Human Intact PTH / Parathyroid Hormone ELISA Kit

Enzyme Immunoassay for the quantification of intact Parathyroid Hormone (PTH) in serum and plasma

Catalog number: ARG80779

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

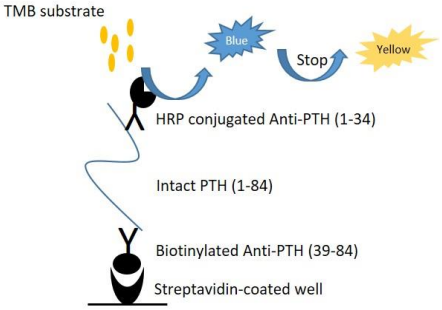
PTH (Parathyroid hormone, Parathormone, Parathyrin) is biosynthesized in the parathyroid gland as a pre-proparathyroid hormone, a larger molecular precursor consisting of 115 amino acids. Following sequential intracellular cleavage of a 25-amino acid sequence, preproparathyroid hormone is converted to an intermediate, a 90-amino acid polypetide, proparathyroid hormone. With additional proteolytic modification, proparathyroid hormone is then converted to parathyroid hormone, an 84 amino acid polypeptide. 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. PTH undergoes proteolysis in the parathyroid glands, but mostly peripherally, particularly in the liver but also in the kidneys and bone, to give N-terminal fragments and longer lived C-terminal and midregion fragments. In subjects with renal insufficiency, C-terminal and midregion PTH assays typically give elevated PTH results, as reflected by impaired renal clearance.

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 primary hyperparathyroidism. In the presence of hypercalcemia, an elevated level of parathyroid hormone virtually establishes the diagnosis. In over 90% of patients 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 of parathyroid hormone or PTH levels within the normal range. When intact PTH

level is plotted against serum calcium, the intact PTH concentration for patients with hypercalcemia of malignancy is almost always found to be inappropriately low when interpreted in view of the elevated serum calcium. Unlike C-terminal and midregion PTH, which typically are grossly elevated in subjects with renal insufficiency, intact PTH assays are less influenced by the declining renal function. 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 ASSAY

This assay employs the sandwich quantitative two-side enzyme immunoassay technique. Streptavidin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells together with Biotin-labeled PTH (39-84) antibody and HRP-labeled PTH (1-34) antibody where a sandwich will be formed between PTH present in the samples and the antibodies. After washing away any unbound substances, a substrate solution (TMB) is then added to the wells and color develops in proportion to the amount of PTH bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of PTH in the sample is then determined by comparing the O.D of samples to the standard curve.



MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Streptavidin-coated microplate	12 X 8 strips	4°C
Standard 0-5 (0, 11.1, 34.1, 105, 302, 971 pg/ml)	6 vials (Lyophilized)	4°C
Controls (1: 37.8-59.8 pg/ml; 2: 194-306 pg/ml)	2 vials (Lyophilized)	4°C
Reconstitution Buffer	5 ml (Ready-to-use)	4°C
Sample Diluent	2 ml (Ready-to-use)	4°C
Biotinylated PTH Antibody	7 ml (Ready-to-use)	4°C
HRP-Conjugated PTH Antibody	7 ml (Ready-to-use)	4°C
20X Wash buffer	30 ml	4°C
TMB substrate	20 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	20 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate Shaker: (One of the suggestions at below)

  Orbital microplate shaker: 3 mm (0.1118 in) shaker at 600 ± 10 rpm or 19 mm (0.75 in) shaker at 170 ± 10 rpm.

  Linear microplate shaker: 25 mm (0.98 in) shaker at 170 ± 10 rpm
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 37°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Store the remaining standards and controls at-20°C as soon as possible after use. It is recommended aliquot and store reconstituted standards and controls at ≤ -20°C and avoid repeated freeze-thaw cycles.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C for up to 4 months. Avoid repeated freeze-thaw cycles.

Note: Based on the customer feedback, EDTA plasma might be also suitable for detecting Intact Parathyroid Hormone with this kit.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 30 ml of 20X Wash buffer +570 ml of distilled water). Diluted wash buffer is stable for 90 days at RT. For long-term storage it should be stored at 4°C.
- Standard 0-5: Reconstitute Standard 0-5 with 0.5 ml reconstitution buffer. Concentration after reconstitution is written on the vial label. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Although the reconstituted standards are stable for 6 weeks when stored at-20°C with up to 3 freeze thaw cycles. It is recommended aliquot and store reconstituted standards at ≤ -20°C and avoid repeated freeze-thaw cycles and use the reconstituted standards as soon as possible.
- Controls: Reconstitute Controls with 0.5 ml reconstitution buffer. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Although the reconstituted controls are stable for 6 weeks when stored at-20°C with up to 3 freeze thaw cycles. It is recommended aliquot and store reconstituted controls at ≤ -20°C and avoid repeated freeze-thaw cycles and use the reconstituted controls as soon as possible.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.

