Rat Total Intact PTH ELISA Kit

Enzyme-Linked ImmunoSorbent Assay (ELISA)
(Coated Plate-Technology)
For the quantitative determination of Rat Total Intact PTH.
****For Research Use Only**

Scantibodies
Laboratory, Inc.

(Part Number: 3KG024)

Store at 2 - 8° C

INTENDED USE

The Scantibodies Rat Total Intact PTH ELISA kit has been designed for the quantitative determination of immunoreactive rat total intact PTH (Rat Total Intact PTH) in rat serum samples. The Total Intact PTH (parathyroid hormone) level is the sum of PTH (1-84) and N-terminal truncated PTH fragments in rat serum.

PHYSIOLOGY

Biologically active rat PTH is an 84 amino acid polypeptide directly synthesized and secreted by rat parathyroid glands. Circulating ionized calcium, phosphate and vitamin D levels are among the most important factors that regulate the circulating rat PTH level (1). PTH (1-84) is metabolized either intra glandular or in the peripheral organs into smaller C-terminal fragments (2, 3). In humans it has been demonstrated that endogenously found large C-terminal fragments (e.g., 7-84 PTH) are produced in the parathyroid glands and secreted with changes in serum calcium and upon administration of vitamin D analogs.

PTH is one of the major calcium and bone regulating hormones. A consistently elevated PTH level in blood circulation would have a catabolic effect resulting in an increased bone resorption and increased serum calcium level (4). On the other hand, if the bone were exposed to a condition of intermittently high dose PTH level, anabolic effects of PTH would be observed.

Administration of human 7-84 PTH in rats has made possible the demonstration that 7-84 PTH has inverse biological actions to 1-84 PTH. 7-84 PTH is hypocalcemic, anti resorptive, inhibits the formation and activation of osteoblasts and osteoclasts (5, 6).

Rat is an optimal small animal model to study calcium regulating hormones and bone mineral metabolism. Precise determination of rat PTH (1-84) and PTH (7-84) levels would be very helpful in research of bone metabolism regulation. Evaluation of rat PTH levels would be important in understanding its impact on calcium and bone metabolism in pre-designed research studies. It has been reported that application of the PTH (1-84)/PTH (7-84) ratio in End Stage Renal Disease (ESRD) patients improved non-invasive assessment of bone turnover (7, 8). Therefore, simultaneous determination of rat total intact PTH level (1-84 PTH plus mainly rat PTH 7-84) and rat whole PTH (1-84) (SLI 3KG025) allows detailed study of bone turnover mechanism in an animal model.

PRINCIPLE OF PROCEDURE

Scantibodies Rat Total Intact PTH ELISA kit is a one-wavelength, two-site Enzyme-Linked ImmunoSorbent Assay (ELISA) utilizing polyclonal antibodies directed against the extended N-terminal and the C-terminal/midregional portions of the rat PTH (parathyroid hormone) peptide. The use of these highly specific antibodies ensures in the Rat Total Intact PTH assay that rat PTH (1-84) and the N-terminal truncated PTH fragments (mainly 7-84 PTH) are detected.

The rat PTH N-terminal antibody is labeled with horseradish peroxidase. The antibody directed against the C-terminal /mid-regions of rat PTH is immobilized onto the surface of the plastic wells of the 96-well ELISA microtiter plates. The rat PTH calibrators, controls and the unknown rat serum samples are simultaneously incubated with the two antibodies in microtiter plate wells. The PTH is captured between the two antibodies and therefore immobilized onto the surface of the plastic wells of the microtiter plates as seen in Figure 1.

After incubation, free and bound label antibodies are separated by simple aspiration and washing steps. During the short incubation with the substrate TMB (tetramethylbenzidine) there is a reaction with the peroxidase conjugated to the tracer antibody resulting in a blue color. The addition of a stopping solution converts the color to a stable yellow solution and the absorbance is read immediately at 450 nm. The concentration of

rat PTH in the samples is directly proportional to the yellow color intensity. The concentration of PTH in the unknown samples and controls is determined by interpolation from the calibration curve which is established with the standards provided.

