Rat C-Terminal PTH ELISA Kit

Immutopics

Immutopics, Inc.

For RESEARCH Use Only
Not for use in diagnostic procedures

96 Tests

Cat.# 60-2200

Store at 2 - 8ºC Upon Receipt

INTENDED USE

This kit intended for research use only in the determination of rat C-Terminal PTH in serum, plasma or cell culture media.

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 from skeletal reserves into the circulation. When levels of serum calcium are increased the secretion of PTH is reduced.

PTH undergoes extensive in-vivo catabolism producing numerous mid-region and C-terminal fragments. These fragments have a much longer half-life in circulation than intact PTH and accumulate to high levels. Since PTH fragments are cleared from circulation by the kidney highly elevated levels can be seen with renal insufficiency.

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. The determination of rat PTH with this kit can provide a precise and sensitive assessment of changes in bone and mineral metabolism.

TEST PRINCIPLE

The Rat C-Terminal PTH ELISA Kit is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of PTH in rat serum, plasma or cell culture media. Goat polyclonal antibodies to rat C-terminal PTH have been purified by affinity chromatography. One antibody is biotinylated for capture and the other antibody is conjugated with the enzyme horseradish peroxidase (HRP) for detection.

A sample containing rat PTH is incubated simultaneously with the biotinylated capture antibody and the HRP conjugated antibody in a streptavidin coated microtiter well. C-terminal PTH contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a “sandwich” complex:

Well/Avidin-Biotin Anti-Rat PTH — Rat C-Term PTH —HRP Anti-Rat PTH

At the end of this incubation period, the well is washed to remove any unbound antibody and other components. The enzyme bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microtiter plate reader. The enzymatic activity of the antibody complex bound to the well is directly proportional to the amount of rat C-terminal PTH in the sample. A standard curve is generated by plotting the absorbance versus the respective rat PTH concentration for each standard on linear or logarithmic scales. The concentration of rat PTH in the sample is determined directly from this curve.

REAGENTS: Preparation and Storage

Store the reagents at 2-8ºC upon receipt. **Store the standards and controls at -20ºC or below after reconstitution.** For the expiration date refer to the label on the kit. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature and mix by gentle swirling and inversion.

1. **STREPTAVIDIN COATED MICROTITER PLATE (40-0010)**
   One plate with 12 eight well strips and frame (96 wells total). This reagent should be stored in the foil pouch with desiccant at 2 - 8°C and is stable until the expiration date on the kit.
2. **BIOTINYLATED RAT C-Terminal PTH ANTIBODY (40-2210)**
   One vial containing 2.7 mL of biotin labeled anti-rat PTH in TRIS buffered saline with protein stabilizers and a non-azide, non-mercury preservative. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit.
3. **HRP CONJUGATED RAT C-Terminal PTH ANTIBODY (40-2220)**
   One vial containing 2.7 mL of horseradish peroxidase conjugated to anti-rat PTH with protein stabilizers and a non-azide, non-mercury preservative. This reagent should be stored at 2 - 8°C protected from light and is stable until the expiration date on the kit.

   NOTE: Make a Working Antibody Solution by pipeting equal volumes of Biotinylated Rat C-Terminal PTH Antibody and HRP Conjugated Rat C-Terminal PTH Antibody prior to use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.
4. **RAT INTACT PTH STANDARDS (40-2531 to 40-2536)**
   Six vials each containing rat intact PTH (1-84) lyophilized in a protein matrix with a non-azide, non-mercury preservative. **Refer to vial label for exact concentration.** Before use reconstitute the vial with the rat intact PTH concentration of 0 pg/mL with 2.0 mL of deionized water. Before use reconstitute each of the other five vials of standards with 1.0 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 when stored at -20ºC or below with up to 3 freeze/thaw cycles.
5. **RAT INTACT PTH CONTROLS I & II (40-2541 & 40-2542)**
   Two vials each containing rat intact PTH (1-84) lyophilized in a protein matrix with a non-azide, non-mercury preservative. **Refer to vial label for control ranges.** Before use reconstitute each control with 1.0 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 when stored at -20ºC or below with up to 3 freeze/thaw cycles.

6. **ELISA WASH CONCENTRATE (40-0041)**
   One vial containing 20 mL of a 20 fold concentrate. Before use dilute the contents to 400 mL with deionized water and mix well. Upon dilution this yields a working wash solution containing a surfactant in saline with a non-azide, non-mercury preservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit.
7. **ELISA HRP SUBSTRATE (40-0021)**
   One bottle containing 11 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 - 8°C protected from light and is stable until the expiration date on the kit.
8. **ELISA STOP SOLUTION (40-0030)**
   One bottle containing 11 mL of 1 M sulfuric acid. This reagent may be stored at room temperature or at 2 - 8°C and is stable until the expiration date on the kit.
9. **PLATE SEALER (20-2000)**
   Two included in kit; use to prevent evaporation and cross-contamination of wells.

SAFETY PRECAUTIONS

Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid (i.e. ELISA HRP Substrate and ELISA Stop Solution). TMB is dissolved in a solution which contains acetone, an irritant to skin and mucous membranes. In case of contact with any of these reagents, wash thoroughly with water. TMB is a suspected carcinogen. Use Good Laboratory Practices. Wash hands before eating. Do not eat, drink or smoke in the work area.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 1.0 mL and 2.0 mL volumetric pipettes for reconstituting standards and controls.
2. Precision pipets capable of delivering 50 µL and 100 µL.
3. Aluminum foil.
4. Repeating dispenser suitable for delivering 350 µL.
5. Aspiration device or suitable microtiter plate washer.
6. Container for storage of wash solution.
7. Spectrophotometric microtiter plate reader capable of reading absorbance at 450 nm and at 595 - 650 nm.
8. Deionized water.
9. Horizontal rotator capable of maintaining 180 – 220 RPM.
10. Timer.

