# Mouse Intact PTH ELISA Kit

Enzyme-Linked ImmunoSorbent Assay (ELISA) for the Quantitative Determination of Mouse Intact Parathyroid Hormone Levels in Plasma or Cell Culture Media

## For RESEARCH Use Only

## Not for use in diagnostic procedures

## **INTENDED USE**

This kit is intended for research use only in the determination of mouse intact PTH levels in plasma or cell culture media.

## INTRODUCTION

Mouse and rat intact parathyroid hormone (PTH) are both 84 amino acid polypeptides produced by the parathyroid gland with their 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 mouse and rat and human physiology relative to calcium metabolism make the mouse and rat excellent live-animal models for studying human skeletal disease and in the pre-clinical evaluation of pharmacologic agents that may alter bone remodeling. Quantitation of biologically active mouse intact PTH with this kit can provide a precise and sensitive assessment of changes in bone and mineral metabolism.

## **TEST PRINCIPLE**

The Mouse Intact PTH ELISA Kit is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of PTH in mouse plasma or cell culture media. Two affinity purified goat polyclonal antibodies have been selected to cross-react and detect the biologically active intact form of both mouse and rat PTH. (See Cross-reactivity section on page 4.) The antibody which recognizes epitopes within the midregion/C-terminal portion (39-84) of the peptide is biotinylated for capture. The other antibody, which recognizes epitopes within the N-terminal region (1-34), is conjugated with the enzyme horseradish peroxidase (HRP) for detection.

A sample containing mouse intact PTH is incubated simultaneously with the biotinylated capture antibody and the HRP conjugated antibody in a streptavidin coated microtiter well. Intact PTH (1-84) contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a "sandwich" complex:

Well/Avidin-Biotin Anti-Mouse PTH - Mouse Intact PTH - HRP Anti-Mouse 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 intact PTH in the sample. A standard curve is generated by plotting the absorbance versus the respective intact PTH concentration for each standard on linear or logarithmic scales. The concentration of mouse intact PTH in the samples is determined directly from this curve. *(Standards are analytically prepared from synthetic Rat Intact PTH 1-84. Mouse PTH values are reported as Rat PTH equivalents.)* 

## **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 allow all reagents to come to room temperature and

**mix by gentle swirling and inversion.** Reagents from different kit lot numbers should not be combined or interchanged.

- 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.
- MOUSE INTACT PTH BIOTINYLATED ANTIBODY (40-2310) One vial containing 2.7 mL of biotin labeled anti-mouse PTH in TRIS buffered saline with protein stabilizers and a non-azide, nonmercury preservative. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit.
- 3. MOUSE INTACT PTH HRP CONJUGATED ANTIBODY (40-2320)

One vial containing 2.7 mL of horseradish peroxidase conjugated to anti-mouse PTH in a stabilized protein solution with 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 pipetting equal volumes of Mouse Intact PTH Biotinylated Antibody and Mouse Intact PTH HRP Conjugated 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 -20°C 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 controls immediately after reconstitution; freeze the unused portion for later use. After reconstitution the controls are stable until the expiration date on the kit when stored at  $-20^{\circ}$ C 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.

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Store at 2 - 8°C Upon Receipt

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96 Test Kit Cat.# 60-2300

#### 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 -  $8^{\circ}$ 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 crosscontamination 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 25  $\mu$ L, 50  $\mu$ L and 100  $\mu$ L.
- 3. Aluminum foil.
- 4. Repeating dispenser suitable for delivering 350 μL.
- 5. Asration 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 595 650 nm.
- 8. Deionized water.
- Horizontal rotator capable of maintaining 180 220 RPM.
   Timer.

