BioVendor Group

ENG

Instructions for Use:

HUMAN FGF-21 ELISA

Catalogue number:

RD191108200R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.006.A	ENG.007.A
Symbol indicating the manufacturer added.	
History of changes added.	

1. INTENDED USE

The RD191108200R Human FGF-21 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human FGF-21 (fibroblast growth factor-21).

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures Human FGF-21 in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is 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 these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

The fibroblast growth factor family (FGFs) are a family of more than 20 small (17-26 kDa) secreted peptides. The initial characterisation of these proteins focused on their ability to stimulate fibroblast proliferation through FGF receptors (FGFRs). Members of FGFs family play important roles in defining and regulating the development and function of endocrine tissues as well as modulating various metabolic processes.
A recently described member of FGFs family, FGF-21, also called Fibroblast growth factor 21 precursor and UNQ3115/PRO10196, has been characterised as a potent metabolic regulator. FGF-21 is preferentially expressed in liver and regulates glucose uptake in human fat cells. Moreover, therapeutic administration of FGF-21 decreased plasma glucose levels and triglycerides to near normal levels in multiple mouse models of type 2 diabetes. Short-term treatment of normal or db/db mice with FGF-21 lowered plasma levels of insulin and improved glucose clearance compared with vehicle after oral glucose tolerance testing. Constant infusion of FGF-21 for 8 weeks in db/db mice nearly normalized fed blood glucose levels and increased plasma insulin levels. When administrated daily for 6 weeks to diabetic rhesus monkeys, FGF-21 caused dramatic decline in fasting plasma glucose, fructosamine, triglicerides, insulin, and glucagon. FGF-21 administration also led to significant improvements in lipoprotein profiles, including lowering of low-density lipoprotein cholesterol and raising of high-density lipoprotein cholesterol as well as beneficial changes in the circulating levels of several cardiovascular risk factors.
FGF-21, when overexpressed, protected animals from diet-induced obesity. These results define a functional role for FGF-21 in vivo and provide evidence that FGF-21 can lower glucose and triglyceride levels in diabetic animals.
In contrast to several members of the FGF family which may induce therapeutically undesirable in vivo proliferation of various cell types, a recent study demonstrated that FGF-21 did not induce mitogenicity, hypoglycemia or weight gain at any dose tested in diabetic or healthy animals or when overexpressed in transgenic mice. Thus, FGF-21 appears to have considerable potential for the treatment of diabetes mellitus.

Areas of investigation:
Lipid metabolism
Diabetes mellitus type 2
Metabolic syndrome

4. TEST PRINCIPLE

In the BioVendor Human FGF-21 ELISA, the standards, quality controls and samples are incubated in microtitrate wells pre-coated with polyclonal anti-human FGF-21 antibody. After 60 min incubation and a washing, biotin-labelled polyclonal anti-human FGF-21 antibody is added and incubated with captured FGF-21 for 60 min. After another washing, the streptavidin- HRP conjugate is added. After 30 min 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-21. A standard curve is constructed by plotting absorbance values against 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
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and 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. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	2x 20 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution (TMB),	ready to use	13 ml
Stop Solution (0.2 M H_2SO_4)	ready to use	13 ml

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- 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.

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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Substrate Solution

Stop Solution

Dilution Buffer

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

Assay reagents supplied concentrated or lyophilized:

Human FGF-21 Master Standard

Refer to the 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 FGF-21 in the stock solution is **1920 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	1920 pg/ml
250 µl of stock	250 µl	960 pg/ml
250 µl of 960 pg/ml	250 µl	480 pg/ml
250 µl of 480 pg/ml	250 µl	240 pg/ml
250 µl of 240 pg/ml	250 µl	120 pg/ml
250 µl of 120 pg/ml	250 µl	60 pg/ml
250 µl of 60 pg/ml	250 µl	30 pg/ml

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

Stability and storage:

The reconstituted Master Standard must be used immediately or stored frozen at –20 °C for 3 months. Avoid repeating freezing/thawing cycles.
Do not store the diluted Standard solutions.

Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Dilution Buffer. Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Dilution Buffer for 1 strip (8 wells).

Stability and storage:
Do not store the diluted Biotin Labelled Antibody solution.

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 with occasional gentle shaking (not to foam).
The reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage
The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 month. Avoid repeated freeze/thaw cycles.

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.

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 FGF-21 in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly 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 serum or plasma samples 2x with Dilution Buffer just prior to the assay, e.g. 75 µl of sample + 75 µl of Dilution Buffer when assaying samples as singlets or preferably 125 µl of sample + 125 µl of Dilution Buffer for duplicates). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
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-21.

