AccuDiag™
Intact PTH
ELISA Kit

INTENDED USE

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

SUMMARY AND EXPLANATION

PTH (Parathyroid hormone, Parathormone, Parathyrin) is biosynthesized in the
parathyroid gland as a pre-parathyroid 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 polypeptide, preproparathyroid 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.

CLINICAL SIGNIFICANCE

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
diminishing 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.

TEST PRINCIPLE

The DAI Intact PTH Immunoassay is a two-site ELISA [Enzyme-Linked
Immunosorbent Assay] for the measurement of the biologically intact 84 amino acid
chain of PTH. Two different goat polyclonal antibodies to human PTH have been
purified by affinity chromatography to be specific for well-defined regions on the
PTH molecule. One antibody is prepared to bind only the mid-region and C-terminal
PTH 39-84 and this antibody is biotinylated. The other antibody is prepared to bind
only the N-terminal PTH 1-34 and this antibody is labeled with horseradish
peroxidase [HRP] for detection. Streptavidin Well - Biotinylated Anti-PTH (39-84) – Intact PTH – HRP conjugated
Anti-PTH (1-34)

Although mid-region and C-terminal fragments are bound by the biotinylated anti-
PTH (39-84), only the intact PTH 1-84 forms the sandwich complex necessary for
detection. The capacity of the biotinylated antibody and the streptavidin coated
microwell both have been adjusted to exhibit negligible interference by inactive
fragments, even at very elevated levels. In this assay, calibrators, 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.

SPECIMEN COLLECTION AND PREPARATION

The determination of Intact PTH should be performed with EDTA
plasma or serum. EDTA plasma has been reported to demonstrate improved PTH stability as
compared to serum.

To assay the specimen in duplicate, 50 μL of serum or EDTA plasma is required.
Collect whole blood without anticoagulant or lavender [EDTA] tube. After allowing
calcium, the intact PTH concentration for patients with 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
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is almost always found to be inappropriately low when interpreted in view of the
elevated serum calcium.

Unlikely C-terminal and midregion PTH, which typically are grossly elevated in
subjects with renal insufficiency, intact PTH assays are less influenced by the
diminishing 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.


MATERIALS AND COMPONENTS

Materials provided with the test kits

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGT 1 = Reagent 1</td>
<td>Biotinylated PTH Antibody</td>
<td>1 x 7.0 mL</td>
</tr>
<tr>
<td>RGT 2 = Reagent 2</td>
<td>Peroxidase (Enzyme) labeled PTH Antibody</td>
<td>1 x 7.0 mL</td>
</tr>
<tr>
<td>RGT B = Reagent B</td>
<td>TMB Substrate [tetramethylbenzidine]</td>
<td>1 x 20 mL</td>
</tr>
<tr>
<td>RGT 3 = Reagent 3</td>
<td>Diluent [aqueous] for Patient Samples</td>
<td>1 x 2 mL</td>
</tr>
<tr>
<td>RGT A = Reagent A</td>
<td>ELISA Wash Concentrate [Saline with surfactant]</td>
<td>1 x 30 mL</td>
</tr>
<tr>
<td>SOLN = Stopping Solution</td>
<td>ELISA Stop Solution [1 N sulfuric acid]</td>
<td>1 x 20 mL</td>
</tr>
<tr>
<td>RGT 4 = Reagent 4</td>
<td>Reconstitution Solution containing surfactant</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>PLA = Microwells</td>
<td>One well with Streptavidin Coated Strips</td>
<td>12 x 8-well strips</td>
</tr>
<tr>
<td>CAL</td>
<td>Labeled antibodies for exact concentrations</td>
<td></td>
</tr>
<tr>
<td>CTRL</td>
<td>Controls 1 &amp; 2 for exact concentrations</td>
<td></td>
</tr>
</tbody>
</table>

Materials required but not provided
- Microplate reader
- Microplate washer [if washer is unavailable, manual washing may be acceptable]
- Precision Pipettors to deliver 25, 100 and 150 μL
- [Optional]: A multi-channel dispenser or a repeating dispenser for 50, 100 and 150 μL
REAGENT PREPARATION AND STORAGE

Store all kit components at 2-8°C.

