

**PRODUCT PROFILE AND INSTRUCTIONS****INTENDED USE**

The Microwell Testosterone ELISA is an enzyme immunoassay system for quantitative determination of Testosterone levels in Swine and related species serum/plasma/urine. 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-nortestosterone, 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 Testosterone antibodies coated on to the plate. The reaction takes place when incubated for 1 hour 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 anti testosterone antibody
2. Enzyme-labeled Testosterone reagent, 12 mL
3. Testosterone reference set: 0, 0.1, 0.5, 1.0, 2.0, 10, 20ng/mL (QC1 (~0.5ng/mL) and QC2 (~ 2.0 ng/mL), 0.5ml/vial
4. Sample diluent, 20 mL
5. TMB Color Reagent, 12 mL
6. Stopping Solution, 6 mL
7. 20 X Wash Buffer, 20 mL.
8. 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 tested 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.
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 (NaN<sub>3</sub>) 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.

## ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. **Pipette 50 ul of standards, samples, and controls into appropriate wells.**
3. Add 100 ul of Testosterone Enzyme Conjugate Solution to each well (except those sets) shake well for 1-2 minutes and incubate at 37°C for 1 hour.
4. 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.
5. Add 100 ul TMB color into each well (including the blanks). Remember for pipetting order.
6. Incubate the plate for 20 minutes at room temperature.
7. Stop reaction by adding 50ul of Stopping Solution to wells in the same sequence that the Substrate Solution was added and gently mixed.
8. Read the absorbance at 450 nm with a micro well reader.

**NOTE:** The substrate (TMB) incubation should be carried out within the temperature range 20-25°C. 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/B0 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/B0% 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 20pg/mL and each clinical laboratory should establish its own normal range based on the experience and animal condition.

## LIMITATIONS OF THE TEST

1. The Testosterone ELISA system designed here is for estimation of Testosterone levels in swine and related species samples 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. Trained and skilled professional only should perform the assay.

## Limitations & Warranty

The present ELISA is designed for helping the scientist to analyze test samples only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the ELISA product. The analyst should always establish the standard curve and a small number of samples before proceeding to analyze a large number of important samples, to save precious samples.

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Swine Testosterone ELISA Test Kit

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