

FGF23 (C-terminal) multi-matrix ELISA

for the quantitative determination of human
FGF23 (C-terminal) in serum, EDTA plasma, heparin plasma, and citrate plasma
Cat. No. BI-20702 . 12 x 8 tests

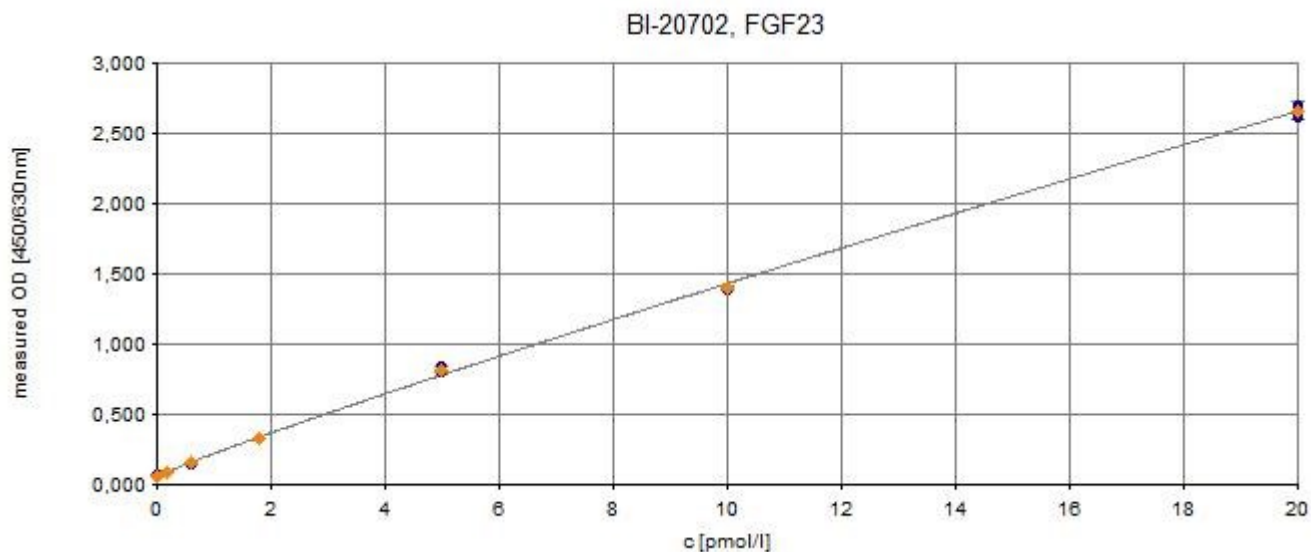
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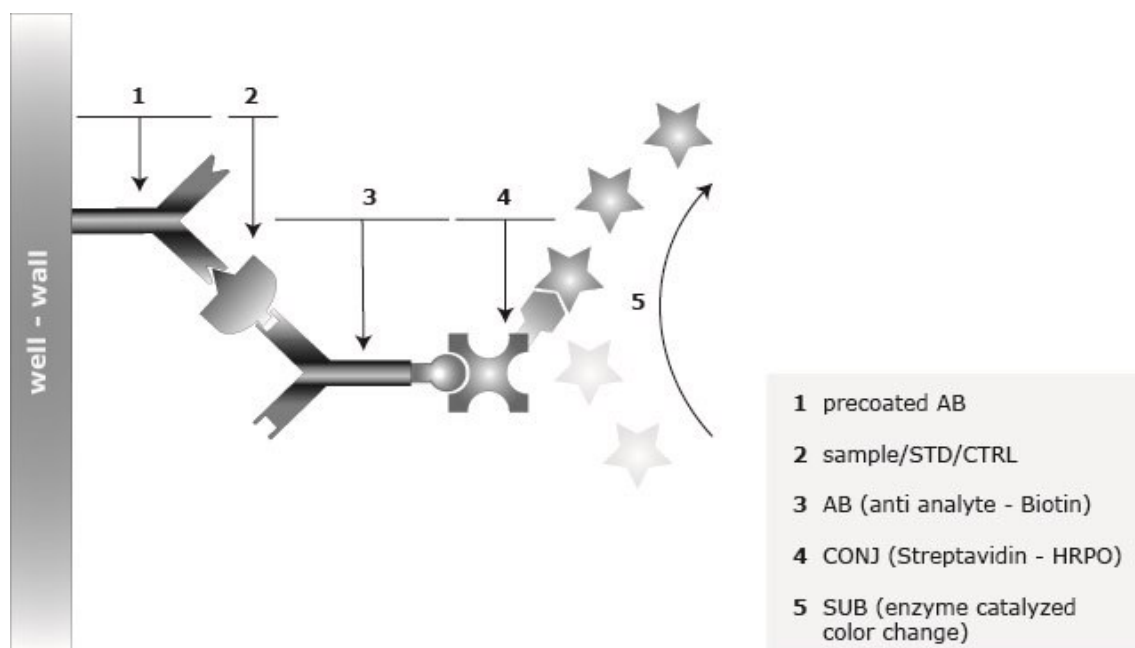
ASSAY CHARACTERISTICS Summary

