Anti-Human PTH (1-34) Antibody ELISA Kit

Immutopics

Immutopics, Inc.

Enzyme-Linked ImmunoSorbent Assay (ELISA) for the Detection of Anti-Human PTH (1-34) Antibodies in Serum, Plasma or Cell Culture Media

96 Test Kit

For RESEARCH Use Only

Cat.# 60-4000

Not for use in diagnostic procedures

Store at 2 - 8ºC Upon Receipt

INTENDED USE

This kit is intended for research use only in the qualitative detection of antibodies to human PTH (1-34) in humans or other animal models. Reference ranges and clinical utility have not been established.

INTRODUCTION

Teriparatide (rhPTH 1-34) is a recombinant human parathyroid hormone derivative consisting of the first 34 amino acids of the hormone. Teriparatide was approved as a drug by the FDA in 2002 and is sold by Eli Lilly & Co. under the brand name Forteo. Teriparatide is indicated for use in patients with osteoporosis. It is currently administered by injection and has an anabolic effect on bone, binding to the PTH/PTHrp Type 1 receptor with the same affinity as the intact molecule, PTH (1-84). This receptor activation results in a series of events leading to the formation of new trabecular and cortical bone. Research is continuing at pharmaceutical and biotech companies to pursue similar drugs and alternate delivery pathways.

Immunogenicity for this drug, defined as the presence of antibodies to human PTH (1-34), was detected in 2.8% of women receiving teriparatide during the clinical trials of Forteo. The detection of these circulating antibodies may be of clinical relevance for the proper assessment of patients.

TEST PRINCIPLE

The Anti-Human PTH (1-34) Antibody ELISA Kit is a two step enzyme-linked immunosorbant assay (ELISA) for the qualitative detection of antibodies to human PTH (1-34) in serum, plasma or cell culture media. The kit contains human PTH (1-34) immobilized onto microtiter plate wells. In the first step, a sample is incubated with the immobilized human PTH (1-34). If specific antibodies are present in the sample, they will bind to the plate well:

Well-PTH (1-34) — Anti-PTH (1-34)

The well is then washed to remove unbound components and in a second step a solution containing human PTH (1-34) conjugated to horseradish peroxidase (HRP) for detection is added to the well and incubated. This reagent binds to the free binding site of any antibody captured in the first step thus forming a bound complex:

Well - PTH (1-34) – Anti-PTH (1-34) – HRP/PTH (1-34)

At the end of this second incubation the well is washed again to remove unbound HRP-human PTH (1-34). The well is then incubated with an enzyme substrate solution in a timed reaction and the absorbance measured in a spectrophotometric microtiter plate reader. A sample containing antibodies to human PTH (1-34) will form a complete bound complex as described above and thus react with the enzyme substrate to produce a color. The intensity of this color is directly proportional to the amount and binding affinity of any captured antibodies from the sample. Negative and positive reference controls are included in the kit and should be run in each assay along with the samples. A sample should be considered positive for anti-human PTH (1-34) antibody if it produces an absorbance value distinctly greater than the negative control and other negative samples.

REAGENTS: Preparation and Storage

Store the kit at 2-8ºC upon receipt. 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. **HUMAN PTH (1-34) COATED MICROTITER PLATE (40-4010)**
   One plate with 12 eight well strips (96 wells total) coated with human PTH (1-34) plus desiccant. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit.
2. **ASSAY BUFFER (40-4060)**
   One vial containing 5.5 mL of assay buffer 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 HUMAN PTH (1-34) (40-4020)**
   One vial containing 10.5 mL of horseradish peroxidase conjugated human PTH (1-34) 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.
4. **ANTI-HUMAN PTH (1-34) ANTIBODY REFERENCE CONTOLS 1, 2, 3, 4 (40-4041, -4042, -4043, -4044)**
   Four vials each containing 1.0 mL of serum with 0.1% sodium azide as a preservative. One vial, labeled Control 1, Negative contains normal human serum with no anti-human PTH (1-34) antibodies. The other three vials contain Positive Control sera: Control 2, 1:100 (diluted 1:100 with normal human serum), Control 3, 1:10 (diluted 1:10 with normal human serum) and Control 4, 1:1 (undiluted).
   The controls should be stored at 2-8°C and are stable until the expiration date on the kit.
5. **ELISA WASH CONCENTRATE (40-0041)**
   Two bottles each containing 20 mL of a 20 fold concentrate. Before use dilute the contents of both bottles to 800 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.
6. **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.
7. **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.
8. **PLATE SEALER (10-2016)**
   Two included in kit to prevent evaporation and cross-contamination.

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.

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," 1999.

MATERIALS REQUIRED BUT NOT PROVIDED

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

SPECIMEN COLLECTION

One hundred microliters of serum, plasma or media is required to assay the sample in duplicate. Samples may be stored at 2-8° C for three days or stored frozen at -20° C or below for longer time periods.

ASSAY PROCEDURE

1. Place a sufficient number of Human PTH (1-34) Coated Strips in a holder to run Anti-human PTH (1-34) Antibody Controls and unknown samples.
2. Pipet 50 µL of control or sample into the designated or mapped well.
3. Pipet 50 µL of Assay Buffer into each well.
4. Cover the plate with a plate sealer and incubate at room temperature for one (1) hour on a horizontal rotator set at 180 - 220 RPM.
5. Remove the 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. Preferably, an automated microtiter plate washer can be used.
6. Pipet 100 µL of Human PTH (1-34) HRP Conjugate into each well.
7. Re-cover the plate with a plate sealer, then cover with aluminum foil to avoid exposure to light.
8. Incubate plate at room temperature for one (1) hour on a horizontal rotator set at 180 - 220 RPM.
9. 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. Preferably, an automated microtiter plate washer can be used.
10. Pipet 100 µL of ELISA HRP Substrate into each well.
11. 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.
12. Remove the aluminum foil and plate sealer. Read the absorbance at 620nm (see Note) within 5 minutes in a microtiter plate reader.
13. Immediately pipet 50 µL of ELISA Stop Solution into each of the wells. Mix on the horizontal rotator for 1 minute.
14. Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader.

If dual wavelength correction is available set the Measurement wavelength to 450 nm and Reference wavelength to absorbance used in Step #12.

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 Peptide and ELISA HRP Substrate) in the original amber bottles or other suitable container which is well protected from light.
3. Store any unused 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. 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.

EXAMPLE DATA

WELL I.D.	ABS (620 nm)	ABS (450 nm)
NEGATIVE	0.010	0.013
	0.009	0.012
POSITIVE (1:100)	0.108	0.293
	0.105	0.289
POSITIVE (1:10)	1.098	3.130
	1.152	3.045
POSITIVE (1:1)	2.154	3.823
	2.201	3.943

Note: Readings at 450 nm enhance assay sensitivity.

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: clientservices@immutopicsintl.com.

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