2. Add 25 µl of controls, standards and samples in duplicate into wells. (keep two wells for blank, refer to step 9 for the final reading announcement)
3. Add 50 µl Biotinylated PTH Antibody into all wells with controls, standards and samples.
4. Add 50 µl HRP-Conjugated PTH Antibody into all wells with controls, standards and samples.
5. Cover wells and incubate for 3 hours at RT (22-28°C) on a microplate shaker at recommended setting (refer to MATERIALS REQUIRED BUT NOT PROVIDED section).
6. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add 150 µl of TMB substrate to each well with controls, standards and samples. Incubate for 30 minutes on a microplate shaker at room temperature in dark.
8. Add 100 µl of Stop Solution to each well with controls, standards and samples. Mix gently. The color of the solution should change from blue to yellow.
9. Before reading, add 250 µl distilled water in the blank wells. Read the OD with a microplate reader at 450 nm immediately. Read again at 405 nm. The second reading is designed to extend the analytical validity of the calibration curve to the value represented by the highest standard. (Please refer to the CALCULATION OF RESULTS selection for the detail)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

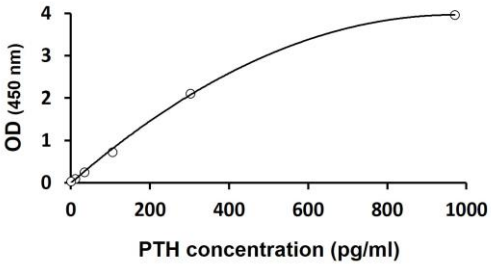
5. Samples with PTH > 300 pg/mL can be quantified against a calibration curve consisting of the readings all the way up to the concentration equivalent to the highest calibrator using the 405 nm reading, away from the wavelength of maximum absorbance.

6. In general, samples and controls should be read using the 450 nm for PTH concentrations up to 300 pg/mL (Using the O.D. value of Standard 0, 1, 2, 3 and 4 reading at 450 nm for calculation). PTH concentrations above 300 pg/mL should be interpolated using the 405 nm reading (Using the OD value of Standard 0, 3, 4 and 5 reading at 405 nm for calculation).

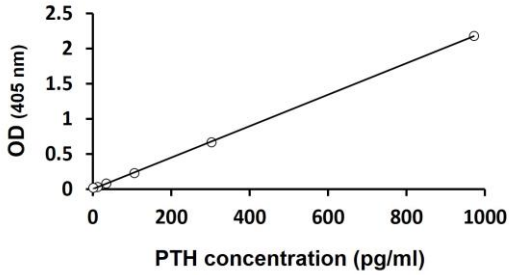
7. Samples with intact PTH levels greater than the highest calibrator (971.0 pg/ml) should be diluted with Sample Diluent and re-assayed for correct values. Or reported as > 971.0 pg/ml.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



Using the O.D. value of Standard 0, 1, 2, 3 and 4 reading at 450 nm for calculation when concentrations of the samples were <300 pg/ml.



Using the O.D. value of Standard 0, 3, 4 and 5 reading at 405 nm for calculation when the concentrations of samples were between 300-971 pg/ml.

QUALITY ASSURANCE

QUALITY CONTROL

Controls should be analyzed with each run of calibrators and patient samples. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample should be considered invalid. If the OD 450 nm of Calibrator 0 after blanking is ≥ 0.100, the curve is invalid and no patient results should be reported.

Sensitivity

The minimum detectable dose (MDD) of total PTH ranged from 1.57-971 pg/ml. The mean MDD was 1.57 pg/ml.

Specificity

The antibodies used in this ELISA kit were purified by affinity chromatography to be specific for well-defined regions on the PTH molecule. The HRP labeled antibody recognizes only the N-terminal region or the 1-34 amino acid sequence of the PTH molecule; whereas the biotinylated antibody is specific to the 39-84 segment. Accordingly, only intact PTH, which requires binding by both the enzyme conjugated and biotinylated antibodies, can be detected by this assay.

Cross-reactivity test:

Human PTH 1-34 (300 pg/mL): <2%

Human PTH 39-84 (300,000 pg/mL): <0.02%

Human PTH 7-84 (1,000 pg/mL): 44.5%

Traceability

The PTH intact calibrators are traceable to the WHO international standard PTH (1-84) recombinant NIBSC 95/646.

1.0 pg/ml = 1.07 pg/ml NIBSC 95/646

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.68-6.08% and inter-assay precision was 2.8-3.6%.

Recovery

95-109%

Linearity

84-109%