Duo PTH Kit

CE

Immunoradiometric (IRMA) Assay
(Coated Bead-Technology)

For the quantitative determination of human Whole and Total Intact PTH

For In Vitro Diagnostic Use.

Scantibodies
Laboratory, Inc.
(Part Number: 3KG601)

Store at 2 - 8° C

INTENDED USE

The Scantibodies Laboratory, Inc. Duo PTH Kit is used for the quantitative determination of human whole parathyroid hormone (PTH 1-84) or Cyclase Activating PTH (CAP) and the total immunoreactive intact PTH (Total Intact PTH) in blood samples and is used as an aid to diagnose Parathyroid disease. The Total Intact PTH level is the sum of Whole PTH(1-84) and a N-truncated PTH fragment.

PHYSIOLOGY

The Whole PTH peptide (1-84) is secreted by parathyroid glands under the regulation of the extra-cellular concentration of ionized calcium, phosphorus, vitamin D and magnesium. PTH acts with respect to calcium on the kidney and the skeleton. PTH binds to receptors, which stimulate adenylate-cyclase to produce cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). The biological activity of PTH resides in the first 3 amino acids of the N-terminal portion of the molecule. PTH is metabolized either intra glandular or in the peripheral organs into fragments. Circulation PTH are immunologically heterogenous. A recent study of circulation immunoreactive PTH showed that significant amounts of a large carboxyl-terminal PTH fragment presented in blood samples from uremic patients. Biologically inactive fragments with molecular weights of 4000 - 7000 Daltons circulate with a half-life of 30 minutes in healthy persons.

cAMP or other PTH dependent processed metabolites (e.g. hypophosphatemia) stimulate the renal hydroxylation of 25-(OH) vitamin D to 1,25-$(OH)_2$ vitamin D. This vitamin D metabolite stimulates calcium absorption by the small intestine. Severe vitamin D deficiency results in an enhanced secretion of PTH compared to the secretion of calcium. Hypomagnesemia in the primary stage stimulates hypocalcemia. Severe hypomagnesemia results in the reduced secretion of PTH.

Primary and secondary hyperparathyroidism, kidney insufficiency, malabsorption-syndrome and pseudo-hypoparathyroidism result in elevated concentrations

of PTH. Decreased concentrations of PTH coincide with high doses of vitamin-D, milk-alkali-syndrome, morbus Boeck, hyperthyreosis, ingestion of thiazide and hypercalcemia of malignancy. PTH concentration is also decreased with absorptive hypercalciuria and hypoparathyroidism.

PRINCIPLE OF PROCEDURE

Scantibodies Laboratory, Inc. Duo PTH Kit contains two immunoradiometric assays (IRMA) for measuring Whole PTH (CAP) and Total Intact PTH values in the blood samples. Both assays share polyclonal anti-PTH (39-84) coated beads as universal solid-phase, two PTH (1-84) calibration sets as assay standards, two assay control sets and universal assay wash buffer. The specificities of these two assays are reached by the specificity of tracer antibodies.

The IRMA for Whole PTH (CAP) utilizes a specific polyclonal PTH antibody directed against the most N-terminal PTH region as tracer antibody. The use of this antibody guarantees that only biologically active Whole PTH (CAP) is detected in the assay system. The anti-PTH N-terminal region specific antibody is labeled with ^{125}I. Whole PTH (CAP)

in patient samples is bound both to the beads and to the ^{125}I-anti N-terminal PTH. After incubation, free ^{125}I-antibodies and bound ^{125}I-antibodies fractions are separated by discarding the supernatant. Simple wash steps reduce the non-specific binding (NSB) to a minimum for increased precision at the low end of the calibration curve. The concentration of Whole PTH (CAP) is directly proportional to the radioactivity bound to the beads after separation. The concentration of PTH in unknown patient samples and controls is determined by interpolation using a calibration curve.

The IRMA for Total Intact PTH is utilizing a specific polyclonal PTH antibodies directed against PTH(1-34) region as tracer antibody. The use of this antibody has demonstrated that both biologically active Whole PTH (CAP) and the N-truncated PTH fragments are detected in the assay system. The anti-PTH(1-34) region specific antibody is labeled with ^{125}I. Both Whole PTH (CAP) and N-truncated PTH fragments in patient samples are bound to the beads and to the ^{125}I-anti PTH antibody. After incubation, free ^{125}I-antibodies and bound ^{125}I-antibodies fractions are separated by discarding the supernatant. Simple wash steps reduce the non-specific binding (NSB) to a minimum for increased precision at the low end of the calibration curve. The concentration of Total Intact PTH is directly proportional to the radioactivity bound to the beads after separation. The concentration of Total Intact PTH in unknown patient samples and controls is determined by interpolation using a calibration curve.