## **SPECIMEN COLLECTION**

The intact PTH molecule is unstable, resulting in decreased immunoreactivity over time. Sample collection and storage procedures should be carried out in an expeditious manner. Due to the variable lability of the molecule, measurement of the mouse intact PTH concentration should be made using EDTA plasma or cell culture media. (Serum is no longer recommended as an appropriate sample.) Fifty microliters of plasma or culture media are required to assay the sample in duplicate. Centrifuge the sample and separate the 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 blood PTH concentrations. It is therefore imperative to use consistent sample collection procedures within studies. (See ref. #4)

## ASSAY PROCEDURE

- 1. Place a sufficient number of Streptavidin Coated Strips in a holder to run PTH standards, controls and unknown samples.
- 2. Pipet 25  $\mu$ L of standard, control, or sample into the designated or mapped well. Freeze the remaining standards and controls as soon as possible after use.
- Pipet 50 μL of the Working Antibody Solution consisting of equal volumes of Mouse Intact PTH Biotinylated Antibody and Mouse Intact PTH HRP Conjugated 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 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  $\mu$ L 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  $\mu\text{L}$  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 595 nm (see Note) within 5 minutes in a microtiter plate reader against the 0 pg/mL Standard wells as a blank.
- 10. Immediately pipet 100  $\mu L$  of ELISA Stop Solution into each of the wells. Mix on a horizontal rotator for 1 minute.
- 11. Read the absorbance at 450 nm within 10 minutes in the microtiter plate reader against a reagent blank of 100  $\mu$ L of Substrate and 100  $\mu$ L 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

- 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.
- Store light sensitive reagents (i.e. HRP Conjugated Antibody, the Working Antibody Solution consisting of combined Biotinylated Antibody and HRP Conjugated 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 immunological 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 highest 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 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 non-specific binding on well surface.

## **CALCULATION OF RESULTS**

The two absorbance readings taken before and after the addition of the ELISA Stop Solution allow for the construction of two standard curves using the rat intact PTH standards contained in the kit. **Refer to the individual vial label for exact concentration.** The primary curve used for calculation of results is the second reading taken after the addition of the ELISA Stop Solution and read at 450 nm. This data utilizes the absorbance values obtained with the first five standards. The first reading taken before the addition of the ELISA Stop Solution and read at 595 nm is intended to extend the analytical range to the value of the sixth (highest) standard provided in the kit. It should be utilized only if sample results extend beyond the value of the fifth standard. Results obtained with the first reading should not replace the on-scale reading at 450 nm. 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.
- Subtract the average absorbance of the 0 pg/mL Standard from the average absorbance of all other readings to obtain corrected absorbance.
- 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 the mouse intact PTH results.

The intact PTH concentration of the controls and samples are 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:

Value of unknown = (unknown) x Value of the 2<sup>nd</sup> Std. (2<sup>nd</sup> Std.)

#### Secondary Procedure — Read at 595 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 intact PTH concentration of samples reading greater than the fifth standard are read directly from the standard curve.

### **EXAMPLE DATA AND STANDARD CURVE**

The following are representative examples of data and the resulting standard curves from the primary and secondary procedures. These curves should not be used in lieu of a standard curve run with each assay.

| PRIMARY ASSAY - 450 nm   |                |                |                  |                  |   |
|--|----------------|----------------|------------------|------------------|---|
| WELL<br>I.D.   | ABS            | AVERAGE<br>ABS | CORRECTED<br>ABS | RESULTS<br>pg/mL | _ |
| Reagent Blank  | 0.000<br>0.000 | 0.000          |                  |                  |   |
| 0 pg/mL  | 0.052<br>0.053 | 0.053          |                  |                  |   |
| 25 pg/mL   | 0.099<br>0.093 | 0.096          | 0.043            |                  |   |
| 80 pg/mL   | 0.188<br>0.182 | 0.185          | 0.132            |                  |   |
| 277 pg/mL  | 0.516<br>0.497 | 0.507          | 0.454            |                  |   |
| 840 pg/mL  | 1.457<br>1.380 | 1.419          | 1.366            |                  |   |
| Control I  | 0.134<br>0.131 | 0.133          | 0.080            | 47               |   |
| Control II   | 0.280<br>0.282 | 0.281          | 0.228            | 140              |   |
| Sample 1   | 0.171<br>0.170 | 0.171          | 0.118            | 71               |   |
| Sample 2   | 0.506<br>0.520 | 0.513          | 0.460            | 281              |   |
| Sample 3   | 1.672<br>1.620 | 1.646          | 1.593            | *                |   |
| <ul> <li>* &gt; 840 pg/mL; calculate using secondary assay.</li> </ul> |                |                |                  |                  |   |



