



# PRIMATE TESTOSTERONE

## PRIMATE TESTOSTERONE ELISA TEST KIT

### PRODUCT PROFILE AND INSTRUCTIONS

The Microwell Testosterone ELISA is an enzyme immunoassay system for quantitative determination of Testosterone levels in Primate/related species serum. 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-rabbit Testosterone antibodies on a limited number of goat anti rabbit IgG antibodies coated on to the plate. After washing away the free antigen, the amount of labeled antigen in the sample is reversibly proportional to the concentration of the unlabeled antigen. The actual concentrations in unknown samples are obtained by means of a standard curve based on known concentrations of unlabeled antigen analyzed in parallel with the unknowns. After washing, TMB substrate solution is added and the enzyme is allowed to react for a fixed time before the reaction is terminated. Absorbencies are measured at 450 nm using ELISA plate reader. A standard curve is produced using values from 5 standards from which absorbency values for blank tubes have been subtracted. 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, 0.25mL each  
0, 0.1, 0.5, 2.0, 5.0 and 20 ng/mL
5. Quality control set of 2 QC1 (<2.0ng/mL) QC2 (10ng/mL)
6. TMB Color Reagent, 12 mL
7. Stopping Solution, 6 mL
8. 20 X Wash Buffer, 20 mL.
9. Sample Diluent , 20 mL
10. 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 Primate/related 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 Primate/related 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.



コスモ・バイオ株式会社

## 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.

## 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.

## ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 10 ul of standards, samples, and QC 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 and read the absorbance at 450 nm with a microwell plate 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 (ng/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.

## REFERENCES

1. Tietz 1970 Fundamentals of Clinical Chemistry
2. Williams 1968 Text book of Endocrinology, 4th edition
3. Sobel CS et al. J Clin Endo 1958 18, 208
4. Zimmerman W 1935 Ztschr Physiol Chem 233, 257
5. Klings K et al. Maternal peripheral testosterone levels during first half of pregnancy Am J Obstet Gynecol.
6. Sanchez RS et al. 1976 Fertility & Sterility 27, 6-20
7. Winter JSD et al. Pituitary gonadal relations in infancy, pattern of serum gonadal steroid concentrations in man from birth to two years of age. J Clin Endocrinol Metab 42, 679 1976
8. Sizoneko PC Endocrinology in preadolescents and adolescents Hormonal changes during normal puberty Am J Dis Child 132 704, 1978
9. Sandoff L. et al. 1974 Obstet and Gynecol 43, 2-13
10. Casey JH et al. 1968 J Clin Endo Metab 28, 479-483
11. Johnson ES 1987 The Lancet April 4, 814
12. Korenman SG et al. 1987 Clin Res 35, 182A
13. Slaats EH et al 1987 Clin Chem 33, 300-302
14. Wilke TJ & Utley DJ 1987 Clin Chem 33, 1372-1375

Revised August 2006



# PRIMATE TESTOSTERONE

---

## PRIMATE TESTOSTERONE ELISA TEST KIT

---

### QUALITY CONTROL DATA

#### Examples of ETI Primate/related Testosterone Standard Curve:

A typical Testosterone ELISA standard curve run as a quality control of each lot is given below:

Primate/related Testosterone concentration ng/mL	Absorbency 450nm
0.0 ng/ml	2.55
0.1 ng/ml	2.12
0.5 ng/ml	1.05
2.0 ng/ml	0.75
5.0 ng/ml	0.55
20 ng/ml	0.26

#### 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 your laboratory primate population.

#### 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.

#### Sensitivity and specificity:

The sensitivity of the assay is 0.2 ng/mL and each laboratory should establish its own base levels based on the species and physiological situation.

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.

#### LIMITATIONS OF THE TEST

1. The present Endocrine's ELISA system designed here is for estimation of testosterone levels in serum/plasma of primate samples only.
2. The wells should be adequately washed to obtain reproducible results.
3. The washing step is extremely important and should be followed according to the instructions..
4. Trained and skilled professional only should perform the assay.
5. Do not mix components with other kits from different sources.

---

[www.endocrinetech.com](http://www.endocrinetech.com) [info@endocrinetech.com](mailto:info@endocrinetech.com)

35325 Fircrest Street Newark, CA 94560-1003

Phone 510-745-0844

---