REAGENTS

The Scantibodies Laboratory, Inc. Duo PTH Kit contains sufficient reagents for 100 single determinations of Whole PTH (CAP) and Total Intact PTH. The kit is stable at 2 - 8° C until the stated expiration date.

PTH CALIBRATORS

Two sets of calibrators consist of seven vials each for Whole PTH and seven vials each for Total Intact PTH containing lyophilized human serum with nominal synthetic PTH concentrations. The lyophilized calibrators are prepared in stabilized human serum containing sodium azide 0.1% (w/v). The PTH concentrations are declared on the vial label.

PTH CONTROLS

Two sets of controls consist of two vials each for Whole PTH and two vials each for Total Intact PTH containing synthetic PTH in lyophilized human serum with 0.1% (w/v) sodium azide. The concentration ranges of PTH are declared on the vial labels.

^{125}I-ANTI N-TERMINAL PTH TRACER

One set of tracer consists of two bottles of ^{125}I-antibodies (sufficient for 100 determinations). Each bottle contains affinity purified goat anti N-terminal PTH antibodies which are labeled with ^{125}I and dissolved in 5 mL phosphate buffered saline with sodium azide 0.1% (w/v) and protein stabilizers. The maximum radioactivity in a bottle is <370 kBq (<10 μCi). This tracer must be used for Whole PTH (CAP) determination. This kit contains ^{125}I (half life: 60 Days), emitting ionizing X (28 keV) and Gamma γ (35,5 keV) radiations.

TOTAL INTACT PTH TRACER

One set of tracer consists of two bottles of ^{125}I-antibodies (Sufficient for 100 determinations). Each bottle contains affinity purified goat anti-PTH(1-34) antibodies which are labeled with ^{125}I and dissolved in 5 mL phosphate buffered saline with sodium azide 0.1% (w/v) and protein stabilizers. The maximum radioactivity in a bottle is <370 kBq (<10 μCi). This tracer must be used for the Total Intact PTH determination. This kit contains ^{125}I (half life: 60 Days), emitting ionizing X (28 keV) and Gamma γ (35,5 keV) radiations.

PTH (39-84) ANTIBODY COATED BEADS

Two bottles contain 100 polystyrene beads (8 mm diameter) each plus desiccant. The beads are coated with affinity purified goat anti-PTH (39-84). Each bottle is sufficient for 100 determinations. The desiccant

contains silica.

WASH CONCENTRATE

Two bottles contain 30 mL each of a 30 fold concentrate of phosphate buffered saline with sodium azide 1.5% (w/v) and detergent.

PREPARATION AND STORAGE OF REAGENTS

PTH CALIBRATORS

The Scantibodies Laboratory, Inc. Duo PTH Kit contains the PTH standards prepared analytically on a mass basis from purified synthetic intact PTH (1-84). These standards are further evaluated against "primary standards" which are stored at –70° C to maintain calibration.

Reconstitute the zero calibrators with 5 mL of distilled or deionized water. Reconstitute the remaining calibrators with 2 mL of distilled or deionized water. After addition of the water, mix each vial thoroughly but gently. Use the reconstituted calibrators within 1 hour. Store the unused portion of the calibrators below -20° C until the stated expiration date. Do not store the calibrators at room temperature for more than one hour at any given time. Do not thaw any calibrator vial more than two times. Do not use calibrators that exhibit precipitation or unusual color.

PTH CONTROLS

Reconstitute the vials of controls with 2 mL of distilled or deionized water. After addition of the water, mix each vial thoroughly but gently. Use the reconstituted controls within 1 hour. Store the unused portion of the controls below -20° C until the stated expiration date. Do not store the controls at room temperature for more than one hour at any given time. Do not thaw any control vial more than two times. Do not use controls that exhibit precipitation or unusual color.

^{125}I-ANTI N-TERMINAL PTH TRACER & TOTAL INTACT PTH TRACER

Both tracers are ready to use. Store both tracers at 2 - 8° C until the stated expiration date. Do not use tracers that exhibit precipitation or unusual color.