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 Controls 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. Pipet **100 µl** of Biotin Labelled Antibody 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. Pipet **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. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
11. Incubate the plate for **15 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-21 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 1920**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 960**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 480**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 240**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 120**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 60**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 30**	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-21 (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. 185.3 pg/ml (from standard curve) x 2 (dilution factor) = 370.6 pg/ml.

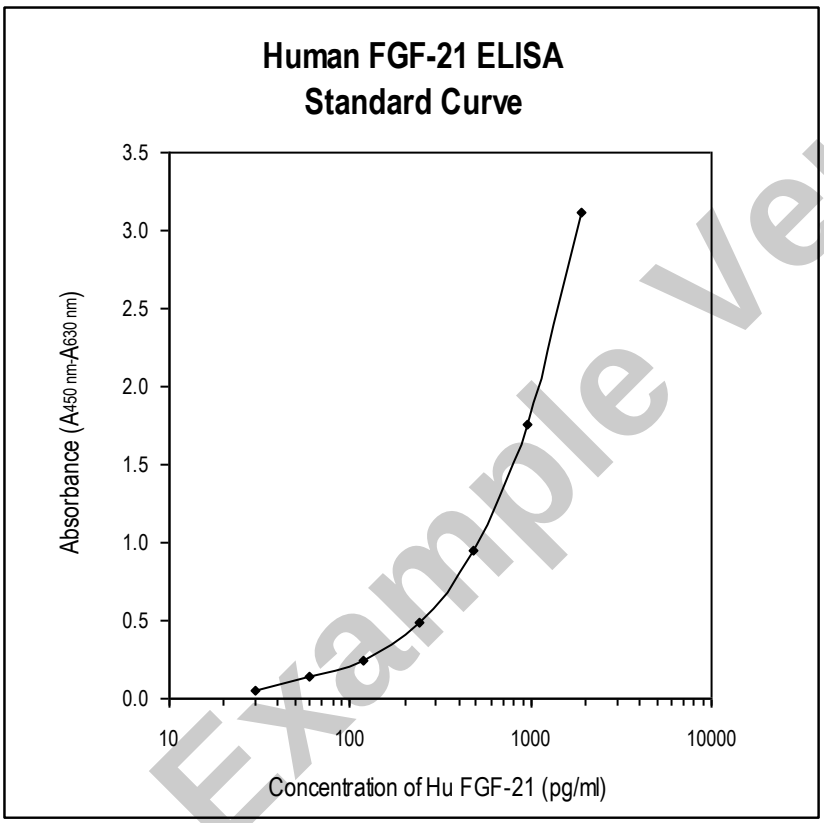

Figure 2: Typical Standard Curve for Human FGF-21 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human FGF-21 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 FGF-21 values in wells and is 7 pg/ml.
*Dilution Buffer is pipetted into blank wells.

Limit of assay

Results exceeding serum/plasma FGF-21 level of 1920 pg/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the FGF-21 concentration.

Specificity

The antibodies used in this ELISA are specific for human FGF-21. No crossreactivity with human FGF-19 and 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
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
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	418.2	9.9	2.4
2	2940.9	46.1	1.6

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

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	249.9	8.8	3.5
2	319.6	10.0	3.1

Spiking Recovery

Serum samples were spiked with different amounts of human FGF-21 and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	172.4	-	-
	920.9	892.7	103.2
	527.4	532.7	102.8
	372.6	352.7	105.6
2	293.3	-	-
	955.2	1013.3	94.3
	631.2	653.3	96.6
	471.7	473.3	99.7

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	-	404.7	-	-
	2x	209.9	202.4	103.7
	4x	98.5	101.2	97.4
	8x	46.4	50.6	91.6
2	-	3017.0	-	-
	2x	1616.0	1508.5	107.1
	4x	785.0	754.3	104.1
	8x	409.8	377.1	108.7

Effect of sample matrix

Heparin, citrate and EDTA 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	76.5	95.3	69.0	106.2
2	250.2	345.9	242.0	359.8
3	165.6	238.1	156.2	209.9
4	188.3	258.5	175.0	276.8
5	299.4	407.1	309.0	461.6
6	634.9	707.2	525.2	798.3
7	244.5	241.2	208.2	317.3
8	182.2	198.5	179.2	223.7
9	103.5	109.6	86.2	142.3
10	48.7	63.1	46.6	81.3
Mean (pg/ml)	**219.4**	**266.5**	**199.7**	**297.7**
Mean Plasma/Serum (%)	**-**	**121.5**	**91.0**	**135.7**
Coefficient of determination R^2	**-**	**0.96**	**0.98**	**0.98**

