Version 06-03/25

User's Manual

Mouse Fgf23(Fibroblast growth factor 23) ELISA Kit

REF **DEIA7416V2**

Σ **48T, 96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit is designed for quantitative detection of Fgf23 in serum, plasma, tissue homogenates and other biological fluids.

General Description

Fibroblast growth factor 23 or FGF23 is a protein that in humans is encoded by the FGF23 gene. FGF23 is a member of the fibroblast growth factor (FGF) family which is responsible for phosphate metabolism.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti- Fgf23 antibody was pre-coated onto 96-well plates. And the biotin conjugated anti-Fgf23 antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Fgf23 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of Fgf23 can be calculated.

Reagents And Materials Provided

1. Micro ELISA Plate(Dismountable) ; 8×6 or 8×12
2. Lyophilized Standard ; 1 vial or 2 vial
3. Sample / Standard dilution buffer ; 10ml/20ml
4. Biotin-detection antibody (Concentrated) ; 60ul/120ul
5. Antibody dilution buffer ; 5ml/10ml
6. HRP-Streptavidin Conjugate(SABC) ; 60ul/120ul
7. SABC dilution buffer ; 5ml/10ml
8. TMB substrate ; 5ml/10ml
9. Stop solution ; 5ml/10ml
10. Wash buffer (25X) ; 15ml/30ml
11. Plate Sealer ; 3/5pieces
12. Product Description ;1 copy

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator

3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

4°C for 6 months

Specimen Collection And Preparation

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.

Sample preparation: Samples should be clear and transparent and be centrifuged to remove suspended solids.

Note: Samples to be used within 5 days may be stored at 4°C,otherwise samples must be stored at -20°C (≤1 month) or -80°C(≤2 months) to avoid loss of bioactivity and contamination.

Hemolyzed samples are not suitable for use in this assay.

Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary in practice. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be make in pre-experiment.

High target protein concentration (10000-100000pg/ml): Dilution: 1:100. (i.e. Add 1μl of sample into 99 μl of Sample / Standard dilution buffer.)

Medium target protein concentration (1000-10000pg/ml): Dilution: 1:10.(i.e. Add 10 μl of sample into 90 μl of Sample / Standard dilution buffer.)

Low target protein concentration (15.6-1000pg/ml): Dilution: 1:2.(i.e. Add 50 μl of sample into 50 μl of Sample / Standard dilution buffer.)

Very low target protein concentration (≤15.6pg/ml): Unnecessary to dilute, or dilute at 1:2.

Reagent Preparation

Bring all reagents to room temperature before use.

1, Wash Buffer:

Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Standard:

1) 000pg/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.
2) 500pg/ml→15.6pg/ml of standard solutions: Label 6 Eppendorf tubes with500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 15.6pg/ml, respectively. Aliquot 0.3 ml of the Sample / Standard dilution buffer into each tube. Add 0.3 ml of the above 1000pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.

3, Preparation of Biotin-detection Antibody working solution

prepare within 1 hour before the experiment.

1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2) Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix

thoroughly.(i.e. Add 1 μl of Biotin-detection antibody into 99 μl of Antibody dilution buffer.)

4, Preparation of HRP-Streptavidin Conjugate (SABC) working solution: prepare within 30min before the experiment.

1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 μl of SABC into 99 μl of SABC dilution buffer.)

Assay Procedure

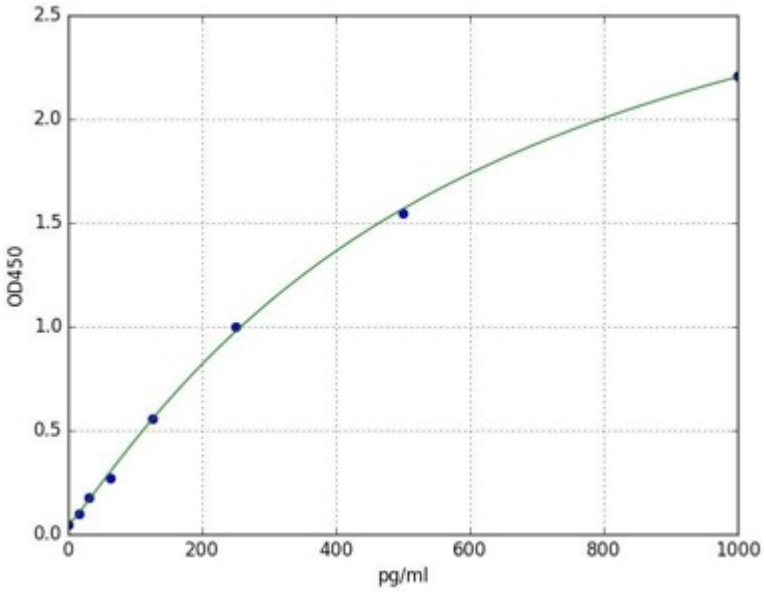
Before adding to wells, equilibrate the SABC working solution and TMB substrate for at least 30 min at room temperature (37 °C). When diluting samples and reagents, they must be mixed completely and evenly. It is

recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Aliquot 0.1ml of1000pg/ml,500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 15.6pg/ml, standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard dilution buffer into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (Mouse serum, plasma, tissue homogenates and other biological fluids.) into test sample wells.
5. Seal the plate with a cover and incubate at 37 °C for 90 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. Do NOT let the wells completely dry at any time. Do Not Wash Plate!
7. Add 0.1 ml of Biotin-detection antibody working solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover, and wash plate 3 times with Wash buffer.
10. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°Cfor 30 min.
11. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.
12. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°Cin dark within 15-30 min. (Note: This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3 -4 wells (with most concentrated Fgf23 standard solutions), the other wells show no obvious color.
13. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
14. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). TheFgf23 concentration of the samples can be interpolated from the standard curve.

Typical Standard Curve



Precision

Intra-Assay: CV<8% Inter-Assay: CV<10%

Detection Range

15.6-1000pg/ml

Sensitivity

<9.375pg/ml

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.