BioVendor Group

ENG

Instructions for use:

HUMAN FGF-19 ELISA

Catalogue number:

RD191107200R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current Version
ENG.004.A	**ENG.005.A**
”History of changes” added.	
Chapter 9: A sentence ”Centrifuge liquid containing microtube vials before opening“ added.	

1. INTENDED USE

The RD191107200R Human FGF-19 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human fibroblast growth factor-19 (FGF-19).

Features

- It is intended for research use only
- The total assay time is less than 3.5 hours
- The kit measures FGF-19 in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard and Quality Controls are recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under this condition, the kit is stable until the expiration date (see the label on the box).
For stability of opened components see Chapter 9.

3. INTRODUCTION

Fibroblast growth factors (FGFs) are a large family of small (17-26 kDa) polypeptide growth factors found in organisms ranging from nematodes to humans. The FGF family has at least 22 members in vertebrates and share 13-71% amino acid identity. The initial characterization of these proteins focused on their ability to stimulate fibroblast proliferation. During embryonic development, FGFs have diverse roles in regulating cell proliferation, migration and differentiation. In the adult organism, FGFs are homeostatic factors and function in tissue repair and response to injury.
FGF signaling is mediated through one of four FGF receptors (FGFR1-FGFR4), a complex family of transmembrane receptor tyrosine kinases. FGFR5 has also been described, but lacks the kinase domain and signaling capability. FGFs have a high affinity for heparin sulfate proteoglycans and require heparin sulfate to activate FGF receptors. Although multiple FGFs interact with each of the four FGFRs, a novel fibroblast growth factor FGF-19 exhibits exclusive binding to only one of FGF receptors (FGFR4).
The normal function of FGF-19 has not been resolved, although its role in inner ear development has been suggested. It has been also found that hepatocyte expression of FGF-19 is induced by the transcription factor, farnesoid X receptor (FXR). FXR is a key regulator of cholesterol metabolism through suppression of the catabolic enzyme cyp7a, the first and rate-limiting step in the biosynthesis of bile acids (BA). A recent study found that, in humans, circulating FGF-19 has a diurnal rhythm controlled by the transintestinal BA flux, and that FGF-19 modulates hepatic BA synthesis. Through its systemic effects, circulating FGF-19 may also mediate other known BA-dependent effects on lipid and carbohydrate metabolism.
In transgenic mice expressing human FGF-19, researchers found a significant increased metabolic rate as well as decreased body weight and adiposity. Additionally, resistance to both diet-induced obesity and insulin desensitization were found. Similar responses have been observed when recombinant FGF-19 was injected into the mice. However, it has been shown too, that FGF-19 transgenic mice develop hepatic adrenocarcinomas with age, and recombinant FGF-19 treated mice exhibit proliferation of hepatocytes.

Areas of investigation:
Cholesterol metabolism
Metabolic syndrome

4. TEST PRINCIPLE

In the Biovendor Human FGF-19 ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human FGF-19 antibody. After a 60 minute incubation followed by washing, biotin labelled polyclonal anti-human FGF-19 antibody is added and incubated with the captured FGF-19 for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of FGF-19. A standard curve is constructed by plotting absorbance values against FGF-19 concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate (TMB) Solution, which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution or the Substrate Solution, wash skin/eyes thoroughly with water and seek medical attention when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (50x)	concentrated	0.3 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Biotin-Ab Diluent	ready to use	13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for samples dilution
- Glassware (graduated cylinder and bottle) for Wash Solution
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). Manual washing is possible but not preferable
- Microplate reader with 450 ±10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Centrifuge liquid containing microtube vials before opening.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label

Assay reagents supplied ready-to-use:

Antibody Coated Microtiter Strips

Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Dilution Buffer

Biotin-Ab Diluent

Substrate Solution

Stop Solution

Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized:

Human FGF-19 Master Standard

Refer to Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human FGF-19 in the stock solution is 800 pg/ml.
Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-----	800 pg/ml
300 µl of stock	300 µl	400 pg/ml
300 µl of 400 pg/ml	300 µl	200 pg/ml
300 µl of 200 pg/ml	300 µl	100 pg/ml
300 µl of 100 pg/ml	300 µl	50 pg/ml
300 µl of 50 pg/ml	300 µl	25 pg/ml
300 µl of 25 pg/ml	300 µl	12.5 pg/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the Standard stock solution and the set of standards.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes occasional gentle shaking (not to foam).
The reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Quality Controls.

