FGF23 (intact) human ELISA

for the quantitative determination of human intact FGF23 in EDTA plasma, heparin plasma, and citrate plasma

Cat. No. BI-20700. 12 x 8 tests

FOR RESEARCH USE ONLY

This ELISA is optimized and validated for human plasma samples. Serum, urine and cell-culture supernatants are compatible with this ELISA.

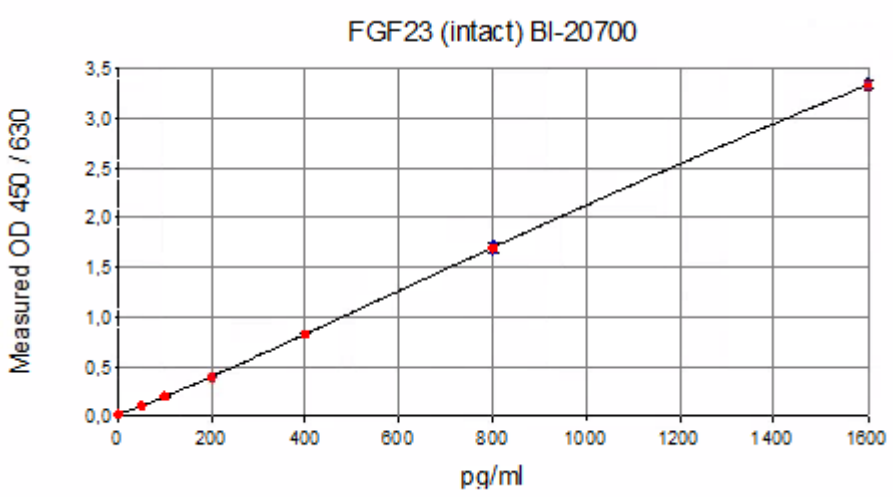
CONTENTS

ASSAY CHARACTERISTICS Summary

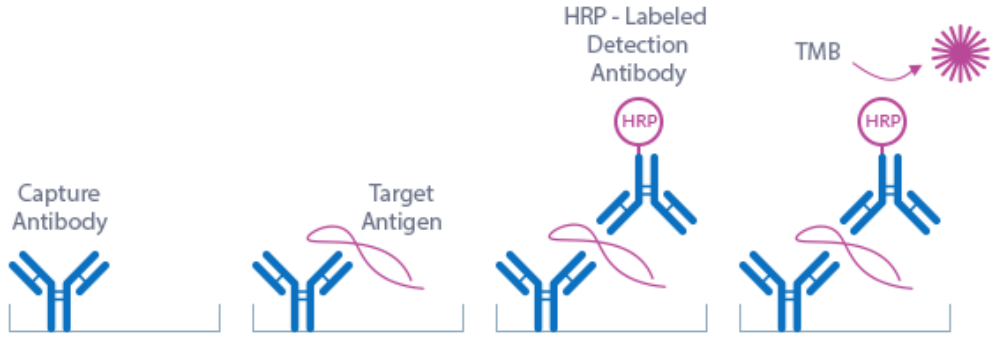
Method	Sandwich ELISA, HRP/TMB, 12x8-well detachable strips			
Sample type(s)*	EDTA plasma, heparin plasma, citrate plasma			
Sample volume	50 µl / well			
Assay time	3 h / 30 min			
Detection limit	5.4 pg/ml (0.21 pmol/l)			
Standard range	0 - 1600 pg/ml (0 – 60.8 pmol/l)			
Conversion factor	1 pg/ml = 0.038 pmol/l; MW: 26 kDa			
Precision		**n**	**CV (%)**	
	Within-run	3	≤8	
	In-between-run	9	≤6	
Accuracy		**n**	**Recovery (%)**	
	EDTA plasma	6	100	
	Heparin plasma	1	79	
	Citrate plasma	1	103	
	Serum	6	91	
Dilution linearity of endogenous intact FGF23		**n**	**Recovery of expected dilution (%)**	
			1+1	**1+3**	**1+7**
	EDTA plasma	5	107	108	111
	Heparin plasma	1	99	97	107
	Citrate plasma	1	143	137	130
	Serum	6	87	74	67
Specificity	Endogenous and recombinant human intact FGF23			
Use	For research use only.			
Values of apparently healthy donors		**n**	**Median intact FGF23 (pg/ml)**	
	EDTA plasma	22	24.4	
	Heparin plasma	22	26.4	
	Citrate plasma	22	17.4	
	Serum	22	14.8	

*This ELISA is optimized and validated for human plasma samples. Serum and other sample types are compatible with this ELISA. For further information on serum and urine measurement and assay performance characteristics, matrix comparisons and stability data please find data in this validation data file or contact our customer service by e-mail info@bmgrp.com or by phone +43/1/29107-45.

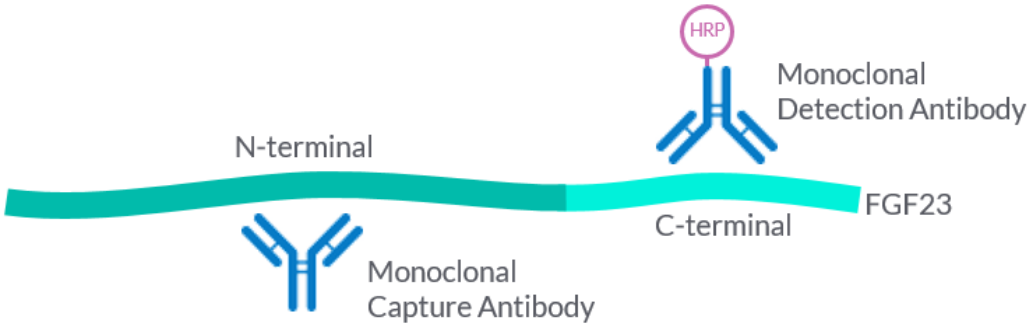
TYPICAL STANDARD CURVE



PRINCIPLE OF THE ASSAY



The FGF23 (intact) human ELISA kit is a sandwich enzyme immunoassay that has been optimized and validated for the quantitative determination of intact FGF23 in human plasma samples. Serum and other sample types are compatible with this ELISA.
In a first step, standard/control/sample and conjugate (monoclonal mouse anti-human FGF23-HRP) are pipetted into the wells of the microtiter strips, which are pre-coated with a recombinant monoclonal anti-human FGF23 antibody. FGF23 present in the standard/control/sample binds to the pre-coated antibody in the well and forms a sandwich with the conjugated anti-human FGF23-HRP antibody. In a washing step all non-specific unbound material is removed. In a second step, the substrate (TMB, tetramethylbenzidine) is pipetted into the wells. The enzyme-catalyzed color change of the substrate is directly proportional to the amount of intact FGF23 present in the sample. This color change is detectable with a standard microplate reader. A dose response curve of the absorbance (optical density, OD at 450 nm) using the values obtained from the standards versus the standard concentration is generated. The concentration of intact FGF23 in the sample is determined directly from the dose response curve.



