Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

Intact-PTH [Parathyroid Hormone]
ELISA *[Enzyme-Linked ImmunoSorbent Assay]*

Specific quantitative assay for the determination of Intact parathyroid hormone in serum

Catalog # S-1226

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

INTENDED USE

This Intact-PTH ELISA is intended for the quantitative determination of Intact-PTH (Parathyroid Hormone) in human serum. This assay is intended for *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

PTH (Parathyroid hormone, Parathormone, Parathyrin) is biosynthesized in the parathyroid gland as a pre-proparathyroid 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 polypetide, proparathyroid 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[1]. 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[2].

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[2]. 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[3].

The most common other cause of hypercalcemia, namely hypercalcemia of malignancy, is associated with suppressed levels of parathyroid hormone[3] or PTH levels within the normal range[4]. 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[3,4,5].

Unlike C-terminal and midregion PTH, which typically are grossly elevated in subjects with renal insufficiency, intact PTH assays are less influenced by the declining renal function[5].

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.

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

PRINCIPLE OF THE TEST

This 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.

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

KIT COMPONENTS

Catalog Number	Kit Components	Description	Quantity
30-1602	Reagent 1	Biotinylated PTH Antibody	1 x 5 mL
30-1615	Reagent 2	Peroxidase (Enzyme) labeled PTH Antibody	1 x 5 mL
30-1611	Reagent 3	TMB Substrate [tetramethylbenzidine]	1 x 15 mL
30-1614	Reagent 4	Diluent [equine serum] for Patient Samples read off-scale	1 x 2 mL
30-1613	Reagent 5	ELISA Wash Concentrate [Saline with surfactant]	1 x 30 mL
30-1612	Reagent 6	ELISA Stop Solution [1 N sulfuric acid]	1 x 10 mL
30-1616	Reagent 7	Reconstitution Solution containing surfactant	1 x 5 mL
30-1601	Microplates	One holder with Streptavidin Coated Strips.	12 x 8-well strips
30-1603 30-1604 30-1605 30-1606 30-1607 30-1608	Calibrators A: 0 pg/mL B: C: D: E: F: Refer to vial labels for exact concentrations	Lyophilized [except zero calibrator] synthetic h-PTH. Zero calibrator [BSA solution with goat serum]. is in liquid form, ready to use. All other calibrators consist of synthetic h-PTH (1-84) in BSA solution with goat serum.	1 x 0.5 mL per level
30-1609 30-1610	Controls 1 & 2 Refer to vial labels for exact ranges	Lyophilized. 2 Levels. Synthetic h-PTH (1-84) in BSA solution with goat serum.	1 x 0.5 mL per level

MATERIAL AND EQUIPMENT 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.

WARNINGS AND PRECAUTIONS FOR USERS

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.

Reagent 6, Stop 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.

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

SAMPLE COLLECTION AND STORAGE

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[6]. 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 blood to clot, the serum or plasma should be promptly separated, preferably in a refrigerated centrifuge, and stored at -20°C or lower. Serum samples may be stored up to 8 hours at 2-8°C. Serum samples frozen at -20°C are stable for up to 4 months.

REAGENT PREPARATION AND STORAGE

Store all kit components at 2-8 ^{o}C except Wash Concentrate and Stop Solution upon receipt prior to use

1. All reagents except the non-zero calibrators, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2-8 ^{o}C, except the Wash Concentrate, which should be kept at room temperature until dilution to avoid precipitation.

2. For each of the non-zero calibrators (Calibrator B through F) and kit controls 1 and 2, reconstitute each vial with 500 µL of Reagent 7 (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 ^{o}C for 6 weeks after reconstitution with up to 3 freeze thaw cycles when handled as recommended in "Procedural Notes" section.

3. Reagent 5: 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.

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

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.

2. Pipet **25 µL** of sample 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 sample.

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°-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 5), 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 3 (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°-28°C).

8. Add or dispense **100 µL** of the Reagent 6 (Stop 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** against 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.

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

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 4 (Sample Diluent) and reassayed. Multiply the result by the dilution factor.

- Reagents from different lot numbers must not be interchanged.

- 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.

