



Rat FGF-23 C-TERMINAL ELISA Kit

Catalog number: FM-E101102

For the quantitation of **Rat FGF-23 C-TERMINAL** concentrations in cell culture supernates, serum and plasma (heparin, EDTA).

This package insert should be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

Rat FGF-23 C-TERMINAL ELISA Kit

Background

Fibroblast growth factor (FGF)23 inhibits renal phosphate reabsorption by activating FGF receptor (FGFR) 1c in a Klotho-dependent fashion. The phosphaturic activity of FGF23 is abrogated by proteolytic cleavage at the RXXR motif that lies at the boundary between the FGF core homology domain and the 72-residue-long C-terminal tail of FGF23. The soluble ectodomains of FGFR1c and Klotho are sufficient to form a ternary complex with FGF23 in vitro. The C-terminal tail of FGF23 mediates binding of FGF23 to a de novo site generated at the composite FGFR1c-Klotho interface. Consistent with this finding, the isolated 72-residue-long C-terminal tail of FGF23 impairs FGF23 signaling by competing with full-length ligand for binding to the binary FGFR-Klotho complex. Injection of the FGF23 C-terminal tail peptide into healthy rats inhibits renal phosphate excretion and induces hyperphosphatemia. In a mouse model of renal phosphate wasting attributable to high FGF23, the FGF23 C-terminal peptide reduces phosphate excretion, leading to an increase in serum phosphate concentration. Our data indicate that proteolytic cleavage at the RXXR motif abrogates FGF23 activity by a dual mechanism: by removing the binding site for the binary FGFR-Klotho complex that resides in the C-terminal region of FGF23, and by generating an endogenous inhibitor of FGF23.

Assay Principle

Rat FGF-23 C-TERMINAL ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Rat FGF-23 C-TERMINAL with a 96-well strip plate that is pre-coated with antibody specific for FGF-23 C-TERMINAL. The detection antibody is a biotinylated antibody specific for FGF-23 C-TERMINAL. The capture antibody is a monoclonal antibody from mouse, the detection antibody is a biotinylated polyclonal antibody.

To measure Rat FGF-23 C-TERMINAL, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Rat FGF-23 C-TERMINAL in the sample. Read the density of the yellow product in each well using a plate reader and benchmark the sample wells' readings against the standard curve to determine the concentration of Rat FGF-23 C-TERMINAL in the sample. For more information on assay principle, protocols, and troubleshooting tips, contact us at techsupport@novateinbio.com or visit our website at <http://www.novateinbio.com/content/56-elisa-troubleshooting>.

Overview

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Product Name	Rat FGF-23 C-TERMINAL ELISA Kit
Reactive Species	Rat
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of rat FGF23C-terminal. 96wells/kit, with removable strips.
Sensitivity	<10pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	31.2pg/ml-2000pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Uniprot ID	Q8VI82

Notice Before Application

Please read the following instructions before starting the experiment.

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. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Materials Provided

Description	Quantity	Volume
Anti-Rat FGF-23 C-TERMINAL Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Rat FGF-23 C-TERMINAL Standard	2	10ng/tube
Rat FGF-23 C-TERMINAL Biotinylated antibody (100x)	1	125 μl
Avidin-Peroxidase Complex (100x)	1	125 μl
Sample Diluent	1	30ml
Antibody Diluent	1	12ml
Avidin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Plate Sealers	4	Piece

Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.

Multichannel pipettes are recommended for large amount of samples.

Deionized or distilled water.

500ml graduated cylinders.

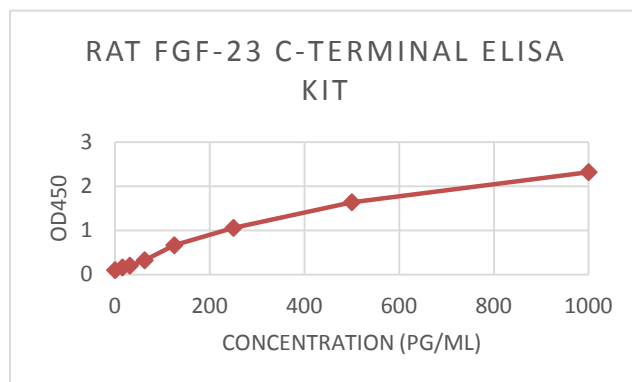
Test tubes for dilution.

Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration 0 (pg/ml)	15.6	31.2	62.5	125	250	500	1000
O.D.	0.104	0.168	0.213	0.335	0.673	1.108	2.412

Rat FGF-23 C-TERMINAL ELISA Kit standard curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability

NovateinBio spends great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

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	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	16	16	16
Mean (pg/ml)	65	235	876	69	298	957
Standard deviation	2.61	13.32	29.48	2.7	15.5	67.3
1CV(%)	4.01%	5.6%	3.4%	3.9%	5.2%	7.0%

Reagent Preparation

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (20-25min) is based on 37°C.
Wash buffer	Dissolve the included powder in 1000ml of deionized water. Excess wash buffer can be stored for up to one week at 4°C.
Biotinylated Anti-Rat FGF-23 C-TERMINAL antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Rat FGF-23 C-TERMINAL Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Rat FGF-23 C-TERMINAL Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Rat FGF-23 C-TERMINAL standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent.

	Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Standard Dilution

1. Number tubes 1-8. Final Concentrations to be Tube # 1 – 2000pg/ml, #2 – 1000pg/ml, #3 – 500pg/ml, #4 – 250pg/ml, #5 – 125pg/ml, #6 – 62.5pg/ml, #7 – 31.25pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 200µl of the reconstituted standard stock solution of 10ng/ml and 800µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay Protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Rat FGF-23 C-TERMINAL antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.