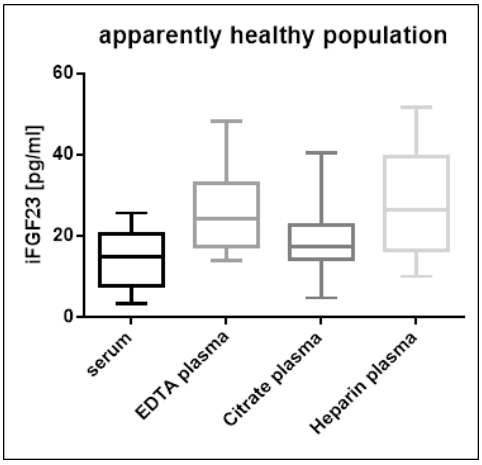
The FGF23 (intact) human ELISA is a sandwich-based immunoassay with a recombinant monoclonal anti-human FGF23 capture antibody recognizing a structural epitope at the N-terminal part of FGF23. Detection is mediated by a HRP-labeled monoclonal anti-human FGF23 antibody directed to the C-terminal part of FGF23. Therefore, specific measurement of intact FGF23 is ensured.

SAMPLE VALUES

Intact FGF23 Values in Apparently Healthy Individuals

To provide values for circulating intact FGF23 (iFGF23), a panel of samples from apparently healthy donors was tested. Each individual donated blood for all tested sample matrices.

	intact FGF23 [pg/ml]			
	EDTA plasma	**Heparin plasma**	**Citrate plasma**	**Serum**
# of samples	n=22	n=22	n=22	n=22
Mean	26.1	27.7	19.7	14.1
Median	24.4	26.4	17.4	14.8
5% Percentile	14.2	10.1	5.8	3.8
95% Percentile	47.5	50.7	40.4	25.0
Minimum	14	10.1	4.8	3.6
Maximum	48.3	51.8	51.8	25.7



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

Intact FGF23 Values in Chronic Kidney Disease and Dialysis Panels

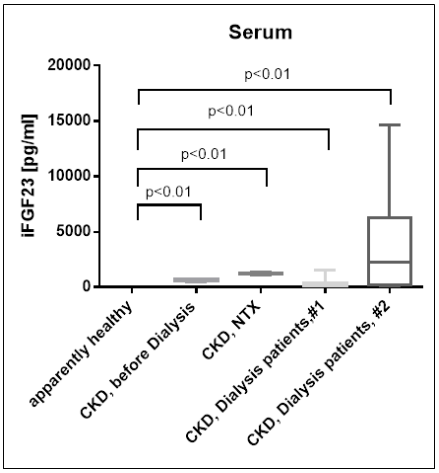
In addition to samples from apparently healthy donors, several panels of samples from chronic kidney disease (CKD) and dialysis patients were tested.

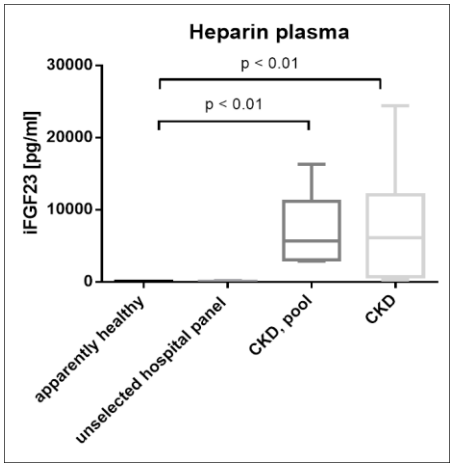
Summary of the results obtained with several chronic kidney disease (dialysis) panels:

	intact FGF23 [pg/ml]			
	Serum CKD	**Serum, CKD;NTX**	**Serum Dialysis #1**	**Serum Dialysis #2**
# of samples	n=6	n=15	n=7	n=9
Mean	623.3	1194	340	4039
Median	**653.1**	**1172**	**136.1**	**2227**
5% Percentile	446.4	1089	39.9	145.5
95% Percentile	685.3	1309	1539	14602
Minimum	446.4	1089	39.9	145.5
Maximum	685.3	1309	1539	14602

	intact FGF23 [pg/ml]		
	Heparin plasma CKD	**Heparin plasma CKD pool**	**Heparin plasma Unselected hospital panel**
# of samples	n=8	n=10	n=14
Mean	7799	7324	27.7
Median	**6104**	**5647**	**32.8**
5% Percentile	255.5	2922	22.3
95% Percentile	24366	16283	126.5
Minimum	255.5	2922	22.3
Maximum	24366	16283	126.5

Comparison of Intact FGF23 Values in Apparently Healthy Individuals and Patients with Chronic Kidney Disease (CKD)/ Dialysis



