Human FGF23 ELISA Kit

Enzyme Immunoassay for the quantification of Human FGF23 in serum, plasma, cell culture supernatants

Catalog number: ARG81256

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INTRODUCTION

This gene encodes a member of the fibroblast growth factor family of proteins, which possess broad mitogenic and cell survival activities and are involved in a variety of biological processes. The product of this gene regulates phosphate homeostasis and transport in the kidney. The full-length, functional protein may be deactivated via cleavage into N-terminal and C-terminal chains. Mutation of this cleavage site causes autosomal dominant hypophosphatemic rickets (ADHR). Mutations in this gene are also associated with hyperphosphatemic familial tumoral calcinosis (HFTC). [provided by RefSeq, Feb 2013] Regulator of phosphate homeostasis. Inhibits renal tubular phosphate transport by reducing SLC34A1 levels. Upregulates EGR1 expression in the presence of KL (By similarity). Acts directly on the parathyroid to decrease PTH secretion (By similarity). Regulator of vitamin-D metabolism. Negatively regulates osteoblast differentiation and matrix mineralization. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for FGF23 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any FGF23 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for FGF23 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-

enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of FGF23 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of FGF23 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 5 ng/vial	4°C
Standard/Sample diluent	1 X 16 ml	4°C
Antibody conjugate concentrate	2 vials (60 µl)	4°C
Antibody diluent buffer	16 ml	4°C
HRP-Streptavidin concentrate	2 vials (60 µl)	4°C (Protect from light)
HRP-Streptavidin diluent buffer	16 ml	4°C
20X Wash buffer	25 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.

- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1000 x g and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.
b) Samples containing sodium azide should not be used in the assay.

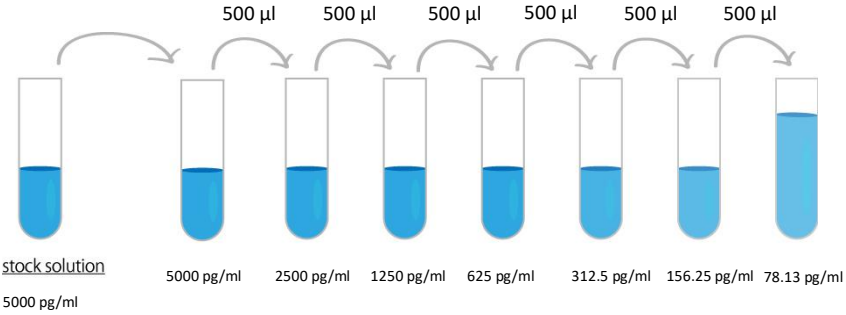
REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- 1X Antibody conjugate: 5-10 minutes before use (freshly prepared is recommended), dilute 100X antibody conjugate concentrate into Antibody diluent buffer with Antibody diluent buffer to yield 1X detection antibody solution.
- 1X HRP-Streptavidin Solution: 5-10 minutes before use (freshly prepared is recommended), dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.
- Sample: For the normal human serum or plasma samples are suggested to make a 1:2 dilution with Standard/Sample diluent. (100 µl of samples + 100 µl of Standard/Sample diluent; dilution factor = 2)

  If the initial assay found samples contain FGF23 higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- Standards: Reconstitute the standard with 1 ml Standard/Sample diluent buffer to yield a stock concentration of 5000 pg/ml. Allow the stock standard to sit for at least 15 minutes with gentle agitation to make sure

the standard is dissolved completely before making serial dilutions. Aliquot and store the reconstituted standard for up to 1 month at -20 °C. Avoid repeated freeze-thaw cycles. The Standard/Sample diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml, 78.13 pg/ml. Diluted standard shall not be reused.



Dilute FGF23 standard as according to the table below:

Standard	FGF23 Conc.	µl of Standard diluent	µl of standard
S7	5000 pg/ml	0	1000 (5000 pg/ml Stock)
S6	2500 pg/ml	500	500 (S7)
S5	1250 pg/ml	500	500 (S6)
S4	625 pg/ml	500	500 (S5)
S3	312.5 pg/ml	500	500 (S4)
S2	156.25 pg/ml	500	500 (S3)
S1	78.13 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. It can be store at 2-8°C for up to 1 month.
2. Add 100 µl of standards, samples and zero controls (Standard/Sample diluent) into wells. Incubate for 1.5 h at 37 °C.
3. Aspirate each well and wash, repeating the process three times for a total four washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl 1X Antibody conjugate into each well. Cover wells and incubate for 1 hour at 37 °C.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well. Cover wells and incubate for 30 minutes at 37 °C.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well. Incubate for 10-20 minutes at 37°C in dark.
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough

mixing

10. Read the OD with a microplate reader at 450nm immediately. (optional: read at 610-650 nm as the reference wave length) It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

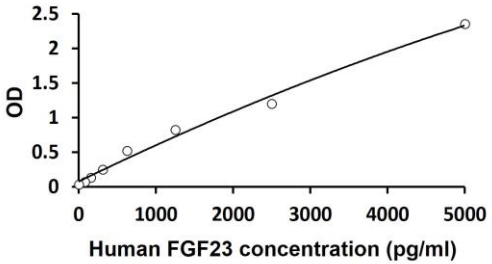
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human FGF23 ranged from 78.1-5000 pg/ml. The mean MDD was 40 pg/ml.

Specificity

This assay recognizes natural and recombinant Human FGF23. No significant cross-reactivity or interference with the factors below was observed:

50 ng/ ml of recombinant proteins:

Human: FGF R1α/Fc Chimera, FGF R2α/Fc Chimera, FGF R3/Fc Chimera, FGF R4/Fc Chimera, Klotho

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.