

Mouse PTH 1-84 ELISA Kit

Enzyme-Linked ImmunoSorbent Assay (ELISA) for the
Quantitative Determination of Mouse Parathyroid
Hormone 1-84 Levels in Plasma or Cell Culture Media

For RESEARCH Use Only

Not for use in diagnostic procedures

Immutopics

Immutopics, Inc.

96 Test Kit
Cat.# 60-2305

Store at 2 - 8°C Upon Receipt

INTENDED USE

This kit is intended for research use only in the determination of mouse PTH 1-84 levels in plasma or cell culture media.

INTRODUCTION

Mouse intact parathyroid hormone (PTH 1-84) is an 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 mouse and human physiology relative to calcium metabolism make the mouse an excellent live-animal model for studying human skeletal disease and in the pre-clinical evaluation of pharmacologic agents that may alter bone remodeling. Quantitation of biologically active mouse PTH 1-84 with this kit can provide a precise and sensitive assessment of changes in bone and mineral metabolism.

TEST PRINCIPLE

The Mouse PTH 1-84 ELISA Kit is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of PTH in mouse plasma or cell culture media. Two different goat polyclonal antibodies have been affinity purified against mouse PTH to detect the biologically active intact form of mouse PTH. 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 PTH 1-84 in the sample. A standard curve is generated by plotting the absorbance versus the respective PTH 1-84 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 Mouse Intact PTH 1-84.)**

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.
- 2. MOUSE PTH 1-84 BIOTINYLATED ANTIBODY (40-2315)**
One vial containing 2.7 mL of biotin labeled anti-mouse 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. MOUSE PTH 1-84 HRP CONJUGATED ANTIBODY (40-2325)**
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 PTH 1-84 Biotinylated Antibody and Mouse PTH 1-84 HRP Conjugated Antibody prior to use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.
- 4. MOUSE PTH 1-84 STANDARDS (40-2331 to 40-2336)**
Six vials each containing mouse 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 mouse intact PTH 1-84 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. MOUSE PTH 1-84 CONTROLS I & II (40-2341 & 40-2342)**
Two vials each containing mouse 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°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 phosphate buffered 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 (40-0026)**
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 (10-2016)

Two included in kit; use to prevent evaporation and cross-contamination of wells.

10. MOUSE PTH 1-84 SAMPLE DILUENT (Optional reagent, must be ordered separately using cat. #30-2331)

One bottle containing 10 mL of a lyophilized protein matrix with a non-azide, non-mercury preservative. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the bottle. Before use reconstitute with 10 mL of deionized water. Allow the bottle to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use. Aliquot and freeze (-20° or below) any unused portion for later use.

SAFETY PRECAUTIONS

Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid (i.e. ELISA HRP Substrate and ELISA Stop Solution). 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 20 µL, 50 µL and 100 µL.
3. Aluminum foil.
4. Automated microtiter plate washer OR
5. Repeating dispenser for delivering 350 µL and suitable aspiration device.
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.
9. Horizontal rotator capable of maintaining 180 - 220 RPM.
10. 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 PTH 1-84 concentration should be made using EDTA plasma or cell culture media.** Forty 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. #1 and #2)

ASSAY PROCEDURE

1. Place a sufficient number of Streptavidin Coated Strips in a holder to run PTH standards, controls and unknown samples.
2. Pipet 20 µL of standard, control, or sample into the designated or mapped well. Freeze the remaining standards and controls as soon as possible after use.
3. Pipet 50 µL of the Working Antibody Solution consisting of equal volumes of Mouse PTH 1-84 Biotinylated Antibody and Mouse PTH 1-84 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. **Using an automated microtiter plate washer 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.**

A suitable aspiration device may also 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 100 µL 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 µL of Substrate and 100 µL of Stop Solution.

If dual wavelength correction is available set the Measurement wavelength to 450 nm and Reference wavelength 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. 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 or optional Sample Diluent 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.
8. Rarely, upon opening the streptavidin plate, small white crystals may be observed in some of the wells. This is entirely cosmetic and will not affect the assay. This condition is reported by other kit manufacturers and results from the final stabilizing buffer used in the coating process.

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 mouse PTH 1-84 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 - 650 nm is intended to extend the analytical range to the value of the sixth (highest) standard provided in the kit. **It should be used only for sample results that fall between the value of the fifth and sixth 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.
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 the mouse PTH 1-84 results.

The PTH 1-84 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:

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

Secondary Procedure — Read at 595 nm – 650 nm

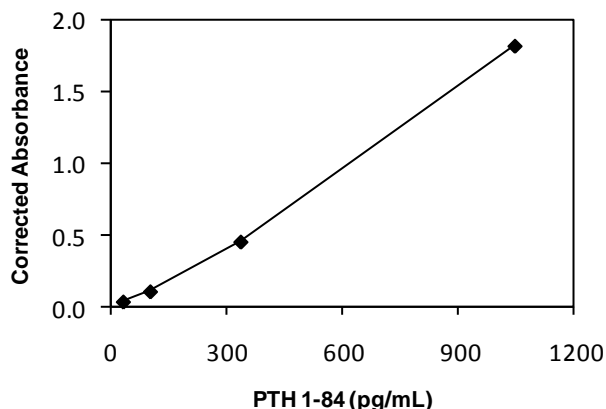
1. Calculate the average absorbance for each pair of duplicate assay wells.
2. The standard curve is generated by plotting the absorbance of the three highest standards on the ordinate against the standard concentration on the abscissa using linear-linear or log-log graph paper.
3. The PTH 1-84 concentration of samples reading only between the fifth and sixth standard are read directly from this standard curve.