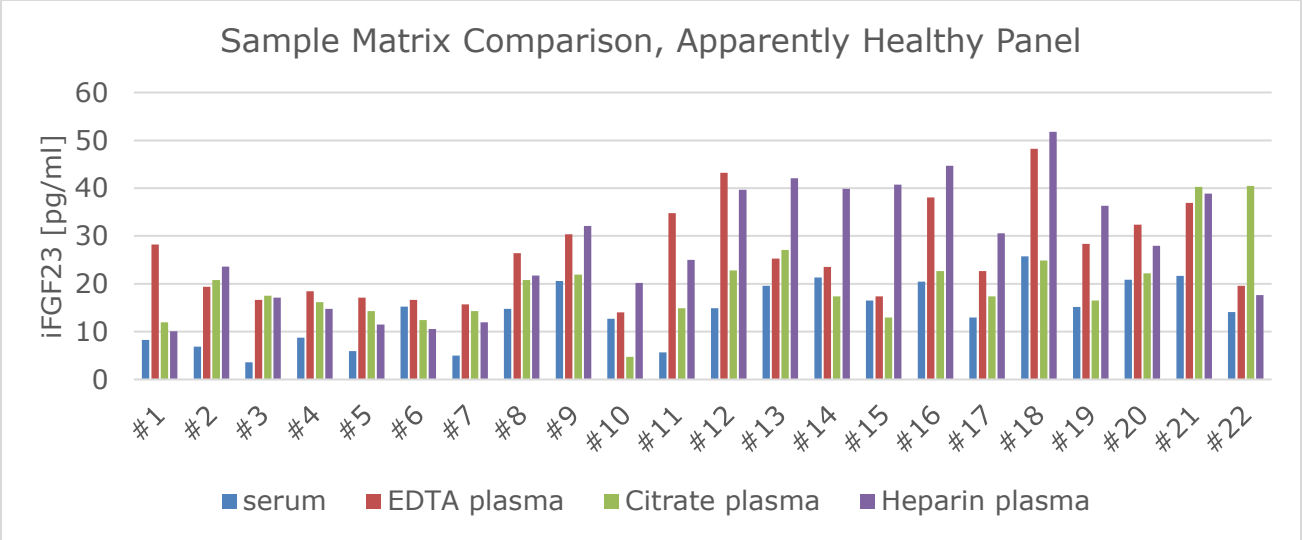


MATRIX COMPARISON

To assess whether all tested matrices behave the same way in the FGF23 (intact) human ELISA, concentrations of intact FGF23 (iFGF23) were measured in EDTA, heparin, citrate plasma and serum samples prepared from 22 apparently healthy donors. Each individual donated blood in all tested sample matrices.

Data and graph for apparently healthy donors:

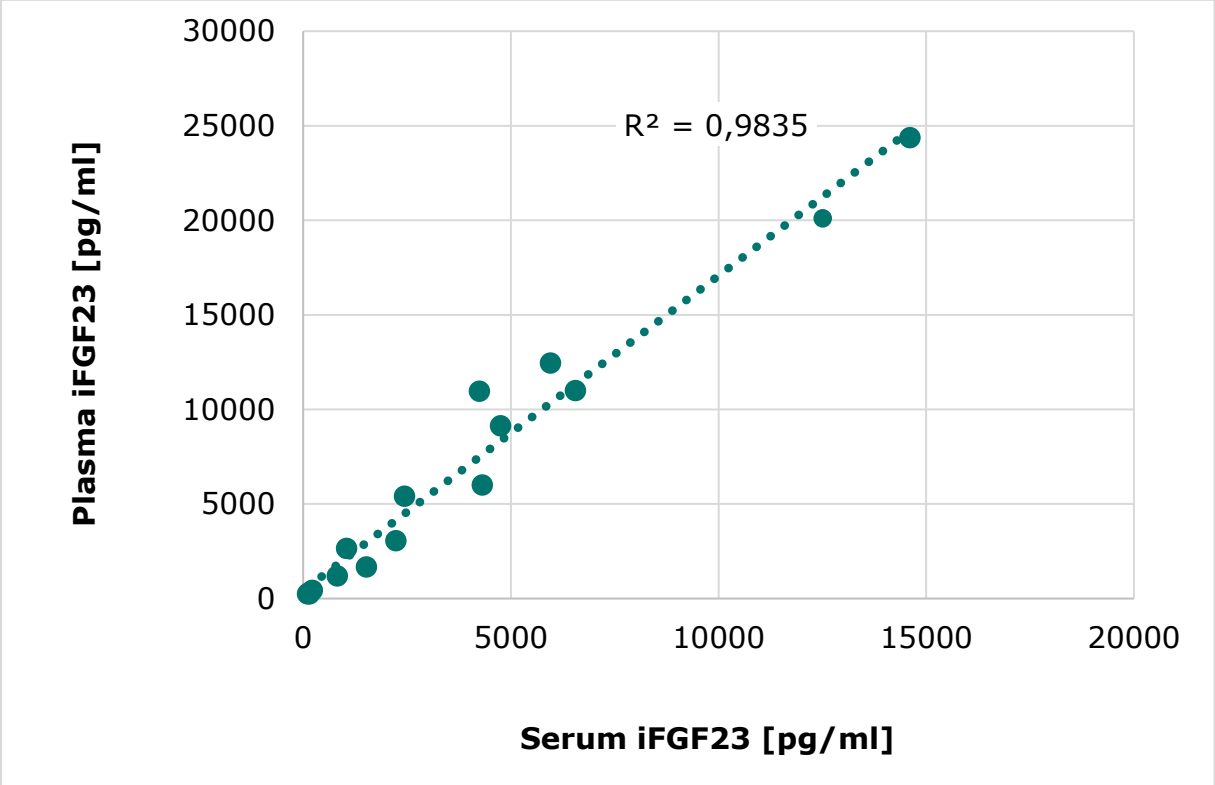
	intact FGF23 [pg/ml]			
Donor ID	**EDTA plasma**	**Citrate plasma**	**Heparin plasma**	**Serum**
#1	28	12	10	8
#2	19	21	24	7
#3	17	18	17	4
#4	18	16	15	9
#5	17	14	11	6
#6	17	12	11	15
#7	16	14	12	5
#8	26	21	22	15
#9	30	22	32	21
#10	14	5	20	13
#11	35	15	25	6
#12	43	23	40	15
#13	25	27	42	20
#14	24	17	40	21
#15	17	13	41	16
#16	38	23	45	20
#17	23	17	31	13
#18	48	25	52	26
#19	28	16	36	15
#20	32	22	28	21
#21	37	40	39	22
#22	20	40	18	14



Intact FGF23 values obtained for serum samples from an apparently healthy cohort (n=22) are lower than those obtained for plasma. It has been shown that FGF23 values differ between serum and plasma. The reasons for this difference are still unclear, however it is assumed that the coagulation process under conditions of serum collection might reduce the accessibility of recognizable determinants.

