Datasheet for ABIN1979131

PNPLA3 ELISA Kit

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Overview

Quantity:	96 tests
Target:	PNPLA3
Reactivity:	Human, Rat, Mouse
Method Type:	Competition ELISA
Detection Range:	0.1-1.000 ng/mL
Minimum Detection Limit:	0.1 ng/mL
Application:	ELISA

Product Details

Purpose:	Human/Mouse/Rat Adiponutrin EIA Kit optimized for serum and cell culture medium. Competition-based ELISA on a 96-well strip plate.
Sample Type:	Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This kit detects both long form (481aa) and short form (477aa) Adiponutrin proteins. No other active isoforms have been reported. Cross Reactivity: This EIA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.
Cross-Reactivity (Details):	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

Product Details

Sensitivity:	21 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Standard Peptide • Assay Diluent(s) • Biotinylated Peptide • HRP-Streptavidin • TMB One-Step Substrate • Stop Solution • Assay Diagram • Positive Control Sample • Capture Antibody • User Manual
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 mL volumes • Adjustable 1-25 mL pipettes for reagent preparation • 100 mL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Orbital shaker • Aluminum foil • Saran Wrap • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • SigmaPlot software (or other software that can perform four-parameter logistic regression models)

Target Details

Target:	PNPLA3
Alternative Name:	Adiponutrin (PNPLA3 Products)
Background:	Adiponutrin (ADPN), Patatin-Like Phospholipase Domane Containing 3 (PNPLA3)
Gene ID:	80339
UniProt:	Q9NST1

Application Details

Application Notes:	Recommended Dilution for serum and plasma samplesHuman: 4X / Mouse: 4X / Rat: 2X
Sample Volume:	100 µL
Assay Time:	5 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed. 2. Add 100 µL detection antibody to each well. 3. Incubate 1.5 h at RT or O/N at 4 °C. 4. Add 100 µL standard or sample to each well. 5. Incubate 2.5 h at RT. 6. Add 100 µL prepared streptavidin solution. 7. Incubate 45 min at RT. 8. Add 100 µL TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL Stop Solution to each well. 11. Read plate at 450 nm immediately.
Reagent Preparation:	1. Keep kit reagents on ice during steps. Equilibrate plate to room temperature before opening the sealed pouch. 2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water. 3. Briefly centrifuge the Anti-Adiponutrin Antibody vial (Item N) before use. Add 50 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently. 4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is your anti-Adiponutrin antibody working solution, which will be used in step 2 of the Assay Procedure. NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure). 5. Briefly centrifuge the vial of Biotinylated Adiponutrin (Item F) before use. Add 5 µL of Item F to 5 mL of the appropriate Assay Diluent. Pipette up and down to mix gently. The final concentration of biotinylated Adiponutrin will be 10 ng/mL. This solution will only be used as the diluent in step 6 of Reagent Preparation. 6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1000 ng/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, 100 pg/mL and 0 pg/mL. Pipette 450 mL of biotinylated Adiponutrin solution into each tube, except for the 1000 ng/mL (leave this one empty). It is very important to make sure the concentration of biotinylated Adiponutrin is 10 ng/mL in all standards. a. Briefly centrifuge the vial of Adiponutrin (Item C). In the tube labeled 1000 ng/mL, pipette 8 µL of Item C and 792 µL of 10 ng/mL biotinylated Adiponutrin solution (prepared in step 5 above). This is your Adiponutrin stock solution (1000 ng/mL Adiponutrin, 10 ng/mL

	biotinylated Adiponutrin). Mix thoroughly. This solution serves as the first standard. b. To make the 100 ng/mL standard, pipette 50 µL of Adiponutrin stock solution the tube labeled 100 ng/mL. Mix thoroughly. c. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 mL of biotinylated Adiponutrin and 50 mL of the prior concentration until 100 pg/mL is reached. Mix each tube thoroughly before the next transfer. d. The final tube (0 pg/mL Adiponutrin, 10 ng/mL biotinylated Adiponutrin) serves as the zero standard (or total binding). 7. Prepare a 10-fold dilution of Item F. To do this, add 2 mL of Item F to 18 mL of the appropriate Assay Diluent. This solution will be used in steps 8 and 10. 8. Positive Control Preparation: briefly centrifuge the positive control vial (Item M). To the tube of Item M, add 101 µL 1x Assay Diluent B. Also add 2 µL of 10-fold diluted Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample that is meant to be a system control (to verify that the detection & kit components are working). It may be diluted further if desired, but be sure the final concentration of biotinylated Adiponutrin is 10 ng/mL. 9. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer. 10. Sample Preparation: Use Assay Diluent A + biotinylated Adiponutrin to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated Adiponutrin as the diluent. It is very important to make sure the final concentration of the biotinylated Adiponutrin is 10 ng/mL in every sample. Example: to make a 4-fold dilution of sample, mix together 2.5 µL of 10-fold diluted Item F (prepared in step 7), 185 mL of appropriate Assay Diluent, and 62.5 µL of your sample, mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate. Do not use Item F diluent from Step 5 for sample preparation. If you plan to use undiluted samples, you must still add biotinylated Adiponutrin to a final concentration of 10 ng/mL. Example: Add 2.5 mL of 10-fold diluted Item F to 247.5 mL of sample. 11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 800- fold with 1X Assay Diluent B. Note: Do not use Assay Diluent A for HRP-Streptavidin preparation in Step 11.
Sample Preparation:	Use Assay Diluent A + biotinylated Adiponutrin to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated Adiponutrin as the diluent. It is very important to make sure the final concentration of the biotinylated Adiponutrin

	is 10 ng/mL in every sample. EXAMPLE: to make a 4-fold dilution of sample, mix together 2.5 µL of 10-fold diluted Item F (prepared in step 7), 185 mL of appropriate Assay Diluent, and 62.5 µL of your sample, mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate. Do not use Item F diluent from Step 5 for sample preparation. If you plan to use undiluted samples, you must still add biotinylated Adiponutrin to a final concentration of 10 ng/mL. EXAMPLE: Add 2.5 mL of 10-fold diluted Item F to 247.5 mL of sample.
Assay Procedure:	1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL anti-Adiponutrin antibody (see Reagent Preparation step 4) to each well. Incubate for 1.5 hours at room 0 temperature with gentle shaking (1-2 cycles/sec). You may also incubate overnight at 4 degrees C. 3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200-300 µL each), Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of each standard (see Reagent Preparation step 6), positive control (see Reagent Preparation step 8) and sample (see Reagent Preparation step 10) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4 °C. 5. Discard the solution and wash 4 times as directed in Step 3. 6. Add 100 µL of prepared HRP-Streptavidin solution (see Reagent Preparation step 11) to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes. 7. Discard the solution and wash 4 times as directed in Step 3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec). 9. Add 50 µL of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately. 1
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance on the y-axis. Draw the best-fit curve through the standard points.

Application Details

Assay Precision:	Intra-Assay: CV < 10 % Inter-Assay: CV < 15 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze/thaw cycles.
Storage:	-20 °C
Storage Comment:	Standard, Biotinylated Adiponutrin peptide, and Positive Control should be stored at -20°C after arrival. Avoid multiple freeze-thaws. The remaining kit components may be stored at 4°C. Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
Expiry Date:	6 months

Publications

Product cited in:	de Andrade, de Castro, Vicente, da Rosa, Nader, Pereira, Fröde: "Evaluation of circulating levels of inflammatory and bone formation markers in axial spondyloarthritis." in: **International immunopharmacology**, Vol. 21, Issue 2, pp. 481-6, (2014) (PubMed). Li, Huang, Tong, Wang, Zhang, Wang, Dai, Li, Lin, Wu: "Comparison of the regulation of ?-catenin signaling by type I, type II and type III interferons in hepatocellular carcinoma cells." in: **PLoS ONE**, Vol. 7, Issue 10, pp. e47040, (2012) (PubMed). Mödder, Roforth, Hoey, McCready, Peterson, Monroe, Oursler, Khosla: "Effects of estrogen on osteoprogenitor cells and cytokines/bone-regulatory factors in postmenopausal women." in: **Bone**, Vol. 49, Issue 2, pp. 202-7, (2011) (PubMed). There are more publications referencing this product on: Product page