**Intact PTH ELISA**

Quantitative immunoenzymatic determination of intact Parathyroid Hormone (PTH) in human serum and plasma

<table>
<thead>
<tr>
<th>IVD</th>
<th>LOT</th>
<th>See external label</th>
<th>2°C</th>
<th>8°C</th>
<th>∑ = 96 tests</th>
<th>REF</th>
<th>DKO157</th>
</tr>
</thead>
</table>

### INTENDED USE

Colorimetric immunoenzymatic method for quantitative determination of intact Parathyroid Hormone (PTH) concentration in human serum and plasma.

**Intact PTH ELISA kit** is intended for laboratory use only.

### 1. CLINICAL SIGNIFICANCE

Parathyroid Hormone (PTH) is a hormone synthesized in the parathyroid gland in a 115 aminoacids form, that, after post-translational modifications, reaches the biologically active form of 84 amino acids (Intact PTH).

PTH is released in the blood, where is rapidly degraded (half-life about 4 minutes); intact PTH concentration in the blood is regulated by a negative feedback mechanism that involves calcium in serum. PTH is degraded inside the parathyroid glands, in liver, kidneys and bones. A high concentration of intact PTH in the blood may indicate kidney dysfunction.

The measurement of the concentration of intact PTH in the blood is an effective way to diagnose the primary hyperparathyroidism and distinguish it from other diseases not related to parathyroid: in fact, intact PTH is elevated in 90% of cases of hyperparathyroidism.

### 2. PRINCIPLE OF THE METHOD

Diametra Intact PTH ELISA measures the concentration of PTH in its biologically active form of 84 amino acids (intact PTH). The assay uses two polyclonal antibodies directed against specific and different regions of human PTH; one antibody is biotinylated (and allows the binding of intact PTH to the microplate, through the binding of biotin with streptavidin coated in the wells of the microplate), the other antibody is linked to horseradish peroxidase (HRP) (and allows the detection of the signal derived from the molecules of captured intact PTH). This method has been set to avoid interference from inactive fragments of PTH; therefore, only the form of 84 amino acids is measured by the assay.

From a procedural point of view, Calibrators, Controls and the serum samples are incubated with both antibodies simultaneously in the wells of the microplate. After a period of incubation (where intact PTH binds to the wells of the microplate through the bridge formed by the two antibodies), the microplate wells are washed with a wash solution to remove unbound material. The detection of the signal that quantifies the associated intact PTH is accomplished by incubation with TMB (tetramethylbenzidine), that reacts with the peroxidase and produces a colorimetric signal. This reaction is stopped by the addition of Stop Solution, a solution of sulphuric acid. The intensity of the color is directly proportional to the amount of intact PTH present in the wells; through a calibration curve, it is possible to determine the exact concentration of intact PTH bound.

### 3. REAGENTS, MATERIALS AND INSTRUMENTATION

#### 3.1. Reagents and materials supplied in the kit

1. **Calibrators** (6 vials, 0.5 mL each)
   - CAL0
   - CAL1
   - CAL2
   - CAL3
   - CAL4
   - CAL5
   
2. **Controls** (2 vial, 0.5 mL each)
   - Control A
   - Control B

3. **HRP Conjugate** (1 vial, 7 mL)

4. **Biotin Conjugate** (1 vial, 7 mL)

5. **Coated Microplate** (1 breakable microplate)

6. **TMB Substrate** (1 vial, 12 mL)

H₂O₂-TMB (avoid any skin contact)
7. Stop Solution (1 vial, 12 mL)
Sulphuric acid 1N (avoid any skin contact)  
REF DCE005/15705-0
8. 20X Conc. Wash Solution (1 vial, 25 mL)
Buffered solution  
REF DCE007/15707-0

3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader (450 nm, 630 nm)

Notes
Store all reagents at 2-8°C in the dark.
Open the bag of Reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use.