In addition, iFGF23 concentrations were assessed in serum and plasma samples from 16 patients with chronic kidney disease (dialysis). Correlations between serum and plasma samples were calculated for each cohort as shown in below graph:

Plasma/Serum Matrix comparison in a CKD cohort (n=16):



ASSAY PERFORMANCE CHARACTERISTICS

ACCURACY / RECOVERY

The accuracy of an ELISA is defined as the precision with which it can recover samples of known concentrations.

The recovery of the FGF23 (intact) human ELISA was measured by adding recombinant intact FGF23 to samples containing a known concentration of endogenous intact FGF23. The %recovery of the spiked concentration was calculated as the percentage of measured compared over the expected value.

This table shows the summary of the recovery experiments in the FGF23 (intact) human ELISA in different sample matrices:

Sample matrix	n	Spike/Recovery [%]			
		+160 pg/ml		+800 pg/ml	
		Mean	Range	Mean	Range
EDTA plasma	6	94	90-103	100	92-109
Heparin plasma	1	82	-	79	-
Citrate plasma	1	97	-	103	-
Serum	6	70	61-78	91	81-101

Experiments:
Recovery of spiked samples was tested by adding 2 concentrations of human recombinant intact FGF23 (160 pg/ml and 800 pg/ml) to different human sample matrices.

Data showing recovery of recombinant intact FGF23 in human EDTA plasma samples:

Sample ID	Spike iFGF23 [pg/ml]			S/R [%]	
	0	160	800	160	800
E1	35	189	886	98	109
E2	23	168	789	92	97
E3	20	163	788	91	97
E4	26	168	818	90	101
E5	56	216	871	103	105
E6	32	172	749	90	92
			Mean S/R [%]	**94**	**100**
			Min	90	92
			Max	103	109

Data showing recovery of recombinant intact FGF23 in human heparin plasma samples:

Sample ID	Spike iFGF23 [pg/ml]			S/R [%]	
	0	160	800	160	800
H1	22	150	646	82	79

Data showing recovery of recombinant intact FGF23 in human citrate plasma samples:

Sample ID	Spike iFGF23 [pg/ml]			S/R [%]	
	0	160	800	160	800
C1	26	178	834	97	103

Data showing recovery of recombinant intact FGF23 in human serum samples:

Sample ID	Spike iFGF23 [pg/ml]			S/R [%]	
	0	160	800	160	800
S1	13	136	742	78	92
S2	7	119	713	71	89
S3	7	116	689	69	86
S4	8	121	757	71	94
S5	18	132	820	72	101
S6	8	104	655	61	81
			Mean S/R [%]	**70**	**91**
			Min	61	81
			Max	78	101

DILUTION LINEARITY & PARALELLISM

Tests of dilution linearity and parallelism ensure that both endogenous and recombinant samples containing intact FGF23 behave in a dose dependent manner and are not affected by matrix effects. Dilution linearity assesses the accuracy of measurements in diluted human samples spiked with known concentrations of recombinant analyte. By contrast, parallelism refers to dilution linearity in human samples and provides evidence that the endogenous analyte behaves in the same way as the recombinant one. Dilution linearity and parallelism are assessed for each sample type and should be within 20% of the expected concentration.

Experiment:
Parallelism was assessed by serially diluting clinical samples containing **endogenous** intact FGF23 with assay buffer.

Summary table below shows the mean recovery and range of serially diluted endogenous intact FGF23 in several sample matrices:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
EDTA plasma	5	107	100-116	108	88-124	111	84-136
Heparin plasma	1	99	-	97	-	107	-
Citrate plasma	1	143	-	137	-	130	-
Serum	6	87	85-90	74	72-79	67	61-74

- *Samples for which the OD value exceeds the highest point of the standard range (STD7) can be diluted 1:11 (1+10, e.g. 10 µl sample + 100 µl ASYBUF).*

Data showing dilution linearity of endogenous intact FGF23 in human EDTA plasma samples:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
E1	538.7	307.7	166.9	91.5	114	124	136
E2	289.5	167.4	88.4	48.7	116	122	135
E3	1248.8	659.4	326.8	154.7	106	105	99
E4	1085.3	547.5	273.6	135.8	101	101	100
E5	1714.1	854.9	377.8	181.0	100	88	84
				Mean R [%]	**107**	**108**	**111**
				Min	100	88	84
				Max	116	124	136

Data showing recovery of endogenous intact FGF23 in a human heparin plasma sample:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
H1	438	216	106	58	99	97	107

Data showing recovery of endogenous intact FGF23 in a human citrate plasma sample:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
C1	143	102	49	23	143	137	130

Data showing dilution linearity of endogenous intact FGF23 in human serum samples:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
S1	1159	520	213	89	90	74	61
S2	991	434	196	84	88	79	68
S3	1013	443	190	82	87	75	65
S4	948	411	172	79	87	72	67
S5	942	399	169	87	85	72	74
S6	899	398	168	76	89	75	68
				Mean R [%]	**87**	**74**	**67**
				Min	**85**	**72**	**61**
				Max	**90**	**79**	**74**

Experiment:
Dilution linearity was assessed by serially diluting samples containing **recombinant** intact FGF23 (iFGF23) with assay buffer.