1. All reagents except the calibrators, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2 °C - 8 °C.
2. For each of the calibrators (Calibrator A through F) and kit controls 1 and 2, reconstitute each vial with 500 μL of Reagent 4 (Reconstitution Solution) and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure 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. Standards and controls are stable at -20 °C for 6 weeks after reconstitution with up to 3 freeze thaw cycles when handled as recommended in "Procedural Notes" section.
3. Reagent A: Wash Concentrate; Mix contents of wash concentrate thoroughly. If precipitate is present in the Wash Concentrate due to storage at lower temperature such as 4 °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.

ASSAY PROCEDURE

1. Place sufficient Streptavidin Coated Strips in a holder to run all six (6) PTH calibrators, A - F of the Intact PTH CALIBRATORS [Exact concentration is stated on the vial label]. Quality Control Sera and patient samples. At a minimum, designate two wells to serve as "blanks". Refer to Step 9 for final plate reading.
2. Pipet 25 μL of calibrators, controls, and samples 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 which already contain the calibrators, controls, and samples.
4. Add or dispense 50 μL of Reagent 2 (Enzyme Labeled Antibody) into each of the same wells. Cover the microplate(s) with aluminum foil or a tray to avoid exposure to light, and place it on an orbital shaker or rotator set at 170 ± 10 rpm for 3 hours ± 30 minutes at room temperature (22 °C – 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 °C – 28 °C).
8. Add or dispense 100 μL of the Stopping Solution into each of the wells. Mix gently.
9. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm. Prior to reading, ensure both "blank wells" as mentioned in Step 1 are filled with 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, patient 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.

PROCEDURAL NOTES

- Intact PTH 1-84 is a very labile molecule. Set up the assay immediately upon the reconstitution or the thawing of all calibrators, controls, and patient samples.
- It is recommended that all calibrators, controls, and patient samples are assayed in duplicate. The average absorbance units of duplicate sets should then be used for reduction of data and the calculation of results.
- The samples should be pipetted into the well with minimum amount of air-bubble. To achieve this, "reverse pipet" described in the package insert of the manufacturers of Pipettors is recommended.
- Patient samples with values greater than the highest calibrator (Calibrator F), which is approximately 700 - 1,000 pg/mL (see exact concentration on vial label), may be diluted with Reagent 3 (Sample Diluent) and reassayed. Multiply the result by the dilution factor.
- If preferred, mix in equal volumes, in sufficient quantities for the assay. Reagent 1 (Biotinylated Antibody) and Reagent 2 (Enzyme Labeled Antibody) in a clean amber bottle, then use 100 μL of the mixed antibody into each well. This alternative method should replace Step (3) and (4), to be followed with the incubation with orbital shaker.

RESULTS

Manual Method

1. For the 450 nm readings, construct a dose response curve (calibration curve) using the first five calibrators provided, i.e. Calibrators A, B, C, D and E. For the 405 nm readings, construct a second dose response curve using the three calibrators with the highest concentrations, i.e. Calibrators D, E and F.
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 A.U. 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.

Automated Method:
Computer programs using cubic spline or 4 PL [4 Parameter Logistics] can generally give a good fit.

Sample Data at 450 nm [raw A.U. readout against distilled or deionized water]
The DAI PTH intact ELISA has a calculated sensitivity of 1.57 pg/mL.

### Specificity and Cross-Reactivity

The antibodies used in the DAI PTH intact ELISA were purified by affinity chromatography to be specific for well-defined regions on the PTH molecule. The peroxidase 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. To further achieve the specificity of this assay, conjugation and biotinylation and the molar ratios thereof, have been optimized to minimize detection of fragments of PTH. Human PTH 1-34 at levels up to 300 pg/mL and the C-terminal 39-84 fragment at levels up to 300,000 pg/mL gave molar cross reactivities to PTH of less than 2% and 0.02%, respectively.

### Human PTH 7-84

Human PTH 7-84 at level up to 1,000 pg/mL showed 44.5% cross-reactivity.

