PTH (Rat) IRMA

For the quantitative determination of rat PTH levels in serum, plasma or cell culture media

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 31-PTHRT-R100
Size: 100 determinations
Version: 10/10 - ALPCO February 8, 2011

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INTENDED USE
This kit is intended for research use only in the determination of rat PTH levels in serum, plasma or cell culture media. This assay has also been validated for use in the determination of mouse PTH levels. (See References, #5)

INTRODUCTION
Rat intact parathyroid hormone (PTH) is an 84 amino acid polypeptide produced by the parathyroid gland with its biological activity residing in the N-terminal region of the peptide. PTH plays an important role in maintaining the concentration of ionized calcium within the limits needed to achieve normal metabolic functions. When serum calcium levels are decreased the parathyroid gland increases secretion of the hormone which results in increased mobilization of calcium from skeletal reserves into the circulation. When levels of serum calcium are increased the secretion of PTH is reduced.

The similarities between rat and human physiology relative to calcium metabolism make the rat an excellent live-animal model for studying skeletal disease and in the pre-clinical evaluation of pharmacologic agents that may alter bone remodeling. Quantitation of the biologically active intact and N-terminal forms of rat PTH with this kit can provide a precise and sensitive assessment of changes in bone and mineral metabolism.

TEST PRINCIPLE
The Rat PTH IRMA Kit is a two-site immunoradiometric assay (IRMA) for the measurement of rat parathyroid hormone in serum, plasma or cell culture media. Two different goat antibodies to the N-terminal region (1-34) of rat PTH have been purified by affinity chromatography. One of the antibodies is immobilized onto plastic beads to capture the PTH molecules and the other antibody is radiolabeled for detection.

A sample containing rat PTH is incubated simultaneously with an antibody coated bead and the 125I labeled antibody. Both intact PTH (1-84) and N-Terminal PTH (1-34) contained in the sample are immunologically bound by the immobilized antibody and the radiolabeled antibody to form a “sandwich” complex:

Bead/Anti-Rat PTH — Rat PTH — 125I Anti-Rat PTH

At the end of the incubation period, the bead is washed to remove any unbound labeled antibody and other components. The radioactivity bound to the bead is then measured in a gamma counter. The radioactivity of the antibody complex bound to the bead is directly proportional to the amount of rat PTH in the sample. A standard curve is generated by plotting the CPM versus the logarithmic scales. The concentration of rat PTH in the samples is determined directly from this curve.

REAGENTS: Preparation and Storage
Store the kit at 2-8°C upon receipt. Store the standards and controls at -20°C or below after reconstitution. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use all reagents may be stored at room temperature and mix by gentle swirling and inversion. Reagents from different kit lot numbers should not be combined or interchanged.

1. RAT PTH ANTIBODY COATED BEADS (40-2010)
   One container of 100 polystyrene beads (6.4 mm diameter) coated with antibody to rat PTH plus desiccant.

2. 125I LABELED RAT PTH ANTIBODY (40-2020)
   Two vials each containing 5.5 mL of 125I labeled anti-rat PTH in 0.6M phosphate buffered saline with protein stabilizers and 0.1% sodium azide. Each vial contains less than 10 uCi (370 kBq) of radioactivity.

3. RAT PTH STANDARDS (40-2031 to 40-2036)
   Six vials each containing synthetic rat PTH (1-34) lyophilized in a protein matrix with 0.1% sodium azide. Refer to vial label for exact concentration. Before use reconstitute the vial with the rat PTH concentration of 0 pg/mL with 4.0 mL of deionized water. Before use reconstitute each of the other five vials of standards with 2.0 mL of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

Use the standards immediately after reconstitution; freeze the unused portion for later use. After reconstitution the standards are stable until the expiration date on the kit box when stored at -20°C or below with up to 3 freeze/thaw cycles.

4. RAT PTH CONTROLS I & II (40-2041 & 40-2042)
   Two vials each containing rat PTH (1-34) lyophilized in a protein matrix with 0.1% sodium azide. Refer to vial label for control ranges. Before use reconstitute each control with 2.0 mL of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

Use the controls immediately after reconstitution; freeze the unused portion for later use. After reconstitution the controls are stable until the expiration date on the kit box when stored at -20°C or below with up to 3 freeze/thaw cycles.