Summary table below shows the mean recovery and range of serially diluted recombinant intact FGF23 in several sample matrices:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
EDTA plasma	6	119	100-116	111	88-124	110	84-136
Heparin plasma	1	133	-	102	-	98	-
Citrate plasma	1	126	-	126	-	118	-
Serum	6	103	85-90	101	72-79	126	61-74

Data showing dilution linearity of recombinant intact FGF23 in human EDTA plasma samples:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
E1	801	461	239	107	115	104	93
E2	765	298	238	117	78	160	157
E3	719	447	235	117	124	105	105
E4	694	447	216	112	129	96	100
E5	783	483	229	117	123	95	97
E6	645	467	242	123	145	104	105
				Mean R [%]	**119**	**111**	**110**
				Min	78	95	93
				Max	145	160	157

Data showing recovery of recombinant intact FGF23 in a human heparin plasma sample:

Sample ID	iFGF23 [pg/ml]						
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
H1	673	448	228	110	133	102	98

Data showing recovery of recombinant intact FGF23 in a human citrate plasma sample:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
C1	834	527	262	123	126	126	118

Data showing dilution linearity of recombinant intact FGF23 in human serum samples:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
S1	892	528	242	134	118	109	120
S2	906	489	238	152	108	105	134
S3	793	418	202	148	105	102	149
S4	791	397	198	130	100	100	132
S5	793	371	186	122	94	94	123
S6	816	373	193	102	91	95	100
				Mean R [%]	**103**	**101**	**126**
				Min	91	94	100
				Max	118	109	149

PRECISION

The precision of an ELISA is defined as its ability to measure the same concentration consistently within the same experiments carried out by one operator (within-run precision or repeatability) and across several experiments using the same samples but conducted by several operators at different locations using different ELISA lots (in-between-run precision or reproducibility).

Within-Run Precision (intra-assay)

Experiment:
2 samples of known concentrations were tested 3 times within 1 kit lot by 1 operator.

Within-run (n=3)	Sample 1	Sample 2
Mean (pg/ml)	99	800
SD (pg/ml)	8.4	10.2
CV (%)	8	1

In-Between-Run Precision (inter-assay)

Experiment:
2 samples of known concentrations were tested 9 times within 2 kit lots by 2 operators.

In-between-run (n=9)	Sample 1	Sample 2
Mean (pg/ml)	103	803
SD (pg/ml)	5.9	15.2
CV (%)	6	2

DETECTION LIMIT & SENSITIVITY

To determine the sensitivity of the FGF23 (intact) human ELISA, experiments measuring the lower limit of detection (LOD) and the lower limit of quantification (LLOQ) were conducted.

The LOD, also called the detection limit, is the lowest point at which a signal can be distinguished above the background signal, *i.e.* the signal that is measured in the absence of FGF23, with a confidence level of 99%. It is defined as the mean back calculated concentration of standard 1 (0 pg/ml of iFGF23, five independent measurements) plus three times the standard deviation of the measurements.

The LLOQ, or sensitivity of an assay, is the lowest concentration at which an analyte can be accurately quantified. The criteria for accurate quantification at the LLOQ are an analyte recovery between 75% and 125% and a coefficient of variation (CV) of less than 25%. To determine the LLOQ, standard 2, i.e. the lowest standards containing recombinant iFGF23, is diluted, measured five times and its concentration back calculated. The lowest dilution, which meets both criteria, is reported as the LLOQ.

The following values were determined for the FGF23 (intact) human ELISA:

LOD	**5.43 pg/ml**
LLOQ	**12.5 pg/ml**

SAMPLE STABILITY

Sample Preparation
Collect venous blood samples by using standardized blood collection tubes. Perform serum or plasma separation by centrifugation according to supplier's instructions of the blood collection devices. **Assay the acquired samples immediately or aliquot and store at -25°C or lower**. Lipemic or haemolyzed samples may give erroneous results. Samples are stable for at least four freeze-thaw cycles. Samples should be mixed well before assaying. We recommend duplicates for all values.

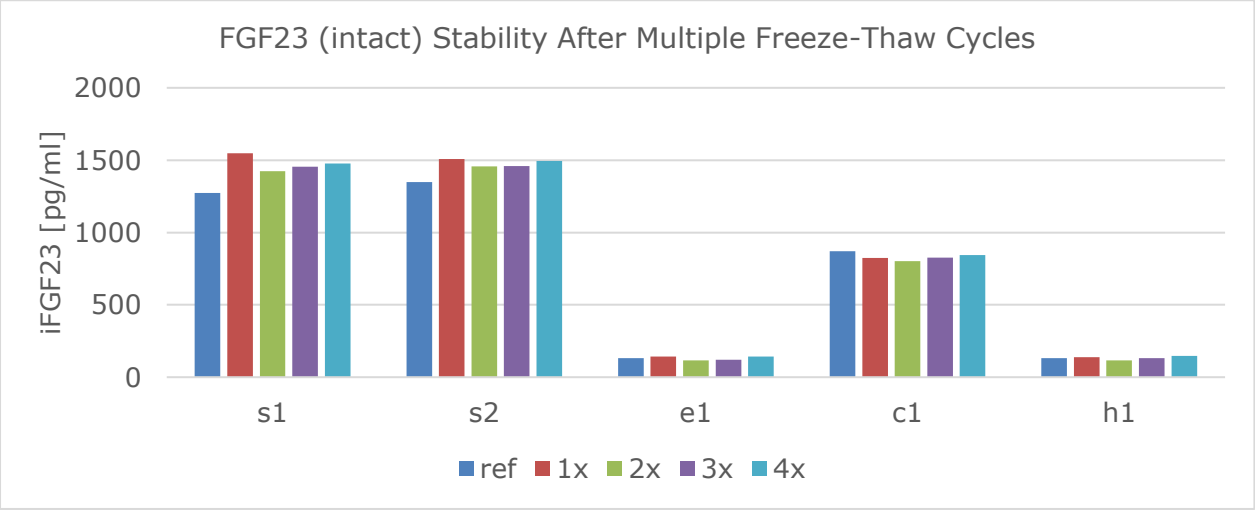
Freeze/Thaw Stability of Samples Containing Endogenous Intact FGF23

The stability of endogenous intact FGF23 (iFGF23) was tested by comparing four measurements in samples that had undergone four freeze-thaw cycles.

For freeze-thaw experiments, samples were collected according to the supplier's instruction using blood collection devices and stored at -80°C. Reference samples were freeze-thawed once. The mean recovery of sample concentration after four freeze-thaw cycles is 109%.

	iFGF23 [pg/ml]					Recovery [%] 4 F/T cycles
Sample matrix ID	**Ref**	**1x**	**2x**	**3x**	**4x**	
Serum #1	1273.7	1547.1	1423.5	1455.6	1477.7	116
Serum #2	1347.8	1507.3	1457.9	1459.5	1494.5	111

EDTA plasma #1	131.0	143.6	116.5	120.5	143.1	109
Citrate plasma #1	870.8	823.4	802.5	825.9	843.9	97
Heparin plasma #1	131.0	137.6	116.0	132.6	148.1	113
					Mean R [%]	**109**



All samples should undergo a maximum of four freeze-thaw cycles.