PTH (39-84) ANTIBODY COATED BEADS

The antibody coated beads are ready to use. Store the beads at 2 - 8° C until the stated expiration date. Allow the beads to equilibrate to ambient temperature prior to opening the bottle. Reseal the bottle immediately after removing the required number of beads.

WASH CONCENTRATE

Mix the contents of the wash concentrate thoroughly with 870 mL of distilled or deionized water (1:30). Store the diluted wash solution at room temperature (18 - 25° C) until the stated expiration date. Do not use wash solution that exhibit precipitation or unusual color.

WARNINGS AND PRECAUTIONS FOR USERS

Use of The Assay

The reagents are for in vitro diagnostic use.

Human Serum Caution

The human serum in this kit has been prepared from human donors and it has been tested by FDA approved immunoassays and found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Anti HIV I/II and Anti HCV. However, it is recommended to consider the calibrators and controls as a potential biohazard and handle them with the same precautions as applied to any untested patient sample.

Radioactivity Warning

This radioactive material may be received, acquired, possessed, or used only by physicians, clinical laboratories, or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a state with which the Commission has entered into an agreement for the exercise of regulatory authority.

All radioactive materials must be disposed of according to the regulations (regulations differ from country to country) and guidelines of the agencies with jurisdiction over the laboratory. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are used. Storage of radioactive materials should be limited to specifically designated and appropriately secured areas. Access to radioactive materials should be limited to authorized and trained personnel only. Do not pipette radioactive solutions by mouth. Avoid direct contact with radioactive materials by using protective articles such as lab coats and disposable gloves. Wash hands thoroughly after use. Radioactive materials must be stored in designated areas in their original containers or in containers providing equivalent radiation protection. A record of disposal of all radioactive materials must be kept. Immediately remove spilled solutions and decontaminate contaminated devices. Check laboratory equipment and glassware regularly to detect contamination with radioisotopes.

Sodium Azide (NaN3) Warning

Some reagents in the Scantibodies Laboratory, Inc. Duo PTH Kit contain Sodium Azide. Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal flush the drain with a large volume of water to prevent azide build-up. Avoid direct contact with skin and mucous membranes.

Dispose of specimens and components of the kit as potentially infectious agents.

SAMPLE PREPARATION AND STORAGE

Specimen Collection

The determination of human Whole and Total Intact PTH should be made on EDTA-plasma only. Eight hundred microliters of plasma are required to assay one sample in duplicate for both Whole PTH (CAP) and Total Intact PTH values. To obtain plasma, collect blood by venipuncture into an EDTA tube. Centrifuge the sample at 2 - 8° C and separate the plasma from the cells within one hour after collection. Plasma should be stored at -20° C or lower if not tested within 2 hours. Avoid repeated freezing and thawing of plasma. Do not use patient samples which have been frozen and thawed more than two times.

Dilution of Patient Samples

Dilute samples with PTH concentrations greater than the highest calibrator with PTH Zero Calibrator before reassay. The dilution factor is applied to the diluted sample assay result in order to determine the PTH concentration in the undiluted sample.

Quality Control

Two levels of controls are provided with each assay kit. The values assigned to these controls are printed on the container label. The control value should fall within the specified range when tested in the same manner as the unknowns. Controls should be included in each assay. If the control values do not meet the established range, the assay may be invalid and should be repeated.

ASSAY PROCEDURE

Materials Provided

The Total Intact PTH assay workstation is located below the Whole PTH assay workstation.

The Scantibodies Laboratory, Inc. Duo PTH Kit (Part No. 3KG601) is supplied with the following:

DESCRIPTION	NUMBER
Whole PTH Calibrators Part Nos. 3CA650, 3CB650, 3CC650, 3CD650, 3CE650, 3CF650, 3CG650	7 vials
Whole PTH Controls Part Nos. 3CA651, 3CB651	2 vials
Total Intact PTH Calibrators Part Nos. 3CA647, 3CB647, 3CC647, 3CD647, 3CE647, 3CF647, 3CG647	7 vials
Total Intact PTH Controls Part Nos. 3CA648, 3CB648	2 vials
PTH (39-84) Antibody Coated Beads Part No. 3KB001	2 bottles of 100 beads each
Total Intact PTH Tracer Part No. 3KL127	2 vials
Goat Anti-N-Terminal PTH ^{125}I Antibody Part No. 3KL022	2 vials