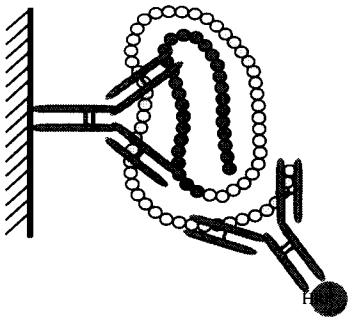


Figure 1

PREPARATION AND STORAGE OF REAGENTS

The Scantibodies Rat Total Intact PTH ELISA kit contains sufficient reagents for 96 single determinations. The kit is stable at 2° - 8° C until the stated expiration date.

SCANTIBODIES ENZYME-LABELED RAT PTH ANTIBODY (3AR167)

Two 8 mL bottles of Peroxidase-labeled Rat Total Intact PTH Antibody-are ready for use. Each contains goat anti-rat PTH conjugate, preservative and protein stabilizers. These conjugate reagents are to be stored at 2° - 8° C and protected from exposure to light.

SCANTIBODIES RAT PTH ANTIBODY COATED MICROTITER PLATE (3KP009)

One holder with Rat PTH Antibody Coated Strips (12x8 well strips) is provided with each kit. The strips are to be stored at 2° - 8° C.

SCANTIBODIES TMB SUBSTRATE (3KL130)

One 16 ml bottle of TMB (tetramethylbenzidine) substrate is provided with each kit. The substrate reagent is ready for use and to be stored at 2° - 8° C and protected from exposure to light.

SCANTIBODIES ELISA WASH CONCENTRATE (3KW018)

One-bottle of wash contains 15 ml of a 20 fold concentrate of phosphate buffered saline with preservative and surfactant. Prior to use, dilute the contents to 300 ml with deionized water and mix well. Store the concentrate at 2° - 8° C until needed.

SCANTIBODIES ELISA STOP SOLUTION (3KL132)

One 12 ml bottle of sulfuric acid (1N) is ready for use and to be store at 2°- 8° C until needed.

RAT TOTAL INTACT PTH STANDARDS (3CA631, 3CB631, 3CC631, 3CD631, 3CE631, 3CF631)

Five vials each containing rat PTH (1-84) peptide lyophilized in a protein matrix with a non-azide, non-mercury preservative are provided. 3CA631 is considered the "zero" standard. Refer to the vial labels for the exact PTH concentrations. Reconstitute each vial of standard with 1.0 ml of deionized water before use. Allow the reconstituted standards to sit for approximately 10 minutes at room temperature with occasional gentle swirling and inversion to assure complete reconstitution prior to use.

Use the standards immediately after reconstitution; freeze the unused portion of the standards for later use. After reconstitution the standards are stable until the expiration date specified on the kit box when stored at -20° C or below with up to 2 freeze/thaw cycles.

RAT TOTAL INTACT PTH CONTROLS I & II (3CA630, 3CB630)

Two vials each containing rat PTH (1-84) peptide lyophilized in a protein matrix with a non-azide, non-mercury preservative are provided. Refer to the vial label for the control ranges representing acceptable recoveries for accepting the values in the assay run. Reconstitute each control with 1.0 mL of deionized water before use. Allow the reconstituted controls to sit for approximately 10 minutes at room temperature with occasional gentle swirling and inversion to assure complete reconstitution prior to use.

Use the controls immediately after reconstitution; freeze the unused portion of the controls for later use. After reconstitution the controls are stable until the expiration date specified on the kit box when stored at -20° C or below with up to 2 freeze/thaw cycles.

PLATE SEALER

Two plastic sealers included in the kit; used to prevent evaporation of the assay mixtures and cross-contamination of the wells during assay incubations.

