

High Sensitivity Human PTH (1-34) ELISA Kit

High Sensitivity (HS) Enzyme-Linked ImmunoSorbent Assay (ELISA)
for the Quantitative Determination of Human Parathyroid Hormone
1-34 Levels in Plasma or Cell Culture Media

Immutopics
Immutopics, Inc.

For RESEARCH Use Only

96 Test Kit
Cat.# 60-3900

Not for use in diagnostic procedures

Store at 2 - 8°C Upon Receipt

INTENDED USE

This kit is intended for research use only in the determination of human PTH (1-34) in plasma or cell culture media. Reference ranges and clinical utility have not been established.

INTRODUCTION

Over the years, studies involving the intermittent dosing of human PTH (1-34) in animals and humans have demonstrated an anabolic effect on bone. This effect was positive for bone mass, size, structure, and strength, demonstrating the antifracture efficacy of the treatment. Recently the FDA has approved the recombinant form of human PTH (1-34) – rhPTH (1-34) or teriparatide, for treatment of many forms of osteoporosis. The measurement of circulating levels of this peptide/drug may be useful in assessing its pharmacological properties and for appropriate dosing and schedule optimization. Additionally, this assay may be useful in screening samples for both endogenous levels of circulating hPTH (1-34) and elevated levels of this peptide as it may interfere to varying degrees with 2-site immunometric assays used for measuring intact PTH (1-84).

Obtaining precise measurements of human PTH (1-34) are complicated by antibody cross-reactivity to endogenous PTH (1-84), requiring either a pre-assay immunoextraction of PTH (1-84) or assaying for both PTH (1-34) and intact PTH (1-84) to account for this cross-reactivity.

This High Sensitivity (HS) Human PTH (1-34) ELISA Kit uses two antibodies that have been prepared for optimal recognition of human PTH (1-34) while minimizing binding to human PTH (1-84). We have determined the cross-reactivity of 1-84 to be less than 3% on a molar basis. However, if cross-reactivity correction is desired, the concurrent use of PTH (1-84) standards is suggested.

TEST PRINCIPLE

The High Sensitivity Human PTH (1-34) ELISA Kit is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of Human PTH (1-34) in plasma or cell culture media. An affinity purified goat polyclonal antibody optimized to bind human PTH (1-34) is biotinylated for capture. Another affinity purified goat polyclonal antibody, specific for a separate portion of the human PTH (1-34) peptide, is conjugated with the enzyme horseradish peroxidase (HRP) for detection.

A sample containing human PTH (1-34) is incubated simultaneously with the biotinylated capture antibody and the HRP conjugated detection antibody in a streptavidin coated microliter well. Human PTH (1-34) contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a “sandwich” complex that is further bound to the microtiter well by the streptavidin-biotin complex:

Well/Avidin — Biotin Anti-h PTH — Human PTH (1-34) — HRP Anti-h 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 human PTH (1-34) in the sample. A standard curve is generated by plotting the absorbance versus the respective human PTH (1-34) concentration for each standard on linear or logarithmic scales. The concentration of human PTH (1-34) 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 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. HIGH SENSITIVITY HUMAN PTH (1-34) BIOTINYLATED ANTIBODY (40-3910)**
One vial containing 2.7 mL of biotinylated anti-human 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. HIGH SENSITIVITY HUMAN PTH (1-34) HRP ANTIBODY (40-3920)**
One vial containing 2.7 mL of horseradish peroxidase conjugated to anti-human 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 High Sensitivity Human PTH (1-34) Biotinylated Antibody and High Sensitivity Human PTH (1-34) HRP Antibody prior to use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.
- 4. HIGH SENSITIVITY HUMAN PTH (1-34) STANDARDS (40-3931 to 40-3937)**
Seven vials each containing human PTH (1-34) lyophilized in a human serum matrix with a non-azide, non-mercury preservative. **Refer to vial label for exact concentration.** Before use reconstitute the vial with the PTH (1-34) concentration of 0 pg/mL with 2.0 mL of deionized water. Before use reconstitute each of the other six 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. HIGH SENSITIVITY HUMAN PTH (1-34) CONTROLS I & II (40-3941 & 40-3942)**
Two vials each containing human PTH (1-34) lyophilized in a human serum 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 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 (40-0022)

One bottle containing 21 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.

10. HUMAN PTH SAMPLE DILUENT (Optional reagent, must be ordered separately using catalog #30-3131)

One bottle containing 10 mL of a lyophilized, treated, stabilized human serum matrix with a non-azide, non-mercury preservative. Reconstitute with 10 mL of deionized water. See insert accompanying product for preparation and storage.

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.

CAUTION: Potential Biohazardous Material

HANDLE ASSAY REAGENTS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.