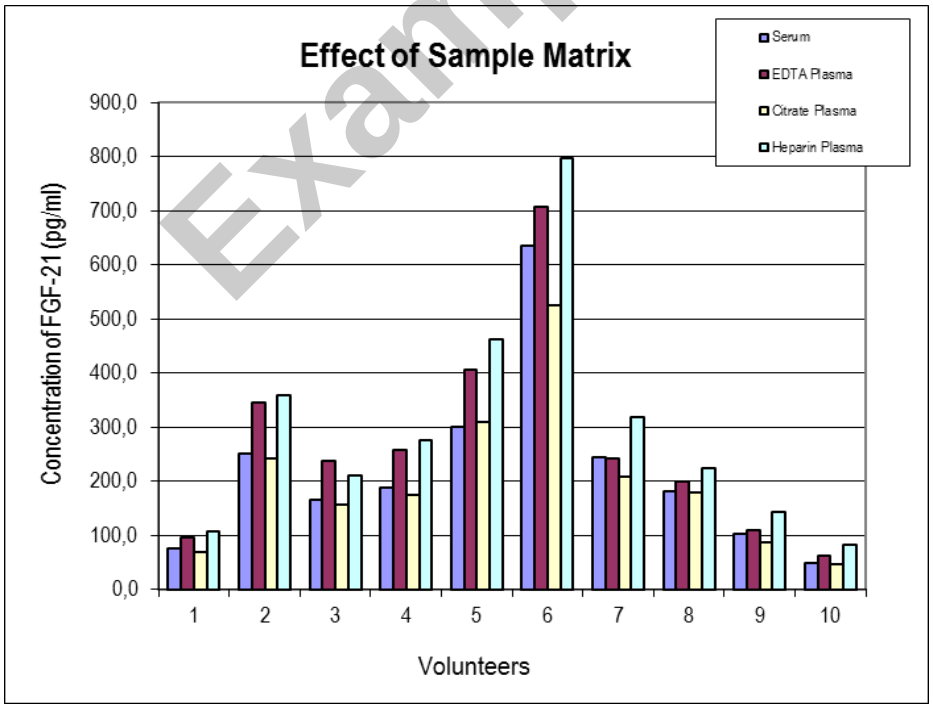

Figure 3: FGF-21 levels measured using Human FGF-21 ELISA in serum, heparin, citrate, and EDTA plasma, respectively, from the same 10 individuals.

Stability of samples stored at 2-8°C

Samples should be stored at –20°C. However, no decline in concentration of FGF-21 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 thimerosal, resulting in the final concentration of 0.03% and 0.05%, respectively.

Sample	Incubation Temp, Period	Serum (pg/ml)	Plasma (pg/ml)		
			Heparin	Citrate	EDTA
1	-20°C	432.9	455.1	290.1	412.5
	2-8°C, 1 day	385.8	427.2	309.0	390.6
	2-8°C, 7 days	389.7	417.6	308.1	325.8
2	-20°C	369.9	382.2	255.3	360.3
	2-8°C, 1 day	239.4	309.0	275.1	339.3
	2-8°C, 7 days	367.5	362.4	279.3	374.7
3	-20°C	324.9	347.1	285.9	356.1
	2-8°C, 1 day	293.1	328.5	293.1	380.7
	2-8°C, 7 days	332.1	370.8	283.8	379.8

Effect of Freezing/Thawing

No decline was observed in concentration of human FGF-21 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)		
			Heparin	Citrate	EDTA
1	1x	221.4	231.6	128.7	232.5
	3x	218.1	260.1	133.5	226.5
	5x	206.4	232.2	118.8	210.9
2	1x	702.0	825.9	597.3	704.7
	3x	696.6	633.3	507.9	686.7
	5x	638.4	599.1	574.2	744.6
3	1x	238.1	218.4	153.6	205.5
	3x	241.2	263.4	141.9	239.7
	5x	226.5	236.4	167.4	196.4

14. DEFINITION OF THE STANDARD

The recombinant protein is used as a Standard in this assay. The recombinant FGF-21 is 195 amino acid residues protein expressed in *E.coli*. The apparent molecular weight is 21 kDa.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 184 unselected donors (108 women + 76 men), 4-84 years old were assayed with Biovendor Human FGF-21 ELISA kit in our laboratory.
The presented data should be regarded only as guideline.

