CANINE TESTOSTERONE

CANINE TESTOSTERONE ELISA TEST KIT (SERUM)

CATALOG NUMBER:ERK C2012 **PRODUCT PROFILE AND INSTRUCTIONS**

Model: 96 Microwell Model
Procedure: 2 STEP INCUBATION
Results: 2 HOURS
Sensitivity: 0.1 ng/mL
Storage: 4-8°C
Shelf Life: 6 months

INTENDED USE

The Microwell Testosterone ELISA is an enzyme immunoassay system for quantitative determination of Testosterone levels in Canine and related species serum/plasma. The test is intended for professional use as an aid in the diagnosis and monitoring of conditions related to serum Testosterone. The test kit is designed to be used by a trained, skilled professional only.

INTRODUCTION

Testosterone is a steroid hormone with secreted from the Leydig cells of the testis in the male, adrenals and the ovaries. The dihydro derivative of Testosterone exerts a potent anabolic action responsible for the post pubescent growth rate and subsequent muscle and bone tissue maintenance of adult males. Testosterone assays are of significance in a number of endocrine dysfunction as in adult Leydig cell or seminiferous cell failure. Testosterone levels in serum may be raised by certain drugs such as 19-nortesterone, epitestosterone, ethisterone and Danazol.

TEST PRINCIPLES

The Testosterone quantitative test is based on a solid-phase enzyme immunoassay based on competitive binding method. A sample (serum/ plasma/urine) containing an unknown amount of Testosterone to be assayed (unlabeled antigen) is added to a standard amount of a conjugated Testosterone(labeled antigen). The labeled and unlabeled antigens are then allowed to compete for high affinity binding sites of anti-rabbit Testosterone antibodies on a limited number of goat anti IgG antibodies coated on to the plate. The reaction takes place when incubated for 2 hours at 37C, during this period a bio-specific reaction takes place After incubation , wash away the free antigen and add TMB substrate solution and incubated for 20 minutes, a blue color developed will be stopped with a stop solution (2N HCl). Absorbencies are measured at 450 nm using ELISA plate reader. A standard curve is produced using values from standards from which absorbency values for blank tubes have been subtracted. The amount of labeled antigen in the sample is reversibly proportional to the concentration of the unlabeled antigen. As the concentration increases in the sample the color intensity decreases proportionately. The results for unknown may be read directly from this standard curve using either manual calculation or by a suitable computer program. This kit is suitable for the direct measurement of Testosterone in serum samples. It may also be used following an extraction procedure, for assaying urinary Testosterone.

Materials Provided

1. Microtiter wells coated with Goat anti rabbit IgG antibody
2. Enzyme-labeled Testosterone reagent, 12 mL
3. Rabbit anti testosterone, 7 mL
4. Testosterone reference set: 0, 0.1, 0.5, 2.0, 6.0, 18 ng/mL
5. Sample diluent, 25 mL
6. TMB Color Reagent, 15 mL
7. Stopping Solution, 6 mL
8. 20 X Wash Buffer, 20 mL.
9. Instructions

Materials Required But Not Provided

1. Semiautomatic pipettes: 20ul and 200ul
2. Disposable pipette tips
3. Microtiter plate shaker
4. Microtiter well reader.
5. Plate washer
6. Absorbant paper
7. 37 C incubator
8. Parafilm to cover plate
9. Distilled water

PRECAUTIONS

1. This kit contains reagents manufactured from animal blood components. The source materials have been tasted by immunoassay for hepatitis B surface antigen and antibodies to HIV virus and found to be negative. Nevertheless, all blood products and samples should be considered potentially infectious and handling should be in accordance with the procedures defined by an appropriate biohazard safety guideline or regulations in your state. 2. The contents of this kit, and their residues, must not come into contact with ruminating animals or swine or other animals. 3. Avoid contact with the Stopping Reagent. It may cause skin irritation and burns. 4.Do not use reagents after expiration date. 5. Do not mix or use components from the kits with different lot numbers.6. Replace caps on reagents immediately. Do not switch caps. 7. Reagents contain sodium azide (NaN3) as a preservative.
   On disposal, flush with a large volume of water to prevent azide build-up. 8. Do not pipette reagents by mouth. 9. Do not use reagents from other kits or mix with other manufactured test kits.

