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

CALBIOTECH

Intact PTH (Parathyroid Hormone) ELISA

Catalog No. PT311T (96 Tests)

INTENDED USE

The Calbiotech Inc., Intact PTH ELISA Kit is intended for the quantitative determination of Intact PTH (Parathyroid Hormone) in human serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

PTH (Parathyroid hormone) is biosynthesized in the parathyroid gland as a pre-proparathyroid hormone, a large molecular precursor consisting of 115 amino acids. This product detects the full-length PTH molecule for research into endocrine physiology, parathyroid gland biology, calcium regulation, and metabolic bone disorder models.

PRINCIPLE OF THE TEST

The Calbiotech Inc., Intact PTH Immunoassay is an adapted two-site sandwich ELISA. In this assay, standards and patient samples are simultaneously incubated with the enzyme labeled detection antibody and a biotin coupled capture antibody 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.

MATERIALS PROVIDED		96 TESTS
1.	Microwells coated with Streptavidin	12x8x1
2.	PTH Standard 6: 1 Vial (lyophilized)	0.75 ml
3.	PTH Controls: 2 Vials (lyophilized)	0.75ml
4.	Anti-PTH-Biotin Reagent: 1 Bottle (Ready to use)	7 ml
5.	Anti-PTH-HRP Enzyme Conjugate: 1 Bottle (Ready to use)	7 ml
6.	Assay Diluent: 1 Bottle (ready to use)	5 ml
7.	TMB Substrate: 1 Bottle (Ready to use)	12 ml
8.	Stop Solution: 1 Bottle (Ready to use)	12 ml
9.	20X Wash Solution: 1 Bottle	25 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA plate 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 test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards 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 standards, control and 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 AND PREPARATION

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

Standards: Reconstitute the lyophilized standards with 0.75 ml distilled or deionized water. Allow it to remain undisturbed until completely dissolved and then mix well by gentle inversion(not to foam),prepare the rest standard set (5-2), by 3-fold serial dilution , from standard.#6 as prescribed in the table below: (Mix each tube thoroughly before the next transfer).

Std No.	Standard. Conc. (pg/mL)	Standard. volume
6	900	reconstitute with 0.75ml of DI water
5	300	0.25ml of Std 6 plus 0.5ml of assay diluent
4	100	0.25ml of Std 5 plus 0.5ml of assay diluent
3	33.3	0.25ml of Std 4 plus 0.5ml of assay diluent
2	11.1	0.25ml of Std 3 plus 0.5ml of assay diluent
1	0	Assay Diluent only

Controls: Reconstitute the lyophilized controls with 0.75 ml distilled or deionized water. Allow them to remain undisturbed until completely dissolved and then mix well by gentle inversion.
Use the standard set and controls as soon as possible upon reconstitution. Freeze (<-20ºC) the remaining standard set and controls immediately after use.

Wash Buffer: 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 25 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 paper 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 the 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	0.029
Standard 2	11.1	0.082
Standard 3	33.3	0.178
Standard 4	100	0.459
Standard 5	300	1.078
Standard 6	900	2.535

LIMITATION OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.