SPECIFICITY

This assay recognizes endogenous (natural) and recombinant human intact FGF23.
The assay does not cross-reat with FGF3, FGF19, and FGF21.

The specificity of an ELISA is defined as its ability to exclusively recognize the analyte of interest. The specificity of the human FGF23 (intact) human ELISA was shown by characterizing both the capture and the detection antibodies through affinity measurements. In addition, the specificity of the ELISA was established through competition experiments, which measure the ability of the antibodies to exclusively bind to FGF23.

Affinities of Coating and Detection Antibodies

Antibody affinities to FGF23 were tested by biolayer interferometry measurements (Octet), which measures the binding of antibodies to a FGF23-coated sensor. Both antibodies used in the FGF23 (intact) human ELISA bind to intact FGF23 with high affinity.

Competition of Signal

Competition experiments were carried out by pre-incubating human samples containing endogenous levels of FGF23 with an excess of capture antibody.
The concentration measured in this mixture was then compared to a reference value, which was obtained from the same sample but without the pre-incubation step.
Mean competition was 92%.

			iFGF23[pg/ml]	Recovery [%] Competition
Sample Matrix	**ID**	**Reference**	**Reference + CAB**	
Serum	#S1	73.7	6.2	92
Serum	#S2	29.3	5.2	82
Serum	#S3	119.4	7.2	94
EDTA plasma	#E1	24.8	2.2	91
EDTA plasma	#E2	65.6	7.2	89
Citrate plasma	#C1	41.3	0.0	100
Heparin plasma	#H1	50.6	2.2	96
Heparin pool	#H2	153.7	7.2	95
Heparin pool	#H3	91.9	6.2	93
Heparin pool	#H4	42.4	7.2	83
Heparin plasma	#H5	502.1	9.2	98
			Mean Comp. [%]	**92**

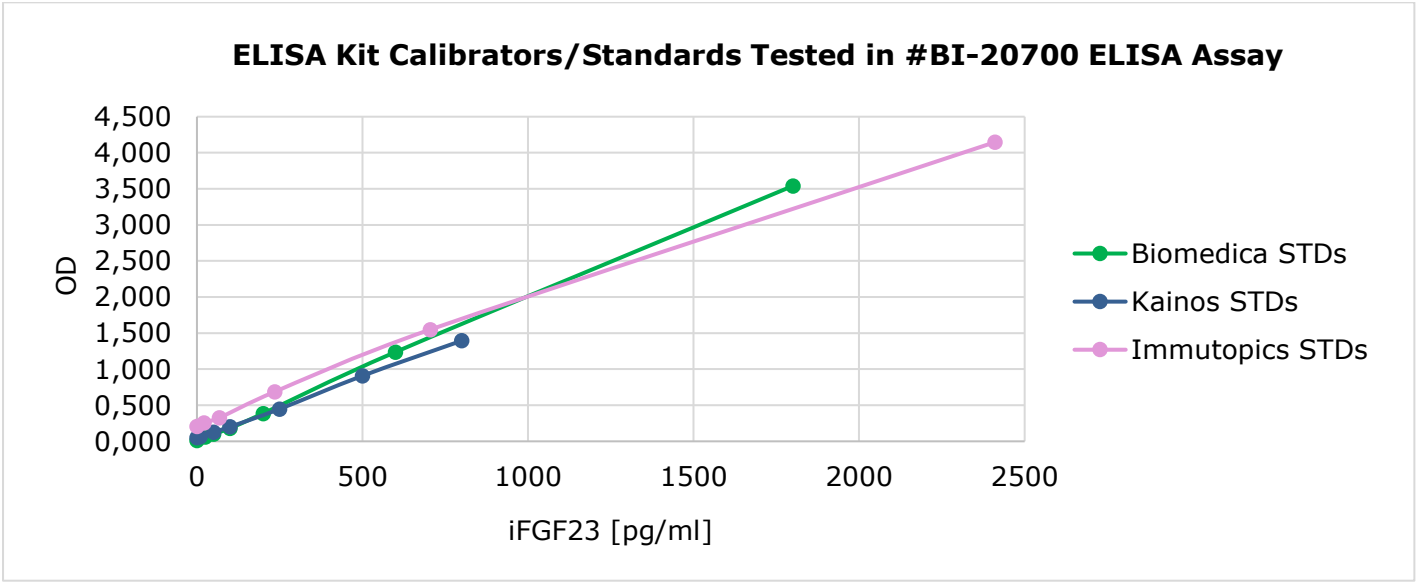
CALIBRATION

The FGF23 (intact) human immunoassay is calibrated against intact FGF23 (AA25-251 of Uniprot Q9GZV9 (https://www.uniprot.org/uniprot/Q9GZV9)).

COMPARISON of the BIOMEDICA FGF23 (intact) human ELISA with other commercially available FGF23 (intact) human ELISA assays

1. Measurement of different human intact FGF23 standards from different ELISA manufacturers in the Biomedica FGF23 (intact) human ELISA:

Three different recombinant intact FGF23 standards/calibrators (Biomedica, Kainos, Immutopics) were measured in the Biomedica FGF23 (intact) ELISA, see below graph:



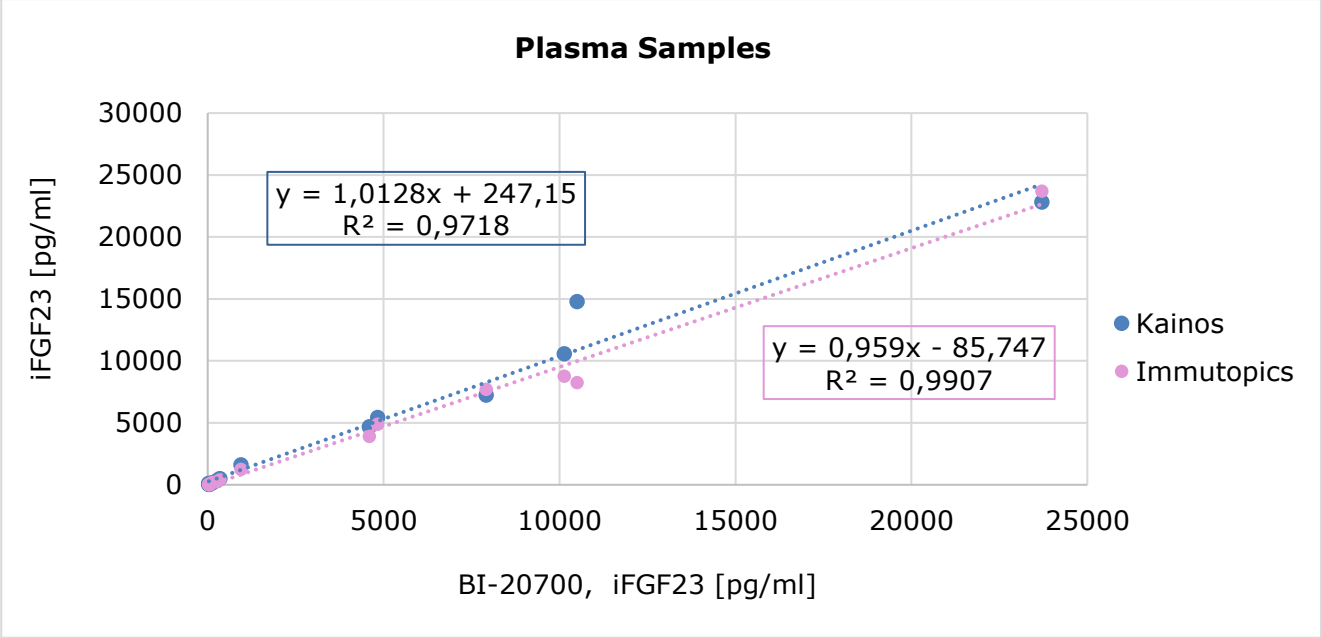
2. Comparison of sample concentrations in different FGF23 (intact) human ELISA kits:

The Biomedica FGF23 (intact) human ELISA was compared with other FGF23 (intact) human ELISA assays using plasma samples from 17 donors, see table and graph below:

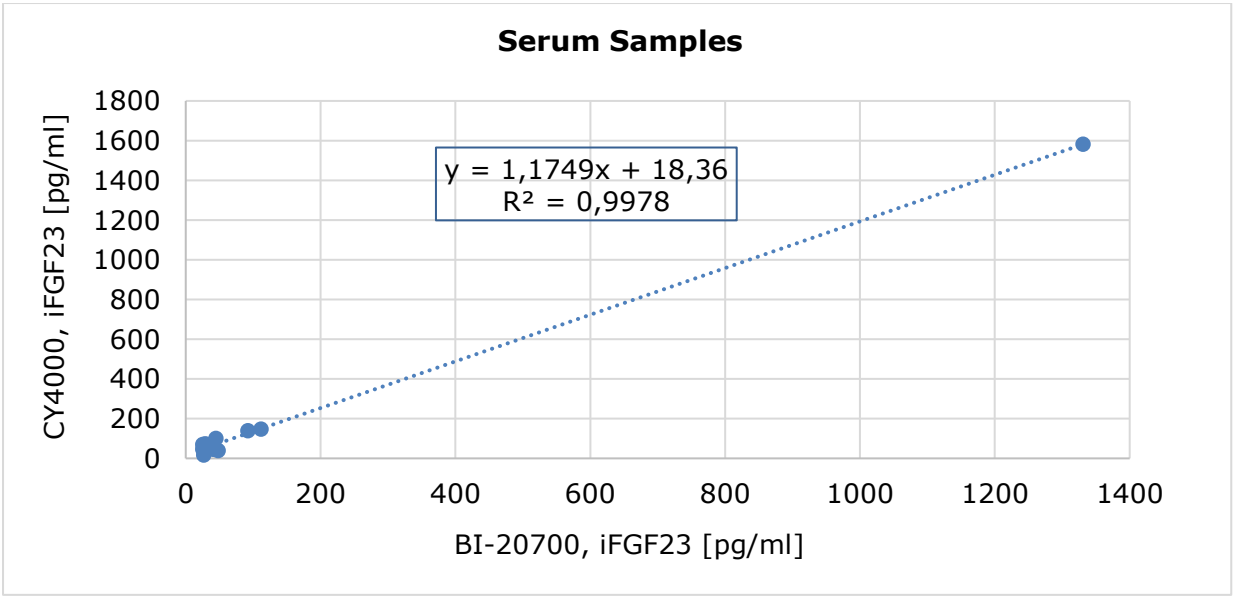
	intact FGF23 [pg/ml]		
Manufacturer	**Biomedica**	**Kainos**	**Immutopics**
Item code	**BI-20700**	**CY-4000**	**60-6600**
Plasma#1	4831	5443	4902
Plasma#2	7915	7257	7706
Plasma#3	30	40	39
Plasma#4	348	504	409
Plasma#5	32	120	15
Plasma#6	69	124	-
Plasma#7	10497	14794	8245
Plasma#8	4593	4675	3909
Plasma#9	10140	10569	8760
Plasma#10	23710	22813	23715
Plasma#11	268	357	279
Plasma#12	128	174	196
Plasma#13	947	1600	1244
Plasma#14	43	51	45
Plasma#15	54	92	64

Plasma#16	50	39	36
Plasma#17	75	92	98

Correlation of plasma samples (app. healthy, clinical and CKD samples) measured in Biomedica (BI-20700, x-axis), Kainos (CY4000, y-axis blue, correlation R^2 = 0.9718) and Immutopics (60-6600, y-axis pink, correlation R^2= 0.9907) FGF23 (intact) ELISA.



Correlation of serum samples measured in Biomedica (BI-20700, x-axis) and Kainos (CY4000, y-axis blue, correlation R^2 = 0.9978) FGF23 (intact) ELISA.



