FGF23 (C-TERMINAL) ELISA ASSAY KIT

Multi-matrix ELISA for the quantitative determination of human FGF23 (C-terminal) in serum, EDTA plasma, heparin plasma, and citrate plasma
Cat. No. BI-20702 . 12 x 8 tests

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Not for Sale in the United States

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Detailed information on the FGF23 (C-terminal) ELISA, e.g. assay performance characteristics, sample matrix comparisons, and stability data is available on our website.

www.bmgrp.com

distributed by:

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1) INTRODUCTION

FGF23 (fibroblast growth factor 23) is a 32 kDa protein with 251 amino acids that is proteolytically processed between arginine\(^{179}\) and serine\(^{180}\) to generate N-terminal and C-terminal fragments. FGF23 is mainly secreted by osteocytes and controls phosphate and 1,25(OH)\(_2\) vitamin D homeostasis.

2) CONTENTS OF THE KIT

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>KIT COMPONENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLATE</td>
<td>Anti FGF23 pre-coated microtiter strips in a strip holder, packed in an aluminium bag with desiccant</td>
<td>12 x 8 tests</td>
</tr>
<tr>
<td>WASHBUF</td>
<td>Wash buffer concentrate 20x, natural cap</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>ASYBUF</td>
<td>Assay buffer, red cap, ready to use</td>
<td>1 x 20 ml</td>
</tr>
<tr>
<td>AB</td>
<td>Goat polyclonal anti FGF23 antibody, biotin labelled, green cap, green dye, ready to use</td>
<td>1 x 6 ml</td>
</tr>
<tr>
<td>STD</td>
<td>Standards 1-7 (0; 0.2; 0.6; 1.8; 5; 10; 20 pmol/l), recombinant human FGF23 in human serum, white caps, lyophilized</td>
<td>7 vials</td>
</tr>
<tr>
<td>CTRL</td>
<td>Controls A + B, yellow cap, lyophilized (exact concentrations after reconstitution see labels)</td>
<td>2 vials</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate, (streptavidin-HRPO), amber bottle, amber cap, ready to use</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>SUB</td>
<td>Substrate (TMB solution), amber bottle, blue cap, ready to use</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>STOP</td>
<td>Stop solution, white cap, ready to use</td>
<td>1 x 7 ml</td>
</tr>
</tbody>
</table>

3) ADDITIONAL MATERIAL IN THE KIT

- 2 self-adhesive plastic films
- Quality control protocol
- Protocol sheet
- Instruction for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 10 µl, 50 µl, 100 µl, 300 µl, 400 µl, and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Graph paper or software for calculation of results

5) REAGENTS AND SAMPLE PREPARATION

All reagents as supplied in the FGF23 ELISA Assay Kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent.

**Sample preparation/dilution:**

Collect venous blood samples by using standardized blood collection tubes for serum or plasma. We recommend performing plasma or serum separation by centrifugation immediately, e.g. 20 min at 2000 x g at 4°C (2-8°C). The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot and store at -25°C or lower. Samples are stable for 4 freeze-thaw cycles. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values.

If samples read higher than STD7, we recommend to dilute serum samples 1:11 (1+10, e.g. 10 µl sample + 100 µl ASYBUF) and plasma samples 1:41 (1+40, e.g. 10 µl + 400 µl ASYBUF) and to test again.

The FGF23 ELISA Assay Kit incorporates sufficient assay buffer (ASYBUF) for a 1:41 dilution of 40 samples. Additional ASYBUF can be ordered free of charge on request.
For further information on sample stability please visit our website www.bmgrp.com (see Technical File) or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.

**Reconstitution/Handling:**

**WASHBUF (Wash buffer):** Dilute the concentrate 1:20, e.g. 50 ml WASHBUF + 950 ml distilled water. Crystals in the buffer concentrate will dissolve at room temperature. The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF when performing the assay.

**STD (Standards) + CTRL (Controls):** Pipette 400 µl of distilled or deionised water into each vial. Leave at room temperature (18-24°C) for 15 min. Vortex gently. The exact concentration is printed on the label. Reconstituted STDs and CTRLs are stable for 4 h at room temperature (18-24°C) or at -25°C or lower until expiry date stated on the label. STDs and CTRLs are stable for 3 freeze-thaw cycles.

6) **PRINCIPLE OF THE ASSAY**

7) **ASSAY PROTOCOL**

All reagents and samples must be at room temperature (18-24°C) before use in the assay.

Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.

Take microtitre strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

1) Pipette 50 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well.

2) Add 50 µl AB (biotinylated anti FGF23 antibody, green cap, green dye) into each well.

3) **Cover tightly and incubate over night (20-24 h) at room temperature (18-24°C).**

4) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). Remove remaining WASHBUF by strongly tapping plate against paper towel after the last wash.

5) Add 100 µl CONJ (Conjugate, amber cap) into each well.

6) **Cover tightly and incubate for 1 hour at room temperature (18-24°C) in the dark.**

7) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). Remove remaining WASHBUF by strongly tapping plate against paper towel after the last wash.

8) Add 100 µl SUB (Substrate, blue cap) into each well.

9) **Incubate for 30 minutes at room temperature (18-24°C) in the dark.**

10) Add 50 µl STOP (Stop solution, white cap) into each well.

11) Measure absorbance immediately at 450 nm with reference 630 nm, if available.
CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with logit-log and 4PL algorithm curve fitting. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered when calculating the final concentration of the sample.

Example typical STD-curve:

The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot. Data for OD obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.50 or more is obtained for the STD with the highest concentration and the values of the CTRLs are in range (target ranges see labels).