WARNINGS AND PRECAUTIONS FOR USERS

USE OF THE ASSAY

The reagents are for Research Use Only.

SAFETY PRECAUTION

Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid (i.e., ELISA HRP Substrate and ELISA Stop Solution). TMB is a suspected carcinogen.

TMB is dissolved in a solution which contains acetone, an irritant to skin and mucous membrane. In case of contact with any of these reagents, wash thoroughly with water.

Use Good Laboratory Practices. Wash hands before eating. Do not eat, drink or smoke in the work area.

SAMPLE PREPARATION AND STORAGE

SPECIMEN COLLECTION

Rat serum samples are used for this rat total intact PTH determination. Fifty microliters of serum are required to assay the sample in duplicate (25 ul is needed for each determination). Collect blood (do not use anticoagulants) and allow it to clot at room temperature. Centrifuge the sample at 600 x g for 15 minutes at room temperature and separate the serum from the cells. Samples should be assayed immediately or stored frozen at -20° C or below. Avoid addition of sodium azide to the samples as sodium azide is reported to inhibit HRP activity. Avoid more than 2 repeated freezing and thawing of specimens.

Use of certain anesthetics on rats from which specimens have been collected has been reported to cause significant elevation in serum PTH concentration (9). It is imperative to use consistent sample collection procedures within studies.

QUALITY CONTROL

Two levels of assay controls are provided with each assay kit for quality control purposes. They should be tested in each assay run. The value ranges assigned to these controls are printed on the control vial labels. In order to consider the results from the assay as valid, the control values determined in each assay run should fall within the specified ranges and the controls must be tested in the same manner as the unknown samples. If the control values do not fall within the established range, the assay may be invalid and should be repeated.

MATERIALS AND EQUIPMENT

MATERIAL PROVIDED

The Kit (Part No. 3KG024) is supplied with the following:

Descriptions	Number
Scantibodies Rat Total Intact PTH Standards Part Nos. 3CA631, 3CB631, 3CC631, 3CD631, 3CE631, 3CF631	6 vials
Scantibodies Rat Total Intact PTH Controls Part Nos. 3CA630, 3CB630	2 vials
Scantibodies Rat PTH Antibody Coated Plate Part No. 3KP009	1 plate
Scantibodies Peroxidase Rat Total Intact PTH Antibody Part No. 3AR167	2 vials
TMB Substrate Part No. 3KL130	1 bottle
Scantibodies Stop Solution Part No. 3KL132	1 bottle
Scantibodies ELISA Wash Concentrate Part No. 3KW018	1 bottle
Directional Insert Part No. 7KI057	1 insert

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. 1.0 mL volumetric pipettes for reconstituting standards and controls.
2. Precision pipettes capable of delivering 25 µL, 100 µL and 150 µL.
3. Repeating dispenser suitable for delivering 100 µL and 150 µL.
4. Aspiration device or suitable microtiter plate washer.
5. Container for storage of wash solution.
6. Spectrophotometric microtiter plate reader capable of reading absorbance at 450 nm.
7. Deionized water.
8. Microplate shaker capable of maintaining a rotational speed of 350 RPM.
9. Timer.
10. Aluminum foil.

ASSAY PROCEDURES

1. Reconstitute each standard and control with 1.0 ml deionized water each.
2. Place a sufficient number of Rat PTH Antibody Coated Strips in a holder to run PTH standards, controls, unknown samples, and blanks.
3. Pipette 25 µL of standard, control, or sample in duplicate into the designated or mapped wells. Freeze the remaining standards and controls as soon as possible (within 2 hours) after

reconstitution or thawing. Leave two wells as the blank duplicate with no reagents will be added to these wells except for wash solution, substrate and stop solution.

