Total Intact PTH Assay

Immunoradiometric Assay (IRMA) Scantibodies
(Coated Bead-Technology) (Part Number: 3KG600)

For the quantitative determination
of human Total Intact PTH.
For in vitro diagnostic use only

INTENDED USE
This kit has been designed for the quantitative
determination of total immunoreactive intact PTH (Total
Intact PTH) in blood samples. The Total Intact PTH
level is the sum of PTH (1-84) and N-truncated PTH
fragments.

PHYSIOLOGY
The cyclase activating PTH peptide (1-84) is secreted
by parathyroid glands under the regulation of the extra-
cellular concentration of ionized calcium, vitamin D and
magnesium. PTH acts with respect to calcium on the
kidneys 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 three 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 carboxy-
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-
hyoparathyroidism 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 Total Intact PTH Coated Bead Kit is an
Immunoradiometric (IRMA) assay utilizing a polyclonal
1-84 PTH antibody with a tendency to bind in the N
terminal region of 1-84 PTH (Label Antibody), and a
polyclonal 1-84 PTH antibody with a tendency to bind in
the C terminal region of 1-84 PTH (Capture Antibody).
The use of these antibodies guarantees that Whole
PTH (1-84 PTH) and truncated PTH fragments are
detected. The Label Antibody is labeled with 125-I. The
Capture Antibody is fixed to the beads. The Total Intact
PTH in patient samples is bound both to the beads and
the Label Antibody. 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 PTH in unknown patient samples
and controls is determined by interpolation using a
calibration curve.

REAGENTS
The Scantibodies Total Intact PTH Kit contains
sufficient reagents for 100 single determinations. The
kit is stable at 2 - 8° C until the stated expiration date.

Scantibodies PTH Calibrators
One set of calibrators consists of seven vials
containing lyophilized human serum with nominal
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.

Scantibodies PTH Controls
One set of controls consists of two vials containing
PTH in lyophilized human serum with 0.1% (w/v)
sodium azide. The concentration ranges of PTH are
declared on the vial labels.

Scantibodies Total Intact PTH Tracer
One set of tracer consists of two bottles of 125I-anti
PTH (1-34). Each bottle contains polyclonal goat anti
PTH (1-34) which is labeled with 125I 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 ).

Scantibodies PTH (39-84) Antibody Coated Beads
One jar contains 100 polystyrene beads (8 mm
diameter) each plus desiccant. The beads are coated
with polyclonal goat anti-PTH (39-84). The desiccant contains silica.

**Scantibodies Wash Concentrate**
One bottle contains 30 ml of a 30 fold concentrate of phosphate buffered saline with sodium azide 1.5% (w/v) and detergent.

**PREPARATION AND STORAGE OF REAGENTS**

**Scantibodies PTH Calibrators**
The Scantibodies Laboratory, Inc. Total Intact PTH Coated Bead Diagnostic 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 calibrator 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.

**Scantibodies 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.

**Scantibodies Total Intact PTH Tracer**
The tracer is ready to use. Store the tracer at 2-8°C until the stated expiration date. Do not use tracer that shows precipitation or unusual color.

**Scantibodies 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.

**Scantibodies Wash Concentrate**
Dilute and thoroughly mix the 30 ml of wash concentrate 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 shows precipitation.

**WARNINGS AND PRECAUTIONS FOR USERS**

**Use of The Assay**
The reagents are for in vitro diagnostic use only.

**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. 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 PTH assay 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.

**SAMPLE PREPARATION AND STORAGE**

**Specimen Collection**
The determination of human PTH should be made on EDTA-plasma. Four hundred microliters of plasma are...
required to assay one sample in duplicate. To obtain plasma, collect blood by venipuncture into a tube containing EDTA. Invert the tube gently 5 - 6 times after collection to ensure adequate mixing. Whole blood may be stored refrigerated for up to 48 hours prior to centrifugation. Centrifuge the sample and separate the plasma from the cells. Plasma should be stored at -20°C or lower if not tested immediately. 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 plasma samples with PTH concentrations greater than the highest calibrator with Scantibodies PTH Zero Calibrator before assay. 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 Scantibodies Total Intact PTH Coated Bead Kit (Part No. 3KG600) is supplied with the following:

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scantibodies PTH Calibrators Part Nos. 3CA647, 3CB647, 3CC647, 3CD647, 3CE647, 3CF647, 3CG647</td>
<td>7 vials</td>
</tr>
<tr>
<td>Scantibodies PTH Controls Part Nos. 3CA648, 3CB648</td>
<td>2 vials</td>
</tr>
<tr>
<td>Scantibodies PTH (39-84) Antibody Coated Beads Part No. 3KB001</td>
<td>1 jar of 100 beads</td>
</tr>
<tr>
<td>Scantibodies Total Intact PTH Tracer Part No. 3KL127</td>
<td>2 vials</td>
</tr>
<tr>
<td>Scantibodies Wash Concentrate Part No. 3KW001</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Directional Insert Part No. 3KI089</td>
<td>1 insert</td>
</tr>
</tbody>
</table>

**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 $^{125}\text{I}$
- Rotator, capable of maintaining 170 ± 10 RPM.

**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.
2. Pipette 0.1 ml of Total Intact PTH Tracer into each tube.
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° angle to prevent splashing.
5. Seal the tubes and incubate them at room temperature (18 - 25°C) for 18 - 24 hours on a rotator at 170 ± 10 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 $^{125}\text{I}$. 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 $^{125}\text{I}$.  

