QUANTITATIVE DETERMINATION OF HUMAN FIBROBLAST GROWTH FACTOR 21 (FGF-21) ELISA

Human Fibroblast Growth Factor 21 ELISA

- > High sensitivity (7 pg/ml)
- > Excellent analytical characteristics
- Validated for human serum and plasma samples (EDTA, citrate, heparin)

LIPID METABOLISM DIABETES MELLITUS TYPE 2 METABOLIC SYNDROME



HUMAN FIBROBLAST GROWTH FACTOR 21 ELISA



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 dietinduced 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.

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BioVendor Human FGF-21 ELISA (RD191108200R)

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).

> 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

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.

Clinical application

- > Lipid metabolism
- Diabetes mellitus type 2
- > Metabolic syndrome

HUMAN FGF-21 ELISA CAT. NO.: RD191108200R			
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit		
Samples	Serum, plasma		
Standards	30 to 1920 pg/ml		
Limit of detection	7 pg/ml		



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

Spiking recovery

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

Sample	Observed	Expected	Recovery O/E
	(pg/mi)	(pg/mi)	(%)
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

Effect of sample matrix

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



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

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

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed	Expected	Recovery
		(P6/ 111)	(P6/ ····/	0/ L (70)
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

Summary of protocol

- · Prepare Standards and QCs
- · Dilute samples 2x
- $\cdot\,$ Add Standards, QCs and samples 100 μI
- · Incubate at RT for 1 hours with shaking 300 rpm
- · Wash plate 3 times
- Prepare Biotin Labelled Antibody Solution
- · Add 100 µl Biotin Labelled Antibody
- \cdot Incubate at RT for 1 hour with shaking 300 rpm
- · Wash plate 3 times
- · Add 100 µl Streptavidin-HRP Conjugate
- · Incubate at RT for 30 min with shaking 300 rpm
- · Wash plate 3 times
- · Add 100 µl Substrate Solution
- Incubate at RT for 15 min
- · Add 100 µl stop solution
- · Read absorbance and calculate results

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



Men (n=76) ■ Women (n=108)

Related products

- RD291108200R Fibroblast Growth Factor 21 Mouse/Rat ELISA
- · RD172108100 Fibroblast Growth Factor 21 Human E. coli
- RD172108100-B Fibroblast Growth Factor 21 Human *E. coli* Tag free
- RD272108100 Fibroblast Growth Factor 21 Mouse E. coli
- RD272108100-B Fibroblast Growth Factor 21 Mouse *E. coli* Tag free

- RD181108100 Fibroblast Growth Factor 21 Human, Rabbit Polyclonal Antibody
- RD184108100 Fibroblast Growth Factor 21 Human, Sheep Polyclonal Antibody
- RD281108100 Fibroblast Growth Factor 21 Mouse, Rabbit Polyclonal Antibody

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