Note:
Concentration of analyte in Quality Control need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Control serves just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody Conc. (50x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (50x) with 49 parts Biotin-Ab Diluent. Example: 20 µl of Biotin Labelled Antibody Concentrate (50x) + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Concentrate (50x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures human FGF-19 in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C or - 70°C. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 3x with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets or 80 µl of sample + 160 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Serum samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of FGF-19.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results!

11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards reconstituted Quality Control and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. (The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C). No shaking!
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550–650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine FGF-19 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 800**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 400**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 200**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 100**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 50**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 12.5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of FGF-19 (pg/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay; e.g. 200 pg/ml (from standard curve) x 3 (dilution factor) = 600 pg/ml.

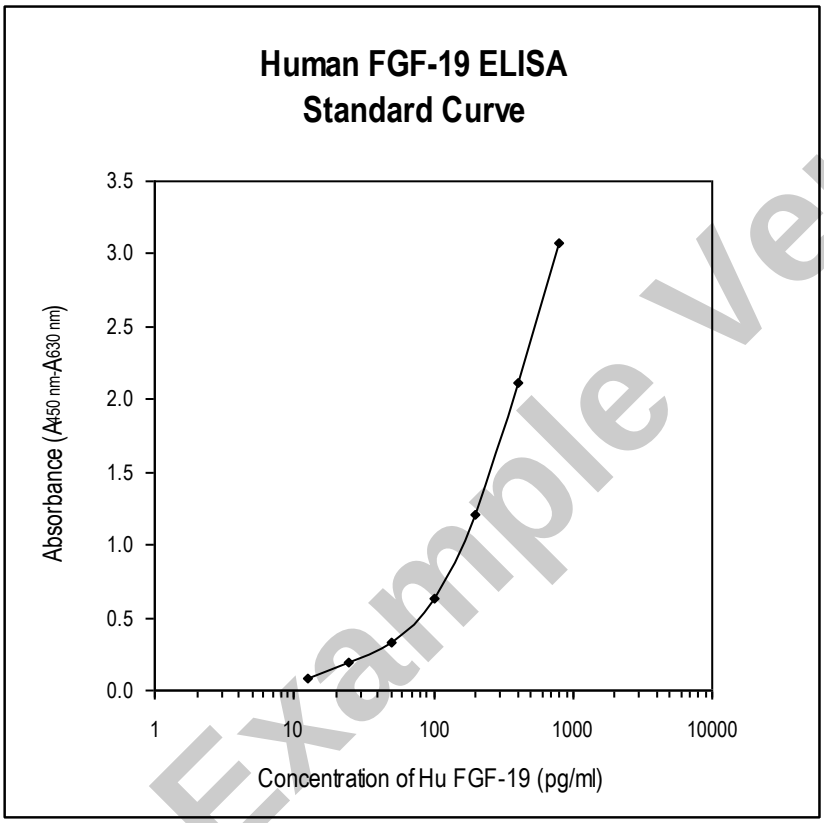

Figure 2: Typical Standard Curve for Human FGF-19 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human FGF-19 ELISA are presented in this chapter

Sensitivity

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real human FGF-19 values in wells and is: 4.8 pg/ml.
* Dilution Buffer is pipetted into Blank wells.

Limit of assay

Results exceeding human FGF-19 level of 800 pg/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the FGF-19 concentration.

