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Product Information

PTH/Parathyroid Hormone EIA Kit
for serum, plasma, culture supernatant, and cell lysates

Catalog Number **RAB0412**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The PTH (Parathyroid Hormone) Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting PTH based on the principle of competitive enzyme immunoassay. In this assay, a biotinylated PTH peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated PTH peptide competes with endogenous (unlabeled) PTH for binding to the anti-PTH antibody. After a wash step, any bound biotinylated PTH then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated PTH peptide and inversely proportional to the amount of endogenous PTH in the standard or samples. A standard curve of known concentration of PTH peptide can be established and the concentration of PTH peptide in the samples can be calculated accordingly.

Components

1. 96-well plate coated with secondary antibody (Item A) - RAB0412A: 96 wells (12 strips × 8 wells) coated with secondary antibody.
2. 20× Wash Buffer (Item B) - RABWASH3: 25 mL.
3. EIA PTH standard (Item C) - RAB0412C: 2 vials, 10 µL/vial.
4. Anti-PTH Detection Antibody (Item N) - RAB0412F: 2 vials, 5 µL/vial.
5. EIA Assay Diluent A (Item D) - RABDIL9: 30 mL, contains 0.09% sodium azide as preservative. Diluent for standards and serum or plasma samples.
6. EIA 5× Assay Diluent B (Item E) - RABDIL10: 15 mL of 5× concentrated buffer. Diluent for standards and cell culture media or other sample types.
7. Biotinylated PTH (Item F) - RAB0412G: 2 vials, 20 µL/vial.
8. HRP-streptavidin (Item G) - RABHRP3: 600 µL of 20× concentrated HRP-conjugated Streptavidin.
9. PTH Positive Control Sample, Lyophilized (Item M) - RAB0412K: 1 vial, 100 µL.
10. TMB Substrate solution (Item H) - RABTMB2: 12 mL of 3,3′,5,5′- tetramethylbenzidine (TMB) in buffered solution.
11. Stop Solution (Item I) – RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Ultrapure water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions
9. Orbital shaker
10. Aluminum foil
11. Plastic wrap

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

If testing plasma or serum samples, use Assay Diluent A to dilute Item F and Item C. If testing cell culture media or other sample types, use Assay Diluent B to dilute Item F and Item C. For sample and positive control dilutions, refer to steps 6, 7, and 8 of Preparation Instructions.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. 5× Assay Diluent B (Item E) should be diluted 5-fold with ultrapure water.

3. Briefly centrifuge the Anti-PTH Antibody vial (Item N) before use. Add 50 µL of 1× 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 1× Assay Diluent B. This is the anti-PTH antibody working solution, which will be used in Procedure, step 2.

Note: the following steps may be done during the antibody incubation procedure (Procedure, step 2).

5. Briefly centrifuge the vial of Biotinylated PTH (Item F) before use. Transfer the entire contents of the Item F vial into a tube containing 10 mL of the appropriate Assay Diluent. This is the Working Stock of Item F. Pipette up and down to mix gently. The final concentration of biotinylated PTH will be 40 pg/mL. This solution will only be used as the diluent in Preparation, steps 6, 7, and 9.

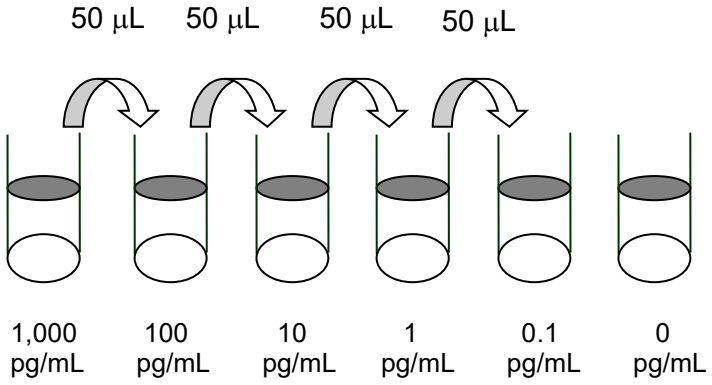
6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1,000 pg/mL, 100 pg/mL, 10 pg/mL, 1 pg/mL, 0.1 pg/mL, and 0 pg/mL. Pipette 450 µL of biotinylated PTH solution into each tube, except for the 1,000 pg/mL (leave this one empty).

   Prepare a 2-fold dilution of Item F. To do this, add 2 mL of Working Stock Item F (see Preparation, step 5) to 2 mL of the appropriate Assay Diluent. The final concentration of biotinylated PTH will be **20 pg/ml**.

   Note: 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 1,000 pg/mL, pipette 8 µL of Item C, 792 µL of 20 pg/mL biotinylated PTH solution prepared above. This is the PTH stock solution (1,000 pg/mL PTH and 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 the 1,000 pg/mL PTH standard into the tube labeled 100 pg/mL. Mix thoroughly.
c. Repeat this step with each successive concentration, preparing a dilution series (see Figure 1). Each time, use 450 µL of biotinylated PTH and 50 µL 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 and 20 pg/mL biotinylated PTH) serves as the zero standard (or total binding).

