Human Fibroblast growth factor 23 ELISA

Cat. No.: BA1019

Enzyme Immunoassay for the quantitative determination of Fibroblast growth factor 23 (FGF23) in human EDTA plasma.

Fibroblast growth factor-23 (FGF-23) is a phosphaturic hormone involved in mineral bone metabolism that helps control phosphate homeostasis and reduces 1,25- dihydroxyvitamin D synthesis. Recent data have highlighted the relevant direct FGF-23 effects on the myocardium, and high plasma levels of FGF-23 have been associated with adverse cardiovascular outcomes in humans, such as heart failure and arrhythmias. Therefore, FGF-23 has emerged as a novel biomarker of cardiovascular risk. experimental data suggest FGF-23 as a direct mediator of cardiac hypertrophy development, cardiac fibrosis and cardiac dysfunction via specific myocardial FGF receptor (FGFR) activation.[1]

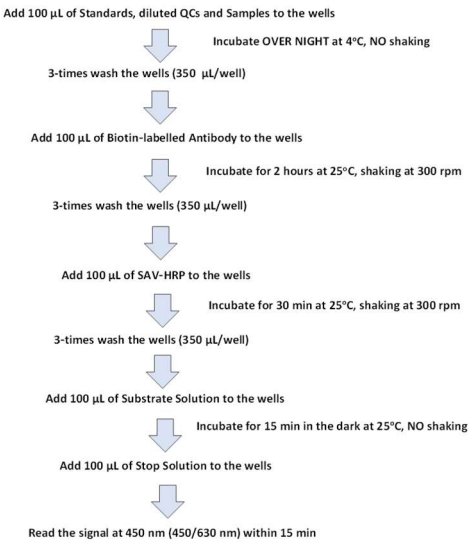
PRINCIPLE OF FGF23 ELISA

The microtiter plate is coated with the antibody specifically binding Fibroblast growth factor 23. The human EDTA plasma is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.

Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of FGF23 in the specimen. The concentration of FGF23 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	***Qty.***
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

Preparation of Standards

Reconstitute lyophilized Human FGF23 Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human FGF23 in Master Standard is 2000 pg/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 2000 pg/mL (lyophilized)	See CofA	2000 pg/mL
Std2	300 µL of Std1	300 µL	1000 pg/mL
Std3	180 µL of Std2	450 µL	400 pg/mL
Std4	300 µL of Std3	300 µL	200 pg/mL
Std5	300 µL of Std4	300 µL	100 pg/mL
Std6	300 µL of Std5	300 µL	50 pg/mL
Blank	-	300 µL	0 pg/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:2 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human EDTA plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:2, i.e., for singlets 75 µL of sample + 75 µL of Dilution Buffer, for duplicates 150 µL of samples + 150 µL of Dilution Buffer, respectively.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **OVER NIGHT** at 4°C ±2°C, NO shaking.
3. Wash the wells 5-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **2 hours** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for **15 min**, at 25°C ±2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1		Bckg	Sa 4	Sa 8	Sa 12	Sa 16	Sa 20	Sa 24	Sa 28	Sa 32	Sa 36
B	Std 2											
C	Std 3		Sa 1	Sa 5	Sa 9	Sa 13	Sa 17	Sa 21	Sa 25	Sa 29	Sa 33	Sa 37
D	Std 4											
E	Std 5		Sa 2	Sa 6	Sa 10	Sa 14	Sa 18	Sa 22	Sa 26	Sa 30	Sa 34	Sa 38
F	Std 6											
G	QCA		Sa3	Sa 7	Sa 11	Sa 15	Sa 19	Sa 23	Sa 27	Sa 31	Sa 35	Sa 39
H	QCB											

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:2 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human FGF23 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 12.5 pg/mL of sample.

2. Precision

Intra-assay

Sample	Mean (pg/mL)	SD	CV (%)
1	1718	100	5.8
2	492	19	3.8

Inter-assay (Run – to – run)

Sample	Mean (pg/mL)	SD	CV (%)
1	41	3	6.3
2	742	90	12.1

3. Accuracy

Dilution linearity

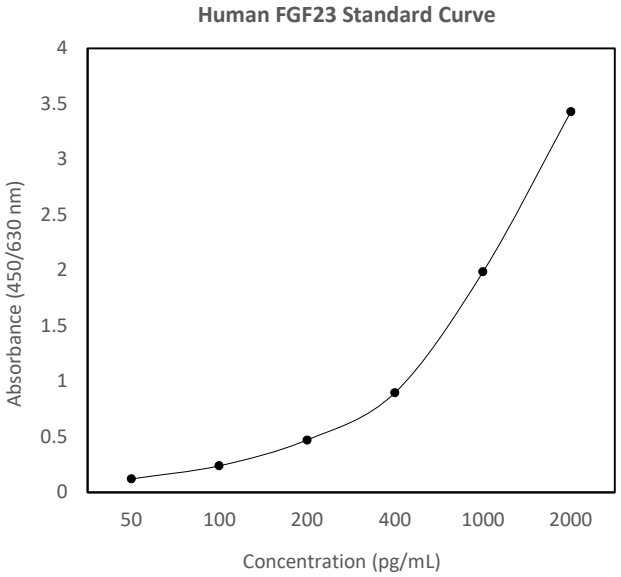
Sample	Dilution	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
1		2003	-	-
	2x	1058	1001	106
	4x	544	501	109
	8x	284	250	113
2		441	-	-
	2x	231	221	105
	4x	111	110	101
	8x	61	55	110

Spiking Recovery

Sample	Spike (pg/mL)	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
1	-	61	-	-
	800	749	861	87
	400	421	461	91
	200	225	261	86

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



RESOURCES

[1] Vázquez-Sánchez S, Poveda J, Navarro-García JA, González-Lafuente L, Rodríguez-Sánchez E, Ruilope LM, Ruiz-Hurtado G. An Overview of FGF-23 as a Novel Candidate Biomarker of Cardiovascular Risk. Front Physiol. 2021 Mar 9;12:632260. doi: 10.3389/fphys.2021.632260. PMID: 33767635; PMCID: PMC7985069.