). The activities of the FGF family are mediated by four receptors, FGF R1 through FGF R4 (2). The receptors are transmembrane proteins with a tyrosine kinase domain and are thought to act as classical receptor tyrosine kinases. FGF R5/FGF RL1 has also been described, but lacks the kinase domain and signaling capability (3).

Human FGF-19 cDNA predicts a 251 aa precursor protein with a 22 aa signal peptide and a 229 aa secreted mature protein with no potential N-linked glycosylation sites (4, 5). It shares approximately 50% aa sequence identity with mouse and rat FGF-15 and 62% aa sequence identity with chicken FGF-19 (4-6). Unlike most FGFs, which bind to and activate more than one FGF receptor, FGF-19 appears to bind only FGF R4 (5). Receptor binding is capable of activating MAP Kinase and inducing the Prolactin promoter (7). FGF-19 has two putative disulfide bonds, one of which is conserved in the FGF family (8). Uniquely, it also has an extended loop structure that may affect heparan sulfate binding (8).

The functional roles of FGF-19 continue to be elucidated and include developmental, metabolic, and tumorigenic activities. During chick embryogenesis, FGF-19 has been shown to act synergistically with Wnt-8c to initiate inner ear development (6). However, the mouse FGF-19 ortholog, FGF-15, is not required for this activity (9). FGF-19 is also expressed in the embryonic chicken eye where it may play a role in lens development (10). Questions regarding the functions of human FGF-19 have been addressed using ectopic expression. Transgenic over-expression of human FGF-19 in mice results in tumor formation, increased energy expenditure, decreased adiposity, and a resistance to weight gain in response to a high fat diet (11, 12). Similar affects have been observed in mice treated intravenously with recombinant FGF-19 (13).

The Quantikine® Human FGF-19 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human FGF-19 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human FGF-19 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human FGF-19 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human FGF-19.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human FGF-19 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human FGF-19 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human FGF-19 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of FGF-19 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human FGF-19 Microplate	892901	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human FGF-19.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human FGF-19 Conjugate	892902	21 mL of a polyclonal antibody specific for human FGF-19 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human FGF-19 Standard	892903	Recombinant human FGF-19 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1S	895137	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:3 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- 500 mL graduated cylinder.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Test tubes for dilution of standards and samples.
- Human FGF-19 Controls (optional; R&D Systems®, Catalog # QC24).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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 or heparin 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: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Cell culture supernate samples may require up to a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5P (diluted 1:3)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

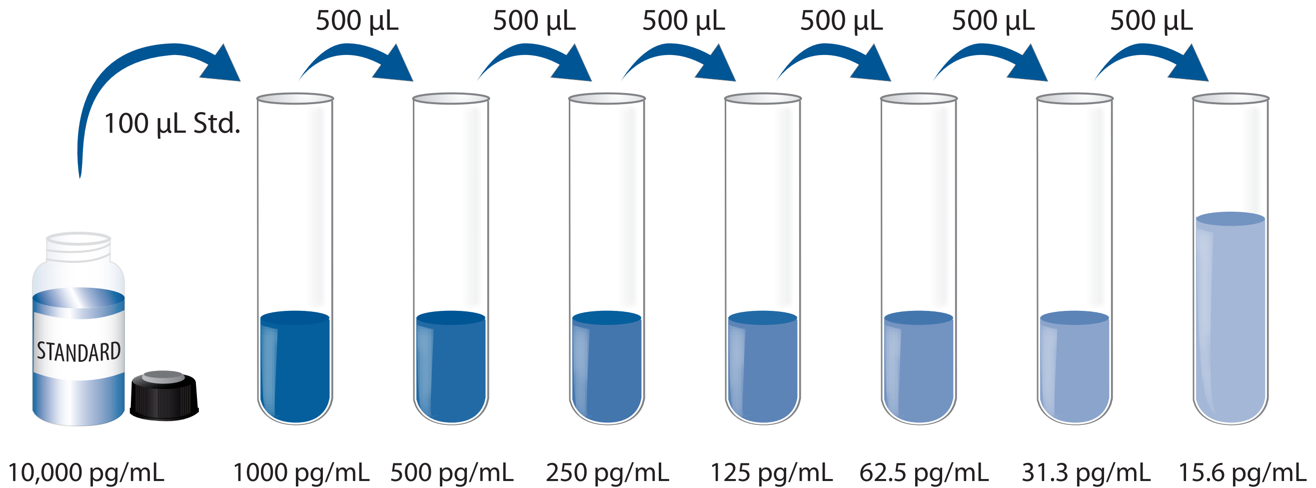
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:3) - Add 10 mL of Calibrator Diluent RD5P Concentrate to 20 mL of deionized or distilled water to prepare 30 mL of Calibrator Diluent RD5P (diluted 1:3).