DESCRIPTION	NUMBER
Wash Concentrate Part No. 3KW001	2 bottles
Directional Insert Part No. (3KI085)	1 insert

Materials And Equipment Required But Not Provided:

Distilled or deionized water
Round-bottomed polypropylene or polystyrene test tubes
(12 x 55, 12 x 75, 12 x 70 mm or equivalent)
Pipettor with disposable tips: 0.2 mL
Repeating dispenser: 0.1 mL
Bead dispenser or plastic tweezers
Wash station
Vortex mixer
Gamma counter calibrated to detect ^{125}I
Dispenser or plastic tweezers

Preparation for Assay

For each assay, prepare the following groups of tubes and place them in a test tube rack (double determination):

2 total count tubes (optional for QC).
2 Bo tubes (NSB)
2 tubes for each calibrator concentration
2 tubes for each control concentration
2 tubes for each patient sample

Pipetting and Incubation Steps

1. Pipette 0.2 mL of calibrators, samples and controls into the corresponding tubes.
   NOTE: For the Whole PTH assay, use the Whole PTH Calibrators and Controls. For the Total Intact PTH assay, use the Total Intact PTH Calibrators and Controls.
2. Pipette 0.1 mL of goat anti-N-terminal PTH ^{125}I antibody into each tube for the determination of Whole PTH (CAP).
   Or Pipette 0.1 mL of Total Intact PTH tracer into each tube for the determination of Total Intact PTH.
3. Gently vortex all tubes.
4. Dispense one antibody coated bead into each tube except for the total count tubes. To add the beads, tilt the test tube rack to approximately a 30 degree angle to prevent splashing.
5. Seal the tubes and incubate them for 18 - 24 hours at room temperature (18 - 25° C) and shaking 170 RPM.
6. Aspirate the supernatant from each tube except for the total count tubes. Wash the beads 3 times with 2 mL of diluted wash solution. After each addition of diluted wash solution aspirate all of the wash solution.
7. Count each tube for at least 1 minute in a gamma counter calibrated to detect ^{125}I. The total count tube should contain approximately 300,000 CPM (assuming the counter has an efficiency of 70% - 80%) when freshly iodinated tracer is used. The total activity of the tracer decreases according to the half-life of ^{125}I.

PIPETTING GUIDE

Additive to Tube	Total Count Tubes	Bo Tubes	Calibrator Tubes	Control Tubes	Sample Tubes
Calibrator	-	200 µl	200 µl		-
Control	-	-	-	200 µl	
Sample	-	-	-	-	200 µl
^{125}I anti-N-terminal PTH **or** Total Intact PTH Tracer	100 µl	100 µl	100 µl	100 µl	100 µl
Beads	-	1	1	1	1
Vortex mix all tubes, except for the total count tubes. Incubate tubes for 18 - 24 hours at room temperature (18° - 25°C) and shaking at 170 RPM.					
Aspirate the supernatant from all of the tubes except the total count tubes. Wash all tubes except the total count tubes by adding 2 mL of diluted wash solution and aspirating the wash solution. Repeat this wash step two more times for a total of three times.					
Count each tube for at least 1 minute in a gamma counter.					

PROCEDURAL COMMENTS

For measuring Whole PTH (CAP), the ^{125}I anti-N-terminal PTH tracer must be used and the Total Intact PTH tracer must not be used.

For measuring Total Intact PTH, the Total Intact PTH tracer must be used and the ^{125}I anti-N-terminal PTH tracer must not be used.

Known Interferences:

- Samples containing up to 250 mg/dl

triglyceride (<20% interference level), 15 mg/dl hemoglobin and 7.5 mg/dl bilirubin do not exhibit any effect on the assay within the medical decision point for this assay.

- Grossly hemolyzed or lipemic samples.
- Samples from patients receiving radioisotopes for diagnostic or therapeutic purposes.
- Contamination of the sample or assay tube with ^{125}I or other radioisotopes.

Reagents from different lot numbers must not be interchanged.

The patient sample or calibrator and tracer should be pipetted carefully into the bottom one-fourth of the assay tube. This is to avoid losing liquid on the surface of the tube as the liquid runs down the tube.

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

Do not handle beads with hands. Use a plastic forceps or equivalent.