Assay Protocol Flow-Diagram

Microplate Well Position	Sample	Calibrators Controls *OR* Patients	Reagent 1 Biotinylated Antibody Solution	Reagent 2 Enzyme Conjugate Antibody	Incubate at room temperature	Working Wash Solution Reagent 5	Reagent 3 TMB Substrate	Incubate at room temp.	Reagent 6 Stop (Acid) Solution	Read Aborbance. At 450 nm and 405 nm
A1	Distilled Water	250 µL								Read against distilled or deionized water
B1	Calibrator A	25 µL	50 µL	50 µL	3 ± ½ hours @ 170 ± 10 rpm	350 µL	150 µL	30 ± 5 minutes @ 170 ± 10 rpm	100 µL	
C1	Calibrator B					Wash 5 times Aspirate				
D1	Calibrator C									
E1	Calibrator D									
F1	Calibrator E									
G1	Calibrator F									
H1	Control 1									
A2	Control 2									
B2	Patient 1									
C2	Patient 2									
D2	ETC.									
E2	ETC.									
F2	ETC.									

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

CALCULATION OF RESULTS

Manual Method

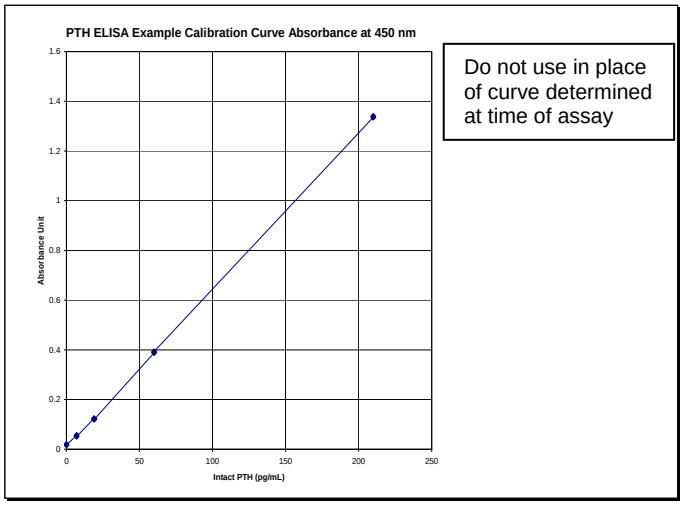
1. For the 450 nm readings, construct a dose response curve (calibration curve) using the first five calibrators provided, i.e. Calibrators A, B, C, D and E. For the 405 nm readings, construct a second dose response curve using the three calibrators with the highest concentrations, i.e. Calibrators D,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

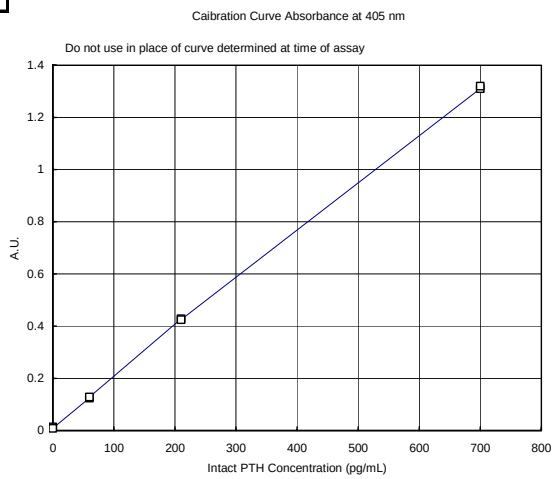
2. Assign the concentration for each calibrator stated on the vial in pg/mL. Plot the data from the calibration curve on linear graph paper with the concentration on the X-axis and the corresponding A.U. on the Y-axis.

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.





Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

Sample Data *at 450 nm* [raw A.U. readout against distilled or deionized water]

Microplate Well	**1st Reading** Absorbance Unit	**2nd Reading** Absorbance Unit	**Average** Absorbance Unit	Intact PTH **pg/mL**	**Intact PTH pg/mL – Result to report**
Calibrator A	0.020	0.016	0.018		0
Calibrator B	0.056	0.051	0.054		7
Calibrator C	0.124	0.119	0.122		18
Calibrator D	0.388	0.393	0.391		55
Calibrator E	1.335	1.340	1.338		210
Control 1	0.200	0.200	0.200	27.6	27.6
Control 2	0.804	0.794	0.799	119	119
Patient Sample 1	0.147	0.136	0.142	19.1	19.1
Patient Sample 2	0.407	0.409	0.408	58.5	58.5
Patient Sample 3	2.375	2.454	2.415	> 200	*
Patient Sample 4	3.725	3.725	3.725	> 200	*

* Because 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.

Sample Data *at 405 nm* [raw A.U. readout against distilled or deionized water]

Microplate Well	**1st Reading** Absorbance Unit	**2nd Reading** Absorbance Unit	**Average** Absorbance Unit	Intact PTH **pg/mL**	**Intact PTH pg/mL – Result to report**
Calibrator A	0.014	0.008	0.011		0
Calibrator D	0.124	0.128	0.126		55
Calibrator E	0.428	0.425	0.427		210
Calibrator F	1.309	1.317	1.313		700
Control 1	0.074	0.066	0.070	< 200	¶
Control 2	0.260	0.251	0.256	121	∏
Patient Sample 1	0.049	0.043	0.046	< 200	¶
Patient Sample 2	0.132	0.133	0.133	< 200	¶
Patient Sample 3	0.758	0.782	0.770	401	401
Patient Sample 4	1.314	1.321	1.318	> 700	⇐

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

¶ For samples with readout < 200 pg/mL, it is recommended to use the data obtained at 450 nm as shown in **Sample Data** *at 450 nm* in the table above. This practice should give the results with optimum sensitivity of the assay.

∏ Although the readout for Control (2) < 200 pg/mL**,** it is recommended that the actual result be read out and recorded for quality control evaluation purposes. Further, absorbance for Control 2 is sufficiently high to be analytically valid.

⇐ The absorbance readout is off-scale or higher than the average absorbance of the highest calibrator. Sample should be repeated with dilution.

NOTE: The data presented are for illustration purposes only and must not be used in place of data generated at the time of the assay.

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.

LIMITATIONS OF THE PROCEDURE

This PTH ELISA kit has exhibited no “high dose hook effect” with samples spiked with 1,000,000 pg/mL of Intact PTH. Samples with intact PTH levels greater than the highest calibrator, however, should be diluted and reassayed for correct values.

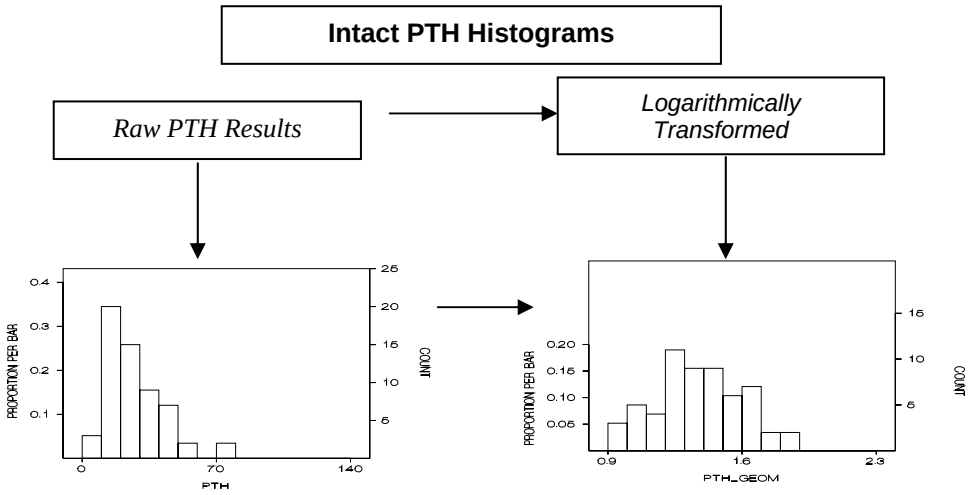
Like any analyte used as a diagnostic adjunct, intact PTH results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

EXPECTED VALUES

Intact PTH levels were measured in fifty-eight (58) apparently normal individuals in the U.S. using this intact PTH ELISA. The values obtained ranged from 8.8 to 76.6 pg/mL. Based on statistical tests on skewness and kurtosis, the population, when transformed logarithmically, follows the normal or Gaussian distribution as shown in the histograms. The geometric mean ± 2 standard deviations of the mean were calculated to be 8.3 to 68.0 pg/mL.