Human FGF-19 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FGF-19 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:3) into the 1000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:3) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1S to each well.
4. Add 100 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours 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 (400 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 200 µL of Human FGF-19 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

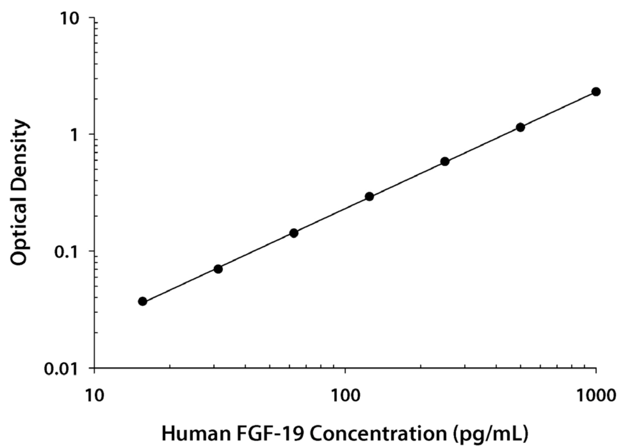
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 a log/log graph. The data may be linearized by plotting the log of the human FGF-19 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.023	0.023	—
15.6	0.059 0.060	0.060	0.037
31.3	0.091 0.094	0.093	0.070
62.5	0.159 0.171	0.165	0.142
125	0.307 0.324	0.316	0.293
250	0.601 0.611	0.606	0.583
500	1.145 1.186	1.166	1.143
1000	2.322 2.330	2.326	2.303

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	113	346	689	119	349	700
Standard deviation	7.26	15.7	24.8	6.46	19.3	31.7
CV (%)	6.4	4.5	3.6	5.4	5.5	4.5

RECOVERY

The recovery of human FGF-19 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	104	93-109%
Serum (n=4)	95	88-100%
EDTA plasma (n=4)	94	85-101%
Heparin plasma (n=4)	92	89-95%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human FGF-19 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	100	101	107	102
	Range (%)	94-105	97-105	102-114	94-106
1:4	Average % of Expected	101	101	99	99
	Range (%)	93-106	98-105	97-101	93-104
1:8	Average % of Expected	100	99	96	94
	Range (%)	94-104	94-104	89-101	85-101
1:16	Average % of Expected	100	94	92	92
	Range (%)	93-105	90-99	85-98	90-95

SENSITIVITY

Fifty-five assays were evaluated and the minimum detectable dose (MDD) of human FGF-19 ranged from 0.53-3.35 pg/mL. The mean MDD was 1.17 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human FGF-19 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human FGF-19 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=36)	189	31-554	125
EDTA plasma (n=36)	179	28-546	118
Heparin plasma (n=36)	183	29-545	122

Cell Culture Supernates:

Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human FGF-19. No detectable levels were observed.

COLO 205 human colon adenocarcinoma cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed on day three, assayed for human FGF-19, and measured 17,072 pg/mL.

HT-29 human colon adenocarcinoma cells were cultured with McCoy's 5a media supplemented with 10% fetal bovine serum until confluent. Aliquots of the cell culture supernate were removed, assayed for human FGF-19, and measured 5714 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human FGF-19.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human FGF-19 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
FGF acidic
FGF basic$_{(146)}$
FGF-3
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-16
FGF-17
FGF-18
FGF-21
FGF-23
FGF R1A
FGF R1B
FGF R2A
FGF R2B
FGF R3A
FGF R4*
FGF R4/Fc Chimera
FGF R5
Flt-3/Flk-2 ligand
Flt-4
G-CSF
GM-CSF
HB-EGF
HGF
HRG-α
IGF-I
IGF-II
KGF (FGF-7)
M-CSF
MSP
MSPβ
β-NGF
PDGF-AA
PDGF-AB
PDGF-BB
PD-ECGF
PlGF
PlGF-2
VEGF$_{121}$
VEGF$_{165}$
VEGF/PlGF
VEGF-D

Recombinant mouse:

FGF-8b
FGF-8c
Flt-3/Flk-2 ligand
G-CSF
GM-CSF
M-CSF
VEGF$_{120}$
VEGF$_{164}$

Recombinant rat:

FGF bp
β-NGF
GM-CSF
PDGF-BB

Other recombinants:

porcine GM-CSF
canine KGF

Natural proteins:

β-Casein
bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF

*Recombinant human FGF R4 did not show interference in the assay when tested using heparin plasma.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

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NOTES

NOTES

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