Specificity

The antibodies used in this ELISA are specific for human FGF-19. No crossreactivity with recombinant human FGF-21 and recombinant human FGF-23 has been observed.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com

Mammalian serum sample	Observed crossreactivity
Bovine	no
Goat	no
Hamster	no
Horse	yes
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

Presented results are multiplied by respective dilution factor

Precision

Intra-assay (Within-Run, n=8)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	196.0	13.7	7.0
2	384.0	19.2	5.0

Inter-assay (Run-to-Run, n=6)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	194.0	16.5	8.5
2	462.0	30.0	6.5

Spiking Recovery

Serum samples were spiked with different amounts of human FGF-19 and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	121.1 859.2 493.5 364.8	- 962.1 587.1 399.6	- 89.3 84.1 91.3
2	415.2 1 128.0 724.5 545.5	- 1 165.2 790.2 602.0	- 96.8 91.7 90.5

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	- 2x 4x 8x	1 023.0 474.6 240.0 133.8	- 511.5 255.7 127.9	- 92.8 93.8 104.6
2	- 2x 4x 8x	1 410.0 651.0 357.0 164.1	- 705.0 352.5 176.2	- 92.3 101.3 93.1

Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

Volunteer No.	Serum (pg/ml)	Plasma (pg/ml)		
		EDTA	Citrate	Heparin
1	156.9	158.4	158.4	157.5
2	155.1	173.1	161.1	140.4
3	264.9	214.8	236.7	241.5
4	413.7	402.9	402.7	423.3
5	447.6	485.9	387.6	424.2
6	117.6	109.5	95.1	119.4
7	227.4	184.2	201.0	210.6
8	256.2	211.8	221.4	237.3
9	191.9	186.9	158.1	186.9
10	373.8	331.2	315.0	343.5
Mean	**260.5**	**245.9**	**233.7**	**248.5**
Mean Plasma/Serum	**-**	**94%**	**90%**	**95%**
Coefficient of determination R^2	**-**	**0.94**	**0.97**	**0.99**

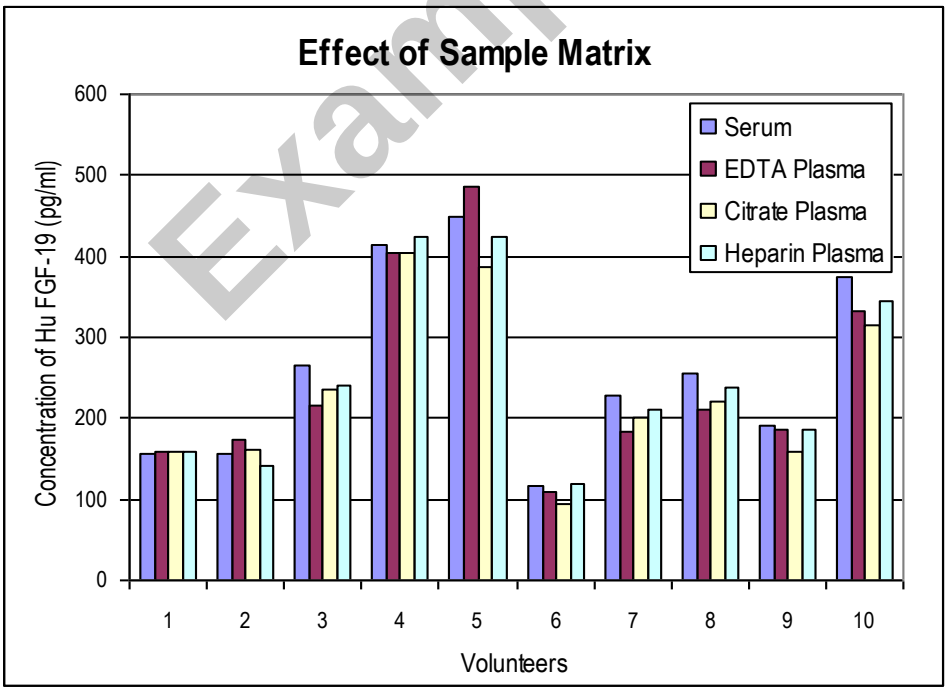

Figure 3: FGF-19 levels measured using Human FGF-19 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