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

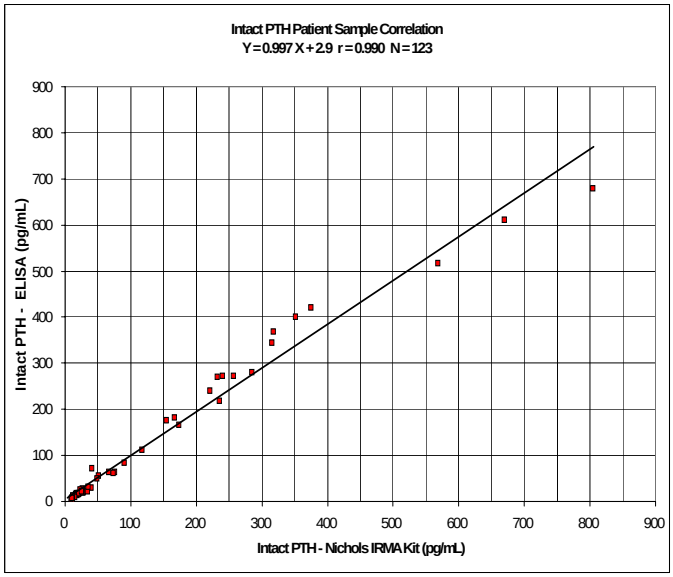


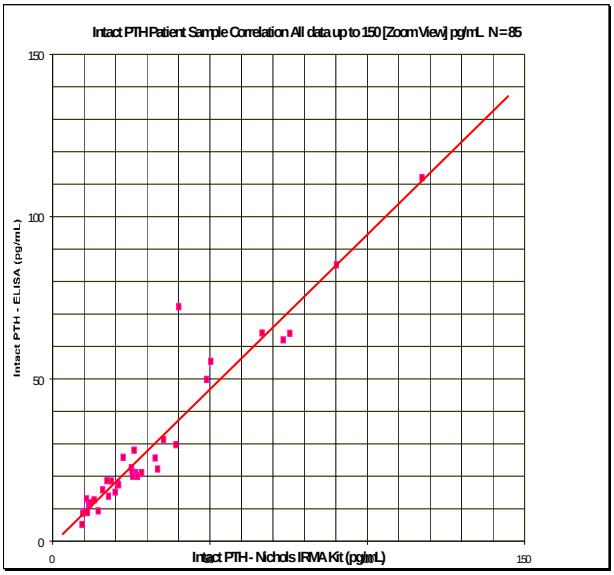
PERFORMANCE CHARACTERISTICS

Accuracy

One hundred twenty-three patient samples, with intact PTH values ranging from 3.2 to 805 pg/mL were assayed by this ELISA procedure and the Nichols PTH Immunoradiometric Assay. Linear regression analysis gives the following statistics:

ELISA = 0.997 1st IRMA Kit + 2.9 pg/mL	r = 0.990	N = 123





Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

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. This PTH ELISA has a calculated sensitivity of 0.9 pg/mL.

Specificity and Cross-Reactivity

The antibodies used in this PTH 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 give molar crossreactivites to PTH of less than 2% and 0.02%, respectively.

Precision and Reproducibility

The precision (intra-assay variation) of this PTH ELISA Test was calculated from 20 replicate determinations on each of the two samples.

