

## MedFrontier<sup>®</sup> FGF23 Intact

### 【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. MedFrontier<sup>®</sup> FGF23 Intact measures ONLY the full-length active form (intact form). MedFrontier<sup>®</sup> FGF23 Intact is for Research Use Only and is not intended for use in diagnostic or therapeutic procedures.

### 【Kit components】 Catalog No. 63278-8

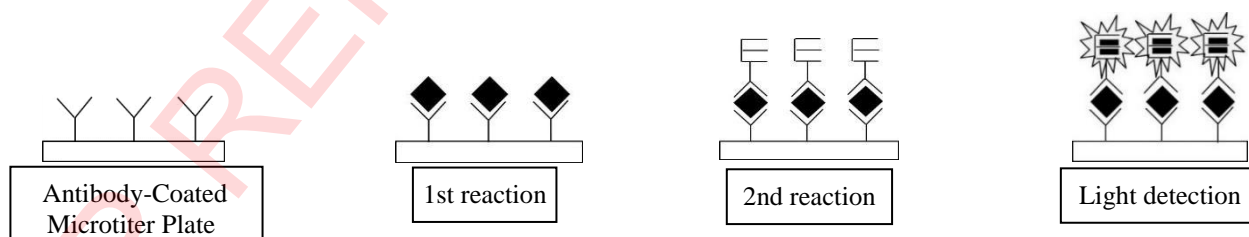
No.	Component	Quantity
1	Monoclonal Antibody (MAb)-Coated Microtiter Plate	96 Wells × 1
2	ALP-Labeled Monoclonal Antibody (MAb) Reagent	13.5 mL × 1
3	Sample Dilution	13.5 mL × 1
4	FGF23 Standard 1 to 6 <sup>*1</sup>	1 mL × 6
5	FGF23 Control L & H <sup>*2</sup>	1 mL × 2
6	ALP Substrate(Lumigen <sup>™</sup> APS-5)	15 mL × 1
7	Wash Buffer Concentrate (20×)	60 mL × 1

\*1. The FGF23 values for Standard 1 to 6 and Control L & H are indicated in the enclosed attachment.

\*2. The use of FGF23 Control L & H along with serum samples is recommended for quality control.

### 【Assay principle】

This is a sandwich Chemiluminescence enzyme immunoassay (CLEIA) kit by using two anti-human FGF23 mouse monoclonal antibodies. When serum is added to a plate well coated with anti-human FGF23 mouse monoclonal antibodies, FGF23 is captured by the immobilized antibodies (1st reaction). After the 1st reaction, the plate is washed. Then, ALP-labeled anti-human FGF23 mouse monoclonal 2nd antibodies against FGF23 react to FGF23 antigens captured by the immobilized antibodies (2nd reaction). After the 2nd reaction, the plate is washed and light detection is performed after adding the luminescence reagent. Each active well of the plate is measured using a luminescence microplate reader and relative light units (RLU) are obtained. The concentration of FGF23 in serum is calculated with a standard curve generated using the FGF23 Standard 1 to 6.



### 【Operating precautions】

#### 1. Sample collection & handling

- (1) Centrifuge serum samples before use, if precipitates are observed.
- (2) Keep serum samples frozen below -20°C if they are not used on the day of collection.

## 2. Other

- (1) Please create a standard curve for each assay.
- (2) Do not scratch or soil the bottom of the plate well.
- (3) Do not combine reagents in kits with different serial numbers.
- (4) Always use purified water; please do not use tap water.
- (5) Only use reagents provided in the same kit.
- (6) Only use consumables described in this package insert.

## 【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. Required instruments, equipment, etc.

- (1) Micropipette tip.
- (2) Precision pipette capable of delivering 30 µL to 200 µL.
- (3) Micropipette tip waste container.
- (4) Pipette, beaker, flask, and cylinder for the preparation of reagents.
- (5) Microplate washer.
- (6) Luminescence microplate reader.
- (7) Purified water.
- (8) Orbital shaker. (ex. orbit: 25mm, speed: 120-220 rpm)

### 3. Assay procedure (For reference)

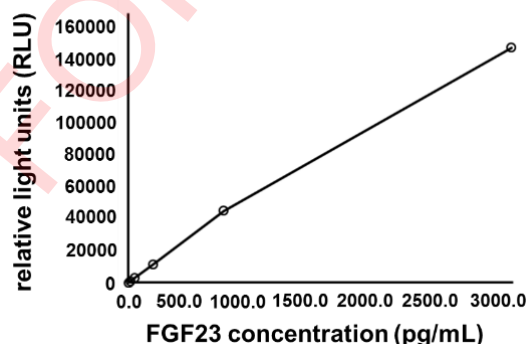
- (1) Add 80 µL of Sample Dilution solution to each well of the Monoclonal Antibody (MAb)-Coated Microtiter Plate and 20 µL each of FGF23 Standard 1 to 6, FGF23 Control L & H, or serum samples.
- (2) Seal the plate and set it aside for 90 minutes at room temperature (18-25°C) with being rotated on a plate shaker.
- (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) with being rotated on a plate shaker.
- (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) with being rotated on a plate shaker.
- (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



### 3. Note

In case of the doubt in sample concentration, dilute the sample.

## 【Using or handling precautions】

### 1. Precautions

- (1) Please wear appropriate protective gear; disposable gloves, lab coat, protective eyewear, etc.
- (2) Use safe laboratory practices.
- (3) Kit component reagents (No.2, 3, 4, 5, 7) contain sodium azide (0.1% or less).
- (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 spray or other antiseptic solutions. In addition, please protect your hands with rubber gloves.

### 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, consume the 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. Gently add ALP Substrate (Lumigen™ APS-5) to each well to avoid foaming.
- (5) Avoid freezing the reagents; store according to the storage method.
- (6) If storing FGF23 Standard 1 to 6 and FGF23 Control L & H in separate containers, do not use glass containers.
- (7) Do not mix reagents with different serial numbers.

### 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 (effective chlorine concentration 1,000 ppm) or glutaraldehyde solution (2%) for 1 hour or more for disinfection.
- (2) Dispose of reagents and equipment according to laws to control waste treatment and clean-up, water pollution, etc.
- (3) Components (No.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 scatters during the disposal process, dilute it with water and wipe it off. Additionally, if serum samples are scattered, wipe thoroughly with 80% alcohol solution spray or other antiseptic solutions. In addition, please protect hands with rubber gloves.
- (5) Do not use kit reagents or containers for any other purpose.

## 【Storage method】

Store at 2-8°C (Store in a cool, dark place; do not freeze.)

## 【Packaging unit】

Product name	Packaging unit	Catalog No.
MedFrontier® FGF23 Intact	96 tests	63278-8

## 【References】

- (1) Yuji Yamazaki, et al. (2002) *J Clin Endocrinol Metab* **87** (11):4957-4960.
- (2) Itsuro Endo, et al. (2008) *Bone* **42**:1235–1239.

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