The human source material used in the preparation of this product has been tested by an FDA approved method for the presence of antibodies to Human Immunodeficiency Virus (HIV I and HIV II) and to Hepatitis C virus (HCV), as well as for Hepatitis B surface antigen (HBsAg) and found to be negative. Because no test method can offer complete assurance that HIV I and HIV II, HCV, HBsAg or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human urine, serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1993.

MATERIALS REQUIRED BUT NOT PROVIDED

- 1.0 mL volumetric pipette for reconstituting standards and controls.
- Precision pipets capable of delivering 50 μ L and 100 μ L.
- Aluminum foil.
- Repeating dispenser suitable for delivering 350 μ L.
- Automated microtiter plate washer or suitable aspiration device.
- Container for storage of wash solution.
- Spectrophotometric microtiter plate reader capable of reading absorbance at 450 nm and at 595-650 nm.
- Deionized water.
- Horizontal rotator capable of maintaining 180 - 220 RPM.
- Timer.

SPECIMEN COLLECTION

Measurement of the PTH (1-34) concentration should be made using EDTA plasma or cell culture media. EDTA plasma is preferred versus serum for enhanced sample stability. Three hundred microliters * (**See Modifications**) of plasma or 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.

ASSAY PROCEDURE

- Place a sufficient number of Streptavidin Coated Strips in a holder to run PTH standards, controls and unknown samples.
- Pipet 150 μ 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 μ L of the Working Antibody Solution consisting of 1 part HRP Antibody and 1 part Biotinylated Antibody into each well.
- Cover the plate with one plate sealer, then cover with aluminum foil to avoid exposure to light.
- Incubate plate at room temperature for three (3) hours on a horizontal rotator set at 180 - 220 RPM.
- Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well five times by dispensing 350 μ L of working wash solution into each well and then completely aspirating the contents. Preferably, an automated microtiter plate washer should be used.
- Pipet 200 μ L* of ELISA HRP Substrate into each of the wells.
- 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.
- 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.
- Immediately pipet 50 μ L of ELISA Stop Solution into each of the wells. Mix on horizontal rotator for 1 minute.
- Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader against a reagent blank of 200 μ L* of Substrate and 50 μ 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.

*** See Modifications**

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.
- Keep light sensitive reagents (i.e. HRP Antibody, the Working Antibody Solution consisting of combined HRP Antibody and Biotinylated Antibody, and ELISA HRP Substrate) in the original amber bottles or other suitable container which is well protected from light.
- Store any unused Streptavidin Coated Strips in the resealable aluminum pouch with desiccant to protect from moisture.
- The sample and all reagents should be pipetted carefully to minimize air bubbles in the wells.
- 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.
- 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, # 2)
- 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 use of 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 the standards provides 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 six 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:

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

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 human PTH 1-34 concentration of samples reading greater than the sixth standard are read directly from the standard curve.

EXAMPLE DATA AND STANDARD CURVE

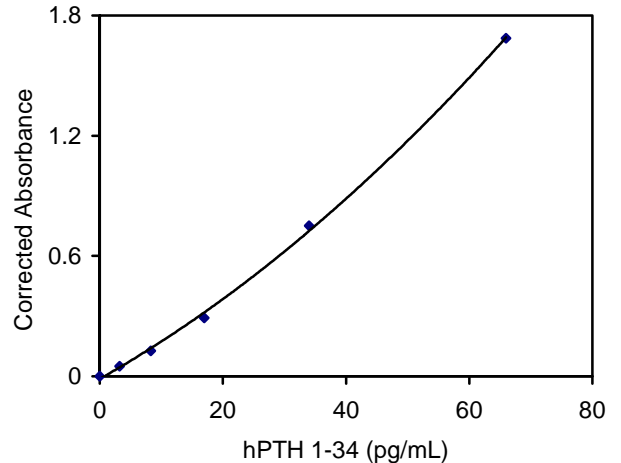
The following are representative examples of data and the resulting standard curves from the primary and secondary procedures. **These curves should not be used in lieu of a standard curve run with each assay.**

PRIMARY ASSAY - 450 nm				
WELL I.D.	AVERAGE ABS	CORRECTED ABS	RESULTS pg/mL	
Reagent Blank	0.000			
0 pg/mL	0.029	0.000		
3.2 pg/mL	0.083	0.079	0.050	
8.3 pg/mL	0.153	0.157	0.126	
17 pg/mL	0.313	0.327	0.291	
34 pg/mL	0.796	0.762	0.750	
66 pg/mL	1.722	1.708	1.686	
Control I	0.216	0.229	0.222	11.9
Control II	0.440	0.444	0.442	21.5
Sample 1	0.115	0.103	0.109	5.2
Sample 2	1.944	1.898	1.921	*

* >66 pg/mL; Calculate using secondary assay.