4. Pipette 150 µL of Peroxidase Rat Total Intact PTH Antibody into each well except the blank duplicate.
5. Cover the plate with one plate sealer and then cover with aluminum foil to avoid exposure to light. Incubate plate at room temperature for two hours on a Microplate Shaker set at speed 350.
6. Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well five times by dispensing 350 µL of diluted wash solution into each well, including the blank duplicate, and then completely aspirate the contents.
7. Pipette 150 µL of TMB Substrate into each of the wells, including the blank duplicate.
8. Recover the plate with a plate sealer and aluminum foil. Incubate at room temperature for 30 minutes on a Microplate Shaker set at speed 350.
9. Remove the aluminum foil and plate sealer. Pipette 100 µL of ELISA Stop Solution into each of the wells. Mix gently.
10. Read the absorbance at 450 nm within 5 minutes in the microtiter plate reader.

PIPETTING GUIDE

Additive to Well	Calibrator Wells	Control Wells	Sample Wells
Blank	-	-	-
Standard	25 µL	-	-
Control	-	25 µL	
Sample	-	-	25 µL
Peroxidase-Rat Total Intact PTH Antibody	150 µL	150 µL	150 µL
Cover plate and shield from light. Shake the plate at speed 350 RPM for 2 hours. Aspirate wells and wash each well with 350 µL diluted wash solution aspirating the contents of the wells after each wash step. Repeat for a total of 5 washes for each well.			
Add 150 µL of TMB Substrate to each well. Cover plate and shield from light. Shake the plate at speed 350 RPM for 30 minutes.			
Add 100 µL of Stop Solution to each well. Read absorbance of the wells at 450 nm within 5 minutes.			

PROCEDURAL COMMENTS

1. The samples and all reagents should be pipetted carefully to minimize air bubbles in the wells. To achieve this, "reverse pipetting" described in the package insert of the manufacturers of Pipettors is recommended.
2. Rat PTH is a very labile molecule. Set up the assay immediately upon the reconstitution or the thawing of all calibrators, controls and samples.
3. It is recommended that all standards, controls, rat samples, and blank be assayed in duplicate. Reagents from different lot numbers must not be interchanged.
4. Store light sensitive reagents (i.e., Peroxidase Conjugated Antibody and TMB Substrate) in the original amber bottles or another suitable container, which is well protected from light.
5. The sequence and timing of each reagent addition is important as both the immunological and enzymatic reactions are in kinetic modes when the assay is in progress. **Aspiration device or suitable microtiter plate washer is strongly recommended.** All pipetting and washing steps should be performed such that the timing is as consistent as possible.
6. Samples with values greater than the highest standard should be diluted with the 0 pg/ml Standard and assayed again.
7. Store any unused Antibody Coated Strips in the resealable aluminum pouch with desiccant to protect from moisture.
8. Freeze/thaw of rat serum may lead to aggregate formation. Therefore, centrifugation of these samples at 600 x g for 15 minutes at room temperature is recommended prior to the assays to remove all particulate matter which can cause variations and random high non-specific binding on well surface.
9. Avoid sample to sample contamination by using a new pipetting tip for each sample.

The unknown samples, standards and controls should be pipetted carefully into the bottom of the assay wells. This is to avoid losing liquid on the side of the wells.

The washing step is an important step in the assay procedure. Accurate dispensing of the wash solution and complete aspiration of the well contents is essential to achieving assay sensitivity, low background and assay precision.

LIMITATIONS OF THE PROCEDURES

This assay is intended for research purposes only and is not intended to be used for human specimens.

The assay procedure must be followed exactly; careful technique must be used to obtain valid results. Any modification to the provided procedure is likely to alter the results.

Grossly hemolyzed, lipemic or icteric samples may give invalid results.

The highest concentration of rat total intact PTH measurable without sample dilution is the concentration of the highest standard. The lowest level measurable is approximately 2 pg/ml.

CALCULATION OF RESULTS

Use the rat PTH standards contained in the kit for construction of this calibration curve. Refer to the individual vial label for exact concentration of rat PTH.

