MedFrontier FGF23

**Introduction**

FGF23 (Fibroblast Growth Factor 23) is a protein belonging to the fibroblast growth factor family. FGF23 is involved in the regulation of phosphorus metabolism. FGF23 has a molecular weight of approximately 32 kDa (ref. 1). FGF23 is produced in bone cells. In vivo, FGF23 is secreted into circulation (ref. 2). A full-length active FGF23 protein may undergo proteolytic cleavage to generate an inactive c-terminal fragment. This mechanism is thought to play a key role in controlling the concentration of circulating bioactive FGF23 in vivo (ref. 3). MedFrontier FGF23 measures ONLY the full-length active form (intact form). FGF23 is being researched in the context of X-linked hypophosphatemic rickets, mineral bone disorder (MBD), chronic kidney disease (CKD), tumor-induced osteomalacia and hyperphosphatemia (ref. 4–7). MedFrontier FGF23 is researched for Research Use Only and is not intended for use in diagnostic or therapeutic procedures.

**Method of operation**

1. Preparation of reagents
   - **(1) Wash Buffer** Dilute 60 mL of Wash Buffer Concentrate (20×) with 1140 mL of purified water at room temperature (18–25°C).
   - **(2) Other reagents** Ready to Use.

2. Assay procedure
   - **(1) Add 80 μL of Sample Dilution solution to each well of the Monoclonal Antibody (MAb)-Coated Microtiter Plate and 20 μL each of FGF23 Standard 1 to 6, FGF23 Control L & H, or serum samples.**
   - **(2) Seal the plate and set it aside for 90 minutes at room temperature (18–25°C).**
   - **(3) After 90 minutes, remove the plate seal, aspirate the reaction solution, and wash each well 5 times with 500 μL of Wash Buffer. It is recommended to overflow the wells with wash buffer.**
   - **(4) Add 100 μL of ALP-Labeled Monoclonal Antibody (MAb) Reagent to each well. Seal the plate and set it aside for 90 minutes at room temperature (18–25°C).**
   - **(5) After 90 minutes, remove the plate seal, aspirate the reaction solution, and wash each well 5 times with 500 μL of Wash Buffer. It is recommended to overflow the wells with wash buffer.**
   - **(6) Add 100 μL of ALP Substrate (Lumigen™ APS-5) solution to each well, and block out light for 1 minute at room temperature (18–25°C).**
   - **(7) Measure relative light units (RLU) within 10 minutes.**

**Calculation and interpretation of results**

1. Calculation of results
   - Calculate the concentration of FGF23 in a sample from a standard curve prepared from FGF23 Standard 1 to 6 at concentrations of approximately 0.0 to 3,000.0 pg/mL.

2. Standard curve example

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**Operating precautions**

1. Sample collection & handling
   - **(1) Use only fresh serum samples or serum samples that were frozen on the day of collection.**
   - **(2) Centrifuge serum samples before use, if precipitates are observed.**
   - **(3) Keep serum samples frozen below –20°C if they are not used on the day of collection.**
   - **(4) Avoid freezing and thawing more than 5 times.**
   - **(5) The use of FGF23 Control L & H along with serum samples is recommended for quality control.**

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**Calculation and interpretation of results**

1. Calculation of results
   - Calculate the concentration of FGF23 in a sample from a standard curve prepared from FGF23 Standard 1 to 6 at concentrations of approximately 0.0 to 3,000.0 pg/mL.

2. Standard curve example
3. Disposal precautions

1. Precautions
When acclimatizing FGF23 Standard 1 to 6 and FGF23 Control L & H to room temperature (18–25°C), serum samples may be infected with HBV, HCV, HIV, etc. Please treat all instruments, waste liquid, dispose of reagents and equipment according to laws to control waste treatment and clean-up, water dilution assay, or other analytical methods. If there is any doubt about the measurements, please verify the results by repeating the assay, dilution assay, or other analytical methods.

2. Handling precautions
(1) Plates and reagents should be used at room temperature (18–25°C).
(2) Do not return reagents dispensed in separate containers to the original containers.
(3) After opening, use this kit as soon as possible.
(4) Handle ALP Substrate (Lumigen™ APS-5) carefully. Do not expose ALP Substrate (Lumigen™ APS-5) to light. ALP Substrate (Lumigen™ APS-5) is light-sensitive. If ALP Substrate (Lumigen™ APS-5) comes in contact with anything other than a clean pipette tip, the reagent must be discarded.
(5) Do not use kit reagents or containers for any other purpose.
(6) If storing FGF23 Standard 1 to 6 and FGF23 Control L & H in separate containers, do not use the same storage method and should be immersed in sodium hypochlorite solution (effective chlorine concentration 1,000 ppm) or glutaraldehyde solution (2%) for 1 hour or more for disinfection.

3. Disposal precautions
(1) Serum samples may be infected with HBV, HCV, HIV, etc. Please treat all instruments, waste liquid, and other materials by one of the following methods, or follow the infectious medical waste disposal manual of each facility.
(1) Instruments and waste should be sterilized at 121°C for at least 20 minutes by autoclaving. However, waste containing sodium hypochlorite solution should not be autoclaved.
(2) Materials should be immersed in sodium hypochlorite solution (efffective chlorine concentration 1,000 ppm) or glutaraldehyde solution (2%) for 1 hour or more for disinfection.
(2) Do not use kit reagents or containers for any other purpose.
(3) Components (2, 3, 4, 5, 7) contain sodium azide (0.1% or less). Sodium azide may react with lead and copper pipes to produce metal azide, which has high explosiveness. Accordingly, if reagents are discarded, please dilute them with a sufficient amount of water to ensure that they do not remain in the drain pipe and rinse appropriately.
(4) If the reagent is spilled, please dilute it with water and wipe it off. If a serum sample is spilled, please wipe thoroughly with 80% alcohol solution spray or other antiseptic solutions. In addition, please protect your hands with rubber gloves.
(5) Do not mix reagents with different serial numbers.
(6) Do not mix reagents with different serial numbers.
(8) In-house data from Kyowa Medex Co., Ltd.

### Table: Assay performance (ref. 8)

<table>
<thead>
<tr>
<th>Serum type</th>
<th>Assigned value (ng/mL)</th>
<th>Measured value (pg/mL)</th>
<th>Accuracy (%)</th>
<th>Reproducibility (CV %; N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>26.6</td>
<td>29.1</td>
<td>109.5</td>
<td>14.6</td>
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<tr>
<td>Middle</td>
<td>296.0</td>
<td>262.0</td>
<td>88.5</td>
<td>4.6</td>
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<tr>
<td>High</td>
<td>1963.5</td>
<td>1912.8</td>
<td>97.4</td>
<td>5.8</td>
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</tbody>
</table>

### References
(8) In-house data from Kyowa Medex Co., Ltd.

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