| SECONDARY ASSAY - 595 nm |                |                |                  |   |
|--------------------------|----------------|----------------|------------------|---|
| WELL<br>I.D.             | ABS            | AVERAGE<br>ABS | RESULTS<br>pg/mL | _ |
| 0 pg/mL                  | 0.000<br>0.000 | 0.000          |                  |   |
| 277 pg/mL                | 0.083<br>0.076 | 0.080          |                  |   |
| 840 pg/mL                | 0.263<br>0.248 | 0.256          |                  |   |
| 2700 pg/mL               | 0.842<br>0.836 | 0.839          |                  |   |
| Sample 3                 | 0.312<br>0.295 | 0.304          | 992              |   |



#### LIMITATIONS OF THE PROCEDURE

- The lowest concentration of mouse intact PTH measurable is 3 pg/mL (assay sensitivity) and the highest concentration of mouse intact PTH measurable without dilution is the value of the highest standard.
- 2. The reagents in this Mouse Intact PTH ELISA kit have been optimized so that the high dose "hook effect" is not a problem for samples with elevated intact PTH values. Samples with mouse intact PTH levels between the highest standard and 200,000 pg/mL will read greater than the highest standard and should be diluted 1:10 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 mouse or rat intact PTH. Immutopics recommends that all assays include the laboratory's own mouse or rat intact PTH controls in addition to those provided with this kit.

## PERFORMANCE CHARACTERISTICS: SENSITIVITY

The sensitivity of the mouse intact PTH assay as determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard is 3 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.

| Mean Value (pg/mL) | Coefficient of Variation |
|--------------------|--------------------------|
| 54                 | 3.9 %                    |
| 157                | 2.5 %                    |

To assess inter-assay precision the mean and coefficient of variation were calculated from duplicate determinations of two samples performed in 20 assays.

| Mean Value (pg/mL) | Coefficient of Variation |
|--------------------|--------------------------|
| 55                 | 8.9 %                    |
| 163                | 7.8 %                    |

## PARALLELISM

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

| SAMPLE | DILUTION  | OBSERVED<br>VALUE | EXPECTED<br>VALUE | % O/E |
|--------|-----------|-------------------|-------------------|-------|
| 1      | undiluted | 87                |                   |       |
|        | 1:2       | 41                | 43                | 95    |
|        | 1:4       | 20                | 22                | 91    |
| 2      | undiluted | 138               |                   |       |
|        | 1:2       | 66                | 69                | 96    |
|        | 1:4       | 32                | 35                | 91    |
| 3      | undiluted | 222               |                   |       |
|        | 1:2       | 106               | 111               | 95    |
|        | 1:4       | 47                | 56                | 84    |

## RECOVERY

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

| SAMPLE | ORIG.<br>VALUE | AMOUNT<br>ADDED | OBSERVED<br>VALUE | EXPECTED<br>VALUE | % O/E |
|--------|----------------|-----------------|-------------------|-------------------|-------|
| 1      | 17             | 29              | 45                | 46                | 98    |
|        |                | 57              | 76                | 74                | 103   |
|        |                | 86              | 100               | 103               | 97    |
| 2      | 19             | 46              | 64                | 65                | 98    |
|        |                | 91              | 112               | 110               | 102   |
|        |                | 137             | 155               | 156               | 99    |
| 3      | 14             | 17              | 30                | 31                | 97    |
|        |                | 34              | 47                | 48                | 98    |
|        |                | 50              | 65                | 64                | 102   |

## CROSS-REACTIVITY

The mouse intact PTH ELISA is specific for the intact 1-84 form of the molecule. N-terminal 1-34 or mid-region and C-terminal 39-84 fragments will not be measured. The antibodies in this kit have been selected to cross-react and detect the biologically active intact form of both mouse and rat PTH. However, the relative affinities of the antibodies to these two peptides are unknown. Cross-reactivity to other mammalian species is not known.

#### 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.

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### **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.

Developed andImmutopics, Inc.Manufactured by:San Clemente, CA 92673

Distributed by:

Immutopics International

San Clemente, CA 92673

## www.immutopicsintl.com

Catalog # 60-2300

90-2300

Effective: 04/07

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