EXAMPLE DATA AND STANDARD CURVE

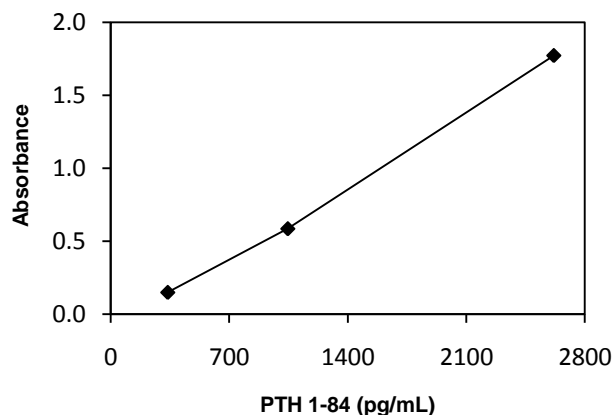
The following are representative examples of data and the resulting standard curves from the primary and secondary procedures. **These curves should not be used in lieu of a standard curve run with each assay.**

PRIMARY ASSAY - 450 nm				
WELL I.D.	ABS	AVERAGE ABS	CORRECTED ABS	RESULTS pg/mL
Reagent Blank	0.000			
	0.000	0.000		
0 pg/mL	0.009			
	0.005	0.007	0.000	
32 pg/mL	0.041			
	0.041	0.041	0.034	
102 pg/mL	0.107			
	0.117	0.112	0.105	
336 pg/mL	0.453			
	0.464	0.458	0.451	
1045 pg/mL	1.837			
	1.805	1.821	1.814	
Control I	0.073			
	0.072	0.073	0.066	64
Control II	0.264			
	0.262	0.263	0.256	204
Sample 1	0.263			
	0.247	0.255	0.248	199
Sample 2	0.178			
	0.169	0.173	0.166	144
Sample 3	2.083			
	2.127	2.105	2.098	*

* > 1045 pg/mL; calculate using secondary assay.

Mouse PTH 1-84 ELISA Primary Assay

SECONDARY ASSAY - 620 nm			
WELL I.D.	ABS	AVERAGE ABS	RESULTS pg/mL
0 pg/mL	0.000		
	0.000	0.000	
336 pg/mL	0.151		
	0.149	0.150	
1045 pg/mL	0.588		
	0.584	0.586	
2616 pg/mL	1.765		
	1.779	1.772	
Sample 3	0.689		
	0.705	0.697	1192

Mouse PTH 1-84 ELISA Secondary Assay**LIMITATIONS OF THE PROCEDURE**

1. The lowest concentration of mouse PTH 1-84 measurable is 4 pg/mL (assay sensitivity) and the highest concentration of mouse PTH 1-84 measurable without dilution is the value of the highest standard.
2. The reagents in this Mouse PTH 1-84 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 500,000 pg/mL will read greater than the highest standard and should be diluted 1:10 with the 0 pg/mL Standard or optional Sample Diluent and reassayed for correct values.

- Grossly lipemic serum or plasma samples may affect the immunological response and it is recommended that results obtained with such samples be scrutinized accordingly.
- 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 intact PTH. Immutopics recommends that all assays include the laboratory's own mouse intact PTH controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS:

SENSITIVITY

The sensitivity of the mouse PTH 1-84 assay as determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard is 4 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
63	5.6 %
198	2.4 %

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
60	5.7 %
209	5.4 %

PARALLELISM

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

SAMPLE	DILUTION	OBSERVED VALUE	EXPECTED VALUE	% O/E
1	undiluted	298		
	1:2	115	149	77
	1:4	53	74	72
	1:8	28	37	76
2	undiluted	156		
	1:2	65	78	83
	1:4	27	39	69
	1:8	15	19	79
3*	undiluted	156		
	1:2	72	78	92
	1:4	33	39	85
	1:8	20	19	105

* Same as Sample #2 except diluted in mouse plasma.

RECOVERY

Various amounts of mouse PTH 1-84 were added to three different mouse plasma samples and assayed. Results in pg/mL are as follows:

SAMPLE	ORIG. VALUE	AMOUNT ADDED	OBSERVED VALUE	EXPECTED VALUE	% O/E
1	46	19	53	65	82
		28	69	74	93
		37	78	83	94
2	46	73	101	119	85
		145	174	191	91
		218	231	264	88
3	61	69	118	130	91
		138	176	199	88
		207	229	267	86

CROSS-REACTIVITY

This Mouse PTH 1-84 ELISA kit uses synthetic mouse PTH 1-84 peptide in the standards and controls and in the affinity purification of the polyclonal antibodies, thus assuring high specificity and affinity to mouse PTH. N-terminal (a.a.1-34) or mid-region and C-terminal (a.a. 39-84) fragments will not be measured. Due to the high homology (90%) between the mouse and rat intact PTH sequences the antibodies in this kit will also cross-react with the biologically active intact form of rat PTH. 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: clientservices@immutopicsintl.com.

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