3KI089, vs. 4
2 June 2005
PIPETTING GUIDE

<table>
<thead>
<tr>
<th>Additive To Tube</th>
<th>Total Count Tubes</th>
<th>Bo Tubes</th>
<th>Calibrator Tubes</th>
<th>Control Tubes</th>
<th>Sample Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td>-</td>
<td>200 µl</td>
<td>200 µl</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200 µl</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200 µl</td>
</tr>
<tr>
<td>Total Intact PTH Tracer</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Beads</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Vortex mix all tubes, except for the TC tubes. Incubate tubes at room temperature (18 - 25° C) for 18 - 24 hours on a rotator at 170 ± 10 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

Known Interferences:
Samples containing up to 250 mg/dl triglyceride (<20% interference level), 15 mg/dl hemoglobin and 15 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 125I 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.

Calculations:
1. Calculate the average CPM for each double determination.
2. Subtract the average CPM of the zero calibrator tubes from the CPM's from all other tubes in order to obtain the corrected CPM for each tube.
3. Corrected CPM = average CPM of duplicate samples - average CPM of duplicate zero calibrators.
4. Draw the calibration curve 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.
5. 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.

Representative Standard Curve:

Automated data reduction can also be used to construct the Scantibodies Total Intact 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

For diagnostic purposes PTH values should be used in addition to other diagnostic data and clinical information available to the physician.
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 non valid results.

The highest concentration of PTH measurable without sample dilution is the concentration of the highest calibrator. The lowest level measurable is approximately 1.23 pg/ml.

**EXPECTED VALUES**

The normal value range was determined following the NCCLS guidelines (C28-A) using 165 samples from apparently healthy individuals. 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.

<table>
<thead>
<tr>
<th>PATIENT CLASSIFICATION</th>
<th>Total Intact PTH RANGE pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14 - 66</td>
</tr>
<tr>
<td>Hyperparathyroidism</td>
<td>&gt; 66</td>
</tr>
</tbody>
</table>

**PERFORMANCE CHARACTERISTICS**

**Accuracy, Recovery**

Different samples with low concentrations of PTH were spiked with 2 amounts of PTH. The % recovery was determined following assay of the spiked samples.

<table>
<thead>
<tr>
<th>Total Intact PTH</th>
<th>Sample Mean value (pg/ml)</th>
<th>Std Dev (pg/ml)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample #</td>
<td>Added (1-84) PTH (pg/ml)</td>
<td>Measured (pg/ml)</td>
<td>Expected (pg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>52.13</td>
<td>88.36</td>
<td>70.54</td>
</tr>
<tr>
<td></td>
<td>185.16</td>
<td>117.69</td>
<td>118.65</td>
</tr>
<tr>
<td>2</td>
<td>177.3</td>
<td>88.36</td>
<td>120.21</td>
</tr>
<tr>
<td></td>
<td>185.16</td>
<td>166.04</td>
<td>181.23</td>
</tr>
<tr>
<td>3</td>
<td>1144.65</td>
<td>88.36</td>
<td>634.51</td>
</tr>
<tr>
<td></td>
<td>185.16</td>
<td>664.91</td>
<td>645.64</td>
</tr>
</tbody>
</table>

**Accuracy, Dilution**

Different samples with high concentrations of PTH were diluted in a sample with low concentrations of PTH. The % recovery was determined following assay of the diluted samples.

<table>
<thead>
<tr>
<th>Total Intact PTH Accuracy, Dilution</th>
<th>Sample Mean value (pg/ml)</th>
<th>Std Dev (pg/ml)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Dilution</td>
<td>Measured value (pg/ml)</td>
<td>Expected value (pg/ml)</td>
<td>% Recovery</td>
</tr>
</tbody>
</table>
The assay does not show any cross-reactivity to the fragments listed below.

<table>
<thead>
<tr>
<th>Total Intact PTH Specificity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide Fragment</td>
<td>Peptide Sample Concentration</td>
<td>Recovery (pg/mL)</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>1 to 34</td>
<td>100,000</td>
<td>6.71</td>
</tr>
<tr>
<td>39 to 68</td>
<td>100,000</td>
<td>0</td>
</tr>
<tr>
<td>53 to 84</td>
<td>100,000</td>
<td>0</td>
</tr>
<tr>
<td>44 to 68</td>
<td>100,000</td>
<td>0</td>
</tr>
<tr>
<td>39 to 84</td>
<td>100,000</td>
<td>0</td>
</tr>
</tbody>
</table>

The total Intact PTH assay has 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.

This correlation was between the Scantibodies Total Intact PTH and the Nichols IRMA iPTH Assay. No correlation was made with the Nichols Advantage iPTH Assay nor the Nichols Bio-intact PTH Assay.

### 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: Harmful Xn
R-phrases: R22, R52/53
S-phrases: S28, S45, S53, S60, S61

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

### PTH LITERATURES

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Scantibodies Laboratory, Inc.
This diagnostic kit complies with IVDD 98/79/CE.


