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Intact FGF23 ELISA - an accurate tool for the detection of iFGF23 in patients with kidney disease.

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SUMMARY AND CONCLUSION

Features of the intact FGF23 sandwich ELISA.

- **One-step ELISA**
- **High precision**
- **Characterized antibodies**
- **Wide assay range**
- **Correlates well** with other assays
- **Cost-effective tool**
- **For plasma and serum**
- **Good sample stability**

We could show that the intact FGF23 ELISA (Biomedica, BI-20700) is a reliable and sensitive tool to specifically measure intact FGF23 in serum and plasma samples.

INTRODUCTION

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone, suppressing renal phosphate reabsorption and vitamin D synthesis, and stimulating calcium re-absorption in distal tubules of the kidney.

The bioactive intact FGF23 contains 251 amino acids and is glycosylated as well as partly phosphorylated. Its activity is mediated by binding to the FGFR/Klotho receptor complex at the target cell surface. Intact FGF23 is cleaved between Arg179 and Ser180 to an N-terminal and a C-terminal fragment.

Secretion of FGF23 is upregulated in patients with chronic kidney disease (CKD) during the course of the disease and the ratio between C-terminal and intact FGF23 is shifted towards the intact protein.

METHODS

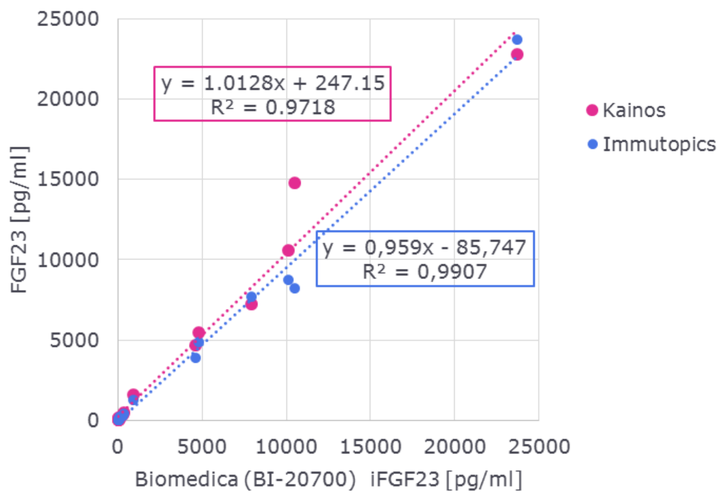
Here, we show the development, characterization and validation of a new intact FGF23 ELISA (Cat. No. BI-20700 Biomedica, Vienna, Austria).

The assay was validated for human plasma samples according to international quality guidelines regarding its specificity, precision, accuracy, robustness, and linearity.

Assay performance as well as sample measurements of apparently healthy and diseased human subjects were compared with other commercially available assays.

COMPARISON OF INTACT FGF23 ELISA ASSAYS

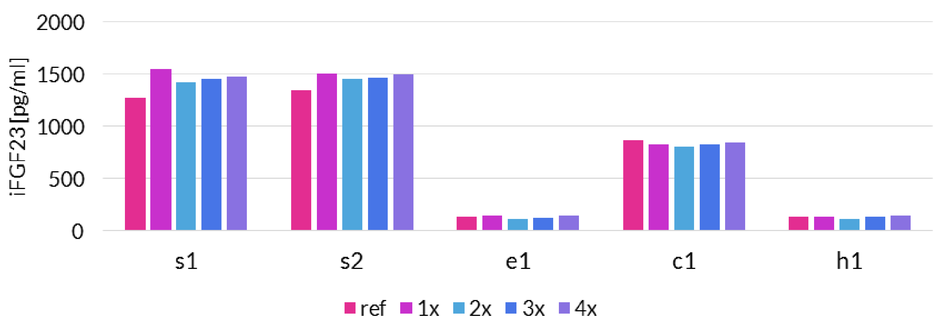
Correlation between plasma samples measured with different intact FGF23 assays



Correlation of plasma samples (9 apparently healthy, 11 clinical and 11 chronic kidney diseased subjects) measured in Biomedica (BI-20700, x-axis), Kainos (CY4000, y-axis pink) and Immutopics (60-6600, y-axis blue) intact FGF23 ELISA. Correlation coefficient is for both comparisons at least R^2>0.97.

