



FGF23 ELISA Kit (Rat)

(OKCD01114)

Lot# KG0043

Instructions for Use

For the quantitative measurement of Rat FGF23 in cell culture supernatants, serum and plasma (heparin, EDTA).

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva's rat FGF23 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for FGF23 has been precoated onto 96-well plates. Standards (Expression system for standard: E.coli, Immunogen sequence: Y25-V251) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for FGF23 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was 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 rat FGF23 amount of sample captured in plate.

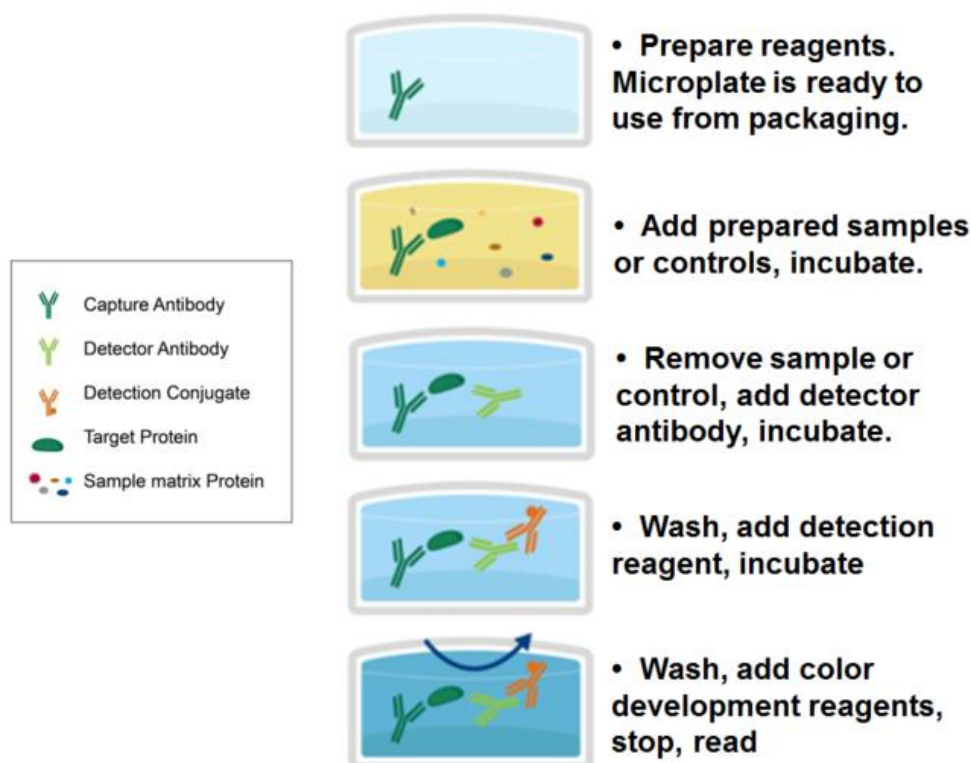
Target Background

Fibroblast growth factor 23 or FGF23 is a protein that in humans is encoded by the FGF23 gene. It is a member of the fibroblast growth factor (FGF) family which is responsible for phosphate metabolism. The main function of FGF23 seems to be regulation of phosphate concentration in plasma. FGF23 is secreted by Osteocytes in response to elevated Calcitriol. And it acts on the kidneys, where it decreases the expression of NPT2, a sodium-phosphate cotransporter in the proximal tubule. Thus, FGF23 decreases the reabsorption and increases excretion of phosphate. Also, FGF23 may suppress 1-alpha-hydroxylase, reducing its ability to activate vitamin D and subsequently impairing calcium absorption.

General Specifications

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Range	15.6pg/ml-1000pg/ml
LOD	<10 pg/ml (Derived by linear regression of OD ₄₅₀ of the Mean Blank + 2xSD)
Specificity	Rat FGF23 UniProt: Q8VI82 Gene ID: 170583
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

- Upon receipt store kit at -20°C until expiration date. Avoid multiple freeze/thaw cycles.

4. Kit Components

- The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
96-Well plate Pre-coated with Anti-Rat FGF23 Antibody	96 Wells (12 x 8 Well Strips)	Store at -20°C until expiration date
Lyophilized Recombinant Rat FGF23 Standard	2 x 10 ng	
100X Biotinylated Anti-Rat FGF23 Antibody	1 x 100 µL	
100X Avidin-Biotin-Peroxidase Complex (ABC)	1 x 100 µL	
Sample Diluent Buffer	1 x 30 mL	
Antibody Diluent Buffer	1 x 12 mL	
ABC Diluent Buffer	1 x 12 mL	
TMB Color Developing Agent	1 x 10 mL	
TMB Stop Solution	1 x 10 mL	
10X Wash Buffer	1 x 30 mL	

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 μ L through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.
- Prior to using the kit, briefly spin component tubes to collect all reagent at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results in inter- intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1 μ L is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates or fibrin strands or which are hemolytic or lipemic might cause inaccurate results due to interfering factors.

