Read carefully before using the product

Research use only

FGF-23 ELISA Kit

【INTRODUCTION】

FGF23 (Fibroblast Growth Factor 23) is an osteocyte-secreted hormone that regulates phosphate, vitamin D and PTH homeostasis. FGF23 is produced as a 226 amino acid polypeptide which has a cleavage site and the flagmented peptides are recognized in serum with full length peptide.

Serum intact FGF23 level are elevated in some patients of hypophosphatemic rickets/osteomalacia (such as tumor-induced osteomalacia, X-linked hypophosphatemic rickets and autosomal dominant hypophosphatemic rickets) and chronic kidney disease.

【CAUTION】

1. The kit is intended only for research purposes, not for use in diagnostic procedures.
2. The reliability of results cannot be guaranteed if the kit is used with a method or for a purpose other than those stipulated.
3. This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions.

【COMPONENTS】

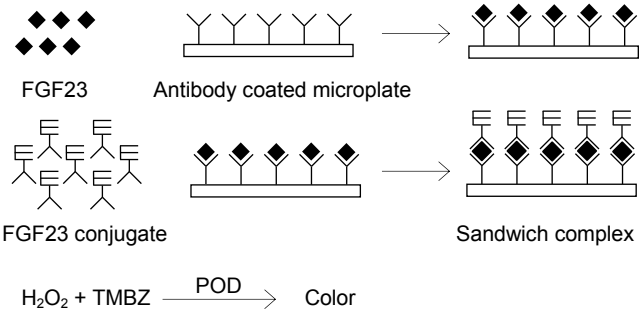
1. **FGF-23 Microplate** (Anti-FGF23 Antibody coated Microplate)
   96 well microplate (12 strips of 8 well) coated with a murine monoclonal antibody against FGF23.
2. **FGF-23 Conjugate** (HRP labeled Anti-FGF23 Antibody)
   12 mL of murine monoclonal antibody against FGF23 conjugated to horseradish peroxidase, with preservatives.
3. **FGF-23 Std1-Std7** (Recombinant Human FGF23)
   7 vials of FGF23 (Std1:12 mL, Std2-7:0.5 mL) in buffered base with preservative.
4. **Assay Diluent**
   1 bottle containing 12 mL of buffer.
5. **Substrate** (Color Reagent)
   1 bottle containing 12 mL of tetramethylbenzidine (TMBZ) with urea hydrogen peroxide.
6. **FGF-23 Wash Buffer** (10x Concentrate)
   1 bottle containing 53 mL (included in this kit and sold separately) of a 10-fold concentrated solution of buffered surfactant with preservative.
7. **Stop Solution**
   1 bottle containing 12 mL of 0.5 mol/L Sulfuric acid.
8. **Plate Sealer**
   3 included in this kit.

【INTENDED USE】

Measurement of intact FGF23 in serum

【PRINCIPLE OF THE EXAMINATION METHOD】

1. **Principle**
   The assay principle is a two-step enzyme-linked immunosorbent assay.In first reaction, a sample containing FGF23 is incubated with the immobilized antibody in a microtiter well. FGF23 in sample is captured by the antibody. In second reaction, this immobilized FGF23 is incubated with HRP labeled antibody to form a "sandwich" complex. Because Peroxidase bound to the solid phase depends on the amount of FGF23, the sample amount of FGF23 can be determined by colorimetrically measuring the amount of detached oxidized (colored) TMBZ using urea hydrogen peroxide (H_2O_2) and 3,3',5,5'-Tetramethylbenzidine (TMBZ) as the substrates.



H_2O_2 + TMBZ $\xrightarrow{POD}$ Color

2. **Feature**
   1) High specificity:
      Measurable without the influence of serum components.
   2) High sensitivity:
      The minimal detectable concentration is 3 pg/mL.

【LIMITATIONS OF THE EXAMINATION PROCEDURE】

1. **Samples**
   1) Serum sample is used for measurement.
   2) After sampling, measure as soon as possible. If samples must be stored, freeze at -20°C or lower.
   3) The intact FGF23 molecule appears to be highly unstable resulting in decreased immunoreactivity over time.
   4) Besides human, analytic species can be measured mouse, rat and monkey.

2. **Interference**
   1) Ascorbic acid (100 mg/dL or lower), bilirubin (50 mg/dL or lower), and chylomicrons (3000 formazine turbidity units or lower), rheumatoid factor (500 IU/mL or lower) each have almost no effect on measurements.
   2) The sample and all reagents should be pipetted carefully to minimize air bubbles in the well.