Method	Sandwich ELISA, HRP/TMB, 12x8-well strips			
Sample type	Serum, EDTA plasma, heparin plasma, and citrate plasma			
Standard range	0 to 20 pmol/l (7 standards and 2 controls in a human serum matrix. Standards: 0/0.2/0.6/1.8/5/10/20 pmol/l)			
Conversion factor	FGF23, C-terminal: 1 pg/ml = 0.133 pmol/l (MW: 7.52 kDa)			
Sample volume	50 µl / well			
Incubation time, temp.	20-24 h / 1 h / 30 min, room temperature			
Sensitivity	LOD: (0 pmol/l + 3 SD): 0.08 pmol/l; LLOQ: 0.1 pmol/l			
Specificity	This assay recognizes endogenous and recombinant human FGF23. The assay measures both intact FGF23 and C-terminal fragments of FGF23.			
Precision	Intra-assay (n=6) ≤ 12% Inter-assay (n=10) ≤ 10%			
Spike/Recovery	<u>Average % recovery spiked with 5 pmol/l</u>	Serum (n=13): 96 EDTA plasma (n=7): 97 Heparin plasma (n=8): 101 Citrate plasma (n=7): 100		
Dilution linearity of endogenous FGF23	<u>Average % of expected of dilution:</u>	1+1	1+3	1+7
	Serum (n=9):	105	100	108
	EDTA plasma (n=4):	103	103	106
	Heparin plasma (n=10):	107	106	104
	Citrate plasma (n=5):	102	106	101
Values of apparently healthy individuals	Median serum (n=35) = 0.8 pmol/l Median EDTA plasma (n=22) = 1.3 pmol/l Median heparin plasma (n=22) = 1.2 pmol/l Median citrate plasma (n=30) = 1.4 pmol/l			

TYPICAL STANDARD CURVE



PRINCIPLE OF THE ASSAY



CAB coating antibody: polyclonal goat IgG
DAB detection antibody: polyclonal rabbit IgG
AG antigen: Fibroblast Growth Factor 23 C-terminal peptide

SAMPLE VALUES

FGF23 levels in an apparently healthy cohort

	FGF23 (C-terminal) [pmol/l]			
	Serum (n=35)	EDTA plasma (n=22)	Heparin plasma (n=22)	Citrate plasma (n=30)
Mean	1.1	1.6	1.5	1.9
Median	0.8*	1.3	1.2	1.4**
Percentile 95%	3.0	4.0	3.8	4.5
Percentile 5%	0.3	0.6	0.5	0.9
Min	0.2	0.3	0.3	0.7
Max	4.2	4.8	4.0	6.8

* Serum and plasma values of FGF23 (C-terminal) show a correlation of $R^2 = 0.8675$ (see data below).

**Various plasma matrices (EDTA, heparin, citrate) show a correlation of $R^2 = 0.9535$ (see data below).

It is recommended to establish the normal range for each laboratory.

