

RODENT TESTOSTERONE

RODENT TESTOSTERONE ELISA TEST KIT LYOPHILIZED STANDARDS

INTENDED USE

The Microwell Testosterone ELISA is an enzyme immunoassay system for quantitative determination of Testosterone levels in rodent and related species serum or 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 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 or plasma) 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 37°C, 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 or samples.

Materials Provided

1. Microtiter wells coated with anti testosterone antibody
2. HRP Conjugate 12 ml
3. Lyophilized standards, 0, 0.1, 0.5, 1.0, 2.0, 10, 20ng/mL (QC1 (~0.5ng/mL) and QC2 (~ 2.0ng/mL),/vial, reconstitute in 1 ml with standard/sample diluent
4. Standard/Sample Diluent, 20mL
5. TMB Color Reagent, 12mL
6. Stopping Solution, 6mL
7. 20 X Wash Buffer, 20mL.

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 (NaN₃) as a preservative. On disposal, flush with 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 OF TEST KIT AND INSTRUMENTATION

Note of Caution: Immediately after receiving the kit all standards, if not used, should be kept at -20°C. Unopened test kits should be stored at 2-8°C. The microtiter plate should always be kept in a sealed bag with desiccants to minimize exposure to damp air at room temperature. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. Do not leave any reagents at room temperature for more than 3 hours.

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. Lyophilized standards should be diluted in 1 ml standard diluent and can be stored at -20C for long term use.

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 microwell 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 Rodent 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. Shipping charges of the replacement kits must be the responsibility of the receiver. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

REFERENCES

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ENDOCRINE TECHNOLOGIES, INC. USA

www.endocrinetech.com

RODENT Testosterone ELISA Test Kit

Product Profile and Instructions

35325 Fircrest Street, Newark, CA 94560-1003 * Phone (800) 745-0843 * (510) 745-0844 * Fax (510) 745-0977



RODENT

TESTOSTERONE

QUALITY CONTROL DATA

Examples of ETI Rodent Testosterone Standard Curve:

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

<u>Rodent Testosterone concentration ng/mL</u>	<u>Absorbency 450nm</u>
0.0 ng/ml	3.2
0.1 ng/ml	2.8
0.5 ng/ml	1.24
1.0 ng/ml	0.82
2.0 ng/ml	0.66
10. ng/ml	0.32
20.0ng/ml	0.23

SENSITIVITY & EXPECTED VALUES

The sensitivity of the assay is 20g/mL and each clinical laboratory should establish its own normal range based on your laboratory animal 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 98.2%. The recovery in the linearity range is about 99.1% and the linear range of the assay is 0-1000pg/mL. The intra-assay variation 9.3% and inter assay variation is about 8.8% The specificity was assessed by determining the cross reactivity of several known steroids in the assay and found less than 0.6% with androsterone and 0.35% with corticosterone but others showed no significant cross-reactivity.

Precision: Inter and Intra assay variation (CV) was determined from ten different pooled male rat serum samples in three different experiments.

Inter-assay variation	Set1: CV= 8.73% (N=10)	Set2: CV= 8.65 % (N=10)	Set3: CV= 9.1 % (N=10)
Intra-assay variation	Set1: CV= 7.42 % (N=10)	Set2: CV= 8.54 % (N=10)	Set3: CV= 8.21 % (N=10)

LIMITATIONS OF THE TEST

1. The present Endocrine's ELISA system designed here is for estimation of testosterone levels in serum/plasma of rodent 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 info@endocrinetech.com

35325 Fircrest Street Newark, CA 94560-1003

Phone 510-745-0844



TECHNOLOGIES, INC.

RODENT TESTOSTERONE ELISA

SPECIFICITY TESTING

CROSS-REACTION WITH OTHER STEROID COMPOUNDS

COMPOUND	% CROSS-REACTION
TESTOSTERONE*	100
PREGNANELONE	<0.1
DIHYDROANDROSTENE	<0.1
5a-ANDROSTENEDIONE	<0.1
TESTOSTERONE PROPIONATE	<0.1
DIHYDROTESTOSTERONE**	<3.8
PROGESTERONE	<0.1

- TESTOSTERONE INHIBITION @ 20NG/ML IS MAXIMUM WHILE OTHER COMPOUNDS EVEN AT 200NG/ML IS NOT EFFECTIVE EXCEPT DHT** WHICH IS ALSO ONLY AT HIGH CONCENTRATION SHOWS 3.8% INHIBITION. WHERE AS DHT AT 20NG/ML HAS NO CROSS-REACTIVITY IN THIS SYSTEM.

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