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Product Information

Intact-PTH (Parathyroid Hormone) ELISA

Catalog Number **SE120107**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
PTH (parathyroid hormone) is biosynthesized in the parathyroid gland as a pre-proparathyroid hormone, a large molecular precursor consisting of 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.

The Intact-PTH (Parathyroid Hormone) ELISA kit is intended for the quantitative determination of Intact-PTH (Parathyroid Hormone) in human serum. The Intact PTH Immunoassay is a two-site ELISA [Enzyme-Linked ImmunoSorbent Assay]. In this assay, standards, controls, or 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 units versus concentration is generated using results obtained from the calibrators. Concentrations of intact PTH present in the controls and samples are determined directly from this curve.

Components

Materials Provided	96 Tests
Microwells coated with Sterptavidin	12 x 8 x 1
Biotinylated PTH Antibody (Reagent 1)	7.0 mL
Peroxidase (Enzyme) labeled PTH Antibody (Reagent 2)	7.0 mL
TMB Substrate (Reagent B)	20 mL
Diluent [equine serum] for Patient Samples read off-scale (Reagent 3)	2 mL
ELISA Wash Concentrate [Saline with surfactant] (Reagent A)	30 mL
Stop Solution (SOLN)	20 mL
Reconstitution Solution containing surfactant (Reagent 4)	5 mL
Calibrators (A, B, C, D, E, F) (CAL)	0.5 mL
Controls 1 and 2 (CTRL)	0.5 mL

Reagents and Equipment Required but Not Provided.
- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately. 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.
2. Avoid multiple freeze-thaw cycles.
3. Prior to assay, frozen sera should be completely thawed and mixed well.
4. Do not use grossly lipemic specimens.

Preparation of non-zero standards/calibrators

For each of the Calibrators (Calibrator A through F) and kit Controls 1 and 2, reconstitute each vial with 500 µL of Reagent 4 and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to ensure complete reconstitution. Use the Calibrators and Controls as soon as possible upon reconstitution. Freeze (–20 °C) the remaining Calibrators and Controls as soon as possible after use. Calibrators and Controls are stable at –20 °C for 6 weeks after reconstitution with up to 3 freeze thaw cycles.

Wash Buffer Concentrate

Mix contents of Wash Concentrate (Reagent A) thoroughly. If precipitate is present in the Wash Concentrate due to storage at lower temperature such as 2–8 °C, dissolve by placing the vial in a 37 °C water bath or oven with swirling or stirring. Add Wash Concentrate (30 mL) to 570 mL of distilled or deionized water and mix. The diluted Working Wash Solution is stable for 90 days when stored at room temperature.

Storage/Stability

Store the kit at 2–8 °C except the Wash Concentrate, which should be kept at room temperature until dilution to avoid precipitation.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

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.

Control serum or serum pools should be analyzed with each run of calibrators and samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the sample may not be valid.

1. Place sufficient Streptavidin Coated Strips in a holder to run all 6 PTH calibrators (A–F) of the Intact PTH Calibrators (Exact concentration is stated on the vial label), Quality Control Sera, and samples.
2. Pipette 25 µL of sample, Calibrators, or Controls into the designated or mapped well. Freeze (–20 °C) the remaining Calibrators and Controls as soon as possible after use.
3. Add or dispense 50 µL of Reagent 1 (Biotinylated Antibody) into each of the wells.
4. Add or dispense 50 µL of Reagent 2 (Enzyme Labeled Antibody) into each well. Cover the microplate(s) with aluminum foil or a tray to avoid exposure to light, And place on an orbital shaker or rotator set at 170±10 rpm for 3 hours±30 minutes at room temperature (22–28 °C).
5. First aspirate the fluid completely and then wash/aspirate each well five (5) times with the Working Wash Solution (prepared from Reagent A), using an automatic microplate washer. The wash solution volume should be set to dispense 0.35 mL into each well.
6. Add or dispense 150 µL of the Reagent B (TMB Substrate) into each of the wells.
7. With appropriate cover to avoid light exposure, place the microplate(s) on an orbital shaker or rotator set at 170±10 rpm for 30±5 minutes at room temperature (22–28 °C).
8. Add or dispense 100 µL of the Stop Solution into each of the wells and mix gently.