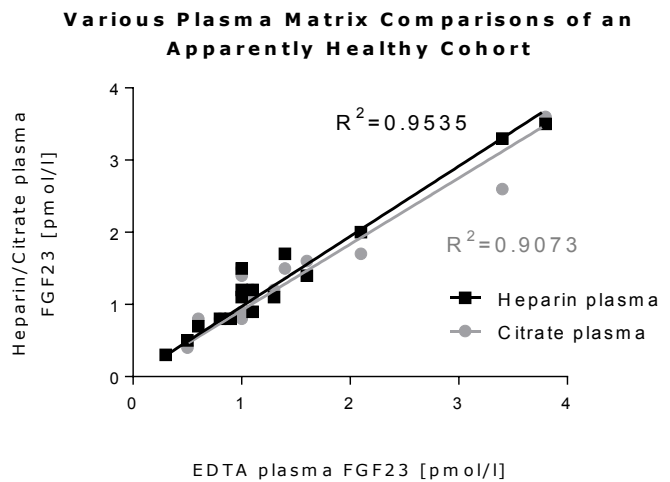
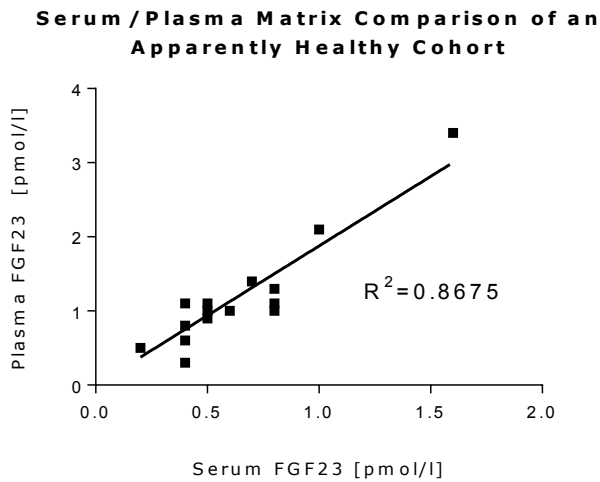
FGF23 levels in various hospital panels

Serum	FGF23 (C-terminal) [pmol/l]		
	CKD Cohort (n=18)	CKD Cohort (dialysis, n=20)	CKD Cohort (dialysis, n=15)
Mean	8.2	89	96
Median	5.7	59	20
Min	1.4	6	3
Max	20.0	310	408

Plasma	FGF23 (C-terminal) [pmol/l]	
	CKD Cohort (dialysis, n=20)	CKD Cohort (dialysis, n=18)
Mean	248	441
Median	177	355
Min	24	150
Max	725	882

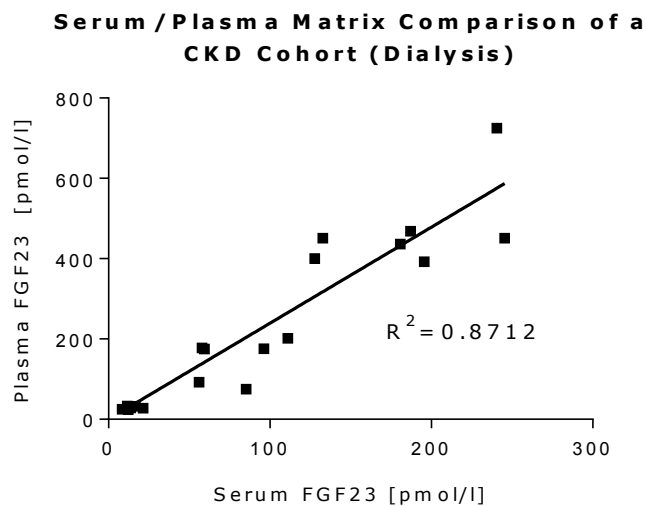
MATRIX COMPARISON

Correlation of serum and plasma samples from apparently healthy individuals



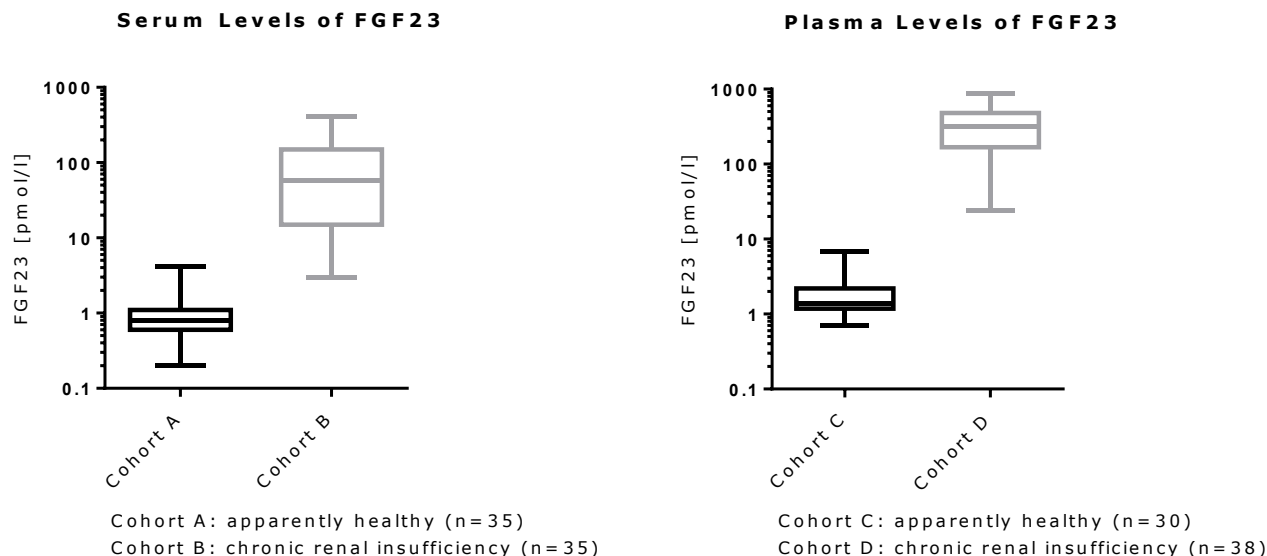
Correlation of serum and plasma samples from a CKD cohort (dialysis)