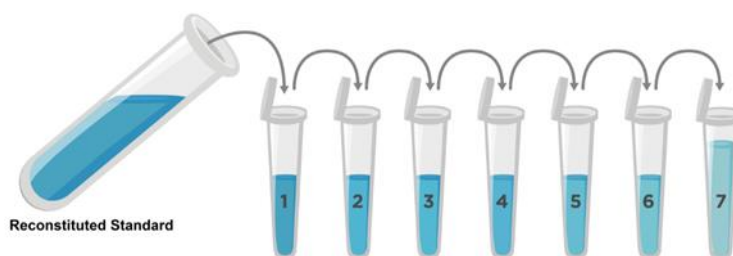
8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and use immediately.

8.1. FGF23 Assay Standards

- 8.1.1. Prepare the FGF23 Standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.
- 8.1.2. Reconstitute one of the provided 10 ng **Lyophilized FGF23 Standard**. Use one for each experiment. Prepare a stock 10,000 pg/mL **Standard** by reconstituting one tube of **Lyophilized FGF23 Standard** as follows:
 - 8.1.2.1. Gently spin or tap the vial to collect all material at the bottom.
 - 8.1.2.2. Add 1 mL of **Sample Diluent Buffer** to the vial.
 - 8.1.2.3. Seal then mix gently and thoroughly.
 - 8.1.2.4. Leave the vial to sit at ambient temperature for 10 minutes.
- 8.1.3. Prepare a set of seven serially diluted Standards as follows:
 - 8.1.3.1. Label tubes with numbers 1 – 8.
 - 8.1.3.2. Add 300 µL of **Sample Diluent Buffer** to Tube #'s 1 – 7.
 - 8.1.3.3. Prepare a **1,000 pg/mL Standard #1** in by adding 100 µL of the **10,000 pg/mL** reconstituted **FGF23 Standard** to 900 µL of **Sample Diluent Buffer** in Tube#1. Mix gently and thoroughly.
 - 8.1.3.4. Prepare **Standard #2** by adding 300 µL of **Standard#1** from Tube #1 to Tube #2. Mix gently and thoroughly.
 - 8.1.3.5. Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.
 - 8.1.3.6. Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.
 - 8.1.3.7. Tube #8 is a blank Standard (only **Sample Diluent Buffer**), which should be included with every experiment.

Standard Number (Tube)	Sample To Dilute	Volume Standard (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
Tube #1	10,000 pg Lyophilized FGF23 Standard	100	900	1,000	1,000 pg/mL
Tube #2	1,000 pg/mL	300	300	600	500 pg/mL
Tube #3	500 pg/mL	300	300	600	250 pg/mL
Tube #4	250 pg/mL	300	300	600	125 pg/mL
Tube #5	125 pg/mL	300	300	600	62.5 pg/mL
Tube #6	62.5 pg/mL	300	300	600	31.2 pg/mL
Tube #7	31.2 pg/mL	300	300	600	15.6 pg/mL
Tube #8	NA	0	300	300	0.0 (Blank)



8.2. 1X Biotinylated Anti-Rat FGF23 Antibody

- 8.2.1. Prepare the **1X Biotinylated Rat FGF23 Antibody** immediately prior to use by diluting the **100X Biotinylated Rat FGF23 Antibody** 1:100 with **Antibody Diluent Buffer**.
- 8.2.2. For each well strip to be used in the experiment prepare 100 μ L by adding 1 μ L of **100X Biotinylated Rat FGF23 Antibody** to 99 μ L **Antibody Diluent Buffer**.
- 8.2.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3. 1X Avidin-Biotin-Peroxidase Complex (ABC)

- 8.3.1. Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC)** 1:100 with **ABC Dilution Buffer**.
- 8.3.2. For each well to be used in the experiment prepare 100 μ L, by adding 1 μ L of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 99 μ L **ABC Dilution Buffer**.
- 8.3.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4. 1X Wash Buffer

- 8.4.1. Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.
- 8.4.2. Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.
- 8.4.3. Seal and mix gently by inversion. Avoid foaming or bubbles.
- 8.4.4. Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5. Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation Guidelines

9.1. Sample Preparation and Storage

- Samples must be tested to determine if the kit is valid.
- Store samples to be assayed at 4°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

General Sample Preparation Guidelines:

- **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 EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Cell culture supernatants** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

9.2. Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

- Prior to performing the full experiment, test a serially diluted representative sample.
 - Alternately, a small pool of several samples can also be used with this same method if sample volume is limited.
- Or
- Refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit
- Dilute samples using **Sample Diluent Buffer**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