Stability of samples stored at 2-8°C

Samples should be stored at -20°C. However, no decline in concentration of human FGF-19 was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Serum (pg/ml)	Plasma (pg/ml)		
			EDTA	Citrate	Heparin
1	-20°C	256.2	211.8	221.4	237.3
	2-8°C, 1 day	224.4	241.5	243.6	263.4
	2-8°C, 7 days	235.8	236.4	209.7	225.3
2	-20°C	191.9	186.9	158.1	186.9
	2-8°C, 1 day	186.9	162.3	169.2	170.4
	2-8°C, 7 days	183.9	176.4	151.8	183.3
3	-20°C	373.8	331.2	315.0	343.5
	2-8°C, 1 day	326.7	383.1	33.6	326.4
	2-8°C, 7 days	372.3	339.3	404.7	374.1

Effect of Freezing/Thawing

No significant decline was observed in concentration of human FGF-19 in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (pg/ml)	Plasma (pg/ml)		
			EDTA	Citrate	Heparin
1	1x	447.6	485.9	387.6	424.2
	3x	463.8	408.3	387.9	459.6
	5x	411.6	439.2	386.1	364.2
2	1x	117.6	109.5	95.1	119.4
	3x	108.9	81.6	93.6	93.3
	5x	111.3	99.0	93.3	112.8
3	1x	227.4	184.2	201.0	210.6
	3x	207.6	207.3	203.4	168.9
	5x	225.6	174.6	188.7	210.6

14. DEFINITION OF THE STANDARD

The recombinant human FGF-19 is used as the Standard. The recombinant human FGF-19, produced in *E.coli*, is 23.03 kDa protein containing 192 amino acid residues of the human FGF-19 and 14 additional amino residues.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 140 unselected donors (70 women + 70 men) 4-85 years old were assayed with the Biovendor Human FGF-19 ELISA in our laboratory.

Age and sex dependent distribution of FGF-19

Sex	Age (years)	n	Mean	SD	Min.	Max.
			FGF-19 (pg/ml)			
Men	4-19	7	155.9	72.8	47.7	266.7
	20-39	16	282.9	189.7	45.9	615.3
	40-59	20	235.3	160.0	55.5	511.8
	60-79	20	304.3	263.0	64.8	1 218.0
	80-85	7	235.5	124.8	66.9	490.5
Women	4-19	8	272.8	151.3	72.0	482.7
	20-39	18	181.9	126.0	56.4	480.6
	40-59	16	208.4	173.2	27.3	733.6
	60-79	20	167.3	83.5	46.5	375.6
	80-82	8	297.7	143.2	79.8	470.4