20 samples from a dialysis cohort were prepared as serum and plasma each deriving from one donor. Samples were assayed and the concentrations of the samples were compared.



► Both serum and plasma can be measured with the FGF23 (C-terminal) multi-matrix ELISA.

FGF23 (C-terminal) levels in serum and plasma samples



ASSAY PERFORMANCE CHARACTERISTICS

RECOVERY

Summary of data showing mean recovery of FGF23:

Matrix	Mean S/R [%]	
	+5 pmol/l	+10 pmol/l
Serum (n=13)	96	89
EDTA plasma (n=7)	97	94
Heparin plasma (n=8)	101	92
Citrate plasma (n=7)	100	90

Experiments:

Recovery of spiked samples was tested by adding 2 concentrations of human recombinant C-terminal FGF23 (5 + 10 pmol/l) to different human sample matrices.

Data showing spike/recovery of human serum samples:

Sample ID	Spike FGF23 [pmol/l]			S/R [%]	
	0	5	10	5	10
#S1	1.3	6.4	10.1	103	87
#S2	0.8	5.9	11.0	102	101
#S3	2.2	7.0	10.4	96	82
#S4	4.8	10.2	13.9	108	91
#S5	2.1	7.1	12.4	100	103
#S6	1.8	6.1	10.6	85	88
#S7	1.2	5.5	7.3	84	60
#S8	1.6	6.2	11.4	92	98
#S9	2.7	7.2	11.7	90	90

#S10	0.8	5.4	10.1	92	92
#S11	1.1	5.9	10.4	95	93
#S12	0.6	5.6	9.3	101	87
#S13	1.9	7.1	10.4	104	85
Mean R [%]				96	89

Data showing spike/recovery of human EDTA plasma samples:

Sample ID	Spike FGF23 [pmol/l]			S/R [%]	
	0	5	10	5	10
#E1	1.3	6.7	11.9	108	106
#E2	1.4	5.0	9.5	73	81
#E3	1.8	6.8	13.2	101	114
#E4	1.2	5.4	8.9	84	77
#E5	3.2	7.6	11.3	88	81
#E6	2.0	7.0	11.6	100	96
#E7	1.3	7.4	11.3	123	101
Mean R [%]				97	94

Data showing spike/recovery of human heparin plasma samples:

Sample ID	Spike FGF23 [pmol/l]			S/R [%]	
	0	5	10	5	10
#H1	1.8	9.9	13.7	161	118
#H2	2.2	7.1	11.5	97	93
#H3	1.3	5.5	9.1	84	78
#H4	2.2	7.1	11.7	98	95
#H5	2.2	6.3	10.2	82	80
#H6	1.2	4.6	8.1	68	69
#H7	1.8	7.3	12.3	110	105
#H8	2.2	7.5	12.1	106	99
Mean R [%]				101	92

Data showing spike/recovery of human citrate plasma samples:

Sample ID	Spike FGF23 [pmol/l]			S/R [%]	
	0	5	10	5	10
#C1	4.7	10.5	14.9	116	101
#C2	2.2	8.8	12.0	132	98
#C3	1.8	8.0	12.1	124	103
#C4	1.8	7.2	12.5	110	107
#C5	1.8	5.4	9.2	74	74
#C6	2.7	5.8	8.6	61	58
#C7	3.9	8.0	12.7	82	88
Mean R [%]				100	90

LINEARITY

Dilution linearity of samples containing endogenous FGF23

Matrix	Mean R of dilution steps [%]		
	1+1	1+3	1+7
Serum (n=9)	105	100	108
EDTA plasma (n=4)	103	103	106
Heparin plasma (n=10)	102	106	104
Citrate plasma (n=5)	102	106	101

► We recommend diluting high measuring samples (outside of the calibration range) in assay buffer.