Age and sex dependent distribution of FGF-21

Sex	Age years	n	Mean	SD	Min.	Max.	Median
			FGF-21 (pg/ml)				
Men	4 - 17	6	101.3	71.9	13.5	204.2	102.8
	21 -49	30	192.7	128.2	13.5	635.5	202.4
	51 -85	40	298.7	227.5	33.6	1021.4	237.4
Women	4 - 18	8	168.7	76.6	30.4	287.5	174.5
	20 -49	37	173.5	148.8	15.2	708.5	122.3
	50 -84	63	322.3	237.3	65.3	1209.8	222.2

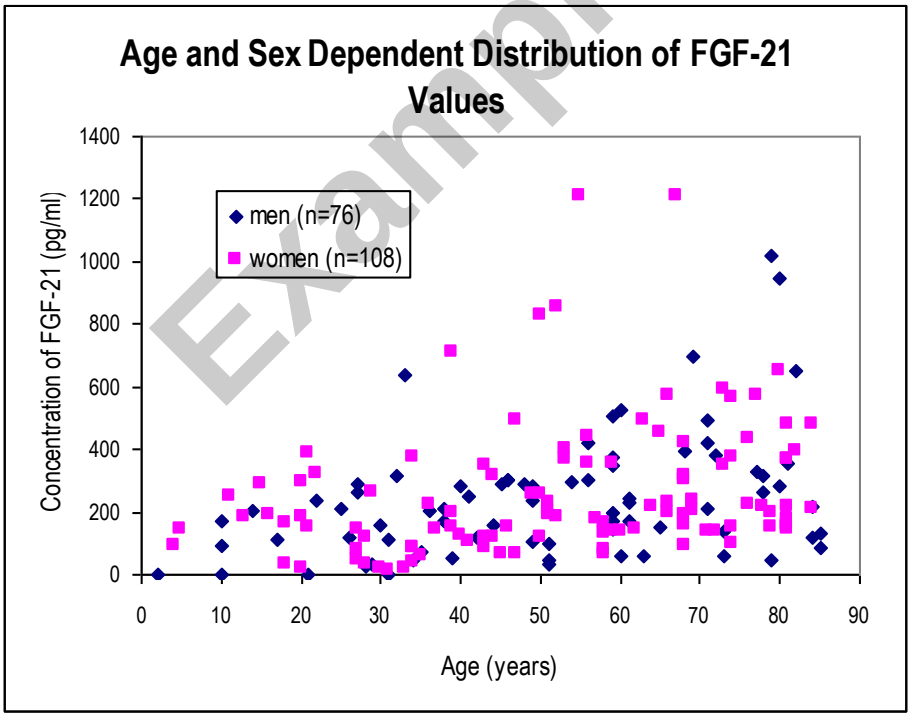

Figure 4: FGF-21 concentration plotted against donor age.

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 samples in the assay. Each laboratory should establish its own normal and pathological references ranges for FGF-21 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human FGF-21 ELISA was compared with independently developed assay by pharmaceutical company. Results are shown in the following correlation graph.

