
RODENT TESTOSTERONE CLIA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS**INTENDED USE**

The Microwell Testosterone CLIA is an enzyme immunoassay system for quantitative determination of Testosterone levels in rodent 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 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

In the rodent testosterone CLIA (Chemiluminisense Immuno Assay) test, a certain amount of anti-testosterone antibody is coated on to a microtiter wells. A measured amount of unknown sample and a constant amount of testosterone conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the testosterone in the test samples and conjugated testosterone compete for the limited binding sites on the anti-testosterone antibody. After 60 minutes of incubation period, at 37°C, the wells are washed 5 times with wash buffer to remove any unbound conjugate. A substrate luminal reagent is then added and incubated for 2 minutes, resulting in the development of light and the emitting light intensity is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled cortisol in the test sample. A series of testosterone standards assayed in the same way, a standard curve is constructed and the concentration of testosterone in the unknown sample is quantified.

This rodent testosterone CLIA kit is suitable for the direct measurement of testosterone in serum/plasma/urine samples. It may also be used following an extraction procedure for assaying urinary testosterone (call for details of the procedure). Note: The testosterone levels should be established in your laboratory using your own set of samples and standards and good laboratory practice should be employed where applicable. .

Materials Provided

1. Microtiter wells coated with anti testosterone antibody
2. Enzyme-labeled Testosterone reagent, 12 mL
3. Testosterone reference set: 0, 0.025, 0.05, 0.5, 1.0, 2.5, 10ng/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. On disposal, flush with a large volume of water.
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.

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. Prepare desired volume of substrate mixing A+B in the dark and must use immediately.

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Pipette 5 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 of freshly prepared substrate should be added in the dark.
6. Incubate the plate for 2 minutes at room temperature.
7. Read in a chemiluminisense plate readr immediately.

CALCULATIONS

1. Calculate the mean light emittance values (RUL) 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 10pg/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 CLIA 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 CLIA 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 CLIA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the CLIA product. 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 CLIA Test Kit

Product Profile and Instructions

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

QUALITY CONTROL DATA

www.endocrinetech.com info@endocrinetech.com

35325 Fircrest Street Newark, CA 94560-1003
Phone 510-745-0844

 **ENDOCRINE**

TECHNOLOGIES, INC.

RODENT TESTOSTERONE CLIA

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
DIHYDROTTESTOSTERONE**	<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.**