High Sensitivity Human PTH 1-34 ELISA

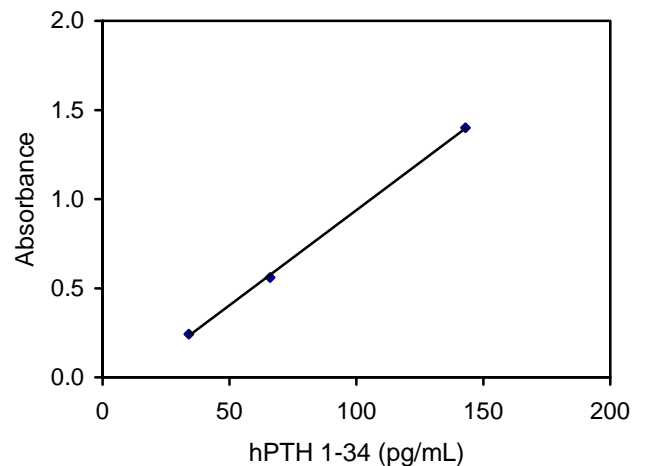
Primary Assay



SECONDARY ASSAY - 620 nm			
WELL I.D.	ABS	AVERAGE ABS	RESULTS pg/mL
0 pg/mL	0.000	0.000	
34 pg/mL	0.245	0.243	
66 pg/mL	0.558	0.563	
143 pg/mL	1.387	1.415	
Sample 2	0.638	0.623	74

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Secondary Assay



LIMITATIONS OF THE PROCEDURE

1. The lowest concentration of human PTH (1-34) measurable is 0.3 pg/mL* (assay sensitivity) and the highest concentration of human PTH (1-34) measurable without dilution is the value of the highest standard. **(See Modifications)**
2. The reagents in this High Sensitivity Human PTH (1-34) ELISA Kit have been optimized so that the high dose "hook effect" is not a problem for samples with elevated PTH (1-34) values. Samples with levels between the highest standard and 300,000 pg/mL will read greater than the highest standard and should be diluted 1:10 or more with the 0 pg/mL Standard and reassayed for correct values.
3. Grossly lipemic 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 human PTH (1-34). Immutopics recommends that all assays include the laboratory's own human PTH (1-34) controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS:

SENSITIVITY

The sensitivity of the High Sensitivity Human PTH (1-34) ELISA assay as determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard is 0.3 pg/mL.* **(See Modifications)**

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
10.1	4.0%
18.6	2.5%

To assess inter-assay precision the mean and coefficient of variation were calculated from duplicate determinations of two samples performed in 11 assays.

Mean Value (pg/mL)	Coefficient of Variation
11.3	4.5%
20.1	4.3%

CROSS-REACTIVITY

The following cross-reactants were diluted in the 0 pg/mL Standard and measured using the High Sensitivity Human PTH (1-34) ELISA Kit. The results are expressed as % cross-reactivity relative to the human PTH (1-34) standards contained in the kit.

CROSS-REACTANT MEASURED	CROSS-REACTIVITY:	
	WEIGHT BASIS	MOLAR BASIS
human PTH (1-34)	100.0	100.0
human PTH (1-84)	6.7	2.9
human PTH (7-84)	<0.001	<0.0004
rat PTH (1-34)	15.0	15.2
rat PTH (1-84)	1.5	0.7
bovine PTH (1-34)	7.4	7.4
bovine PTH (1-84)	0.7	0.3

MODIFICATIONS

When sampling from rats or mice it is often necessary to use a smaller sample volume.

This assay can be easily modified to use either a 25 µL or a 50 µL sample size by lowering the volume of standard, control and sample pipetted in Step #2 of the Assay Procedure **AND** reducing the volume of ELISA HRP Substrate pipetted in Step #7 to 100 µL. All other procedural steps remain the same.

Reducing the sample size will change the reported sensitivity.

As determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard, the sensitivity for each sample size is as follows:

25 µL	50 µL	150 µL
1.8 pg/mL	0.8 pg/mL	0.3 pg/mL

RECOVERY

Seven, presumed normal, EDTA plasma samples were spiked with 3.5 picograms of hPTH (1-34) to yield an expected concentration of 23.3 pg/mL. The mean recovery was 103% (24.0 pg/mL) ± 11% (2.6 pg/mL).

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.

REFERENCES

1. Reeve J, "Teriparatide [rhPTH (1-34)] and Future Anabolic Treatments in Osteoporosis", In: Favus MJ, Ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Washington, D.C.; American Society for Bone and Mineral Research, Sixth Edition, 2003; 344-349.
2. Gao P, Schmidt-Gayk H, Dittrich K, Nolting B, Maier A, Roth H J, Seemann O, Reichel H, Ritz E, Schilling T, "Immunochemiluminometric assay with two monoclonal antibodies against the N-terminal sequence of human parathyroid hormone", *Clinica Chimica Acta*, Vol. 245, 1996; 39-59.

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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