The single wavelength absorbance reading taken after the addition of the ELISA Stop Solution allows for the construction of the only calibration curve needed for this test. The average absorbance of the blank duplicate should be subtracted from the average absorbance of the standards, controls and unknown duplicates prior to construction of the calibration curve and calculation of the total intact PTH concentrations in the controls and unknowns.

MANUAL METHOD

1. Read the plate at 450 nm. Subtract the blank from the average absorbance of each duplicate. Construct a calibration curve using the whole six calibrators provided, i.e., Calibrators A, B, C, D, E and F.
2. Assign the concentration for each standard 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 "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.

AUTOMATED METHOD

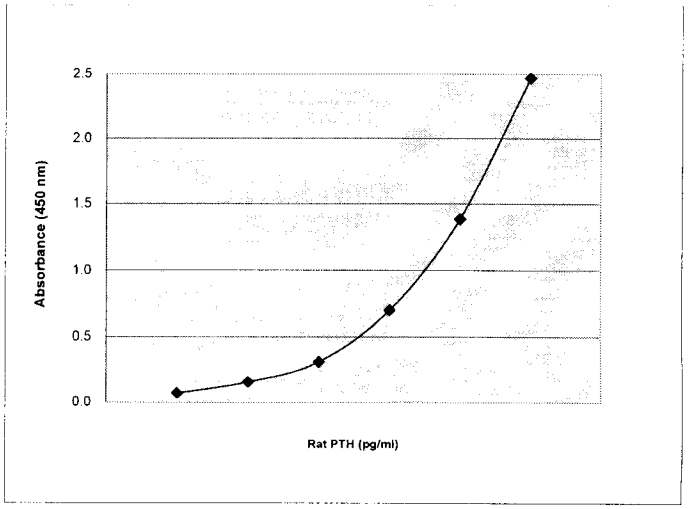
SoftMax Pro 5 computer program (Molecular Devices, CA, USA) or equivalent is used for data collection, data analysis, data reduction and graphing. 4 Parameter Logistic (4PL) is applied to analyze the calibration for acceptance with a criterion that the correlation coefficient must be greater than 0.975 ($R > 0.975$ or $R^2 > 0.950$). The representative calibration curve presented in the following section gives an R value of 0.9996.

SAMPLE DATA AT 450 NM (ABSORBANCE UNIT A.U. READOUT)

Microplate Well	1st Reading : Adjusted AU	2nd Reading : Adjusted AU	Average: Adjusted AU	Total Intact PTH (pg/ml)
Standard A	0.007	0.015	0.011	0
Standard B	0.092	0.093	0.092	33.5
Standard C	0.243	0.241	0.242	90
Standard D	0.614	0.616	0.615	238
Standard E	1.214	1.221	1.218	482
Standard F	2.427	2.482	2.454	927
Control I	0.149	0.152	0.151	50.08
Control II	0.727	0.739	0.733	280.26
Rat Sample #1	0.490	0.484	0.487	186.44
Rat Sample #2	0.314	0.321	0.318	121.83
Rat Sample #3	0.621	0.616	0.619	236.51
Rat Sample #4	0.449	0.440	0.445	170.25

***NOTE**: When the absorbance readout of a sample is off-scale or higher than the average absorbance of the highest standard, the sample should be repeated with dilution. The data presented are for illustration purposes only and must not be used in place of data generated at the time of the assay.

REPRESENTATIVE CALIBRATION CURVE



EXPECTED VALUES

The expected values were determined with 30 x 3 serum samples collected from 30 apparently normal Sprague-Dawley rats (15 female and 15 male, age 6 - 8 weeks). These values represent the 95% confident range.