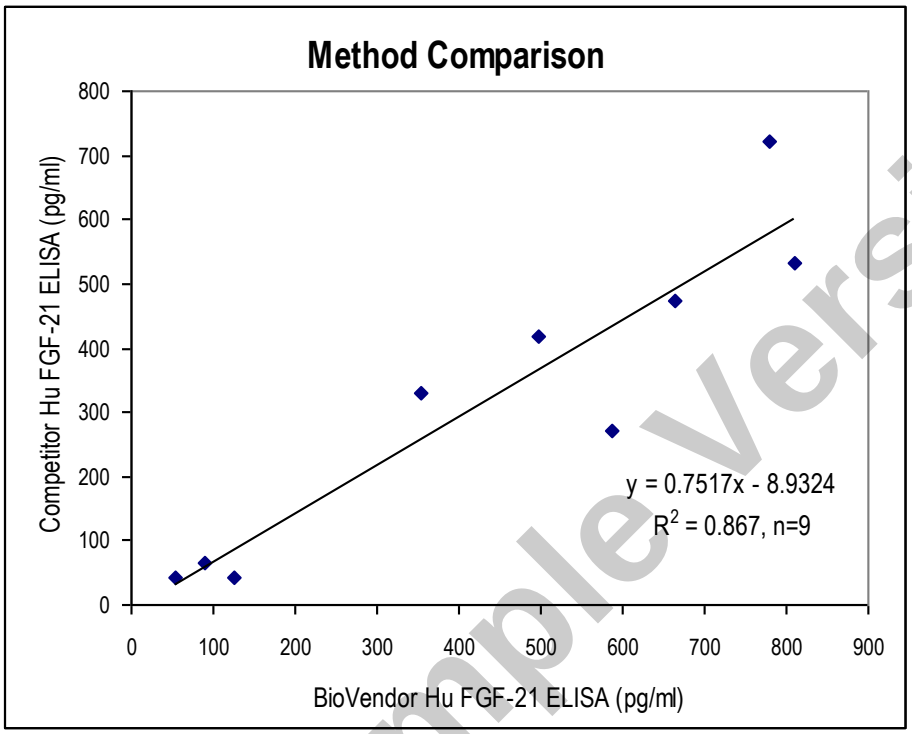

Figure 5: Method comparison.

