Datasheet for ABIN612700

# FGF21 ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | FGF21 |
| Reactivity: | Human |
| Method Type: | Sandwich ELISA |
| Minimum Detection Limit: | 0.03 ng/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | The AssayMax Human FGF21 ELISA kit is designed for detection of human FGF21 in plasma, serum, and cell culture supernatants |
| Brand: | AssayMax |
| Sample Type: | Plasma, Cell Culture Supernatant |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Components: | FBG21 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against FGF21. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. FGF21 Standard: Human FGF21 in a buffered protein base (2 ng, lyophilized). Biotinylated FGF21 Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against human FGF21 (80µl). MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). |

## Product Details

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| | Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml). |
| Material not included: | Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water |

## Target Details

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| Target: | FGF21 |
| Abstract: | FGF21 Products |
| Background: | Fibroblast growth factor 21 (FGF21) is a member of endocrine FGF subfamily, along with FGF19 and FGF23. The secreted human FGF21 is expressed in liver, pancreas, and white adipose tissue. It contains 209 amino acids in the precursor and 181 amino acids in the mature protein with a molecular mass of about 20 kDa, and has 75% homology with mouse FGF-21. FGF21 signals through cell-surface tyrosine kinase FGF receptors complexed with a cofactor - Klotho. FGF21 is a novel metabolic regulator involved in glucose metabolism, lipolysis, and ketogenesis and triglyceride clearance, growth hormone signaling, and metabolic diseases. In rodent models of diabetes, it stimulates glucose uptake in adipocytes, protects animals from diet- induced obesity, and lowers blood glucose and triglyceride. Serum FGF21 levels are increased in patients with metabolic diseases including nonalcoholic fatty liver disease, type 2 diabetes, gestational diabetes, obesity, Cushing's syndrome, HIV-1-induced lipodystrophy, and chronic kidney hemodialysis (5-7). Conversely, circulating FGF21 concentrations were reduced in subjects with anorexia nervosa. FGF21 is a biomarker for metabolic diseases and a candidate for the treatment of insulin resistance. |
| Pathways: | RTK Signaling |

## Application Details

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| Sample Volume: | 50 µL |
| Assay Time: | < 5 h |
| Plate: | Pre-coated |
| Protocol: | This assay employs a quantitative sandwich enzyme immunoassay technique that measures FGF21 in less than 5 hours. A polyclonal antibody specific for FGF21 has been pre-coated onto a microplate. FGF21 in standards and samples is sandwiched by the immobilized antibody and |

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| | a biotinylated polyclonal antibody specific for FGF21, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured. |
| Reagent Preparation: | Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. MIx Diluent Concentrate (10x): Dilute MIx Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 2 ng of Human FGF21 Standard with 1 ml of MIx Diluent to generate a stock solution of 2 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the FGF21 standard solution (2 ng/ml) 1:2 with MIx Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625, and 0.0313 ng/ml solutions. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [FGF21] (ng/ml) P1 1 part Standard (2 ng/ml) 2.0000 P2 1 part P1 + 1 part MIx Diluent 1.0000 P3 1 part P2 + 1 part MIx Diluent 0.5000 P4 1 part P3 + 1 part MIx Diluent 0.2500 P5 1 part P4 + 1 part MIx Diluent 0.1250 P6 1 part P5 + 1 part MIx Diluent 0.0625 P7 1 part P6 + 1 part MIx Diluent 0.0313 P8 MIx Diluent 0.0000 Biotinylated FGF21 Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIx Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute Wash Buffer Concentrate 1:20 with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIx Diluent. Any remaining solution should be frozen at -20°C. |
| Sample Collection: | Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:2 with MIx Diluent. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:2 into MIx Diluent. Store serum at -20°C or below. Avoid repeated freeze-thaw cycles Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles 2 |
| Assay Procedure: | Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store |

## Application Details

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| | in a vacuum desiccator. Add 50 µL of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated FGF21 Antibody to each well and incubate for two hours. Wash the microplate as described above. Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. 3 Add 50 µL of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings. |
| Calculation of Results: | Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed. |
| Assay Precision: | Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.4% respectively. |
| Restrictions: | For Research Use only |

## Handling

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| Handling Advice: | Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents. 1 The kit should not be used beyond the expiration date. |

## Handling

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| Storage: | 4 °C/-20 °C |
| Storage Comment: | Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent |

## Publications

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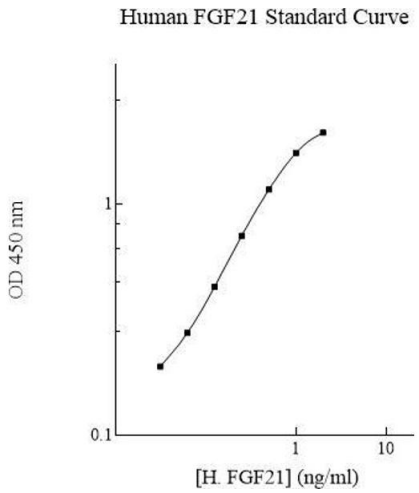
| Product cited in: | Dushay, Chui, Gopalakrishnan, Varela-Rey, Crawley, Fisher, Badman, Martinez-Chantar, Maratos-Flier: "Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease." in: **Gastroenterology**, Vol. 139, Issue 2, pp. 456-63, (2010) (PubMed). |

## Images




**ELISA**

**Image 1.**