5. WASH CONCENTRATE (40-0050)
   One bottle containing 30 mL of a 30 fold concentrate. Before use dilute the contents to 900 mL with deionized water and mix well. Upon dilution this yields a working wash solution containing a surfactant in 0.01M phosphate buffered saline.

Store at 2 - 8° Upon Receipt
with 0.05 % sodium azide. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. RAT PTH SAMPLE DILUENT (Optional reagent, must be ordered separately using catalog # 30-2031)

One bottle containing 20 mL of a protein matrix with 0.1% sodium azide in liquid, ready-to-use form. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the bottle.

SAFETY PRECAUTIONS

Some of the reagents in this kit contain sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup (Manual Guide-Safety Management, Explosive Metal Azides. On disposal, flush with a large volume of water to prevent azide buildup (Manual Guide-Safety Management, Explosive Metal Azides). For practitioners or institutions receiving radioisotopes under a specific license:

This radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals, and is only for in vitro 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 conditions of your specific license.

1. Storage of radioactive material should be limited to a specifically designated area.
2. Access to radioactive materials must be limited to authorized personnel only.
3. Do not pipette radioactive material by mouth.
4. Do not eat or drink within designated radioactive work area.
5. Areas where spills may occur should be wiped up and then washed with an alkali detergent or radiological decontamination solution. Any glassware used must be rinsed completely with water before washing with other laboratory glassware.

For practitioners or institutions receiving radioisotopes under a specific license:

The receipt, use, transfer and disposal are subject to the regulations and conditions of your specific license.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Polystyrene or polypropylene tubes, 12 x 75 mm.
2. Test tube rack.
3. Marking pen for labeling tubes.
4. 2.0 mL and 4.0 mL volumetric pipettes for reconstituting standards and controls.
5. Precision pipets capable of delivering 100 µL and 200 µL.
6. Forceps or suitable bead dispenser.
7. Parafilm® or equivalent for covering tubes.
8. Repeating dispenser suitable for delivering 2.0 mL.
9. Aspiration device or suitable bead washer.
10. Container for storage of wash solution.
11. Gamma counter.
12. Deionized water.
13. Vortex mixer.
14. Timer.

SPECIMEN COLLECTION

Measurement of the rat PTH concentration may be made using serum, plasma, or cell culture media. Four hundred microliters of serum, plasma, or culture media are required to assay the sample in duplicate. If obtaining serum, collect blood and allow it to clot at room temperature. Centrifuge the sample and separate the serum, plasma or media from the cells. Samples should be assayed immediately or stored frozen at -20°C or below. Avoid repeated freezing and thawing of specimens.

The use of various anesthetics can cause significant elevations in serum and plasma PTH concentrations. It is therefore imperative to use consistent sample collection procedures within studies. (See References, #4)

ASSAY PROCEDURE

1. Pipet 200 µL of standard, control or sample into appropriately labeled tubes.
2. Pipet 100 µL of 125I Labeled Rat PTH Antibody into all tubes.
3. Vortex all tubes.
4. Using forceps or appropriate bead dispenser, add one bead to each tube. Tilt tube rack to approximately a 30 degree angle to prevent splashing. Cover tube rack with Parafilm® or equivalent.
5. Incubate tubes at room temperature for 18 to 24 hours.
6. Aspirate the contents of each tube. Wash beads three times by dispensing 2 mL of wash solution into each tube and then completely aspirating the contents.
7. Count each tube in a gamma counter for one minute and record the counts.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and 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.
2. The sample and the 125I Labeled Rat PTH Antibody should be pipetted carefully into the bottom one-fourth of the tube.
3. The washing step is an important part of the total assay procedure. Accurate dispensing of the wash solution and thorough and complete aspiration of the tube contents is essential. The length of time the wash solution sits in each tube is also an important factor. The washing procedure should be performed such that this timing is as consistent as possible.
4. If data reduction requires total count tubes, label duplicate tubes appropriately and pipet 100 µL of the 125I Labeled Rat PTH Antibody into each tube and cap.
5. Samples with values greater than the highest standard should be diluted 1:10 with the 0 pg/mL Standard or the optional Sample Diluent reagent and reassayed. Multiply the result by 10. (See Limitations, #1 and #2)
6. Plasma or cell culture media samples may contain fibrin clots or cellular debris. Freeze/thaw of plasma samples may accelerate clot formation. These samples must be centrifuged and decanted prior to assay to remove all particulate material which can cause random high nonspecific binding on tube or bead surface.
CALCULATION OF RESULTS
The standard curve is generated using the rat PTH standards contained in the kit. Refer to individual vial labels for exact concentrations. Generate the curve as follows:

1. Calculate the average CPM for each pair of duplicate assay tubes.
2. Subtract the average CPM of the 0 pg/mL Standard from all other average CPMs to obtain corrected CPM.
3. The standard curve is generated by plotting the corrected CPM of each standard level on the ordinate against the standard concentration on the abscissa using log-log paper. Appropriate computer assisted data reduction programs may also be used for calculation of rat PTH results.

The rat PTH concentration of the controls and samples are read directly from the standard curve using their respective corrected CPM. Samples having a corrected CPM between the 0 pg/mL Standard and the next highest standard should be calculated by the formula:

\[
\text{Value of unknown} = \frac{\text{Corrected CPM (unknown)}}{\text{Corrected CPM (2nd Std.)}} \times \text{Value of the 2nd Std.}
\]

EXAMPLE DATA AND STANDARD CURVE
The following are representative examples of data and the resulting standard curve. This curve should not be used in lieu of a standard curve run with each assay.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Tube ID.</th>
<th>CPM</th>
<th>Average Corrected CPM</th>
<th>Results pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 pg/mL</td>
<td>351</td>
<td>345</td>
<td>348</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>345</td>
<td>348</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23 pg/mL</td>
<td>1863</td>
<td>1887</td>
<td>1539</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>1910</td>
<td>1877</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>78 pg/mL</td>
<td>5210</td>
<td>5402</td>
<td>5054</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>5593</td>
<td>5402</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>230 pg/mL</td>
<td>13177</td>
<td>13361</td>
<td>13013</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>13545</td>
<td>13361</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>780 pg/mL</td>
<td>40138</td>
<td>40143</td>
<td>39795</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>40147</td>
<td>40143</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1900 pg/mL</td>
<td>70091</td>
<td>70567</td>
<td>70219</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>71042</td>
<td>70567</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Control I</td>
<td>3467</td>
<td>3568</td>
<td>3220</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>3669</td>
<td>3568</td>
<td>49</td>
</tr>
<tr>
<td>15</td>
<td>Control II</td>
<td>26738</td>
<td>27164</td>
<td>26816</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>27590</td>
<td>27164</td>
<td>493</td>
</tr>
<tr>
<td>17</td>
<td>Sample 1</td>
<td>2082</td>
<td>2021</td>
<td>1673</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>1960</td>
<td>2021</td>
<td>24</td>
</tr>
<tr>
<td>19</td>
<td>Sample 2</td>
<td>23061</td>
<td>22942</td>
<td>22594</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>22823</td>
<td>22942</td>
<td>408</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE PROCEDURE
1. The lowest concentration of rat PTH measurable is 1.0 pg/mL (assay sensitivity) and the highest concentration of rat PTH measurable without dilution is the value of the highest standard. Due to the inherent statistical variation at the highest standard as observed in any IRMA, a sample with a concentration exceeding the highest standard may, on occasion, read less than the highest standard.
2. The reagents in this Rat PTH IRMA Kit have been optimized so that the high dose “hook effect” is not a problem for samples with elevated rat PTH values. Samples with rat PTH levels between the highest standard and 100,000 pg/mL will read greater than the highest standard and should be diluted 1:10 with the 0 pg/mL Standard or the optional Sample Diluent reagent and reassayed for correct values.
3. Grossly lipemic serum or plasma samples may affect the immunological response and it is recommended that results obtained with such samples be scrutinized accordingly.
4. Differences in protein concentration and protein type between samples and standards in an immunoassay contribute to “protein effects” and dose biases. When measuring low protein concentration culture media samples against high protein concentration standards, it is recommended that like samples be assayed together in the same assay to minimize this bias.
5. The synthetic rat intact 1-84 molecule and the synthetic 1-34 fragment are highly susceptible to protease degradation. Dilution into untreated serum, plasma or protein matrix may result in erroneous and variable results depending on the level of protease present.