3. **Others**
   1) A calibration curve must be prepared for each assay.
   2) If multiple samples assayed, make sure to keep the intervals to add each reagent constant so that each reaction completes in the defined time period.
   3) The washing step is also an important part of the total assay procedure. If you using an automated microtiter plate washer, please completely removal of Wash Buffer Solution.
   4) Avoid the well from drying out during the wash process.
      Make sure the well bottoms do not get scratched or soiled.
   5) In case FGF23 concentration in the sample exceeds the measurable range, dilute the sample with FGF-23 Std1 and repeat the assay.
   6) Some samples may cause reaction with other substances and interfere with the assay. When measured value and result have question, confirm the results in retest or other method.

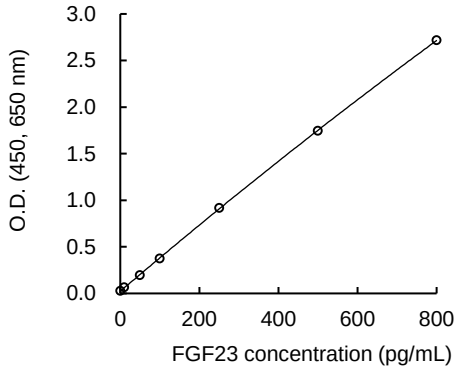
【EXAMINATION PROCEDURE】

1. **Preparation of Reagents**
   1) Wash Buffer Solution
      Before using, dilute the contents to 10-fold with deionized water and mix well.
   2) Reagents other than the above
      Ready to use.

2. **Material Required but not Provided**
   1) Automatic pipettes, Multi-channel pipettes, Disposable pipette tips
   2) Plate mixer
   3) Microtiter plate reader (λ=450, 600-650 nm)

3. **Assay Method**
   1) Add 50 μL of Assay Diluent into all wells to be used.
   2) Add 50 μL of FGF-23 Standards or sample into each well, and cover the plate with a plate sealer.
   3) Incubate plate at room temperature for 120 minutes on a plate mixer.
   4) Remove the plate sealer.
      After removing the reaction solution, add 300 μL of Wash Buffer Solution into each well and then discard the Wash Buffer Solution. Repeat this process 4 times. Remove any droplets remaining in the wells by tapping the plate on paper towel or similar absorbent material.
      [Note 1] In order to avoid wells from drying out when adding Wash Buffer Solution, work quickly with a multi-channel pipette or a similar device.
      [Note 2] Make sure the pipette tips do not scratch the well bottoms trying to remove the solution from the well.
   5) Add 100 μL of FGF-23 Conjugate into each well and cover the plate with a plate sealer. Incubate plate at room temperature for 60 minutes on a plate mixer.
   6) Repeat in the same manner 4).
   7) Add 100 μL of Substrate into each well and cover the plate with a plate sealer.
      Incubate plate at room temperature for 30 minutes in dark.
   8) Remove plate sealer.
      Immediately add 100 μL of Stop Solution into each well.
      Read the absorbance at 450 nm (secondary wavelength 600-650 nm) within 10 minutes by microtiter plate reader.

4. **Calculation of Test Result**
   1) The standard curve is generated by plotting absorbance for the FGF-23 Std1-Std7 versus the corresponding concentration.
   2) Calibration Curve



【WARNINGS AND PRECAUTIONS】

1. **Warnings**
   1) Serum samples should be handled as potentially infectious as they may contain infectious agents including HBV, HCV, HIV, etc.
   2) Any instruments that have come to contact samples should also be treated potentially infectious.
   3) Wear protective gloves to avoid infection.
   4) In case any of the reagents contact eyes, mouth and/or skin, immediately flush with copious amount of water then consult a physician as necessary.

2. **Handling Procedures**
   1) Do not freeze.
   2) Use any opened reagents as soon as possible.
      Store any unused Antibody coated Strips in the resealable aluminum pouch with desiccant to protect from moisture.
      Wash Buffer Solution shall be stored at 2-10°C and used within 4 weeks after dilution.
   3) The kit should be used before the expiration date printed on the product label.
   4) Do not pool reagents even if the Lot No. of kits are the same.
      Do not combine reagents from different kits.

3. **Disposal Procedures**
   1) Dispose of used reagent bottles as medical waste or industrial waste according to the rules stipulated for waste materials.
   2) Wipe off if the released amount is small. Flush with copious amount of water if a large volume is released.

【STORAGE】

Store at 2-10°C in dark.

【WARRANTY】

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. KAINOS LABORATORIES, INC. disclaims any implied warranty of merchantability or fitness for a particular purpose, and in no event shall KAINOS LABORATORIES, INC. be liable for consequential. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal right and you may have other rights which vary from state to state.

【LITERATURE REFERENCES】

1. Yuji Yamazaki, J Clin Endocriniol Metab, 87 (11), 4957-4960 (2002)

【CUSTOMER SERVICE】

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