### Precision and Reproducibility

The precision (intra-assay variation) of the DAI PTH intact ELISA Test was calculated from 25 replicate determinations on each of the two samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Value (pg/mL)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>32.6</td>
<td>3.6</td>
</tr>
<tr>
<td>B</td>
<td>179.2</td>
<td>6.88</td>
</tr>
</tbody>
</table>

The total precision (inter-assay variation) of the DAI PTH intact ELISA Test was calculated from data on two samples obtained in 21 different assays by three technicians on three different lots of reagents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Value (pg/mL)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30.3</td>
<td>2.6</td>
</tr>
<tr>
<td>B</td>
<td>159.1</td>
<td>2.8</td>
</tr>
</tbody>
</table>

### Recovery

Various amounts of PTH 1-84 were added to three different patient sera to determine the recovery. The results are described in the following table:

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>PTH Endogenous (pg/mL)</th>
<th>PTH added (pg/mL)</th>
<th>Expected Value (pg/mL)</th>
<th>Measured Value (pg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>32.7</td>
<td>132</td>
<td>165</td>
<td>168</td>
<td>102%</td>
</tr>
<tr>
<td>B</td>
<td>68.5</td>
<td>132</td>
<td>201</td>
<td>191</td>
<td>95%</td>
</tr>
<tr>
<td>C</td>
<td>19.9</td>
<td>132</td>
<td>152</td>
<td>165</td>
<td>106%</td>
</tr>
</tbody>
</table>

### Average 100%

### Linearity of Patient Sample Dilutions: Parabolid

Four patient serum samples were diluted with Reagent 3 (the Diluent for Patient Samples read off-scale). Results in pg/mL are shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Expected</th>
<th>Observed</th>
<th>% Observed + Expected</th>
<th>Linear (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1:2</td>
<td>77.3</td>
<td>77.2</td>
<td>77.2</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1:3</td>
<td>51.7</td>
<td>51.6</td>
<td>51.6</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1:4</td>
<td>25.8</td>
<td>25.6</td>
<td>25.6</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1:5</td>
<td>13.3</td>
<td>13.2</td>
<td>13.2</td>
<td></td>
</tr>
</tbody>
</table>

The DAI PTH intact ELISA kit has exhibited no “high dose hook effect” with samples spiked with 2,100,000 pg/mL of Intact PTH. Samples with intact PTH levels greater than the highest calibrator, however, should be diluted and reasayed for correct values.

Like any analyte used as a diagnostic adjunct, intact PTH results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests. The PTH intact ELISA will detect non-Intact PTH (1-84) such as PTH fragment (7-84). PTH fragment (7-84) may cause falsely elevated Intact PTH results in patients with abnormal renal function because these patients may have various concentrations of PTH fragment (7-84) in their blood. In patients with abnormal renal function, 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 in the table below.

### QUALITY CONTROL

Control serum or serum pools should be analyzed with each run of calibrators and patient 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 patient sample may not be valid.

### PERFORMANCE CHARACTERISTICS

#### Traceability

The DAI 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

#### Accuracy

Three hundred and nine (309) patient samples, with intact PTH values ranging from 1.0 to 833 pg/mL were assayed by the previous DAI PTH kit and the updated DAI PTH kit. Linear regression analysis gives the following statistics:

DAI ELISA = 1.06 - 1.49 pg/mL \( r = 0.998 \) N = 309

#### Sensitivity

The sensitivity, or minimum detection limit, of this assay is defined as the smallest single value, which can be distinguished from zero at the 95% confidence limit.
renal function please interpret the Intact PTH results with caution and do not make patient management decisions on the Intact PTH result alone. Samples from patients routinely exposed to animal or animal serum products may contain heterophilic antibodies causing atypical results. This assay has been formulated to mitigate the risk of this type of interference. However, potential interactions between rare sera and test components can occur.

**EXPECTED RESULTS**

Intact PTH levels were measured in 148 apparently normal individuals in the U.S. with the Intact PTH ELISA.

The values obtained ranged from 9.0 to 94 pg/mL for serum.

Based on statistical tests on skewness and kurtosis, the population, when transformed logarithmically, follows the normal or Gaussian distribution.

The geometric mean + 2 standard deviations of the mean were calculated to be 10.4 to 66.5 pg/mL for serum.

**PRECAUTIONS**

Although the reagents provided in this kit has been specifically designed to contain no human blood components, the human patient samples, which might be positive for HBsAg, HbcAg or HIV antibodies, must be treated as potentially infectious biohazard. Common precautions in handling should be exercised, as applied to any untested patient sample.

Stopping Solution consists of 1 N Sulfuric Acid. This is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves and eye protection, with appropriate protective clothing. Any spill should be wiped immediately with copious quantities of water. Do not breath vapor and avoid inhalation.

**REFERENCES**