9. Read the absorbance of the solution in the wells within 15 minutes, using a microplate reader set to 450 nm against 250 μL of distilled or deionized water. Read the plate again with the reader set to 405 nm against distilled or deionized water.
Note: The second reading is designed to extend the analytical validity of the calibration curve to the value represented by the highest calibrator, which is approximately 700–1,000 pg/mL. Hence, samples with PTH >200 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. In general, patient and control samples should be read using the 450 nm for PTH concentrations up to 200 pg/mL. PTH concentrations above 200 pg/mL should be interpolated using the 405 nm reading.
10. By using the final absorbance values obtained in the previous step, construct a calibration curve via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of the intact PTH.

Results
Calculations
1. For the 450 nm readings, construct a dose response curve (calibration curve) using the first five standards provided (*i.e.*, 1, 2, 3, 4, and 5). For the 405 nm readings, construct a second dose response curve using the three calibrators with the highest concentrations (*i.e.*, 4, 5, and 6).
2. Assign the concentration for each calibrator stated on the vial in pg/mL. Plot the data from the calibration curve on linear graph paper with the concentration on the x-axis and the corresponding absorbance units on the y-axis.
3. 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. Patient and control samples should be read using the 450 nm for PTH concentrations up to 200 pg/mL. PTH concentrations above 200 pg/mL should be interpolated using the 405 nm reading.

Example of a Standard Curve (450 nm)
Sample Data at 450 nm [raw Absorbance Units readout against distilled or deionized water]

Microplate Well	1st Reading (Absorbance Units)	2nd Reading (Absorbance Units)	Average Absorbance Units	Intact PTH (pg/mL)	Intact PTH (pg/mL) Result to report
Calibrator A	0.020	0.016	0.018		0
Calibrator B	0.056	0.051	0.054		7
Calibrator C	0.124	0.119	0.122		18
Calibrator D	0.388	0.393	0.391		55
Calibrator E	1.335	1.340	1.338		210
Control 1	0.200	0.200	0.200	27.6	27.6
Control 2	0.804	0.794	0.799	119	119
Sample 1	0.147	0.136	0.142	19.1	19.1
Sample 2	0.407	0.409	0.408	58.5	58.5
Sample 3	2.375	2.454	2.415	>200	*
Sample 4	3.725	3.725	3.725	>200	*

Because the concentration readout is >200 pg/mL, it is recommended to use the data obtained at 405 nm as shown in Sample Data at 405 nm.

Example of a Standard Curve (405 nm)

Sample Data at 405 nm (raw Absorbance Units readout against distilled or deionized water).

Microplate Well	1st Reading Absorbance Units	2nd Reading Absorbance Units	Average Absorbance Units	Intact PTH (pg/mL)	Intact PTH (pg/mL) Result to report
Calibrator A	0.014	0.008	0.011		0
Calibrator D	0.124	0.128	0.126		55
Calibrator E	0.428	0.425	0.427		210
Calibrator F	1.309	1.317	1.313		700
Control 1	0.074	0.066	0.070	<200	–
Control 2	0.260	0.251	0.256	121	–
Sample 1	0.049	0.043	0.046	<200	–
Sample 2	0.132	0.133	0.133	<200	–
Sample 3	0.758	0.782	0.770	401	401
Sample 4	1.314	1.321	1.318	>700	–

For samples with readout <200 pg/mL, it is recommended to use the data obtained at 450 nm (Sample Data at 450 nm). This practice should give the results with optimal sensitivity of the assay. Although the readout for Control 2 is <200 pg/mL, it is recommended the actual result be read out and recorded for quality control evaluation purposes. Further, absorbance for Control 2 is sufficiently high to be analytically valid.

The absorbance readout is off-scale or higher than the average absorbance of the highest calibrator. Sample should be repeated with dilution.

Note: The data presented are for illustration purposes only and must not be used in place of data generated at the time of the assay.

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