Monobind Inc.
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AccuBind
ELISA Microwells

Parathyroid Hormone (PTH) Test System

Product Code: 9025-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

PTH (Parathyroid hormone) is biosynthesized in the parathyroid gland as a pre-proparathyroid hormone: a large molecular precursor consisting of 115 amino acids. After two intra-cellular proteolytic cleavage steps, the parathyroid gland secretes the final active form consisting of an 84 amino acid peptide.

In healthy individuals, the 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, 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. 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.

3.0 PRINCIPLE

The Intact PTH Immunoassay is an adapted two-site sandwich ELISA. In this assay, standards and patient samples are simultaneously incubated with the enzyme labeled detection antibody and a biotin coupled capture 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. Standards are used to generate a dose response curve of absorbance unit vs. concentration. Concentrations of intact PTH present in the controls and patient samples are determined directly from this curve.

4.0 REAGENTS

Materials Provided:

A. PTH Calibrators – 0.5 ml/vial
Six (6) vials of references for PTH at levels of 0(**1**), 15(**2**), 30(**3**), 100(**4**), 300(**5**) and 1000(**6**) pg/ml. Store at 2-8°C.

B. PTH Controls – 0.5 ml/vial
Two (2) vials of reference controls for PTH. Store at 2-8 °C.

C. Anti-PTH Biotin Reagent – 7 ml/vial
One (1) vial containing anti-PTH biotin reagent. Store at 2-8°C.

D. PTH Enzyme Conjugate – 7 ml/vial
One (1) vial containing anti-PTH conjugate reagent. Store at 2-8°C.

E. Streptavidin Coated Microwells – 96 wells
One 96-well microplate coated with streptavidin. Store at 2-8°C.

F. Wash Solution Concentrate (20x) – 25 ml/vial
See Reagent Preparation section. Store at 2-8°C.

G. TMB Substrate – 12 ml/vial
One (1) vial containing tetramethylbenzidine (TMB). Store at 2-8°C.

H. Stop Solution – 12 m/vial
One (1) vial containing a strong acid. Store at 2-8°C.

I. 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

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025ml (25µl) and 0.050ml (50µl) 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.

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 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. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8 °C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, 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. When assayed in duplicate, 0.050 ml (50 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 by adding the contents of the bottle (25 ml, 20x) to 475 ml of distilled or deionized water in a suitable storage container. Store diluted buffer at room temperature (20-27 °C).

Note: Do not use reagents that are contaminated or have bacteria 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 0.025 ml (25 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.50 ml (50 µl) of the Biotin Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Add 0.50 ml (50 µl) of the Conjugate Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
5. Incubate 90 minutes at room temperature on a plate shaker (500-600rpm)
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.300 ml (300 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat three (3) additional times for a total of four (4) 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 three (3) additional times.**
8. 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
9. Incubate at room temperature for fifteen (15) minutes.
10. 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.**
11. 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 reassaying specimens with concentrations greater than 1000 pg/ml, dilution should be performed.

10.0 CALCULATION OF RESULTS

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

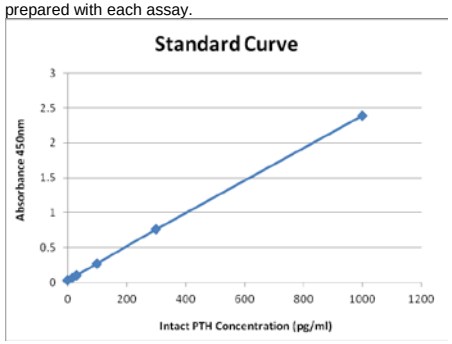
1. Plot the absorbance for each duplicate serum reference versus the corresponding PTH concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of PTH 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).

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.	Conc. (pg/ml)	Abs
Cal 1	0	0.032
Cal 2	15	0.066
Cal 3	30	0.102
Cal 4	100	0.270
Cal 5	300	0.759
Cal 6	1000	2.392

*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.



11.0 Q.C. PARAMETERS

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

1. Maximum Absorbance (Calibrator 'F') ≥1.2
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, 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 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, Readers, 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. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.
7. The PTH ELISA kit has exhibited no high dose hook effect with high dose spiked samples. Samples with Intact PTH levels greater than the highest calibrator, however, should be diluted and reassayed for correct values.

13.0 EXPECTED RANGES OF VALUES

A study of an apparently normal adult population was undertaken to determine expected values for the PTH test system and is presented in Table 1

TABLE 1
Expected Values for the PTH Test System

Population	PTH Values (in pg/ml)
Normal	9.0-94

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 precisions of the PTH AccuBind® ELISA Test System were determined by analyses on different levels of pool control sera. The number (N), mean values (X), standard deviation (σ) and coefficient of variation (CV) for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Serum 1	16	51.8	3.29	6.3%
Serum 2	16	244	9.33	3.8%

TABLE 3
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Serum 1	16	19.6	1.61	8.2%
Serum 2	16	56.9	3.88	6.8%
Serum 3	16	136	9.1	6.7%

14.2 Sensitivity

The PTH AccuBind® ELISA Test System has a sensitivity of 0.49. The sensitivity (detection limit) was ascertained by determining the variability f the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The PTH AccuBind® ELISA Test System was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 110. The least square regression equation and the correlation coefficient were computed for the PTH test method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Least Square Regression Analysis	Correlation Coefficient
This Method (X) Reference (Y)	y = 2.7005 +1.0124(X)	0.996

Only slight amounts of bias between the PTH AccuBind® ELISA Test System and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

15.0 REFERENCES

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6. Kruger, L.., Rosenblum, S., Zaazra, J. and Wong, J. "Intact PTH is stable in unfrozen EDTA plasma for 48 hours prior to laboratory Analysis". *Clin. Chem*. 41:6: page S47, 1995.

Revision: 0 **Date: 2016-JAN-01** **DCO: N/A**
Product Code: 90*25-300*
MP9025

Size		96(A)
Reagent (fill)	A)	0.5ml set
	B)	0.5ml set
	C)	1 (7ml)
	D)	1 (7ml)
	E)	1 plate
	F)	1 (25ml)
	G)	1 (12ml)
	H)	1 (12ml)

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