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Bio-intact PTH (Parathyroid Hormone) ELISA

Catalog No. PT360T (96 Tests)

INTENDED USE

The Calbiotech, Inc. Bio-intact PTH (BiPTH) ELISA Kit is intended for the quantitative determination of 1-84aa PTH (Parathyroid Hormone) in human serum or plasma. The kit has zero cross reaction to 7-84aa PTH fragment. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Measures the biologically active (1–84) form of parathyroid hormone. Research applications include bone remodeling pathways, calcium–phosphate homeostasis, PTH receptor signaling, and endocrine feedback regulation studies.

PRINCIPLE OF THE TEST

The Bio-intact PTH (BiPTH) immunoassay is an adapted two-site sandwich ELISA. In this assay, standards and patient samples are simultaneously incubated with the enzyme labeled detection antibody (specific to 39-84 aa residue) and a biotin coupled capture antibody (specific to 1-6 aa residue) in a streptavidin-coated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components and the enzyme bound to the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stopping solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of intact PTH in the sample. Standards are used to generate a dose response curve of absorbance unit vs. concentration. Concentrations of intact PTH present in the controls and patient samples are determined directly from this curve.

MATERIAL PROVIDED	96 TESTS
1. Microwells coated with Streptavidin	12x8x1
2. PTH Standards: 6 Vials (Ready to use)	0.5 mL
3. Anti-PTH-Biotin Reagent: 1 Bottle (Ready to use)	7 mL
4. Anti-PTH-HRP Enzyme Conjugate: 1 Bottle (Ready to use)	7 mL
5. TMB Substrate: 1 Bottle (Ready to use)	12 mL
6. Stop Solution: 1 Bottle (Ready to use)	12 mL
7. 20X Wash Solution: 1 Bottle	25 mL

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8° C
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents to heat, sun, or strong light.

RUO **Calbiotech, Inc.**
1935 Cordell Ct., El Cajon, CA 92020 USA
Tel (619) 660-6162 | Fax (619) 660-6970 | Web www.calbiotech.com

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrators contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that serum samples be run in duplicate.
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION.

1. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

1. Allow materials and reagents to reach room temperature.
2. Place the desired number of strips in the plate holder.
3. Pipet 50 µl of standards, controls, and samples into the designated wells.
4. Add 50 µl of anti-PTH-Biotin Reagent to all wells.
5. Add 50ul of anti-PTH-HRP Conjugate to all wells
6. Cover the plate and incubate at room temperature for 90 minutes on a plate shaker (500 - 600rpm).
7. Remove liquid from all wells. Wash wells 4 times with 300 µl of 1X wash buffer. Blot on absorbent towels.
8. Add 100 µl of TMB Substrate to all wells.
9. Incubate for 15 minutes at room temperature.
10. Add 50 µl of Stop Solution to all wells. Mix gently.
11. Read absorbance on ELISA Reader at 450nm within 15 minutes after adding the stopping solution. Use 630nm as a reference.

CALCULATION OF RESULTS

1. Assign the concentration for each standard stated on the vial in pg/ml. Plot data from the standard curve on linear graph paper with the concentration on the X-axis and the corresponding A.U. on the Y-axis.
2. Draw a straight line between 2 adjacent points. This mathematical algorithm is commonly known as the "point-to-point" calculation. Obtain the concentration of the sample by locating the absorbance unit on the Y-axis and finding the corresponding concentration value on the X-axis.

Example of a Standard Curve

	Conc. pg/mL	OD 450nm
Standard 1	0	0.03
Standard 2	15	0.07
Standard 3	30	0.11
Standard 4	100	0.32
Standard 5	300	0.83
Standard 6	1000	2.46

LIMITATIONS OF THE PROCEDURE

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.
2. The PTH ELISA kit has exhibited no “high dose hook effect” with high dose spiked samples. However, samples with intact PTH levels greater than the highest calibrator, should be diluted and re-assayed for correct values.