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 (IFU, package insert)
Material Safety Data Sheet (MSDS)
FGF23 (intact) human ELISA – Info Leaflet /Flyer

Measurement of Intact FGF23 in Human Urine Samples

The recovery of the FGF23 (intact) human ELISA was measured by adding recombinant intact FGF23 to urine samples containing a known concentration of endogenous intact FGF23. The recovery of the spiked concentration was calculated as the percentage of measured compared over the expected value.

Below table shows the summary of the recovery experiments in the FGF23 (intact) human ELISA in urine samples:

Sample ID	Spike iFGF23 [pg/ml]		S/R [%]
	0	300	
U1	44.6	316.0	98
U2	8.3	284.8	94
U3	2.9	293.3	97
U4	0.0	254.5	85
		Mean S/R [%]	**93**

Dilution linearity was assessed by serially diluting urine samples containing spiked concentrations of recombinant intact FGF23 with assay buffer.

Below table shows the mean recovery and range of serially diluted recombinant intact FGF23 in urine samples:

Sample ID	iFGF23 [pg/ml]			R [%]	
	Ref	1+1	1+3	1+1	1+3
U1	316.0	155.6	85.8	98	109
U2	284.8	148.4	73.5	104	103
U3	293.3	152.9	82.5	104	113
U4	254.5	143.2	78.6	113	124
			Mean R %]	**105**	**112**

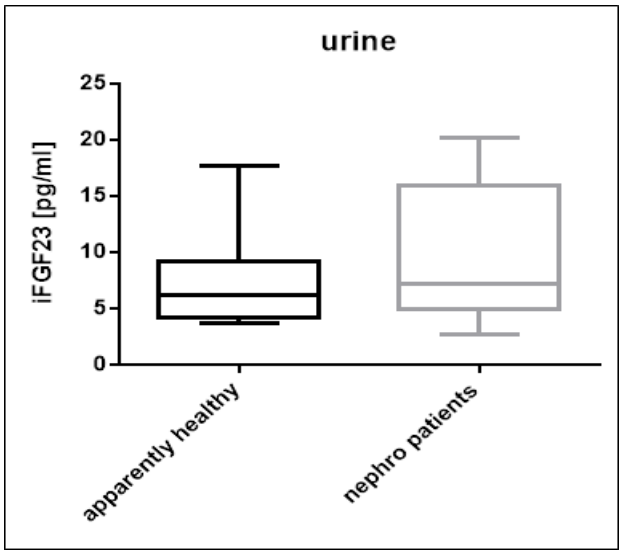
Parallelism was assessed by serially diluting urine samples containing endogenous levels of intact FGF23 with assay buffer.

Below table shows the mean recovery and range of serially diluted endogenous intact FGF23 in urine samples:

Sample ID	iFGF23 [pg/ml]				R [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
U1	44.6	14.0			63		
U2	3403.1		957.3	473.8		113	111

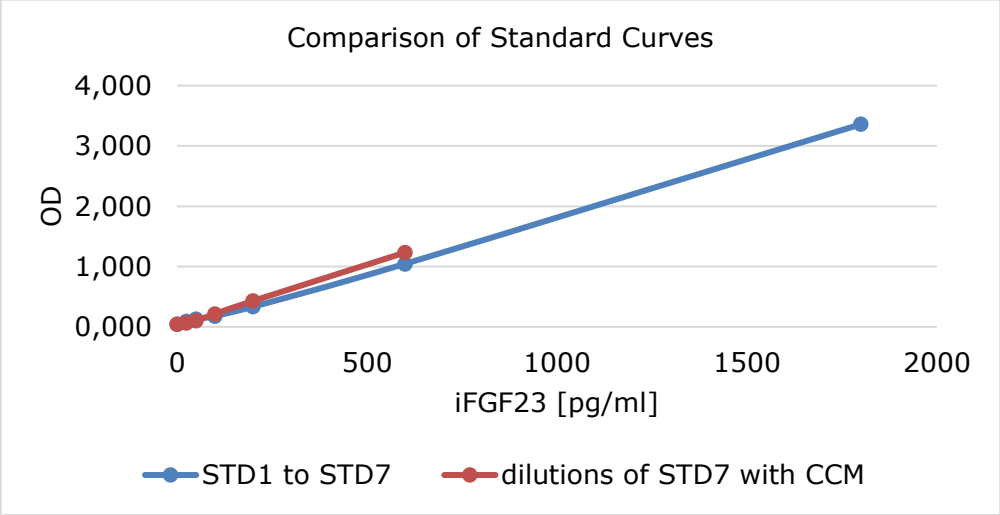
Intact FGF23 urine values (pg/ml) in various cohorts:

	iFGF23 [pg/ml]	
	app. healthy donors	**CKD cohort**
Number of samples	7	9
Mean	**7.7**	**9.5**
Minimum	3.7	2.7
25% Percentile	4.2	4.9
Median	**6.2**	**7.2**
75% Percentile	9.2	15.9
Maximum	17.7	20.2
5% Percentile	3.7	2.7
95% Percentile	17.7	20.2



Measurement of Intact FGF23 in Human Cell Culture Supernatants

Comparison of the FGF23 (intact) ELISA standard curve with the calibrator (STD7) resuspended and diluted in cell culture medium (CCM).



Experiments showing intact FGF23 values from cell culture supernatants (CCS) from two human breast cancer cell lines that were conditioned for 48 h.

CCS were spiked with recombinant human intact FGF23 and %recovery was calculated as the percentage of measured compared over the expected value.

Dilution linearity experiments were performed with spiked CCS and CCM.

Sample ID	Spike [OD]		R [%] compared to spike in STD1
	0	+900pg/ml	
CCS#1	0.045	1.899	123
CCS#2	0.023	1.824	118
CCM	0.045	1.724	111
STD1	0.045	1.547	100
		Mean R [%]	**117**

Sample ID	Spike [OD]			R % recovery	
	ref	1+1	1+3	1+1	1+3
CCS#1	1.899	0.974	0.512	100	105
CCS#2	1.824	0.891	0.456	95	98
CCM	1.724	0.891	0.456	101	103
			Mean R [%]	**99**	**102**