

Canine Intact PTH ELISA Kit

Immutopics

Immutopics, Inc.

For RESEARCH Use Only

96 Test Kit

Cat. # 60-3800

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 canine PTH in serum, plasma or cell culture media.

REAGENTS: Preparation and Storage

Store the reagents at 2-8°C 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 CANINE PTH ANTIBODY (40-3815)

One vial containing 2.7 mL of biotin labeled anti-canine 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 CANINE PTH ANTIBODY (40-3825)

One vial containing 2.7 mL of horseradish peroxidase conjugated to anti-canine PTH in TRIS buffered saline with protein stabilizers and 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 Canine PTH Antibody and HRP Conjugated Canine PTH Antibody prior to use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.

4. HUMAN INTACT PTH ZERO STANDARD (40-3031)

One vial containing lyophilized human serum matrix with a non-azide, non-mercury preservative. Before use reconstitute the vial with the intact PTH concentration of 0 pg/mL with 2.0 mL of deionized water. Allow the vial to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

5. CANINE PTH INTERMEDIATE STANDARD (40-3851)

One vial containing synthetic canine 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 1.0 mL of deionized water. Allow the vial to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

Prepare a series of working standards by diluting the canine PTH intermediate standard with the PTH zero standard to achieve PTH levels of approximately 10 pg/mL to 2,000 pg/mL.

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 (40-0021)

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°C and is stable until the expiration date on the kit.

9. PLATE SEALER (20-2000)

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). 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. 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.

ASSAY PROCEDURE

- Place a sufficient number of Streptavidin Coated Strips in a holder to run PTH standards, controls and unknown samples.
- Pipette 50 μ L of standard, control, or sample into the designated or mapped well.
- Pipette 50 μ L of the Working Antibody Solution consisting of equal volumes of Biotinylated Canine PTH Antibody and HRP Conjugated Canine PTH 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. Alternatively, an automated microtiter plate washer can be used.
- Pipette 100 μ 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 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 μ L 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 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.

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.

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:

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

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

SENSITIVITY

The sensitivity of the canine intact PTH assay as determined on 16 duplicate determinations of the 0 pg/mL Standard is 3 pg/mL.

CROSS-REACTIVITY

The antibodies used in this assay are two different affinity purified polyclonal preparations isolated from goats immunized with human PTH. Both the HRP conjugated detection antibody, which recognizes amino terminal epitopes, and the biotinylated capture antibody, which recognizes c-terminal epitopes, exhibit excellent recognition to canine PTH.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Immunotopics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Immunotopics, 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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To place an order or for technical assistance, contact Immunotopics International at (800) 681-6665 or (949) 369-9207 or FAX to (949) 369-9405 or e-mail: info@immunotopicsintl.com.

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