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Product Information

Intact-PTH (Parathyroid Hormone) ELISA kit

Catalog Number: EA100878
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Intact-PTH ELISA Kit is intended for the quantitative determination of Intact-PTH (Parathyroid Hormone) in human serum or plasma.

Background

PTH (Parathyroid hormone) is biosynthesized in the parathyroid gland as a pre-proparathyroid hormone, a large molecular precursor consisting 115 amino acids. In healthy individuals, regulation of parathyroid hormone secretion normally occurs via a negative feedback action of serum calcium on the parathyroid glands. Intact PTH is biologically active and clears very rapidly from the circulation with a half-life of less than four minutes. Intact PTH assays are important for the differentiation of primary hyperparathyroidism from other (non-parathyroid-mediated) forms of hypercalcemia, such as malignancy, sarcodosis and thyrotoxicosis. The measurement of parathyroid hormone is the most specific way of making the Diagnosis of primay hyperparathyroidism. In the presence of hypercalcemia, an elevated level of parathyroid hormone virtually establishes the diagnosis. In over 90% of patents with primary hyperparathyroidism, the parathyroid hormone will be elevated. The most common other cause of hypercalcemia, namely hypercalcemia of malignancy, is associated with suppressed levels parathyroid hormone or PTH levels within the normal range. PTH values are typically undetectable in hypocalcemia due to total hypoparathyroidism, but are found within the normal range in hypocalcemia due to partial loss or inhibition of parathyroid function.

Principle of the Test

The Intact PTH Immunoassay is a two-site ELISA [Enzyme-Linked ImmunoSorbent Assay]. In this assay, standards, controls, or patient samples are simultaneously incubated with the enzyme labeled antibody and a biotin coupled 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. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of intact PTH present in the controls and patient samples are determined directly from this curve.

Components

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 and Equipment Required but 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.

Warnings and Precautions

1. This test kit is intended for the quantitation of PTH in human serum or plasma.
2. Potential biohazardous materials:
3. 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
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that serum samples be run in duplicate.

7. 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

1. Collect blood specimens and separate the serum immediately.
2. Specimens should be stored frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Reagent Preparation

S**tandards**: 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).
Patient samples with values greater than the highest calibrator (standard 6), which is approximately 700 – 1000 pg/mL (see exact concentration on vial label), may be diluted with assay diluent and re-assayed. Multiply the result by the dilution factor.

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

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 value.

Performance Characteristics

Correlation with a Reference ELISA kit:

A total of 18 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.996	1.0124	2.7005

Precision

Intra-Assay

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Coefficient of Variation (%)
1	16	51.8	3.29	6.3%
2	16	244	9.33	3.8%

Inter-assay

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Coefficient of Variation (%)
1	16	19.6	1.61	8.2%
2	16	56.9	3.88	6.8%
3	16	136	9.1	6.7%

Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero standard	20	0.1	0.194	0.49

Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following normal range for PTH (US adult patients) may be used as initial guideline only: 10-65pg/ml[7]

References

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