Rat serum specimens	No. of samples tested	Mean tPTH (pg/ml)	Expected tPTH range (pg/ml)
Apparently normal rats	90	99.2	25.1 – 216.6

PERFORMANCE CHARACTERISTICS

RECOVERY, SPIKE

Rat PTH (1-84) at various concentrations was spiked into two rat serum samples containing different amounts of rat Total Intact PTH as indicated below. The percent recoveries were then calculated.

Sample tPTH Conc. (pg/ml)	Added (pg/ml)	Expected (pg/ml)	Measured (pg/ml)	Recovery (%)
35.1	50	85.1	91.6	107.6
35.1	250	285.1	309.5	108.6
35.1	500	535.1	507.5	94.8
35.1	750	785.1	792.8	101.0
181	50	231	244	105.4
181	250	431	424	98.4
181	500	681	722	106.1
181	750	931	898	96.4

RECOVERY, DILUTION

Three rat serum samples were diluted with the Rat PTH zero standard. The % recovery was determined following assay of the diluted samples.

Sample	Kit Lot	Dilution	Measured Value (pg/mL)	Expected Value (pg/mL)	% Recovery
1	E1	Neat	863.5	863.5	
		1:2	444.4	431.8	102.9
		1:4	210.6	216.0	97.5
		1:8	96.5	108.0	89.4
		1:16	40.3	54.0	74.6
	E2	Neat	732.2	732.2	
		1:2	406.5	366.1	111.0
		1:4	203.6	183.1	111.2
		1:8	106.7	91.5	116.6
		1:16	53.4	45.8	116.6
		1:32	25.6	22.9	111.8
	E3	Neat	674.7	674.7	
		1:2	383.5	337.4	113.7
		1:4	187	168.7	110.8
		1:8	100.9	84.3	119.7
		1:16	51.4	42.2	121.8
		1:32	23.8	21.1	112.8
				Average	**107.9**
2	E1	Neat	851.0	851.0	
		1:2	394.9	425.5	92.8
		1:4	219.0	213.0	102.8
		1:8	110.4	106.4	103.8
		1:16	47.8	53.2	89.8
		1:32	26.0	26.6	97.7
	E2	Neat	832.7	832.7	
		1:2	396.0	416.4	95.1
		1:4	196.7	208.1	94.5
		1:8	96.5	104.0	92.8
		1:16	46.9	52.0	90.2
		1:32	19.8	26.0	76.2
	E3	Neat	853.5	853.5	
		1:2	411.7	426.8	96.5
		1:4	181.0	213.4	84.8
		1:8	102.2	107.0	95.5
		1:16	50.6	53.3	94.9
		1:32	22.7	26.7	85.0
				Average	**92.8**

PRECISION

Intra-assay coefficient of variation was determined by performing 20 replicate determinations on three rat serum samples.

Sample	Mean Value (pg/ml)	SD (pg/ml)	Coefficient of Variation
1	47.63	1.58	3.3%
2	308.47	11.62	3.8%
3	648.87	17.94	2.8%

ACCURACY

Inter-assay coefficient of variation was determined by performing 12 different assays on three rat serum samples.

Sample	Mean Value (pg/ml)	SD (pg/ml)	Coefficient of Variation
1	58.2	3.6	6.2%
2	327.5	11.3	3.5%
3	649.8	35.3	5.4%

SENSITIVITY

The detection limit of the assay is defined as the lowest measurable value distinguishable from zero by the test of greater than 2SD from the mean of the zero point. This sensitivity was determined by assaying the zero standard 20 times in the same assay. The detection limit was found to be approximately 2 pg/ml.

SPECIFICITY

Synthetic human PTH and rat PTH peptide fragments were spiked into rat serum and tested for cross-reactivity in the assay.

