Monobind Inc.
Lake Forest, CA 92630, USA

AccuLite
CLIA Microwells

Parathyroid Hormone, Intact (PTH) 2nd Generation Test System

Product Code: 9075-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Intact PTH Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

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].

3.0 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 tracer-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 = Tracer 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 tracer 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.

4.0 REAGENTS

Materials Provided:

A. PTH Calibrators – 1.0 ml/vial (Lyophilized) Icons [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 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) Icons [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 hours at 2-8 °C. To store for a longer period, aliquot the reconstituted controls into cryo vials and store at -20°C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.

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

D. PTH Light Reaction wells – 96 wells – Icon
One 96-well white 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. Signal Reagent A – 7 ml/vial – Icon C^A
One (1) vial containing luminol in buffer. Store at 2-8°C. *See Reagent Preparation section.*

G. Signal Reagent B– 7 ml/vial – Icon C^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. *See Reagent Preparation section.*

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 one 96-well microplate

4.1 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 luminometer.
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.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

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 known 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.

6.0 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.

7.0 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.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of Wash Solution Concentrate 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
2. **Working Signal Reagent Solution –** Store at 2 - 8°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

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

9.0 TEST 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 Tracer 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 45 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 four (4) additional times for a total of five (5) 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 four (4) additional times.**
7. Add 0.100 ml (100 µl) of working signal reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE PLATE AFTER SIGNAL ADDITION
8. Incubate at room temperature for five (5) minutes.
9. Read the relative light units (RLUs) in each well for 0.2 – 1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal reagent solution.**

Note1: **Do not use the working signal reagent solution if older than 36 hours.**
Note 2: **Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycles.**
Note 3: **It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the microwells at an angle while touching the side of the well**
Note 4**: 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**.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PTH in unknown specimens.
1. Plot the RLUs 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 RLUs 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 RLUs (23535) intersects the dose response curve at 263 pg/ml PTH concentration (See Figure 1).

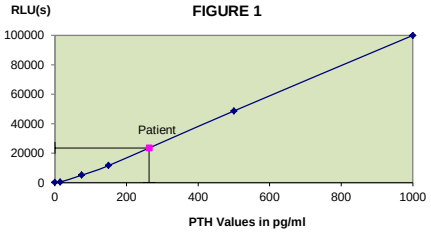
Note: Computer data reduction software designed for CLIA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (pg/ml)
Cal A	A1	36	36	0
	B1	35		
Cal B	C1	369	375	15
	D1	382		
Cal C	E1	4971	5055	75
	F1	5135		
Cal D	G1	11617	11560	150
	H1	11503		
Cal E	A2	48447	48641	500
	B2	48835		
Cal F	C2	99798	100000	1000
	D2	100202		
Patient	E2	22743	23535	**263**
	F2	24327		

*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. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

FIGURE 1



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

11.0 Q.C. PARAMETERS

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

1. The dose response curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

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

12.1 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; therefore the reagents should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate luminometers 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 Monobind'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, luminometers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 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, Monobind 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 AccuLite® CLIA 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.

13.0 EXPECTED RANGES 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.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the PTH AccuLite® Microplate EIA Test System were determined by analyses on six different levels of pool human sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2.

TABLE 2
Precision data for the PTH Test System

	Mean Value (pg/ml)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Sample 1	11.3	0.92	8.14	1.22	10.84
Sample 2	60.6	2.77	4.57	3.94	6.50
Sample 3	126.3	5.50	4.35	9.45	7.49
Sample 4	236.0	4.10	1.74	11.84	5.02
Sample 5	419.4	6.56	1.56	21.35	5.09
Sample 6	661.0	16.69	2.52	33.69	5.10

*As measured in forty experiments in duplicate over a twenty day period.

14.2 Sensitivity

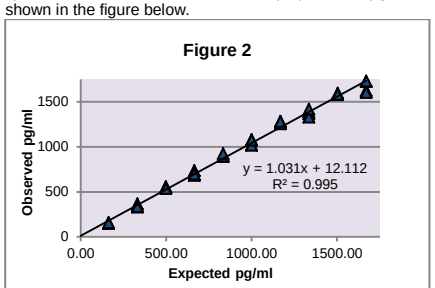
The Intact PTH AccuLite® CLIA test system has a LoB=1.894 pg/ml and a LoD=LoQ=2.967 pg/ml.

14.3 Accuracy

14.3.1 Linearity

The linearity of the Intact PTH AccuLite® CLIA test system was tested by diluting human serum samples containing a high level of PTH (up to>1600 pg/ml) with the "0 pg/ml" serum reference. The system demonstrates excellent linearity up to 1670 pg/ml as shown in the figure below.

Figure 2



14.3.2 Recovery

The recovery of the Intact PTH AccuLite® Microplate CLIA Test System was calculated for five patient samples spiked with 50, 150, 250, 550, and 750 pg/ml PTH. Recoveries were determined to be within 15% of the expected values for all samples.

14.4 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

15.0 REFERENCES

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MP9075 **Product Code: 9075-*300***

Size		96(A)	192(B)
Reagent (fill)	A)	1.0ml set	1.0ml set
	B)	1.0ml set	1.0ml set
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)

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Glossary of Symbols
(EN 980/ISO 15223)