

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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TABLE OF CONTENTS

Contents	Page
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	1
INTENDED USE	1
REAGENTS SUPPLIED	1
OTHER MATERIALS REQUIRED, BUT NOT PROVIDED	1
STORAGE	2
PREPARATION OF REAGENTS	2
PREPARATION OF STANDARDS AND SAMPLES	2
ASSAY PROCEDURE	3
CALCULATION	3
TYPICAL STANDARD CURVE	4
ASSAY CHARACTERISTICS	5
SUMMARY OF ASSAY PROCEDURE	6

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. Microtiter Strips (96 wells), coated with a polyclonal antibody against human FGF-23, sealed
2. 10×Wash buffer, 50 mL
3. 5×Assay buffer, 25 mL
4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against human FGF-23, 0.12 mL
5. Human FGF-23 standard, 5000 pg of recombinant human FGF-23, lyophilized
6. 200×STP-HRP solution, 0.06 mL
7. Substrate solution, 12 mL, ready for use
8. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips

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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 microtiter plate, 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 REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (25 mL) with 100 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 mL) with 450 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

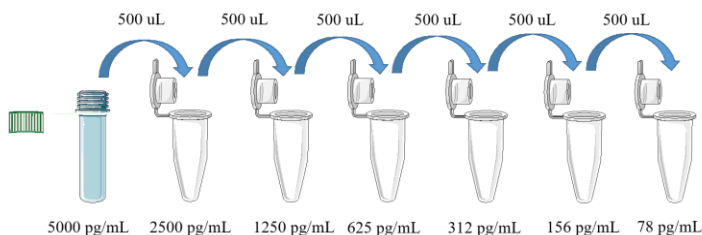
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µL of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human FGF-23 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer 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. Pipette 500 µL of 1×Assay buffer to 2500, 1250, 525, 312, 156, 78 pg/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 pg/mL). The reconstituted standard stock should be aliquoted and stored at -80°C for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **5-fold** dilution in the 1×Assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

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

1. Add 100 µL of standard or sample per 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 300 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 hour.
4. Wash each well 3 times as 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 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 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.

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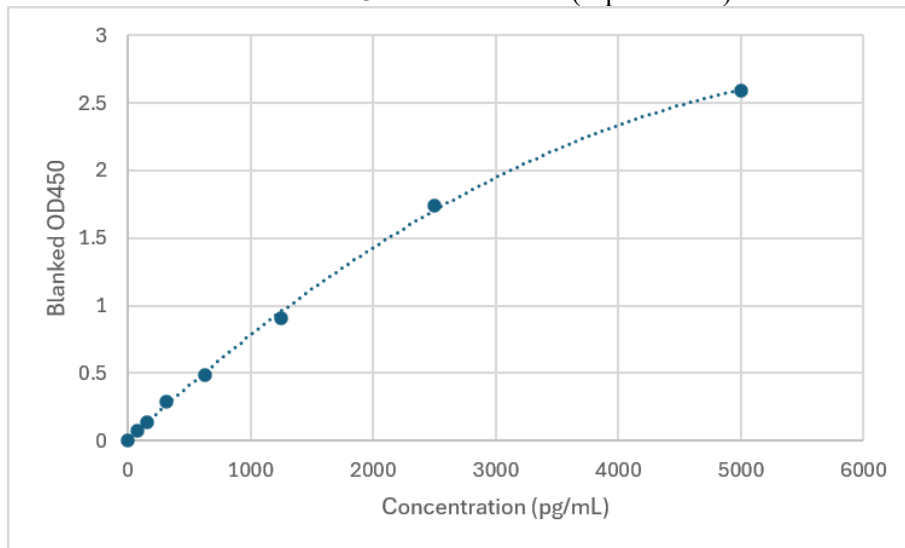
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TYPICAL STANDARD CURVE

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	0.096	0
78	0.171	0.075
156	0.233	0.137
312	0.386	0.29
625	0.581	0.485
1250	0.999	0.903
2500	1.837	1.741
5000	2.687	2.591

Human FGF-23 standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of human FGF-23 that can be detected by this assay is 30.74 pg/mL.

B. Precision

Intra-assay Precision (Precision within an assay) C.V. <3.1%.

Inter-assay Precision (Precision between assays) C.V. <6.1%.

SUMMARY OF ASSAY PROCEDURE