- The ABC Working Solution, TMB Color Developing Agent and TMB Stop Solution must be kept warm at 37°C for 30 minutes prior to use. Equilibrate all other reagents and materials to ambient room temperature prior to use in the procedure.
- To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

- 10.1.** Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.
- 10.2.** Cover the plate with the plate sealer and incubate at 37°C for 90 minutes.
- 10.3.** Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
- 10.4.** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.5.** Add 100 µL of prepared **1X Biotinylated Anti-Rat FGF23 Antibody to each well.**
- 10.6.** Cover with the plate sealer and incubate at 37°C for 60 minutes.
- 10.7.** Wash plate 3 times with **1X Wash Buffer** as follows:
 - 10.7.1. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
 - 10.7.2. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
 - 10.7.3. Add 300 µL of **1X Wash Buffer** to each assay well.
 - 10.7.4. Incubate for 1 minute.
 - 10.7.5. Repeat steps 10.7.1 through 10.7.4 **two** more times.
- 10.8.** Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC)** into each well, cover with plate sealer and incubate at 37°C for 30 minutes.
- 10.9.** Wash plate 5 times with **1X Wash Buffer** as follows:
 - 10.9.1. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
 - 10.9.2. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
 - 10.9.3. Add 300 µL of 1X Wash Buffer to each assay well.
 - 10.9.4. Incubate for 1 minute.
 - 10.9.5. Repeat steps 10.9.1 through 10.9.4 **four** more times.
- 10.10.** Add 90 µL of **TMB Color Developing Agent** to each well, cover with plate sealer and incubate at 37°C in the dark for 15-25 minutes.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)
- 10.11.** Add 100 µL of **TMB Stop Solution** to each well. Well color should change to yellow immediately.
- 10.12.** Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD₄₅₀** for each test or standard well as follows:

$$(\text{Relative OD}_{450}) = (\text{Well OD}_{450}) - (\text{Mean Blank Well OD}_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD₄₅₀** of each standard serial dilution point vs. the respective standard concentration. The **FGF23** concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD₄₅₀** against the standard curve. This is best achieved using curve fitting software. A standard curve should be generated each time the test is performed.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

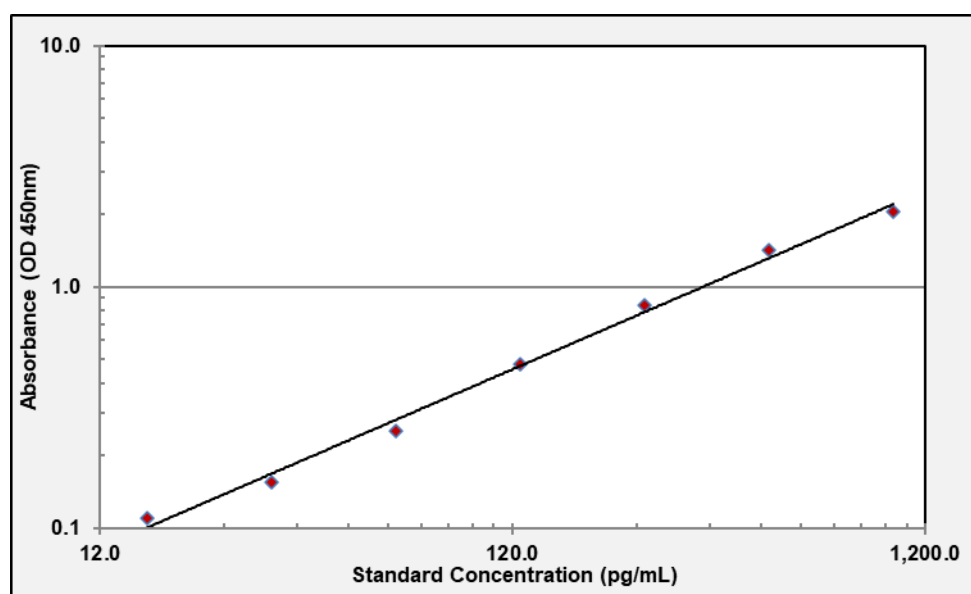
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1. Typical absorbance values. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD₄₅₀.

Standard Number	8	7	6	5	4	3	2	1
Concentration (pg/ml)	0	15.6	31.2	62.5	125	250	500	1,000
OD ₄₅₀	0.036	0.110	0.155	0.254	0.479	0.841	1.418	2.047

12.2. Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3. Reproducibility

	Intra-Assay			Inter-Assay		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	55	244	925	59	248	978
Standard Deviation	2.81	13.18	39.88	2.9	16.6	77.3
CV(%)	5.1	5.4	4.3	4.9	6.7	7.9

13. Technical Resources

13.1 Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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