

Intact PTH [Parathyroid Hormone] ELISA

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

Product Number: PTH2016BA (96 Tests)

1 INTENDED USE

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

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. After two intra-cellular proteolytic cleavage steps, the parathyroid gland secretes the final active form consisting of an 84 amino acid peptide. 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, sarcoidosis 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 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.

MATERIAL PROVIDED	96 TESTS
1. Microwells coated with Streptavidin	12x8x1
2. PTH Standards: 6 Vials (Ready to use)	0.5 ml
3. PTH Controls: 2 Vials (Ready to use)	0.5ml
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. TMB Substrate: 1 Bottle (Ready to use)	12 ml
7. Stop Solution: 1 Bottle (Ready to use)	12 ml
8. 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.

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

Product Number: PTH2016 BA

2. This test kit is intended for the quantitation of PTH in human serum or plasma.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. 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 may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store 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.
 - a)
 - b) 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 (18-26°C).

ASSAY PROCEDURE

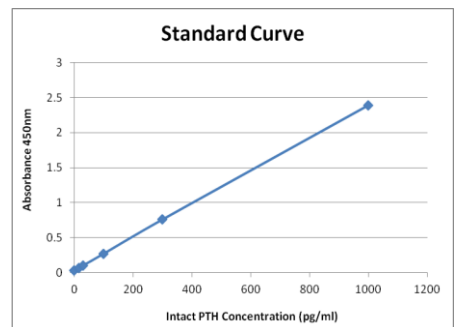
1. Allow materials and reagents to reach room temperature.
2. Place the desired number of strips in the plate holder.
3. Pipet 25 µl of standards, controls, and samples into the designated wells.
4. Add 50 µl of anti-PTH-Biotin Reagent to all wells.
5. Add 50ul of anti-PTH-HRP Conjugate to all wells
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	0.032
Standard 2	15	0.066
Standard 3	30	0.102
Standard 4	100	0.270
Standard 5	300	0.759
Standard 6	1000	2.392



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 reassayed for correct values.

Product Number: PTH2016 BA

PERFORMANCE CHARACTERISTICS

1. Correlation with a Reference ELISA kit:

A total of 110 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

<i>Correlation</i>	<i>Slope</i>	<i>Intercept</i>
0.996	1.0124	2.7005

Precision

Intra-Assay

Serum	No. of Replicates	Mean mIU/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 mIU/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 mIU/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 patients) may be used as initial guideline only: 9.0 to 94 pg/ml

REFERENCES

- Segre, G.V., Niall H.D., Habener J.F. et. al. : Metabolism of parathyroid hormone: physiological and clinical significance. Am. J. Med. 56: 774,1974.
- Mallete, L.E., Gagel, R.F.: Parathyroid Hormone and Calcitonin. In: Murray J.F. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 65-69, 1990.
- Bilezikian, J.P.: Primary Hyperparathyroidism. In: Murray J.F. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 109-111, 1990.
- Stewart, A.F.: Humoral Hypercalcemia of Malignancy. In: Murray J.F. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 115-118, 1990.
- Mallette, L.E.: The parathyroid polyhormones: New concepts in the spectrum of peptide hormone action. Endocrin. Rev. 12:110-117, 1991.
- Kruger, L., Rosenblum, S., Zaazra, J. and Wong, J. Intact PTH is stable in unfrozen EDTA plasma for 48 hours prior to laboratory Analysis. Clin. Chem. 41:6: page S47, 1995.

Symbols Key/ Symbolschlüssel/ Explication des symboles / Legenda / Símbolos	
	Manufactured by / Hergestellt von/ Fabriqué par/ Prodotto da/ Fabricado por
	In Vitro Diagnostic Medical Device/ In Vitro Diagnosticum/ Dispositif médical de diagnostic <i>in vitro</i> / Diganostico <i>in vitro</i> / Producto para diagnóstico In vitro
	Lot Number/ Chargenbezeichnung/ Numéro de lot/ Lotto/ Número de lote
	Expiration Date/ Verfallsdatum/ Date de péremption/ Scadenza/ Fecha de caducidad
	Storage Temperature/ Lagertemperatur/ Température de conservation/ Temperatura di conservazione / Temperatura de almacenamiento
	CE Mark/ CE-Zeichen/ Marquage CE / Marchio CE/ MarcaCE
	Catalogue Number/ Katalog Nummer/ Référence du catalogue/ Numero di codice/ Número de Catálogo
	Consult Instructions for Use/ Gebrauchsanweisung beachten/ Consulter la notice d'utilisation/ Consultare le istruzioni/ Consulte las Instrucciones de Uso
	Contains sufficient for "n" tests/ Ausreichend für "n" Tests/ Contenu suffisant pour "n" tests/ Contenuto sufficiente per "n" saggi/ Contenido suficiente para "n" tests

bioactiva diagnostica GmbH

Louisenstr. 137, 61348 Bad Homburg, Germany
 Tel: +49 (0) 6172 17102-0 Fax: +49 (0) 6172 17102-29
 bioactiva@bioactiva.de
 www.bioactiva.de