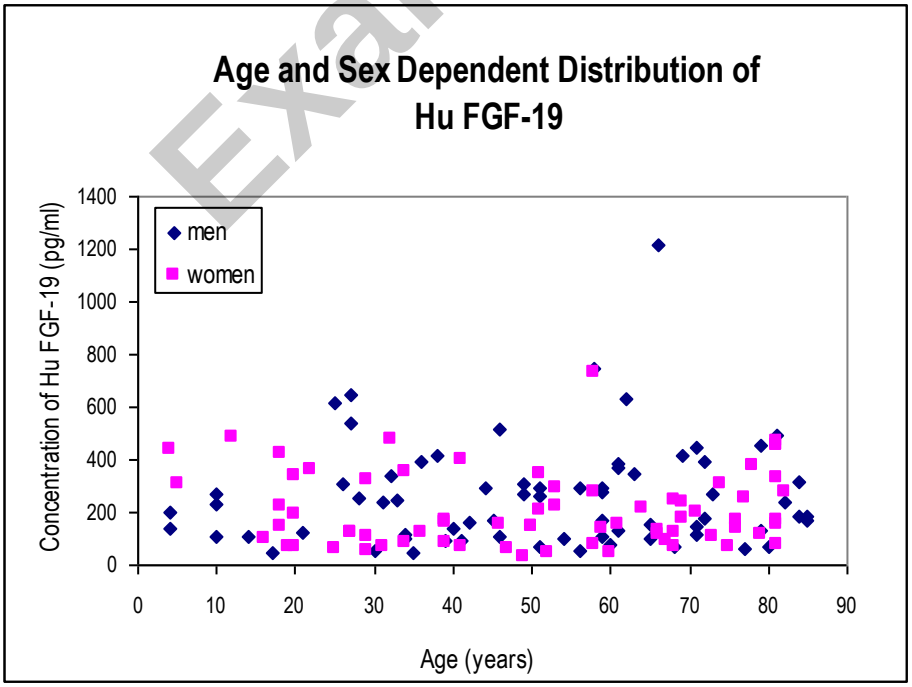

Figure 4: Human FGF-19 concentration plotted against donor age and sex.

Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should estabilish its own normal and pathological reference ranges for FGF-19 levels with the assay.

16. METHOD COMPARSION

The Biovendor Human FGF-19 ELISA was compared to the other commercial ELISA immunoassay, by measuring 39 serum samples. The following correlation graph was obtained:

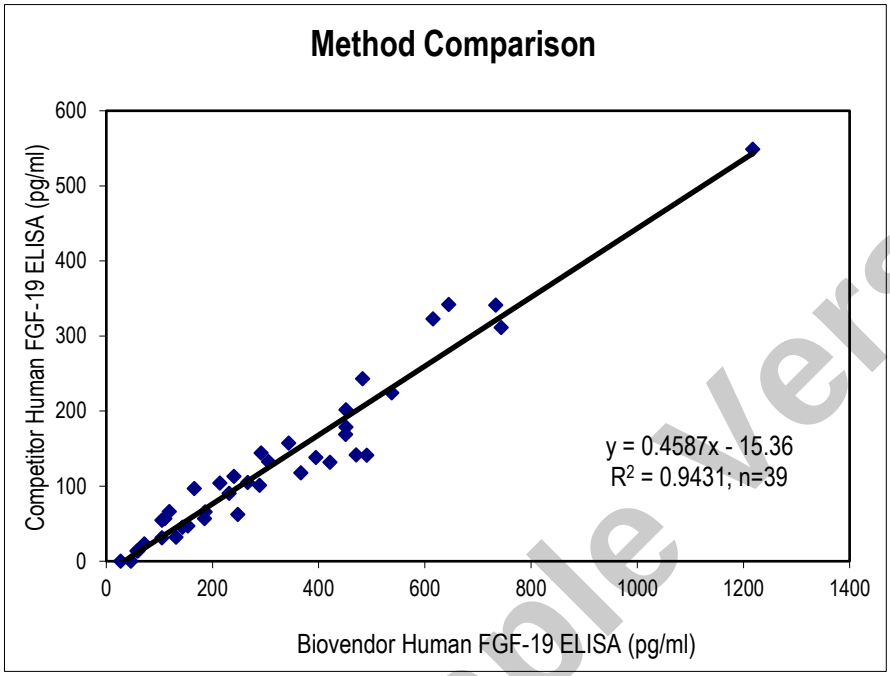

Figure 5: Method comparison.

17. TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:
- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:
- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

18. REFERENCES

References to human FGF-19:

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- Strack AM and Myers RW: Modulation of metabolic syndrome by fibroblast growth factor 19 (FGF-19)? Endocrinology 145, 2591-2593 (2004), Review
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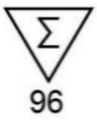
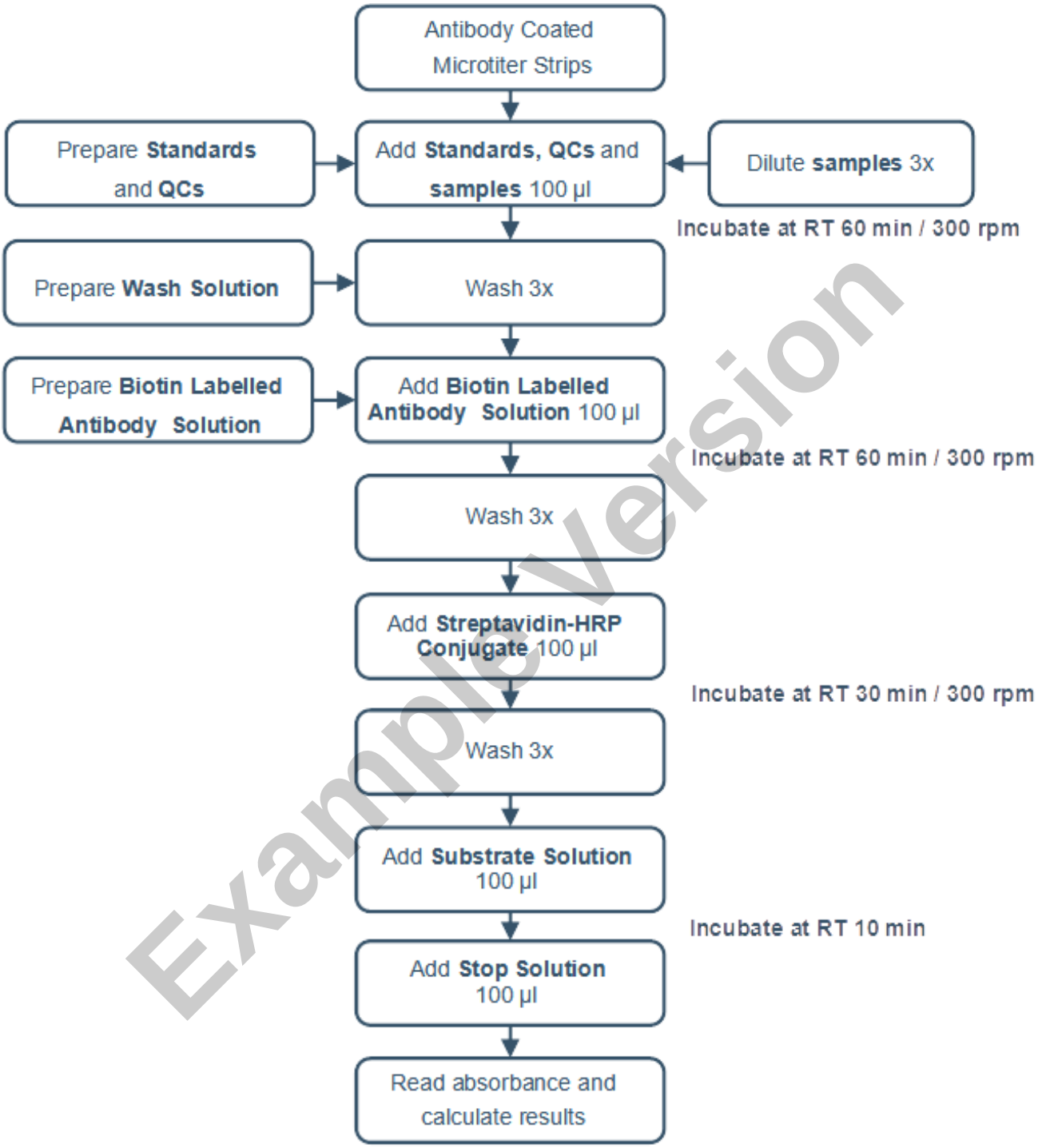
- Tomlinson E, Fu L, John L, Hultgren B, Huang X, Renz M, Stephan JP, Tsai SP, Powell-Braxton L, French D and Stewart TA: Transgenic mice expressing human fibroblast grwth factor-19 display increased metabolic rate and decreased adiposity. Endocrinology 143, 1741-1747 (2002)
- Ornitz DM and Itoh N: Fibroblast growth factors. Genome Biology 2(3), 3005.1-3005.12 (2001), Review