It is recommended that calibrators and patient samples be assayed in duplicate. The average counts per minute of each duplicate should then be used for data reduction and the calculation of results.

When adding the beads to the tubes, tilt the test tube rack to a 30° angle to avoid splashing.

Calibrators must be frozen immediately after use and may only be thawed and reused a maximum of two times provided acceptable control results are obtained.

Avoid sample to sample contamination by using a new pipette tip for each sample.

CALCULATION OF RESULTS

Evaluation

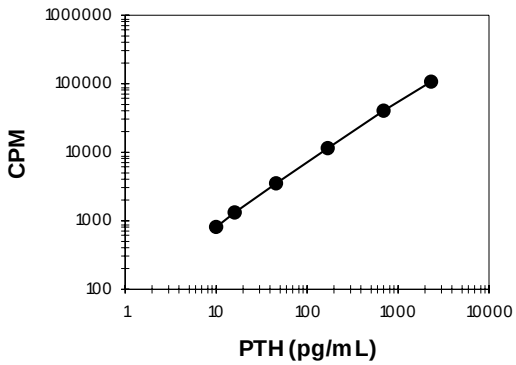
1. Calculate the average CPM for each double determination.
2. Subtract the average CPM of the zero calibrator tubes from the average CPM from all duplicate samples and controls in order to obtain the corrected CPM. Corrected CPM = average CPM of duplicate samples - average CPM of duplicate zero calibrators.
3. Draw separate calibration curves for each assay by plotting the average corrected CPM from each duplicate calibrator level (ordinate) against the respective concentration declared on the calibrator vial (absolute) using log-log graph paper. Obtain sample concentrations by interpolation of average sample CPM on the calibration curve. **NOTE**: The calibrator and control values may be different for each assay.
4. If samples were run with dilution, multiply the diluted sample assay results from the curve by the appropriate dilution factors to obtain the undiluted sample assay results.

SAMPLE DATA

Tube	CPM	Ave. CPM	Corrected CPM
Total Activity	338952 341760	340356	
0 pg/mL	626 619	622	
10 pg/mL	1223 1217	1220	598
20 pg/mL	1899 1815	1857	1235
48 pg/mL	4012 4147	4080	3458
190 pg/mL	12647 12791	12719	12097
850 pg/mL	45506 45520	45513	44891
2100 pg/mL	107182 106731	106956	106334

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

REPRESENTATIVE STANDARD CURVE



Automated data reduction can also be used to construct the PTH calibration curve. To program automated data reduction systems or to adapt an existing program consult the data processor manufacturer or the programmer.

LIMITATIONS OF THE PROCEDURE

PTH values should be used in addition to other diagnostic data and clinical information available to the physician.

The kit is for use in clinical labs by a technician trained to perform immunoradiometric assays.

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

Grossly hemolyzed, lipemic or icteric samples are likely to give invalid results.

The highest concentration of PTH measurable without sample dilution is the concentration of the highest calibrator. The lowest level measurable is approximately 1.0 pg/mL for Whole PTH (CAP) IRMA and 1.23 pg/mL for Total Intact PTH IRMA.

Do not use kit past stated expiration date.

EXPECTED VALUES

It is recommended that each laboratory establish its own range of normal values. The values given are only indicative and may vary from other published data.

The EDTA-plasma normal range of Whole PTH (CAP) was 5 - 39 pg/mL based on 128 normal donors determined following NCCLS guidelines (C28-A). The sample Whole PTH (CAP) value greater than 39 pg/mL is considered as hyperparathyroidism.

The EDTA-plasma normal range of Total Intact PTH was 14 - 66 pg/mL based on 165 normal donors determined following NCCLS guidelines (C28-A) to calculate the central 95% reference interval. The sample Total Intact PTH value greater than 66 pg/mL is considered as hyperparathyroidism.

The N-truncated PTH fragment level could be calculated by substraction of Whole PTH (CAP) value from Total Intact PTH value. This N-truncated PTH fragment level may not reflect the true PTH value in the sample and the clinical relevance has not been determined.

PERFORMANCE CHARACTERISTICS

Recovery Accuracy

Different samples containing known concentrations of PTH were spiked with an equal volume of a PTH containing sample. The expected value is the total diluted concentration of PTH. The % recovery was determined following assay of the samples.

