Human FGF-23 ELISA Kit

(Catalog Number: 31295)

For the quantitative determination of Human FGF-23 concentrations in serum, plasma or cell culture supernatant samples

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INTRODUCTION

FGF-23 is a 32-kDa protein that is secreted mainly by osteocytes in bone. It has been identified that has a physiological role in regulating mineral homeostasis. FGF-23 exerts its biological functions by binding to its cognate fibroblastic growth factor receptor (FGFR) in the presence of its coreceptor Klotho. FGF-23 is produced and secreted in response to hyperphosphatemia and increased 1,25 dihydroxyvitamin D3 levels. FGF-23 concentrations increase progressively as glomerular filtration rate (GFR) declines and the level can be 1000-fold higher in patients with end-stage renal disease compared with healthy individual. This increase is considered as one of the earliest biochemical abnormality in chronic kidney disease. Therefore, FGF-23 concentration is a kind of standard to determine the existence of kidney failure.

PRINCIPLE OF THE ASSAY

This assay is a rapid quantitative sandwich ELISA. The immunoplate is pre-coated with a polyclonal antibody specific for human FGF-23. Standards or samples and a biotin labelled polyclonal antibody specific for human FGF-23 are pipetted into the wells and any human FGF-23 present is bound by the immobilized antibody. After washing away any unbound substances, streptavidin-HRP conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human FGF-23 bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human FGF-23, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This human FGF-23 ELISA kit is designed for quantification of human FGF-23 in serum, plasma and cell culture supernatant samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)-Coated with a polyclonal antibody against human FGF-23, sealed.
2. 10×Wash buffer-50 ml.
3. 5×Assay buffer-20 ml.
4. 1×Sample diluent-20 ml.
5. 100×Detection antibody solution-A biotin labelled polyclonal antibody against human FGF-23, 0.12 ml.
6. Human FGF-23 standard-5000 pg of recombinant human FGF-23 in a buffered protein base, lyophilised.
7. 40×STP-HRP solution-0.3 ml.
8. Substrate solution- 12 ml, ready for use.
9. Stop solution- 12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human FGF-23 microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF STANDRADS AND SAMPLES

Human FGF-23 standards: Reconstitute the lyophilised standard with 1 ml of 1×Sample diluent to generate a standard stock solution of 5000 pg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.

Prepare serially diluted standards using 1×Sample diluent as follows:

Standard volume	Volume of 1×Sample diluent	Concentration
5000 pg/ml stock	-	5000 pg/ml
250 µl of 5000 pg/ml	250 µl	2500 pg/ml
250 µl of 2500 pg/ml	250 µl	1250 pg/ml
250 µl of 1250 pg/ml	250 µl	625 pg/ml
250 µl of 625 pg/ml	250 µl	312.5 pg/ml
250 µl of 312.5 pg/ml	250 µl	156.25 pg/ml
250 µl of 156.25 pg/ml	250 µl	78.125 pg/ml

1×Sample diluent serves as the zero standard (0 pg/ml).

Note: The reconstituted standard stock should be aliquoted and stored at -80ºC for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required a 5-fold dilution in 1×Sample diluent. Samples which generate values which are greater than the most concentrated standard should be further diluted with the appropriate sample dilution buffer.

ASSAY PROCEDURE

It is recommended that all standards and samples should be assayed in duplicate.

1. Add 100 µl of standard or sample to each well, incubate at room temperature for 2 hours.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 320 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 2 hours.
4. Wash each well 3 times as described in step 2.
5. Add 100 µl of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 3 times as described in step 2.
7. Add 100 µl of Substrate solution to each well, incubate at room temperature for 20 minutes. Protect from light.
8. Add 50 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

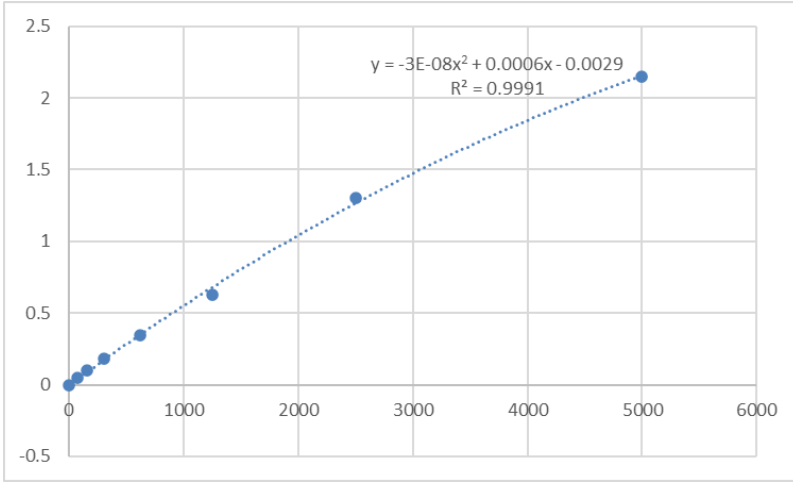
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human FGF-23 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human FGF-23 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Human FGF-23 (pg/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.076	0
78.125	0.123	0.047
156.25	0.173	0.097
312.5	0.256	0.180
625	0.425	0.349
1250	0.707	0.631
2500	1.376	1.300
5000	2.225	2.149

Human FGF-23 standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. 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: Ablank + 2xSDblank) is calculated from the real FGF-23 values in wells and is 30.74 pg/ml.

