AccuDiag™ Intact PTH ELISA Kit

REF 1737-P2

IVD See External Label 2°C 8°C 96 Tests

Intact PTH ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-1000 pg/ml
Sample	50 µl
Incubation Time	90 minutes
Shelf Life	12-14 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The DAI Intact PTH ELISA is intended for the Quantitative Determination of Intact PTH Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric.

SUMMARY AND EXPLANATION

Parathyroid hormone (PTH) is a polypeptide composed of 84- amino acids and vital to calcium homeostasis[1] regulating blood serum calcium (Ca^{2+}) in concert with Vitamin D and Calcitonin. Secreted by the parathyroid gland in response to low Ca^{2+}, PTH stimulates calcium release in the bone marrow, production in the intestines and kidney[2] and minimizes urinary excretion. Meanwhile calcitonin has the opposing effect to increase urinary excretion and reduce blood calcium when Ca^{2+} is at elevated levels[3].

Intact PTH clears quickly from the bloodstream with half-life of less than four minutes. Detecting elevated PTH levels is imperative in monitoring bone metabolism especially in the presence of hypercalcemia[4], which virtually makes the primary diagnosis of hyperparathyroidism, as the vast majority (>90%) of such patients have elevated PTH. Differentiation from other forms of (non-parathyroid-mediated) hypercalcemia such as malignancy (the second most common cause), sarcoidosis, and thyroid toxicosis are associated with suppressed levels of parathyroid hormone or PTH in normal range. In cases of hypocalcemia, PTH levels may not be detectable due to total hypoparathyroidism but are found in normal range in hypocalcemia due to partial loss or inhibition of parathyroid function. Clinical significance of parathyroid hormone has increased in conjunction with the etiology of hypocalcemia and hypercalcemia. Initial studies revealed parathyroid hormone is synthesized as a prohormone followed by significant cleavage and modification, with these fragments comprising the majority of circulating parathyroid hormone. However, PTH fragments lack biological activity, and intact PTH (IPTH) spanning residues 1-84 is responsible for calcium regulation. The N-terminus of PTH is necessary in receptor docking, while the C-terminal residues are responsible for PTH receptor activation [5,6]. Thus, separation of whole parathyroid hormone from fragmented peptides is integral in osteometabolic analysis[7].

ASSAY PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of x-PTH antibody (C terminal epitope) coated on the well.

Upon mixing the enzyme-labeled antibody (N-terminal epitope) and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{PTH} + Ab_{Well} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{PTH} - Ab_{Well}$$

$Ab_{(well)}$ = Antibody coated on well (Excess Quantity)
Ag_{PTH} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{PTH} - Ab_{(well)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

After sufficient time results, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS

Materials provided with the test kit

A. PTH Calibrators – 1.0 ml/vial (Lyophilized) [A – F]

Six (6) vials of references for PTH at levels of 0(**A**), 15(**B**), 75(**C**), 150(**D**), 500(**E**) and 1000(**F**) pg/ml. Store at 2-8°C. ***Reconstitute each vial with 1.0ml of distilled or deionized water***. The reconstituted calibrators are stable for 24-48 hours at 2-8°C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20°C. ***DO NOT FREEZE/ THAW MORE THAN TWICE***. A preservative has been added.
Note: The calibrators, human serum based, are traceable to the WHO 1st IS standard 95/646.

B. PTH Controls – 1.0 ml/vial (Lyophilized) [M&N]
Two (2) vials of reference controls for PTH. Store at 2-8°C. ***Reconstitute each vial with 1.0ml of distilled or deionized water***. The reconstituted controls are stable for 24-48 hours at 2-8°C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20°C. ***DO NOT FREEZE/ THAW MORE THAN TWICE***. A preservative has been added.

C. PTH Enzyme Reagent 2nd Gen – 6ml/vial
One (1) vial contains anti-PTH conjugate reagent. Store at 2-8°C.

D. PTH Antibody Coated Plate – 96 wells
One 96-well microplate coated with x-PTH antibody. Store at 2-8°C.

E. Wash Solution Concentrate (20x) – 20 ml/vial
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C. See Reagent Preparation section.