Peptides	Endogenous tPTH (pg/ml)	Peptide added (pg/ml)	Meas. values (pg/ml)	cross-reactivity (%)
Rat PTH (1-34)	85.3	1,000	84.3	-0.09
Rat PTH (1-34)	85.3	2,000	86.7	0.07
Rat PTH (39-84)	85.3	1,000	91.5	0.11
Rat PTH (39-84)	85.3	2,000	90.0	0.24
Human PTH (7-84)	85.3	1,000	95.6	1.03
Human PTH (7-84)	85.3	2,000	94.5	0.46
Human PTH (1-84)	85.3	1,000	91.9	0.66
Human PTH (1-84)	85.3	2,000	93.6	0.42

HIGH DOSE HOOK

Samples spiked with increasing amounts of rat PTH (1-84) read higher absorbance than the highest standard.

	Rat PTH concentration (pg/ml)	OD 450 nm
Highest standard	927	2.188
Hook sample	>20,000	3.958

INTERFERENCE SUBSTANCE

Rat hemoglobin was spiked at increasing concentrations to a rat serum sample to study its interference to rat total intact PTH determination.

Endogenous Rat tPTH (pg/ml)	Hemoglobin Conc. (mg/dl)	Meas. Rat tPTH (pg/ml)	% change
330.1	0	330.1	N/A
330.1	1.875	336.0	1.79
330.1	3.75	338.7	2.61
330.1	7.5	317.9	-3.70
330.1	15	317.0	-3.97

PTH LITERATURE

1. Jara A, Bover J, Lavigne J, Felsenfeld A: Comparison of Two Parathyroid Hormone Assays for the Rat: The New Immunoradiometric and the Older Competitive Binding Assay. *J. Bone Mineral Res,* Vol. 9, No 10, P. 1629, 1994.
2. Segre GV, Perkins AS, Witters LA, and Potts JT Jr: Metabolism of Parathyroid Hormone by Isolated Kupffer Cells and Hepatocytes. *Journal Clinical Investigations* 67: 449-457, 1981.
3. Segre GV, D'Amour PD, Hultman A, and Potts JT Jr: Effect of Hepatectomy and Nephrectomy Uremia on Metabolism of Parathyroid Hormone in the Rat. *J. Clinical Investigations,* 67:439-448, 1981.
4. Slatopolsky E, Finch JL, Martin D, Sicard G, Gao P, Cantor TL: A Novel Mechanism for Skeletal Resistance in Uremia. *J. American Society of Nephrology* 10:625A, 1999.
5. Divieti P, John MR, Juppner H, Bringhurst FR: Human PTH-(7-84) Inhibits Bone Resorption in Vitro Via Actions Independent of the Type 1 PTH/PTHrP Receptor. *Endocrinology* 143(1): 171-176, 2002.
6. Langub MC, Monier-Faogere M-C, Wang G, Williams JP, Koszewski NJ, Malluche HH: Administration of PTH-(7-84) Antagoniszes the Effects of PTH-(1-84) on Bone in Rats with Moderate Renal Failure. *Endocrinology* 144(4): 1135-1138.
7. Monier-Faugere M, Geng Z, Mawad H, Friedler RM, Gao P, Cantor TL, and Malluche HH: Improved Assessment of Bone Turnover by the PTH (1-84)/Large C-PTH Fragments Ratio in ESRD Patients. *Kid. International,* Vol. 60, 1460-68, 2001.
8. Gao P, ScheibelS, D'Amour P, Cantor TL: Measuring the Biologically Active or Authentic

Whole Parathyroid Hormone (PTH) with a Novel Immunoradiometric Assay Without Cross-reaction to the PTH (7-84) Fragment. *J. Bone and Mineral Res.* 14:S446, 1999.

9. Schultz VL, Boass A, Garner SC, Toverud SU: Several Anesthetics, but not Diethyl Ether, Cause Marked Elevation of Serum Parathyroid Hormone Concentration in Rats. *J. Bone Mineral Res,* Vol. 10, No 9, P. 1298, 1995

SCANTIBODIES LABORATORY, INC.
9336 Abraham Way, Santee, CA 92071
Phone 1-619-258-9300, Fax 1-619-258-9366