B. Precision:

Intra-assay Precision (Precision within an assay)

Two samples of known concentration were tested 20 times on one plate.

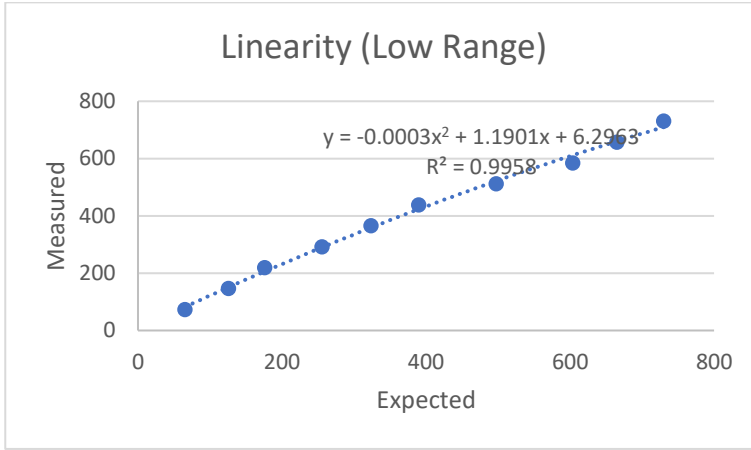
Sample	n	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	20	1100.7	35.1	3
2	20	388.2	23.1	6

C. Linearity:

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human FGF-23 were serially diluted with the 1×Sample diluent to produce samples with values within the dynamic range of the assay.

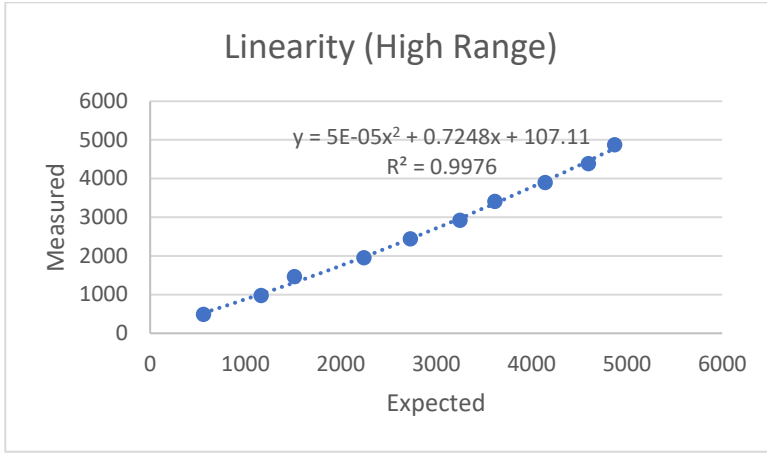
Low Range

Dilution	Measured	Expected	Recovery
1	729.934	729.934	100%
0.9	665.125	656.9406	101%
0.8	603.839	583.9472	103%
0.7	497.406	510.9538	97%
0.6	389.817	437.9604	89%
0.5	323.502	364.967	89%
0.4	255.595	291.9736	88%
0.3	175.904	218.9802	80%
0.2	125.898	145.9868	86%
0.1	65.1536	72.9934	89%



High Range

Dilution	Measured	Expected	Recovery
1	4870.822	4870.822	100%
0.9	4598.373	4383.74	105%
0.8	4143.354	3896.658	106%
0.7	3615.286	3409.575	106%
0.6	3251.289	2922.493	111%
0.5	2730.108	2435.411	112%
0.4	2241.409	1948.329	115%
0.3	1512.905	1461.247	104%
0.2	1165.669	974.1644	120%
0.1	560.093	487.0822	115%



Linearity of Human FGF-23 ELISA range from 72.9934 to 4870.822 pg/ml
The recovery rate ranges from 80% to 120%

Spiking

				Serum	Assay Buffer	
	Serum	Conc.	Add	Test result	Expected	Recovery
1st trial	0	0	0	0	0	/
	0	1250	116.35	87.03	116.35	75%
	0	2500	235.08	216.89	235.08	92%
	0	5000	422.9	472.68	422.9	112%
2nd trial	0	0	0	0	0	/
	0	1250	118.29	101.73	118.29	86%
	0	2500	234.98	209.13	234.98	89%
	0	5000	438.52	420.98	438.52	96%

The recovery rate ranges from 75% to 112%

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of Standard or sample to each well.
↓
Incubate at room temperature for 2 hours.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 2 hours.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of Substrate solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Add 50 µl of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation