Datasheet for ABIN1979230

PTH ELISA Kit

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Overview

Quantity:	96 tests
Target:	PTH
Reactivity:	Rat, Human, Mouse
Method Type:	Competition ELISA
Detection Range:	1-1.000 pg/mL
Minimum Detection Limit:	1 pg/mL
Application:	ELISA

Product Details

Purpose:	Human/Mouse/Rat Parathyroid Hormone 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:	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.
Sensitivity:	1.27 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments

Product Details

	• 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:	PTH
Alternative Name:	PTH (PTH Products)
Target Type:	Hormone
Background:	Parathyroid Hormone (PTH)
Gene ID:	5741
UniProt:	P01270
Pathways:	cAMP Metabolic Process, Regulation of Carbohydrate Metabolic Process

Application Details

Application Notes:	Recommended Dilution for serum and plasma samplesHuman: 2X / Mouse: 2X / 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-PTH 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-PTH 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 PTH (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 PTH will be 20 pg/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 pg/mL, 100 pg/mL, 10 pg/mL, 1 pg/mL, 0.1 pg/mL and 0 pg/mL. Pipette 450 mL of biotinylated PTH solution into each tube, except for the 1000 pg/mL (leave this one empty). It is very important to make sure the concentration of biotinylated PTH is 20 pg/mL in all standards. a. Briefly centrifuge the vial of PTH (Item C). In the tube labeled 1000 pg/mL, pipette 8 µL of Item C and 792 µL of 20 pg/mL biotinylated PTH solution (prepared in step 5 above). This is your PTH stock solution (1000 pg/mL PTH, 20 pg/mL biotinylated PTH). Mix thoroughly. This solution

	serves as the first standard. b. To make the 100 pg/mL standard, pipette 50 µL of PTH stock solution the tube labeled 100 pg/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 PTH and 50 mL of the prior concentration until 0.1 pg/mL is reached. Mix each tube thoroughly before the next transfer. d. The final tube (0 pg/mL PTH, 20 pg/mL biotinylated PTH) 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 PTH is 20 pg/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. 1000 100 10 1 0.1 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 50 mL 50 mL 50 mL 50 mL 10. Sample Preparation: Use Assay Diluent A + biotinylated PTH to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated PTH as the diluent. It is very important to make sure the final concentration of the biotinylated PTH is 20 pg/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 PTH to a final concentration of 20 pg/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 1000-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 PTH to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated PTH as the diluent. It is very important to make sure the final concentration of the biotinylated PTH is 20 pg/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 PTH to a final concentration of 20 pg/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-PTH antibody (see Reagent Preparation step 4) to each well. Incubate for 1.5 hours at room 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 Parathyroid Hormone (PTH) 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:	Maditz, Smith, Miller, Oldaker, Tou: "Feeding soy protein isolate and oils rich in omega-3 polyunsaturated fatty acids affected mineral balance, but not bone in a rat model of autosomal recessive polycystic kidney disease." in: **BMC nephrology**, Vol. 16, pp. 13, (2015) (PubMed). Prahalad, Hickey, Huang, Hoelz, Dobrolecki, Murthy, Winata, Hock: "Serum proteome profiles identifies parathyroid hormone physiologic response." in: **Proteomics**, Vol. 6, Issue 12, pp. 3482-93, (2006) (PubMed). Bieglmayer, Prager, Niederle: "Kinetic analyses of parathyroid hormone clearance as measured by three rapid immunoassays during parathyroidectomy." in: **Clinical chemistry**, Vol. 48, Issue 10, pp. 1731-8, (2002) (PubMed).