QUALITY CONTROL
To assure the validity of the results each assay should include adequate controls with known levels of rat PTH. ALPCO recommends that all assays include the laboratory’s own rat PTH controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS:
SENSITIVITY
The sensitivity of the rat PTH assay as determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard is 1.0 pg/mL.

PRECISION
To assess intra-assay precision the mean and coefficient of variation were calculated from 20 duplicate determinations of two samples each performed in a single assay.

<table>
<thead>
<tr>
<th>Mean Value (pg/mL)</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4.0 %</td>
</tr>
<tr>
<td>485</td>
<td>4.3 %</td>
</tr>
</tbody>
</table>
To assess inter-assay precision the mean and coefficient of variation were calculated from duplicate determinations of two samples performed in 20 assays.

<table>
<thead>
<tr>
<th>Mean Value (pg/mL)</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>4.3 %</td>
</tr>
<tr>
<td>486</td>
<td>4.7 %</td>
</tr>
</tbody>
</table>

**PARALLELISM**

Rat serum samples were diluted with the 0 pg/mL Standard and assayed. Results in pg/mL are as follows:

<table>
<thead>
<tr>
<th>SAMPLE DILUTION</th>
<th>OBSERVED VALUE</th>
<th>EXPECTED VALUE</th>
<th>% O/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 undiluted</td>
<td>441</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>217</td>
<td>221</td>
<td>98</td>
</tr>
<tr>
<td>1:4</td>
<td>108</td>
<td>110</td>
<td>98</td>
</tr>
<tr>
<td>1:8</td>
<td>51</td>
<td>55</td>
<td>93</td>
</tr>
<tr>
<td>1:2</td>
<td>149</td>
<td>147</td>
<td>101</td>
</tr>
<tr>
<td>1:4</td>
<td>70</td>
<td>74</td>
<td>95</td>
</tr>
<tr>
<td>1:8</td>
<td>37</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>2 undiluted</td>
<td>294</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>149</td>
<td>147</td>
<td>101</td>
</tr>
<tr>
<td>1:4</td>
<td>70</td>
<td>74</td>
<td>95</td>
</tr>
<tr>
<td>1:8</td>
<td>37</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>3 undiluted</td>
<td>211</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>106</td>
<td>106</td>
<td>100</td>
</tr>
<tr>
<td>1:4</td>
<td>52</td>
<td>53</td>
<td>98</td>
</tr>
<tr>
<td>1:8</td>
<td>26</td>
<td>26</td>
<td>100</td>
</tr>
</tbody>
</table>

**RECOVERY**

Various amounts of rat PTH were added to different rat serum samples and assayed. Results in pg/mL are as follows:

| SAMPLE Origin. VALUE AMOUNT ADDED OBSERVED VALUE EXPECTED VALUE % O/E |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1 12             | 184             | 194             | 196             | 99              |
|                 | 380             | 405             | 392             | 103             |
|                 | 570             | 568             | 582             | 98              |
| 2 60             | 177             | 223             | 237             | 94              |
|                 | 355             | 400             | 415             | 96              |
|                 | 533             | 590             | 593             | 100             |
| 3 90             | 170             | 234             | 260             | 90              |
|                 | 338             | 403             | 428             | 94              |
|                 | 507             | 590             | 597             | 99              |

**CROSS-REACTIVITY**

The following cross-reactants were diluted in a PTH-free protein matrix and measured using the Rat PTH IRMA kit. The results are expressed as % cross-reactivity relative to the rat PTH (1-34) standards contained in the kit.

| CROSS-REACTANT MEASURED CROSS-REACTIVITY: MEASURED CROSS-REACTIVITY: WEIGHT BASIS MOLAR BASIS |
|--------------------------------|----------------------------------|----------------------------------|----------------------------------|
| rat PTH (1-84)                | 47 %                             | 109 %                            |
| human PTH (1-34)              | 103 %                            | 104 %                            |
| human PTH (1-84)              | 29 %                             | 67 %                             |

The antibodies used in this kit have also been shown to recognize PTH in other mammalian species including mouse, dog, pig and monkey. This kit has been validated for detecting mouse PTH as reported in Reference # 5 by Meyer et al. Since these other PTH peptides are not available in purified form, actual cross-reactivities cannot be established.

**WARRANTY**

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. ALPCO DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall ALPCO be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights which vary from state to state.

**REFERENCES**