ASSAY CHARACTERISTICS

<table>
<thead>
<tr>
<th>Method:</th>
<th>Sandwich ELISA, HRP/TMB, 12x8-well strips</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample type:</td>
<td>Serum, EDTA plasma, heparin plasma, and citrate plasma</td>
</tr>
<tr>
<td>Standard range:</td>
<td>0 to 20 pmol/l (7 standards and 2 controls in a human serum matrix)</td>
</tr>
<tr>
<td>Conversion factor:</td>
<td>FGF23, C-terminal: 1 pg/ml = 0.133 pmol/l (MW: 7.52 kDa)</td>
</tr>
<tr>
<td>Sample volume:</td>
<td>50 µl / well</td>
</tr>
<tr>
<td>Incubation time:</td>
<td>20-24 h / 1 h / 30 min</td>
</tr>
<tr>
<td>Sensitivity:</td>
<td>LOD: (0 pmol/l + 3 SD): 0.08 pmol/l; LLOQ: 0.1 pmol/l</td>
</tr>
<tr>
<td>Specificity:</td>
<td>This assay recognizes endogenous and recombinant human FGF23. The assay measures both intact and C-terminal fragments of FGF23.</td>
</tr>
<tr>
<td>Precision:</td>
<td>Intra-assay (n=6) ≤ 12%, Inter-assay (n=10) ≤ 10%</td>
</tr>
<tr>
<td>Spike/Recovery (average recovery spiked with 5 pmol/l rec. FGF23):</td>
<td>Serum (n=13) = 96% Heparin plasma (n=8) = 101%</td>
</tr>
<tr>
<td></td>
<td>EDTA plasma (n=7) = 97% Citrate plasma (n=7) = 100%</td>
</tr>
<tr>
<td>Dilution linearity (average recovery of expected FGF23 after a 1+1; 1+4; 1+7</td>
<td>Dilution: 1+1 1+4 1+7</td>
</tr>
<tr>
<td></td>
<td>Serum (n=9) 105% 100% 108%</td>
</tr>
<tr>
<td></td>
<td>EDTA plasma (n=4) 103% 103% 106%</td>
</tr>
<tr>
<td></td>
<td>Heparin plasma (n=10) 107% 106% 104%</td>
</tr>
<tr>
<td></td>
<td>Citrate plasma (n=4) 119% n.a. n.a.</td>
</tr>
</tbody>
</table>
Values from apparently healthy individuals:

- Median serum (n=35) = 0.8 pmol/l
- Median EDTA plasma (n=22) = 1.3 pmol/l
- Median heparin plasma (n=22) = 1.2 pmol/l
- Median citrate plasma (n=30) = 1.4 pmol/l

Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.

For further information on assay characteristics please visit our website **www.bmgrp.com** (s. Technical File) or contact our customer service by e-mail **export@bmgrp.com** or by phone +43/ 1/ 29107-45.

**10) PRECISION**

Intra-assay: 2 samples of known concentration were tested 6 times within 1 kit lot by 1 operator.

Inter-assay: 2 samples of known concentration were tested 10 times within 2 different kit lots by 4 different operators.

<table>
<thead>
<tr>
<th>Intra-assay (n=6)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Inter-assay (n=10)</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (pmol/l)</td>
<td>0.6</td>
<td>10.0</td>
<td>Mean (pmol/l)</td>
<td>0.6</td>
<td>9.9</td>
</tr>
<tr>
<td>SD (pmol/l)</td>
<td>0.07</td>
<td>0.6</td>
<td>SD (pmol/l)</td>
<td>0.06</td>
<td>0.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>12</td>
<td>6</td>
<td>CV (%)</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Detailed information on the FGF23 (C-terminal) ELISA, e.g. assay performance characteristics, matrix comparisons, and stability data is available on our website **www.bmgrp.com**.

**11) TECHNICAL HINTS**

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
12) PRECAUTIONS

All test components of human source were tested against HIV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed as if they were infectious. All liquid reagents contain ≤ 0.1% Proclin 300 as preservative. Avoid contact with skin and mucous membrane. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Wear gloves, glasses and lab coat while performing this assay.
- Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. Flush with water if contact occurs!!

13) LITERATURE

**BI-20702 FGF23 (C-TERMINAL) ASSAY PROTOCOL AND CHECKLIST**

**PREPARATION OF REAGENTS:**
- Bring all reagents to room temperature (18-24°C).
- Prepare reagents and samples as instructed.
- Bring unused and prepared components to the storage temperature mentioned in the package insert.
- Take microtiter strips out of the aluminium bag and mark positions on the protocol sheet.

**TEST PROCEDURE:**
- **Step 1)** Pipette 50 µl STD/SAMPLE/CTRL (standard/sample/control) into all wells.
- **Step 2)** Add 50 µl AB (biotinylated anti FGF23, biotin labelled, green cap) into all wells.
- **Step 3)** Cover tightly and incubate over night (20-24 h) at room temperature (18-24°C).
- **Step 4)** Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- **Step 5)** Add 100 µl CONJ (Conjugate, amber cap) into each well.
- **Step 6)** Cover tightly and incubate for 1 hour at room temperature (18-24°C), in the dark.
- **Step 7)** Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- **Step 8)** Add 100 µl SUB (Substrate, blue cap) into each well.
- **Step 9)** Incubate for 30 minutes at room temperature (18-24°C), in the dark.
- **Step 10)** Add 50 µl STOP (Stop solution, white cap) into each well.
- **Step 11)** Read Optical Density at 450 nm with reference 630 nm, if available.
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