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

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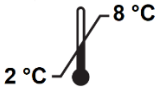
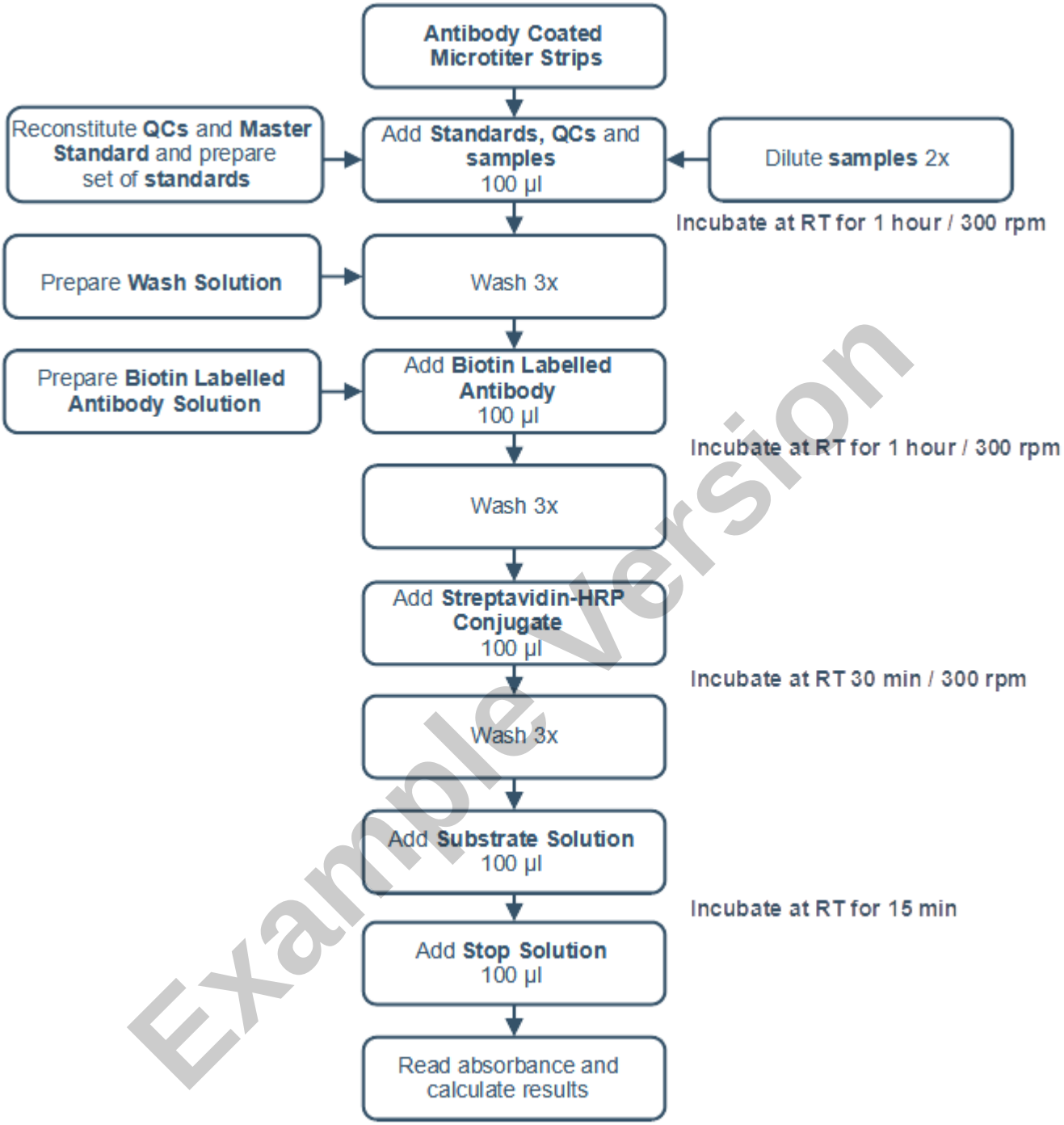
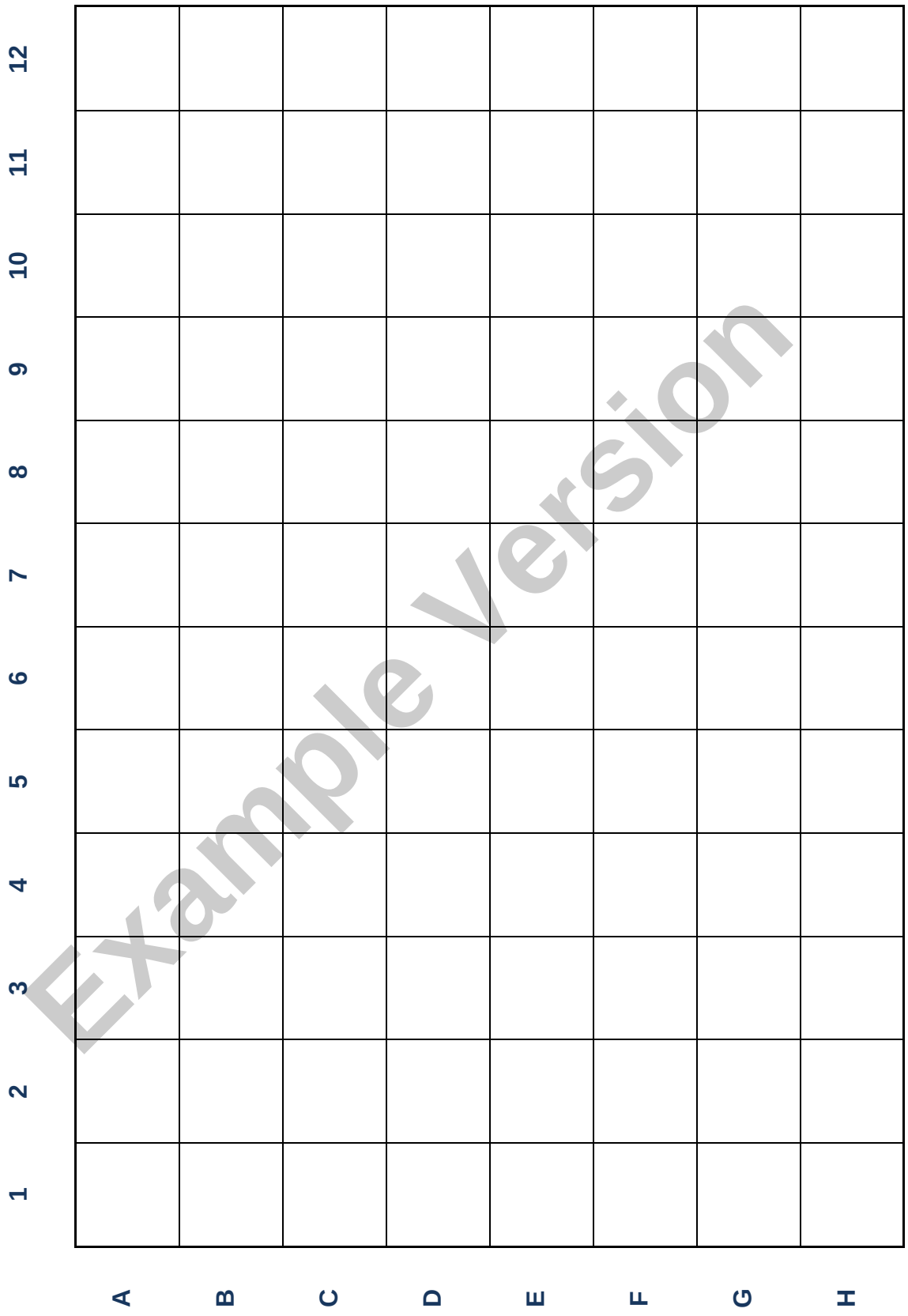
For more references on this product see our web pages at www.biovendor.com.

19. EXPLANATION OF SYMBOLS

	Catalogue number
	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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