F. Substrate Reagent – 12 ml/vial
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C

G. Stop Solution – 8 ml/vial
One (1) vial contains a strong acid (H_2SO_4). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not expose reagents to heat, sun, or strong light.
Note 3: The above components are for a single 96-well microplate.

Materials required but not provided

1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

PRECAUTIONS

For In Vitro Diagnostic Use
Not for internal or external Use in Human or Animal.
All products that contain human serum have been found to be non-reactive for hepatitis B surface Antigen. HIV 1& 2 and HCV Antibodies by FDA licensed reagents. Since no know test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control /national Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or EDTA plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants for serum or EDTA containing tubes for plasma. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

If the specimen(s) cannot be assayed immediately after blood withdrawal, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing (a maximum of two freeze/thaw cycles prior to use). When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve 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. 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.

REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacteria growth.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
Test Procedure should be performed by a skilled individual or trained professional

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the PTH Enzyme Reagent 2nd gen to each well. It is very important to dispense all reagents close to the bottom of the coated well.
4. Swirl the microplate gently for 20-30 seconds, cover and incubate for 60 minutes at room temperature.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.

An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

7. Add 0.100 ml (100 µl) of TMB Substrate to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION.**
8. Incubate at room temperature for twenty (20) minutes.
9. Add 0.050 ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
10. Read the absorbance in each well at 450nm (using a reference wavelength of 630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 1000 pg/ml, dilution should be performed in human serum or plasma with low PTH values and multiplied accordingly.

RESULTS

A dose response curve is used to ascertain the concentration of PTH in unknown specimens.

1. Plot the absorbance for each duplicate serum reference versus the corresponding PTH concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of PTH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.800) intersects the dose response curve at 419 pg/ml PTH concentration (See Figure 1).

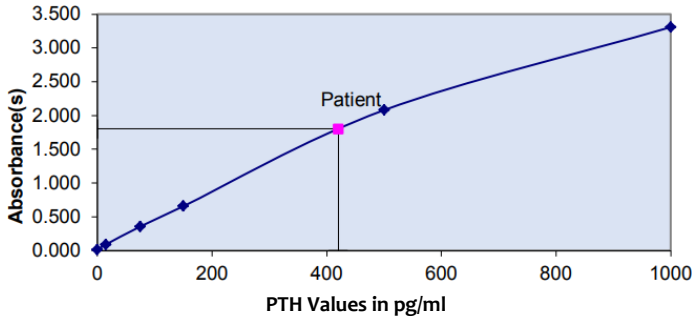
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	0.013	0.015	0
	B1	0.017		
Cal B	C1	0.082	0.091	15
	D1	0.106		
Cal C	E1	0.370	0.357	75
	F1	0.355		
Cal D	G1	0.677	0.657	150
	H1	0.647		
Cal E	A2	2.103	2.079	500
	B2	2.065		
Cal F	C2	3.265	3.308	1000
	D2	3.360		
Patient	E2	1.801	1.800	419
	F2	1.800		

The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

Figure 1



*If the absorbance readout is off-scale or higher than the average absorbance of the highest calibrator, sample should be repeated with dilution.

Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Calibrator 'F') ≥1.3
2. Four out of six quality control pools should be within the established ranges.

RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Diagnostic Automation, Inc.

ASSAY PERFORMANCE

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from DAI's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11. It is important to calibrate all the equipment e.g. pipettes, readers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

INTERPRETATION

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, DAI shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The reagents for DAI ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33)*. For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history, and, all other clinical findings.

EXPECTED RANGE OF VALUES

Intact PTH levels were measured in fifty-eight (58) apparently normal individuals. The values obtained ranged from 9.0 to 94 pg/ml. Based on statistical tests on skewness and kurtosis, the population, when transformed logarithmically, follows the normal or Gaussian distribution as shown in histograms. The geometric mean ± 2 standard deviations of the mean were calculated to be 10.4 to 66.5 pg/ml. It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

SPECIFICITY

The following fragments of PTH were tested and found to be non-reactive.

Peptide	Conc (pg/ml)	% Reactivity
1-34 fragment	100,000	0.001
1-44 fragment	100,000	0.005
7-34 fragment	100,000	0.002

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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Brand Name	**AccuDiag™**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands** www.cepartner4u.com
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