IRMA for Whole PTH (CAP):				
Sample value (pg/mL)	**Added PTH (pg/mL)**	**Expected value (pg/mL)**	**Measured value (pg/mL)**	**Recovery (%)**
36.05	- 50.07 141.51	- 43.06 88.78	- 43.11 90.62	- 100 102
77.09	- 41.34 130.31	- 59.22 103.7	- 66.9 108.72	- 113 105
126.2	- 39.23 130.33	- 82.72 128.27	- 91.42 127.38	- 111 99

IRMA for Total Intact PTH:					
Sample #	**Sample value (pg/mL)**	**Added (1-84) PTH (pg/mL)**	**Measured value (pg/mL)**	**Expected value (pg/mL)**	**Recovery (%)**
1	52.13	43.72 146.63	46.77 99.89	47.92 99.38	98 101
2	177.3	43.72 146.63	113.56 179.98	110.51 161.97	103 111
3	1144.65	43.72 146.63	588.16 649.48	594.19 645.64	99 101

Dilution Accuracy

Different samples with high concentrations of PTH were diluted with PTH zero calibrator. The % recovery was determined following assay of the diluted samples.

IRMA for Whole PTH (CAP):				
Sample	**Dilution**	**Measured value (pg/mL)**	**Expected value (pg/mL)**	**Recovery (%)**
1	Neat	2057.5		
	1:2	1053.58	1028.75	102
	1:4	519.13	514.38	101
	1:8	273.32	257.19	106
2	Neat	1595.35		
	1:2	758.28	797.68	95
	1:4	395.67	398.84	99
	1:8	210.98	199.42	106
3	Neat	1006.36		
	1:2	485.9	503.18	97
	1:4	256.29	251.59	102
	1:8	140.6	125.80	112
4	Neat	646.09		
	1:2	333.07	323.05	103
	1:4	168.56	161.52	104
	1:8	87.11	80.76	108

IRMA for Whole PTH (CAP):				
Sample	**Dilution**	**Measured value (pg/mL)**	**Expected value (pg/mL)**	**Recovery (%)**
5	Neat	573.91		
	1:2	303.99	286.96	106
	1:4	159.69	143.48	111
	1:8	86.04	71.74	120
6	Neat	181		
	1:2	97.82	90.50	108
	1:4	48.02	45.25	106
	1:8	20.42	22.63	90
7	Neat	153.12		
	1:2	91.05	76.56	119
	1:4	49.59	38.28	130
	1:8	22.28	19.14	116

IRMA for Total Intact PTH:				
Sample	**Dilution**	**Measured Value (pg/mL)**	**Expected value (pg/mL)**	**Recovery (%)**
1	Neat	173.62	-	-
	1:2	94.41	86.81	109
	1:4	44.91	43.41	103
2	Neat	148.41	-	-
	1:2	73.17	74.21	99
	1:4	32.13	37.10	87
3	Neat	310.76	-	-
	1:2	160.64	155.38	103
	1:4	80.63	77.69	104
	1:8	38.59	38.85	99

IRMA for Total Intact PTH:				
Sample	**Dilution**	**Measured Value (pg/mL)**	**Expected value (pg/mL)**	**Recovery (%)**
4	Neat	1165.97	-	-
	1:2	588.30	582.99	101
	1:4	290.79	291.49	100
	1:8	141.69	145.75	97
5	Neat	1583.56	-	-
	1:2	758.60	791.78	96
	1:4	418.53	395.89	106
	1:8	216.64	197.95	109
6	Neat	2052.41	-	-
	1:2	1061.19	1026.21	103
	1:4	533.87	513.10	104
	1:8	296.02	256.55	115

High Dose Hook Response

The high dose hook response of Whole PTH (CAP) Specific Kit and the Total Intact PTH Kit were determined as 20,000 pg/mL of synthetic PTH(1-84) and/or PTH(7-84). Samples greater than the highest standard (approximately 2300 pg/mL) and up to 20,000 pg/mL PTH will read CPM values greater than that of the highest standard.

Precision

Intra-assay coefficient of variation was evaluated by performing 20 replicate determinations on two samples in the same assay.

IRMA for Whole PTH (CAP):			
Sample	**Mean value (pg/mL)**	**SD (pg/mL)**	**% CV**
1	30.3	1.5	4.94
2	283.87	7.49	2.64

IRMA for Total Intact PTH:			
Sample	**Mean value (pg/mL)**	**SD (pg/mL)**	**% CV**
1	21.50	1.04	4.83
2	327.87	10.38	3.17

Inter-assay coefficient of variation was evaluated by performing 20 different assays on two samples.