Experiment:

Dilution linearity was assessed by serially diluting samples containing endogenous FGF23 with assay buffer.

Data showing the dilution of endogenous FGF23 in serum samples:

Sample ID	FGF23 [pmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#S1	3.8	2.1	1.1	0.6	111	117	124
#S2	16.2	8.1	3.6	1.9	99	89	92
#S3	7.0	3.5	1.8	0.9	100	103	101
#S4	9.0	4.6	2.1	1.0	102	95	91
#S5	8.5	4.7	2.7	1.3	111	126	123
#S6	6.0	3.4	1.3	0.9	113	90	121
#S7	15.7	7.3	3.5	1.9	93	89	96
#S8	20.4	10.9	5.1	2.6	106	101	103
#S9	10.6	5.6	2.5	1.6	105	93	121
Mean R [%]					105	100	108

Data showing the dilution of endogenous FGF23 in EDTA plasma samples:

Sample ID	FGF23 [pmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#E1	3.8	2.4	1.1	0.6	127	120	121
#E2	23.3	13.6	6.7	3.2	117	115	112
#E3	8.9	4.5	2.4	1.4	101	109	124
#E4	20.7	7.0	3.6	1.8	67	69	68
Mean R [%]					103	103	106

Data showing the dilution of endogenous FGF23 in heparin plasma samples:

Sample ID	FGF23 [pmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#H1	7.5	4.1	2.2	1.1	108	117	119
#H2	5.8	2.9	1.7	1.0	102	117	132
#H3	11.4	6.4	3.3	1.6	113	117	112
#H4	9.0	4.9	2.6	1.3	113	117	112
#H5	7.7	3.6	1.8	0.9	92	93	90

#H6	10.7	5.3	2.6	1.2	100	97	91
#H7	7.6	3.9	2.1	1.0	102	111	108
#H8	6.4	3.2	1.5	0.8	99	93	95
#H9	3.7	1.8	1.0	0.4	98	109	93
#H10	8.6	4.1	2.0	1.0	96	95	90
Mean R [%]					102	106	104

Data showing the dilution of endogenous FGF23 in citrate plasma samples:

Sample ID	FGF23 [pmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#C1	17.0	8.0	4.0	2.0	94	93	93
#C2	13.0	7.1	4.0	1.9	109	123	118
#C3	12.6	6.3	3.1	1.6	100	100	101
#C4	10.0	5.3	2.6	1.2	106	106	97
#C5	9.7	4.8	2.6	1.2	100	108	97
Mean R [%]					102	106	101

Dilution linearity of samples containing recombinant FGF23

Dilution	Rec FGF23 1,000 pmol/l		
	Expected [pmol/l]	Obtained [pmol/l]	R [%]
1+49	20	19.2	91
1+99	10	12.9	118
1+199	5	7.0	118
1+399	2.5	3.9	112

Recommendations for sample dilution

- High measuring samples outside of the calibration range of the curve should be diluted in assay buffer (ASYBUF- provided in the kit).
- Low measuring samples (for validation studies) measuring <1 pmol/l, should be diluted with STD1 (provided in the kit). The kit standard matrix is a human serum matrix containing 0 pmol/l FGF23 concentrations.
- The differences in using different dilution media for high and low measuring samples are due to the matrix differences (serum versus assay buffer).

PRECISION

Intra-assay precision & Inter-assay precision

Intra-assay (n=6) \leq 12%, Inter-assay (n=10) \leq 10%

Intra-assay: 2 samples of known concentrations were tested 6 times within 1 kit lot by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 10 times within 2 different kit lots by 4 different operators.

