SP316

DETECTION OF INTACT FGF23 USING A NOVEL WELL-CHARACTERIZED ELISA

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SUMMARY AND CONCLUSION

Special features of the intact FGF23 sandwich ELISA.

- **One-step ELISA**
- **High precision**
- **Characterized antibodies**
- **Wide assay range**
- **Good correlation** 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 and also 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.

Increased serum concentrations of intact FGF23 are a hallmark of renal phosphate-wasting diseases such as autosomal domininant hypophosphatemic rickets (ADHR), X-linked hypophosphatemia (XLH), tumor-induced osteomalacia, and autosomal recessive hypo-phosphatemic rickets.

METHODS

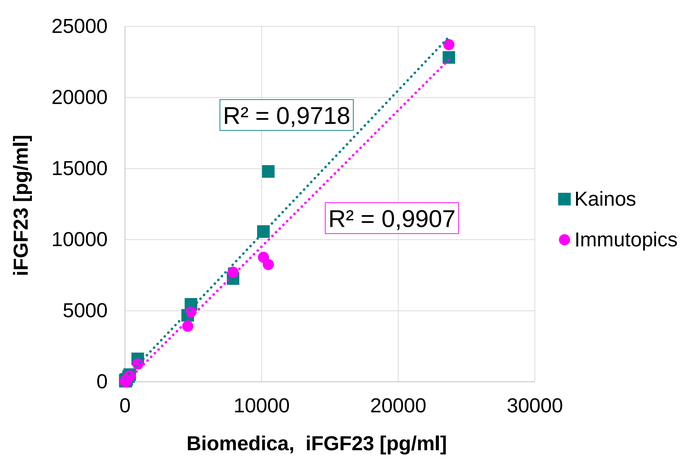
Here, we show the development, characterization and validation of a new intact FGF23 ELISA (cat. no. BI-20700 Biomedica, Vienna, Austria).

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

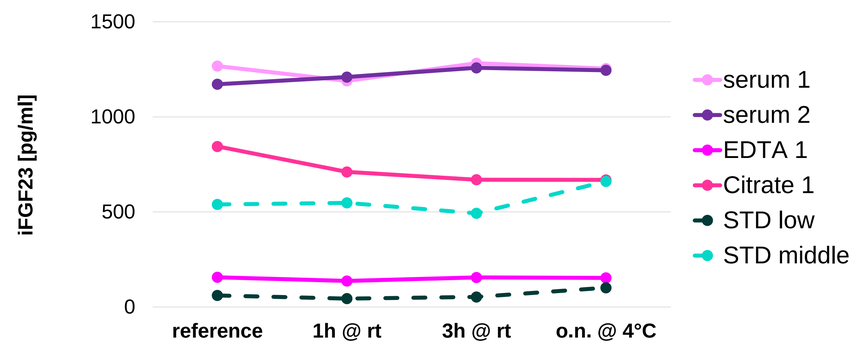
Correlation between plasma samples measured with different intact FGF23 assays



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 green) and Immutopics (60-6600, y-axis pink) intact FGF23 ELISA. The assays show a very good correlation with at least R^2>0.97.

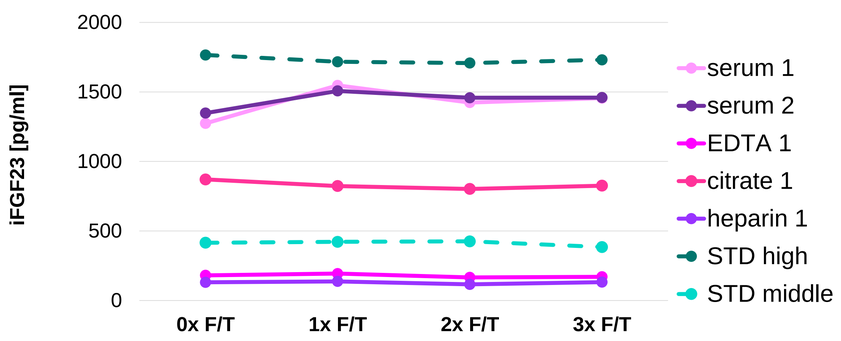
STABILITY OF INTACT FGF23

Benchtop stability of human samples



Benchtop stability of human samples (serum, EDTA and citrate plasma, purple lines) containing endogenous intact FGF23 and two standards (STD; recombinant intact FGF23 in human plasma matrix, green dashed lines). Samples were stored for 1 or 3 hours at room temperature (rt) or overnight (o.n.) at 4° C and compared with the reference resulting in less than 20% measurement alterations.

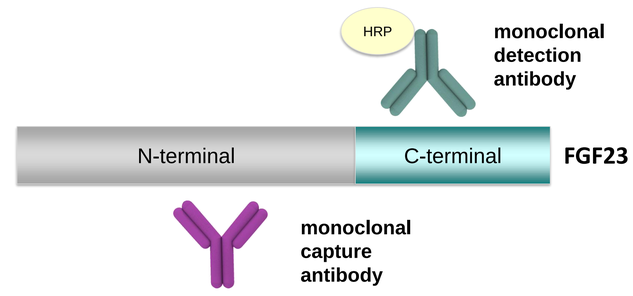
Freeze-thaw stability of human samples



Freeze-thaw stability (F/T) (x-axis) of five human samples containing endogenous intact FGF23 (serum, EDTA, citrate and heparin plasma, purple lines) and two standards (STD; recombinant intact FGF23 in human plasma matrix, green dashed lines). Samples are stable for at least 3x freeze-thaw cycles.

INTACT FGF23 ELISA CHARACTERISTICS

Principle of the detection of intact FGF23



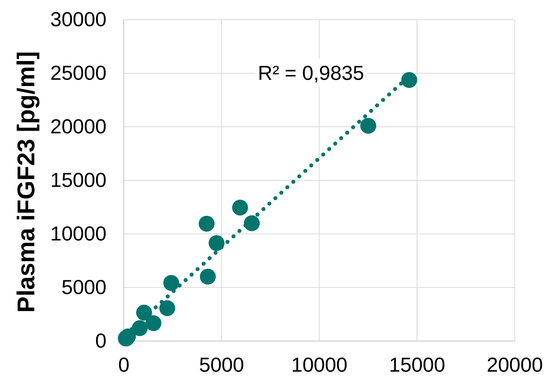
The intact FGF23 ELISA is a sandwich-based immunoassay with a recombinant monoclonal anti-human FGF23 capture antibody (pink) recognizing a structural epitope at the N-terminal part of FGF23. Detection is mediated by a HRP-labeled monoclonal anti-human FGF23 antibody directed to the C-terminal part of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

Good 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 OF INTACT FGF23 ELISA

Sensitivity:	5.4 pg/ml
Intra-assay precision:	≤ 5%
Inter-assay precision:	≤ 8%
Accuracy:	93% (79% - 103%)
Dilution linearity:	111% (87% - 147%)
Cross-reactivity:	No cross-reactivity with rh FGF3, rh FGF19 and rh FGF21 and with mouse FGF23

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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