STABILITY OF INTACT FGF23

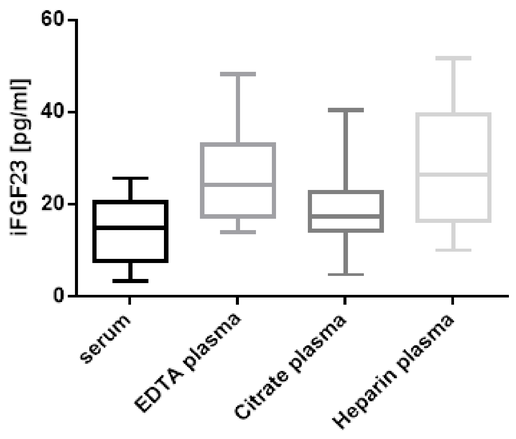
Freeze-thaw stability of human samples



Freeze-thaw stability (F/T) (x-axis) of five human samples containing endogenous intact FGF23 (serum (s), EDTA (e), citrate (c) and heparin (h) plasma). Samples are stable for at least four freeze-thaw cycles.

SAMPLE VALUES

Intact FGF23 values in apparently healthy individuals

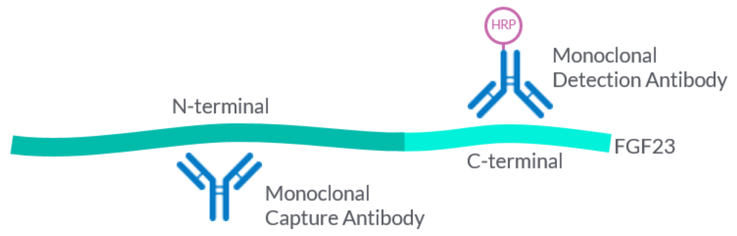


Intact FGF23 values in apparently healthy individuals (n=22). Each individual donated blood for all tested matrices.

Median values:
Serum: 14.1 pg/ml
EDTA plasma: 26.1 pg/ml
Citrate values: 19.7 pg/ml
Heparin plasma: 17.4 pg/ml

INTACT FGF23 ELISA CHARACTERISTICS

Principle of the detection of intact FGF23



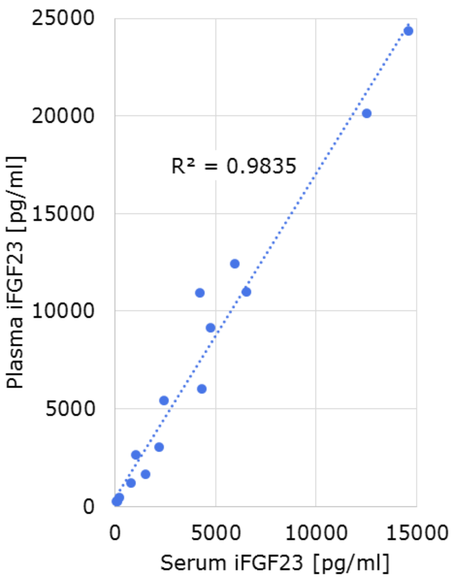
The intact FGF23 ELISA is a sandwich-based immunoassay with a recombinant monoclonal anti-human FGF23 capture antibody (pink) recognizing a structural epitope at the N-terminal part of FGF23. Detection is mediated by a HRP-labeled monoclonal anti-human FGF23 antibody directed to the C-terminal part of FGF23. Therefore, specific measurement of intact FGF23 is ensured.

One-step assay protocol

50 µl sample / standard / control + 50 µl detection antibody
3h @ 18-26°C
5x washing
100 µl substrate
30 min @ 18-26°C (dark)
50 µl stop solution
read OD (450 nm)

Samples, standards (0 – 1600 pg/ml) and controls are pipetted into the antibody coated plate and incubated together with the labelled detection antibody for 3 hours. After washing TMB substrate is added for 30 minutes, followed by stopping the reaction and OD measurement at 450 nm.

Correlation between plasma and serum



Correlation between serum (x-axis) and plasma (y-axis) samples from 16 different donors with kidney disease (R^2= 0.98). Intact FGF23 serum values are lower in serum than in plasma samples.

VALIDATION DATA (EDTA PLASMA)

Sensitivity:	5.4 pg/ml
Within-run precision:	≤ 6% CV
In-between-run precision:	≤ 8% CV
Accuracy:	+160 pg/ml: 94% + 800 pg/ml: 100%
Parallelism:	1+1: 107% 1+3: 108% 1+7: 111%
Dilution linearity:	1+1: 119% 1+3: 111% 1+7: 110%

LITERATURE

Vervloet M, (2019): Nature Rev 15: 109-119. Bachetta J, Bardet C, Prié D (2019): Metabolism. Epub ahead of print.
Erben RG, Andrukhova O (2017): FGF23-Klotho signaling axis in the kidney. Bone 100: 62-68.

CONTACT

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