Human Bioactive PTH 1-84 ELISA Kit

Immutopics

Immutopics, Inc.

For RESEARCH Use Only

96 Test Kit

Cat. # 60-3000

Not for use in diagnostic procedures

Store at 2 - 8ºC Upon Receipt

INTENDED USE

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

REAGENTS: Preparation and Storage

Store the reagents at 2-8ºC upon receipt. 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 HUMAN PTH 39-84 ANTIBODY (40-3015)**
   One vial containing 2.7 mL of biotin labeled 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. **HRP CONJUGATED HUMAN PTH 1-3 ANTIBODY (40-3025)**
   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 combining equal volumes of Biotinylated Human PTH Antibody and HRP Conjugated Human PTH Antibody prior to use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.
4. **HUMAN INTACT PTH STANDARDS (40-3031 to 40-3036)**
   Six vials each containing synthetic human PTH (1-84) 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 intact PTH concentration of 0 pg/mL with 2.0 mL of deionized water. Before use reconstitute each of the other five lyophilized 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. **HUMAN INTACT PTH CONTROLS I & II (40-3041 & 40-3042)**
   Two vials each containing human intact PTH (1-84) 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 -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 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.

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. 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.

ASSAY PROCEDURE

1. Place a sufficient number of Streptavidin Coated Strips in a holder to run PTH standards, controls and unknown samples.
2. Pipette 50 µL of standard, control, or sample into the designated or mapped well.
3. Pipette 50 µL of the Working Antibody Solution consisting of equal volumes of Biotinylated Human PTH 39-84 Antibody and HRP Conjugated Human PTH 1-3 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. **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. Pipette 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 pipette 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 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. 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 re-sealable 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. 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.
7. 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 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:

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

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 PTH concentration of samples is read directly from the standard curve.

CROSS-REACTIVITY

This assay may detect with varying degrees of cross-reactivity the PTH from other mammalian species.

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's at (800) 681-6665 or (949) 369-9207 or FAX to (949) 369-9405 or e-mail: clientservices@immutopicsintl.com.

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