Intra-assay (n=6)	Sample 1	Sample 2	Inter-assay (n=10)	Sample 1	Sample 2
Mean (pmol/l)	0.6	10.0	Mean (pmol/l)	0.6	9.9
SD (pmol/l)	0.07	0.06	SD (pmol/l)	0.06	0.5
CV (%)	12	6	CV (%)	10	5

SENSITIVITY

Limit of detection (LOD)

The LOD is defined as the mean value of the back calculated concentration plus three times the standard deviation. The LOD of the FGF23 (C-terminal) ELISA is **0.08 pmol/l**.

Lower limit of quantification (LLOQ)

The lower limit of quantification is defined as the accuracy of the back calculated concentrations and shall not exceed $\pm 25\%$ (acc. to ICH [Ref. 1]).

For the FGF23 (C-terminal) ELISA the LLOQ is **0.1 pmol/l**.

SAMPLE STABILITY

Sample preparation

We recommend performing serum or plasma separation by centrifugation as soon as possible, e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C).

The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C, for long time storage at -80°C. All samples should undergo only 4 freeze-thaw cycles.

Freeze/thaw of serum samples containing endogenous FGF23

Serum samples can undergo 4 freeze-thaw cycles.

The mean recovery of sample concentrations stressed by 4 F/T cycles is 101%.

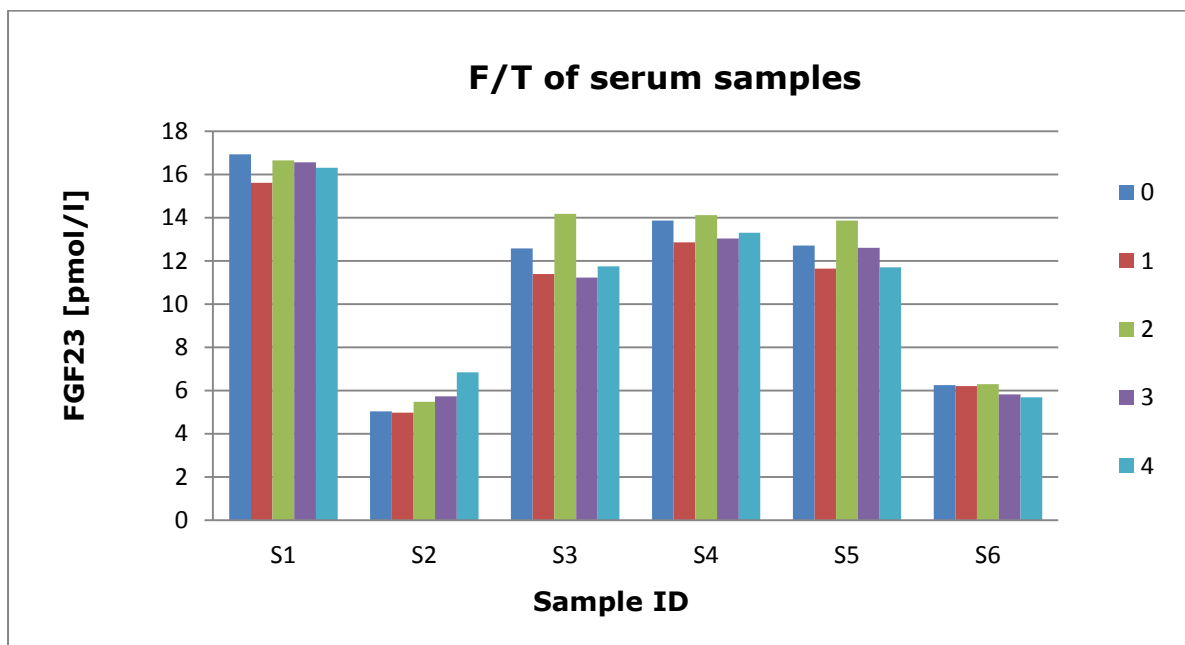
The mean CV of sample concentrations (not stressed and stressed up to 4 times by freeze-thaw cycles) is 6%.

Serum samples are stable for at least 4 freeze-thaw cycles.

Plasma samples behave in a similar fashion.

FGF23 concentrations of samples after freeze-thaw cycles:

no of F/T cycles	Ref 0	1	2	3	4			
Sample ID	FGF23 [pmol/l]					Mean [pmol/l]	CV [%]	R [%] 4 F/T vs ref
S1	16.9	15.6	16.7	16.6	16.3	16.4	3	96
S2	5.0	5.0	5.5	5.7	6.8	5.6	12	136
S3	12.6	11.4	14.2	11.2	11.7	12.2	9	93
S4	13.9	12.9	14.1	13.0	13.3	13.4	4	96
S5	12.7	11.6	13.9	12.6	11.7	12.5	7	92
S6	6.3	6.2	6.3	5.8	5.7	6.1	4	91
						Mean [%]	6	101



SPECIFICITY

This assay recognizes endogenous (natural) and recombinant human FGF23. The assay measures both intact FGF23 and C-terminal fragments of FGF23.