STORAGE & STABILITY CONDITIONS

1. Store the kit at 2-8 C upon receipt and when it is not in use. **Do not Freeze.**
2. Keep microtiter wells in a sealed bag with desiccants to minimize exposure to damp air.
3. Allow all the reagents to reach to room temperature before setting up the assay.
4. Remove only desired number of wells and seal the bag and store at 2-8 C as before.
5. Do not at any time mix or use components with other manufacturers kits. Do not use the kit components after expiration date and discard according to the state and local regulations.

INSTRUMENTATION

A microtiter well reader with bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 405 nm wavelength is acceptable for use in absorbency measurement.

SPECIMEN COLLECTION AND PREPARATION

1. This kit is suitable for use with serum or heparin plasma samples. The use of hemolytic or lipemic samples and samples with bilirubin will affect results and may interfere with the assay.
2. No special preparation of the samples is required. Avenous blood sample (enough to produce about 0.5 ml serum) is collected aseptically.

3. If the sample is not tested immediately refrigerate at 2-8 C. If the storage period greater than 3 days are anticipated, the specimen should be frozen and repeated thawing and freezing should be avoided.
4. If the sample is turbid or contain precipitate may give false results. Such samples should be centrifuged before use.

REAGENT PREPARATION

1. Prepare Wash buffer by diluting 1 part with 19 parts of distilled water, excess amount may be stored at 2-8 C for couple of weeks. 2. Dilute highly concentrated specimen samples with sample dilution buffer and mix well before use in the assay. **3 Preparation of the samples: Mix 0.05mL of serum with 0.20mL of sample diluent and add 0.05mL per well. The samples diluted can be stored at –20C for further use. Dilution of sample will eliminate adding very low volume (10ul).**

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 50 ul of standards, diluted samples, and controls into appropriate wells.
3. Add 100 ul of Testosterone Enzyme Conjugate Solution to each well (except those set for blanks).
4. Add 50 ul of rabbit anti testosterone antibodies and mix well for 30 sec. and incubate at 37C for 2 hours. You may use parafilm to cover the wells or use appropriate zip-lock bag to store the plate during the incubation.
5. Discard the contents of the wells and wash the plate 5 times with Wash Solution (250-300ul) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture.
6. Add 100 ul TMB color into each well (including the blanks). Remember for pipetting order.
7. Incubate the plate for 20 minutes at room temperature .
8. Stop reaction by adding 50ul of Stopping Solution to wells in the same sequence that the Substrate Solution was added and gently mixed.
9. Read the absorbance at 450 nm with a microwell reader.

NOTE: The substrate incubation should be carried out within the temperature range 20-25C. For temperature outside this range, the duration of the incubation should be adjusted.

CALCULATIONS

1. Calculate the mean absorbance values (A) for each set of reference standards, controls, samples and blanks.
2. Subtract the value for blanks from those for standards, control and unknown samples.
3. Calculate the B/B)% values by dividing each value by the value for the zero-standard.
4. For the standards, plot a graph on semi-log graph paper with B/BO% values on the ordinate and the Testosterone concentrations (pg/mL) on the abscissa.
5. Using the graph read off the Testosterone concentrations for the unknown samples.
6. The values above the readable and below the readable range should be repeated using appropriate dilution.

SENSITIVITY & EXPECTED VALUES

The sensitivity of the assay is 0.1 ng/mL and each clinical laboratory should establish its own normal range based on different samples.

QUALITY CONTROL

Good Laboratory practice requires that quality control specimens be run with each standard curve to establish assay performance characteristics such as recovery, linearity, precision and specificity. The average recovery in this assay is in the range of 96.6% The recovery in the linearity range is about 98.5% and the linear range of the assay is 0-1000pg/mL. The intra-assay variation 9.3% and inter assay variation is about 9.6% The specificity was assessed by determining the crossreactivity of several known steroids in the assay and found less than 0.4% with androsterone and 0.25% with corticosterone but others showed no significant crossreactivity.

LIMITATIONS OF THE TEST

1. The Testosterone ELISA system designed here is for estimation of Testosterone levels in Canine and related speciessamples only. 2. The wells should be adequately washed to obtain reproducible results. The washing step is extremely important and should be followed according to the instructions. 3. The assay should be performed by trained and skilled professional only.

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