SPECIMEN COLLECTION

The measurement of the rat C-terminal PTH concentration may be made using serum, plasma or cell culture media. One 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 -20ºC or below. Avoid repeated freezing and thawing of specimens.

ASSAY PROCEDURE

1. Place a sufficient number of Streptavidin Coated Strips in a holder to run PTH standards, controls and unknown samples.
2. Pipet 50 µL of standard, control, or sample into the designated or mapped well.
3. Pipet 50 µL of the Working HRP Antibody Solution consisting of equal parts of Biotinylated Rat C-Terminal PTH Antibody and HRP Conjugated Rat C-Terminal PTH Antibody into each well.
4. Cover the plate with one plate sealer, then cover with aluminum foil to avoid exposure to light.
5. Incubate plate at room temperature for three (3) hours on a horizontal rotator set at 180 - 220 RPM.
6. Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well five times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microtiter plate washer can be used.
7. Pipet 100 µL of ELISA HRP Substrate into each of the wells.
8. Re-cover the plate with the plate sealer and aluminum foil. Incubate at room temperature for 30 minutes on a horizontal rotator set at 180 - 220 RPM.
9. Remove the aluminum foil and plate sealer. Read the absorbance at 620 nm (see Note) within 5 minutes in a microtiter plate reader against the 0 pg/mL Standard wells as a blank.
10. Immediately pipet 50 µL of ELISA Stop Solution into each of the wells. Mix on horizontal rotator for 1 minute.
11. Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader against a reagent blank of 100 µL of Substrate and 50 µL of Stop Solution.
    If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set to absorbance used in step #9.

    NOTE: Absorbance may be read at wavelengths from 595 nm to 650 nm depending upon available filters.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and samples be assayed in duplicate. The average absorbance reading of each duplicate should then be used for data reduction and the calculation of results.
2. Keep light sensitive reagents (i.e. HRP Conjugated Antibody, the Working HRP Antibody Solution consisting of combined HRP Conjugated Antibody and Biotinylated Antibody, and ELISA HRP Substrate) in the original amber bottles or other suitable container which is well protected from light.
3. Store any unused Streptavidin Coated Strips in the resealable aluminum pouch with desiccant to protect from moisture.
4. The sample and all reagents should be pipetted carefully to minimize air bubbles in the wells.
5. The sequence and timing of each reagent addition is important as both the immunologic and enzymatic reactions are in kinetic modes. The washing step is also an important part of the total assay procedure. The use of an automated microtiter plate washer is strongly recommended. All pipeting and washing steps should be performed such that the timing is as consistent as possible.
6. Samples with values greater than the higest standard should be diluted 1:10 with the 0 pg/mL Standard and reassayed. Multiply the result by 10. (See Limitations, #1 and #2)

7. Plasma or cell culture media samples may contain fibrin clots orcellular 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 non-specific binding on well surface.

CALCULATION OF RESULTS

The use of the two absorbance measurements, the first at 595 to 650 nm and the second after the addition of the ELISA Stop Solution at 450 nm, combined with the range of standards above provides for two ways to calculate results. The first allows the curve to be extended to the highest standard for measuring high dose samples while the second shifts the response back towards the low end of the curve to provide better sensitivity for measuring low dose samples.

Each curve should be generated as follows:

Primary Procedure — Read at 450 nm

1. Calculate the average absorbance for each pair of duplicate assay wells.
2. Subtract the average absorbance of the 0 pg/mL Standard from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by plotting the corrected absorbance of the first five standard levels on the ordinate against the standard concentration on the abscissa using linear-linear or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The PTH concentration of the samples is read directly from the standard curve using their respective corrected absorbance. If log-log graph paper or computer assisted data reduction programs utilizing logarithmic transformation are used, samples having corrected absorbance between the 0 pg/mL Standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected Absorbance (unknown)}}{\text{Corrected Absorbance (2nd Std.)}} \times \text{Value of the 2nd Std.}$$

Secondary Procedure — Read at 595 nm to 650 nm

1. Calculate the average absorbance for each pair of duplicate assay wells.
2. The standard curve is generated by plotting the absorbance of the three highest standards on the ordinate against the standard concentration on the abscissa using linear-linear or log-log graph paper.
3. The PTH concentration of the samples is read directly from the standard curve.

LIMITATIONS OF THE PROCEDURE

1. The lowest concentration of rat C-terminal PTH measurable is 5 pg/mL (assay sensitivity) and the highest concentration of rat C-terminal PTH measurable without dilution is the value of the highest standard.
2. The reagents in this Rat C-Terminal PTH ELISA kit have been optimized so that the high dose “hook effect” is not a problem for samples with elevated rat C-terminal PTH values. Samples with rat C-terminal PTH levels between the highest standard and 2,000,000 pg/mL will read greater than the highest standard and should be diluted 1:10 or greater with the 0 pg/mL Standard 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.

QUALITY CONTROL

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

CROSS-REACTIVITY

The affinity purified antibodies used in this assay both recognize epitopes within the C-terminal (53-84) portion of rat PTH. Because of this C-terminal recognition by the antibodies the assay is not able to differentiate between C-terminal fragments and the intact form of PTH and will detect both if present in the sample. In addition, the C-terminal portion of the PTH molecule is highly conserved between rat and mouse PTH.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Immutopics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Immutopics, Inc. 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.

CLIENT SERVICES

To place an order or for technical assistance, contact Immutopics International at (800) 681-6665 or (949) 369-9207 or FAX to (949) 369-9405 or e-mail: info@immutopicsintl.com.

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Distributed by: *Immutopics International* *San Clemente, CA 92673*

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60-2200

Effective: 09/05