CALIBRATION

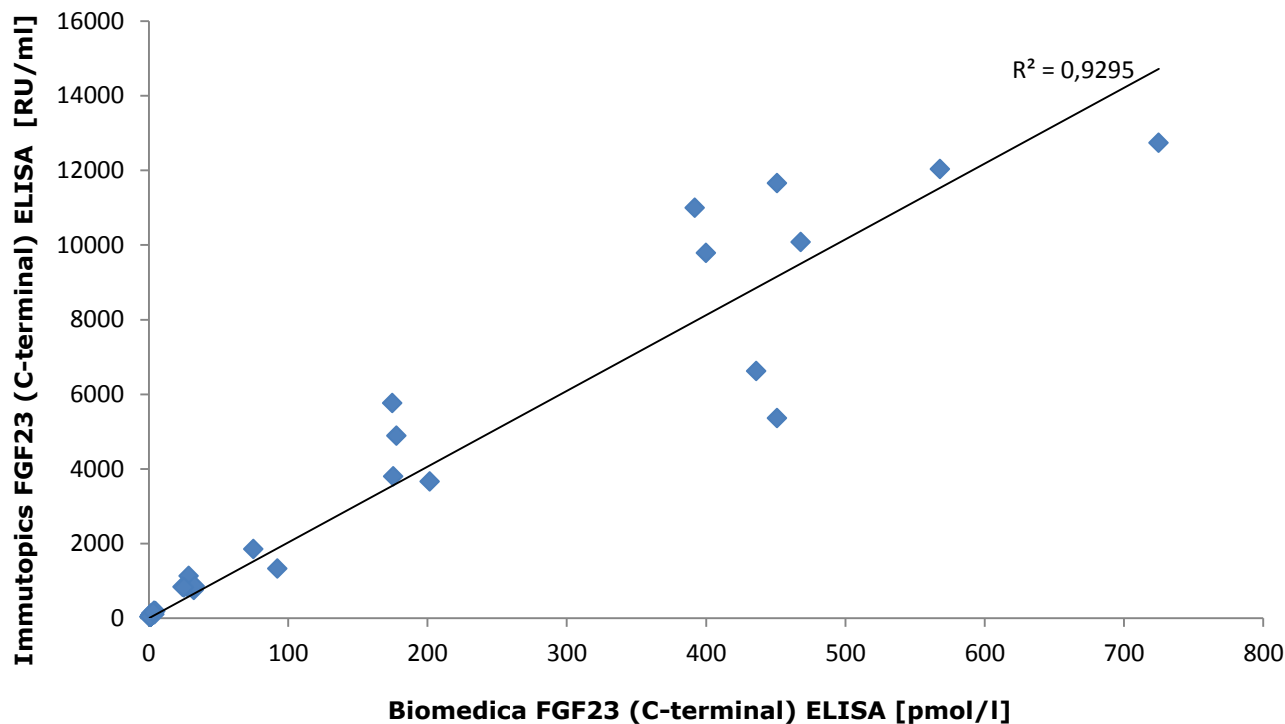
This immunoassay is calibrated against recombinant human FGF23 (C-terminal) peptide.

COMPARISON of FGF23 (C-terminal) ELISAs

Biomedica ELISA Cat.No. BI-20702 vs Immutopics ELISA Cat.No. 60-6100

Sample matrix: EDTA plasma, apparently healthy cohort (n=33), CKD cohort (dialysis, n=20)

**FGF23 (C-terminal) ELISA Comparison in EDTA Plasma
Biomedica vs. Immutopics**



Validation

The assay is fully validated according to ICH Q2 (R1), Ref. [1].

[1] CPMP/ICH/381/95 ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology” including:

ICH Q2A “Text on Validation of Analytical Procedures”

ICH Q2B “Validation of Analytical Procedures: Methodology”

Available on our Website www.bmgrp.com

Instructions for Use (package insert)

Material Safety Data Sheet

FGF23 (C-terminal) multi-matrix ELISA – Info Leaflet

Date: March 2015