4. WARNINGS
- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS
- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of Calibrators and Controls
Calibrators and Controls are ready to use.
The Calibrators have the following concentration of intact PTH:

<table>
<thead>
<tr>
<th>pg/mL</th>
<th>C0</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>100</td>
<td>300</td>
<td>1000</td>
</tr>
</tbody>
</table>

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Sample
The determination of intact PTH with this kit can be performed in human serum or plasma.
The samples are stable at 2-8°C for 5 days; store at -20°C for longer time (samples are stable at -20°C for 1 month).
Avoid freezing and thawing cycles.
Do not use Sodium Azide as preservative, because it could inhibit the HRP activity.

6.3. Preparation of Wash Solution
Dilute the content of each vial of the "20X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect
the 1:20 dilution ratio. The diluted wash solution is stable for 90 days at room temperature (22-28°C).

6.4. Procedure
- Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes. At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C₀-C₅), two for each Control, two for each sample.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Calibrator</th>
<th>Sample/ Control</th>
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</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td>C₀-C₅</td>
<td>25 µL</td>
</tr>
<tr>
<td>Sample/ Control</td>
<td></td>
<td>25 µL</td>
</tr>
<tr>
<td>Biotin Conjugate</td>
<td></td>
<td>50 µL</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td></td>
<td>50 µL</td>
</tr>
</tbody>
</table>

Cover the plate and incubate at room temperature (22-28°C) for 90 minutes gently shaking (500-600 rpm). Remove the reaction mixture. Wash the wells 4 times with 300 µL of diluted wash solution.

Important note: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

Automatic washer: in case you use an automatic washer, it is advised to do 6 washing steps.

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 minutes</td>
<td>in the dark at room temperature (22-28°C).</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
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</table>

Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 630 nm within 5 minutes.

7. QUALITY CONTROL
Each laboratory should assay controls at normal, high and low levels range of intact PTH for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS
1. Calculate the mean of the absorbance (Em) for each point of the calibration curve (C₀-C₅) and of each sample.
2. Plot the mean value of absorbance (Em) of the calibrators (C₀-C₅) against concentration.
4. Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in pg/mL.
5. For Intact PTH concentrations greater than the Cal 5 concentration (1000 pg/mL) dilute the sample 1:4 with Cal 0 (for example: to obtain 100 µL of diluted sample mix 25 µL of original sample with 75 µL of Cal 0) and test again.

9. REFERENCE VALUES
The following range can be used as initial guideline:

Expected range: 9 - 94 pg/mL

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision
10.1.1. Intra Assay
Within run variation was determined by replicate (16x) the measurement of two different sera in one assay. The within assay variability is ≤ 6.3%.

10.1.2. Inter Assay
Between run variation was determined by replicate (21x) the measurement of three different sera in different lots. The between assay variability is ≤ 8.2%.
10.2. Specificity

PTH antibodies used in this method have been selected so that Diametra Intact PTH ELISA kit recognizes the intact form of PTH, and do not recognize the PTH fragments derived from post-translational modifications on intact PTH. Therefore, we calculated the specificity compared to PTH fragments:

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Tested concentration</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact PTH (1-84)</td>
<td>1000 pg/mL</td>
<td>100%</td>
</tr>
<tr>
<td>PTH 7-84</td>
<td>1000 pg/mL</td>
<td>107%</td>
</tr>
<tr>
<td>PTH 1-13</td>
<td>100 ng/mL</td>
<td>0%</td>
</tr>
<tr>
<td>PTH 1-34</td>
<td>100 ng/mL</td>
<td>0%</td>
</tr>
<tr>
<td>PTH 39-84</td>
<td>1 ng/mL</td>
<td>0.01%</td>
</tr>
</tbody>
</table>

10.3. Sensitivity

The lowest detectable concentration of intact PTH that can be distinguished from the Calibrator zero is 0.49 pg/mL at the 95% confidence limit.

10.4. Correlation

Diametra Intact PTH ELISA kit was compared to a commercially available kit. 110 serum samples were analysed.

The linear regression equation was calculated:

\[ Y = 1.0124 \times X + 2.7005 \]

\[ r^2 = 0.996 \]

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY


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