Figure 1.
Dilution Series for Standards



7. Positive Control Preparation: Briefly centrifuge the positive control vial (Item M). Add 100 µL of Working Stock Item F (see Preparation, step 5) to 100 µL of the prepared Positive Control (Item M). Mix thoroughly. This is a 2-fold dilution of the Positive Control. The positive control is a cell culture medium sample with an expected signal between 10–30% of total binding (70–90% of competition) if diluted as described. It may be diluted further if desired, but be sure the final concentration of biotinylated PTH is 20 pg/mL.

8. Sample Preparation: Use Assay Diluent A plus biotinylated PTH to dilute serum/plasma samples. For cell culture medium and other sample types, use 1× Assay Diluent B plus biotinylated PTH as the diluent.

   Note: It is very important to make sure the final concentration of the biotinylated PTH is 20 pg/mL in every sample. To perform a 2-fold dilution of the sample, add 125 µL of Working Stock Item F (see Preparation, step 5) to 125 µL of prepared sample. The final concentration of biotinylated PTH will be **20 pg/ml**. For a higher dilution of the sample, dilute the sample with the appropriate Assay Diluent before performing above step.

   For example, to make a 4-fold dilution of sample, dilute sample 2-fold (62.5 µL of sample plus 62.5 µL of 1× Assay Diluent B). Mix together 125 µL of 2-fold diluted Item F, 125 µL of the prepared sample above; mix gently. The total volume is 250 µL, enough for duplicate wells on the microplate.

   Do not use Item F diluent from Preparation, step 6 for sample preparation.

9. If Item B (20× Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into ultrapure water to yield 400 mL of 1× Wash Buffer.

10. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 20-fold with 1× Assay Diluent B.

    Note: Do not use Assay Diluent A for HRP-Streptavidin from Preparation, step 10.

Storage/Stability

Standard PTH, Biotinylated PTH, and Positive Control should be stored at –20 °C or –70 °C (recommended at –70 °C) after arrival. Avoid repeated freeze-thaw cycles.

The remaining kit components may be stored at –20 °C.

Opened microplate strips and Item N may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

The kit remains active for up to 1 year.

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 Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or incubate overnight at 4 °C.

3. Discard the solution and wash wells 4 times with 1× 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 Preparation, step 6), positive control (see Preparation, step 7), and sample (see Preparation, step 8) 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 Preparation, step 10) 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.

Results

Calculations

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 (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

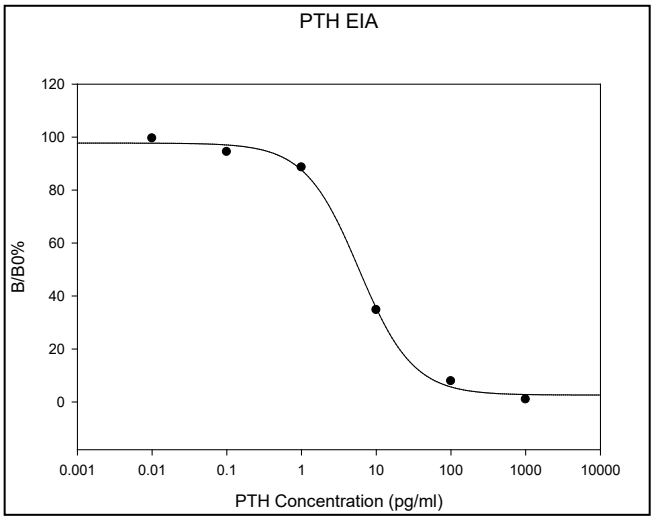
Percentage absorbance =

$$(B - \text{blank OD})/(B_0 - \text{blank OD})$$

B = OD of sample or standard
B_0 = OD of zero standard (total binding)

Typical Data

Standard curve(s) is for demonstration only. Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable concentration of PTH is 1.27 pg/mL.

Standard Curve Range: 1-1,000 pg/mL

Reproducibility:

Intra-Assay: CV <10%
Inter-Assay: CV <15%

Specificity

Cross-Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY, and APC.

References

1. Bieglmayer, C. et al., Kinetic analyses of parathyroid hormone clearance as measured by three rapid immunoassays during parathyroidectomy. Clin. Chem., **48**(10), 1731–8 (2002).
2. Prahalad, A.K. et al., Serum proteome profiles identify parathyroid hormone physiologic response. Proteomics, **6**(12), 3482–93 (2006).
3. Poole, K., and Reeve, J., Parathyroid hormone - a bone anabolic and catabolic agent. Curr. Opin. Pharmacol., **5**(6), 612–7 (2005).
4. Costanzo, L.S., BRS Physiology, (Lippincott, Williams, & Wilkins: 2007) p. 260. ISBN 978-0781773119.

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to overnight
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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