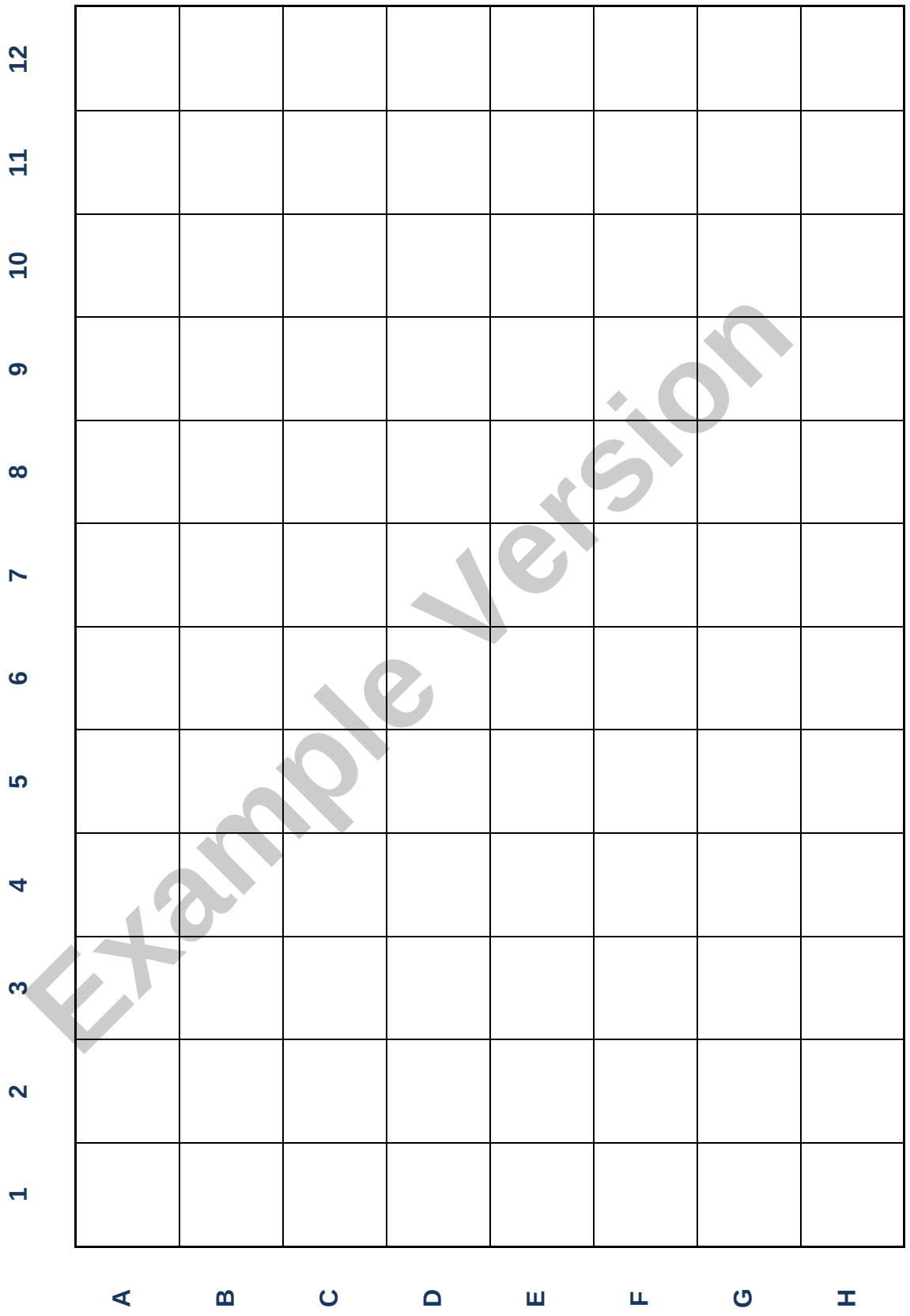
For more references on this product see our web pages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
LOT	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

20. ASSAY PROCEDURE - SUMMARY





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