Intra-Assay Variation

Sample	Mean Value (pg/mL)	N	Coefficient of Variation %
A	39.6	20	3.2
B	171	20	1.8

The total precision (inter-assay variation) of this PTH ELISA Test was calculated from data on two samples obtained in 21 different assays, by three technicians on two different lots of reagents, over a three-week period

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

Inter-Assay Variation

Sample	Mean Value (pg/mL)	N	Coefficient of Variation %
A	32.7	21	7.7
B	132	21	7.0

Recovery

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

Serum Sample	**Endogenous**	**PTH**	**Expected**	**Measured**	**Recovery**
	PTH	added	Value	Value	(%)
	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	
A	65.9	--	--	--	--
	59.3	132	191	197	103.1%
	52.7	264	317	330	104.2%
	46.1	396	442	448	101.3%
B	102	--	--	--	--
	91.80	132	223.80	234.00	104.6%
	81.60	264	345.60	353.00	102.1%
	71.40	396	467.40	484.00	103.6%
C	35.6	--	--	--	--
	32.04	132	164.04	174.00	106.1%
	28.48	264	292.48	307.00	105.0%
	24.92	396	420.92	417.00	99.1%

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

Linearity of Patient Sample Dilutions: Parallelism

Four patient serum samples were diluted with Reagent 4 (the Diluent for Patient Samples read off-scale). Results in pg/mL are shown below:

Sample	Dilution	Expected	Observed	% Observed ÷ Expected
A	Undiluted	-	641	-
	1:2	321	309	96%
	1:4	160	138	86%
	1:8	80.1	78.2	98%
B	Undiluted	-	>700	-
	1:2	-	356	-
	1:4	178	166	93%
	1:8	89.0	96.7	109%
C	Undiluted	-	410	-
	1:2	205	161	79%
	1:4	103	81.2	79%
	1:8	51.3	49.4	96%
D	Undiluted	-	649	-
	1:2	325	300	92%
	1:4	162	132	81%
	1:8	80.1	74.5	93%

Peninsula Laboratories International, Inc.

305 Old County Road, San Carlos, CA 94070
Tel: (650) 801-6090
Fax: (650) 595-4071

REFERENCES:

1. Segre, G.V., Niall H.D., Habener J.F. et. al. : Metabolism of parathyroid hormone: physiological and clinical significance. **Am. J. Med.** 56: 774,1974.

2. Mallete, L.E., Gagel, R.F.: Parathyroid Hormone and Calcitonin. In: Murray J.F. (ed) **Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism**. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 65-69, 1990.

3. Bilezikian, J.P.: Primary Hyperparathyroidism. In: Murray J.F. (ed) **Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism**. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 109-111, 1990.

4. Stewart, A.F.: Humoral Hypercalcemia of Malignancy. In: Murray J.F. (ed) **Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism**. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 115-118, 1990.

5. Mallette, L.E.: The parathyroid polyhormones: New concepts in the spectrum of peptide hormone action. Endocrin. Rev. 12:110-117, 1991.

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.

Further Suggested Reading:

1. Raisz, L.G., Yajnik, C.H., Bockman, R.S., and Bower, B.B.: Comparison of commercially available parathyroid hormone immunoassay in the differential diagnosis of hypercalcemia due to primary hyperparathyroidism or malignancy. **Ann. Intern. Med.** 91:739-740, 1979.

2. Endres, D., Brickman, A., Goodman, W., Maloney, D., and Sherrard, D.: N-Terminal PTH radioimmunoassays in assessment of renal osteodystrophy. **Kidney International**. 21:132, 1982.

3. Dambacher, M.A., Fischer, J.A., Hunziker, W.H., et.al.: Distribution of circulating immunoreactive components of parathyroid hormone in normal subjects and in patients with primary and secondary hyperparathyroidism: the role of kidney and of the serum calcium concentration. **Clin. Sci.** 57:435,1979.

4. Kao, P.C., Jiang, N.S., Klee, G.G., and Purnell, D.C.: Development and validation of a new radioimmunoassay for parathyrin (PTH). **Clin. Chem.** 28:69, 1982.

5. Endres, D.B., Villanueva, R., Sharp, C.F. Jr, Singer, F.R.: Measurement of parathyroid hormone. **Endocrinol Metab. Clin. North Am.** 18:611-629,1989.

6. Kao, P.C., van Heerden, J.A., Grant, C.S., Klee, G.G., Khosla S: Clinical performance of parathyroid hormone immunometric assays. **Mayo Clin. Proc.** 67:637-645, 1992.

7. Marcus, R.: Laboratory diagnosis of primary hyperparathyroidism. **Endocrinol Metab. Clin. North Am.** 18:647-658, 1989.