IRMA for Whole PTH (CAP):			
Sample	**Mean value (pg/mL)**	**SD (pg/mL)**	**% CV**
1	32.75	2.54	7.76
2	285.05	11.81	4.14

IRMA for Total Intact PTH:			
Sample	**Mean value (pg/mL)**	**SD (pg/mL)**	**% CV**
1	25.92	1.75	6.75
2	314.95	11.36	3.61

Sensitivity

The detection limit of the assay is defined as the lowest measurable value distinguishable from zero. This sensitivity was determined by assaying the zero calibrator 20 times in the same assay. The detection limits determined is approximately 1.0 pg/mL for Whole PTH (CAP) IRMA and 1.23 pg/mL for Total Intact PTH IRMA at 2 standard deviation above the PTH zero calibrator.

The functional sensitivity is defined as being the measured concentration by imprecision profile for a CV equal to 20%. It has been assessed as being 5 pg/mL.

Specificity

This Whole PTH (CAP) PTH assay does not show any cross-reaction to PTH (7-84) fragment when the synthetic PTH (7-84) peptide is serially diluted with standard zero matrix and assayed.

PTH (7 -84) Conc. Sample (pg/mL)	Measured PTH conc. (pg/mL)
2500	undetectable
5000	undetectable
10000	undetectable
20000	undetectable

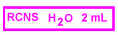
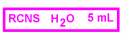
The Total Intact PTH assay, however, showed almost 100% cross-reaction to PTH (7-84) fragment.

A high degree of correlation exists between the PTH levels of duplicate samples measured by a commercially available predicate PTH Kit and those levels measured by the Scantibodies Laboratory, Inc. (SLI) Total Intact PTH Specific IRMA Assay. A correlation coefficient (r) of 0.955 (n=68) was obtained with a slope of 1.08 and intercept of 0.041 where x represents the predicate device data and y represents the SLI data. Calculations were made with samples ranging from 9 - 71 pg/mL.

For Whole PTH (1-84) (CAP) IRMA Assay, the correlation coefficient (r) of 0.98 (n=223) was obtained with a slope of 1.47 and intercept of -13.65 where x represents the predicate device data and y represents the SLI data. Calculations were made with samples ranging from 9.6 - 1808 pg/mL.

Chemical Characterization:	1) Antibodies coated on to polystyrene Beads (or tubes).
	2) Radioactive Isotope containing Iodine-125 with radioactivity <10 μCi and Sodium Azide @ 0.1%.
	3) Calibrators & Controls – Human Serum containing Sodium Azide @ 0.1%
	4) Wash Concentrate containing sodium azide @ 1.5%.
Hazardous Ingredients:	Radioactive Isotope (Iodine-125) @ <10 μCi/Vial (<370 kBq) CAS Number: 7553-56-2 Symbols: Harmful Xn R-phrases: R22, R52/53 S-phrases: S28, S45, S53, S60, S61
	Sodium Azide @ 0.1% CAS Number: 026628-22-8 Symbols: N/A R-phrases: N/A S-phrases: N/A
	Sodium Azide @ 1.5% CAS Number: 026628-22-8 Symbols: Very Toxic T+; N R-phrases: R28, R32, R50/53 S-phrases: S28, S45, S53, S60, S61

Symbol	Used for	Symbol	Used for
	Do Not Reuse		Use By YYYY-MM-DD or YYYY-MM
	Batch Code		Serial Number
	Date of Manufacture		Sterile

Symbol	Used for	Symbol	Used for
	Sterilized Using Aseptic Processing Technique		Catalog Number
	Caution, Consult Accompanying Documents		Biological Risks
	Manufacturer		Authorized Representative in the European Community
	Contains Sufficient for < n > Tests		For IVD Performance Evaluation Only
	*In vitro* Diagnostic Medical Device		Upper Limit of Temperature
	Lower Limit of Temperature		Temperature Limitation
	Consult Instructions for Use		Positive Control
	Control		Negative Control
	Antibody Coated Beads		Antibody Coated Tubes
	Radioactive Iodine Tracer		Wash Solution
	Reconstituted with 2 mL Water		Reconstituted with 5 mL Water
	European Conformity Mark		Radioactive
	Toxic		Harmful

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