
TESTOSTERONE (TEST) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL1104-2

INTENDED USE

The Autobio testosterone CLIA test kit is intended for the quantitative determination of testosterone concentration in human serum.

INTRODUCTION

Testosterone is formed in the leydig cell, then capillaries and veins carry it to the periphery or it traverses testicular myoid cells and enters the seminiferous tubules where it is involved in spermatogenesis. In the seminiferous tubules testosterone stimulates primary spermatocytes to form secondary spermatocytes and finally young spermatocytes. Testicular secretion accounts for 95 percent of the circulating testosterone present in men. In female, the ovary and adrenal secrete small amounts of testosterone; however, the majority of testosterone in the blood derives from metabolism of androstenedione.

The measurement of serum testosterone in man and boy is related to the investigation of testicular dysfunction and is used to monitor the treatment of patients with congenital adrenal hyperplasia. In women, serum testosterone is useful evaluating hirsutism, alopecia and menstrual disorder. The plasma concentration of testosterone also is part of the evaluation of newborns or young infants with ambiguous genitalia, and isolated micropenis.

The traditional assay of plasma testosterone involves extraction of steroids, chromatographic purification, RIA and ELISA. This system is used to establish an CLIA method for detection of total testosterone in serum.

PRINCIPLE OF THE TEST

In the Test CLIA test procedure, the testosterone standard or patient serum is incubated with the testosterone antibody and the testosterone-horseradish peroxidase conjugate in the anti-mouse IgG coated well. In this solid-phase system, the antibody bound testosterone will remain on the well while unbound testosterone will be removed by washing. A chemiluminescence reaction is developed when the CLIA substrate is mixed with the antibody bound testosterone-horseradish peroxidase enzyme conjugate. The Related Light Unit (RLU) is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Test in the sample. By reference to a series of Test standards assayed in the same way, the concentration of Test in the unknown sample is quantified.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with goat anti-mouse IgG (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled testosterone (Test) in Stabilizing Buffer (1 vial, 3.0ml/6.0 ml)
3. Anti-Test Reagent: Mouse monoclonal antibodies to Test (anti-Test MAb) in Stabilizing Buffer (1 vial, 3.0ml/6.0ml)
4. Reference Standards: 0, 0.5, 1, 2.5, 10 and 20ng/ml (6 vials, 0.5ml/ea)
5. Substrate A: (1 vial, 1.8ml/3.5ml)
6. Substrate B: (1 vial, 1.8ml/3.5ml)
7. PBS-T Powder: PBS-Tween (1 bag, 5g)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Precision pipettes and tips, 0.025ml, 0.05ml
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper
6. Luminometer

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 ~ 8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2 ~ 8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing. Multiple freeze-thaw cycles should be avoided.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18 ~ 25°C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Prepare Wash Solution: add 1 bag of PBS-T Powder to 500ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for 2 months.

IMPORTANT NOTES

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU values.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 25µl of Reference Standards, specimens, and controls into appropriate wells.
2. Dispense 50µl of Anti-Test Reagent into each well.
3. Dispense 50µl of Enzyme Conjugate Reagent into each well. Gently mix for 30 seconds.
4. Incubate at room temperature for 60 minutes.
5. At the end of the incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with wash buffer. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of each well is about 300µl.
6. Dispense 25µl of substrate A, then 25µl of substrate B into each well. Gently mix for 10 seconds.
7. Incubate at room temperature in the dark for 10 minutes without shaking, then read the RLU values with a Luminometer.

CALCULATION OF RESULTS

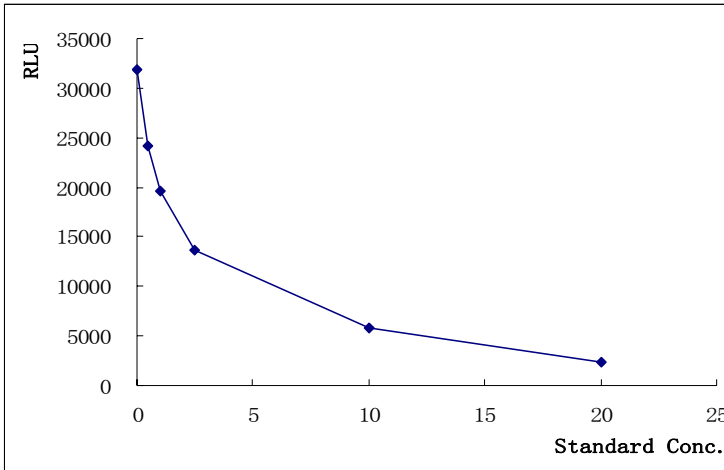
1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.

2. On linear graph paper plot the RLU (ordinate) for each Reference Standard against the corresponding concentration of TEST in ng/ml (abscissa) and draw a calibration curve through the Reference Standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

T (ng/ml)	RLU
0	31888.0
0.5	24214.3
1	19563.3
2.5	13576.8
10	5837.0
30	2286.0



EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on randomly selected clinical laboratory samples:

Testosterone Level (ng/ml)

	Normal Healthy Range
Male	2.5 ~ 12
Female	0 ~ 2.0

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration correspondent to the mean RLU of standard diluent (based on 10 replicate analyses) subtract 2 SD. Therefore, the sensitivity of the Autobio Test CLIA kit is not higher than 0.2ng/ml.

B. Specificity

No interference was detected with the performance of Autobio Test CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
Progesterone	100ng/ml
Estradiol	1000pg/ml

C. Precision

a. Intra-assay Precision

Intra-assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	1.74	0.10	5.75
High titer	20	9.31	0.39	4.19

b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	1.86	0.14	7.53
High titer	10	9.43	0.45	4.77

E. Accuracy

For samples in the range of 0.2ng/ml to 20ng/ml, the relationship between the Autobio Test CLIA and the Beckman Access 2:180 Test[®] assay, is described by the equation below:

Method	No. of Specimens	Linear Equation	Correlation Coefficient
Beckman Access 2 [®]	180	$y = 0.9383x + 0.089$	0.9687

LIMITATIONS

1. For diagnostic purposes, results should be used in conjunction with other data; e.g. symptoms, results of other tests, clinical impressions, etc.
2. If the testosterone results are inconsistent with clinical evidence, additional testing is suggested to confirm the results.
3. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. Additional information may be required for diagnosis.
4. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION
	CATALOGUE NUMBER
	CONSULT INSTRUCTIONS FOR USE

REFERENCES

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2. M Sanchez-Carbayo, M Mauri, R Alfayate, C Miralles, and F Soria. Elecsys Testosterone Assay Evaluated. Clin Chem 44(8): 1744-1746,1998
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6. SJ Winters, DE Kelley and B Goodpaster. The Analog Free Testosterone Assay: Are the Results in Men Clinically Useful? Clin Chem 44(21): 2178-2182, 1998

For order and inquires, please contact

AUTOBIO DIAGNOSTICS CO., LTD.

ADD: No.87 Jingbei Yi Road, National Eco & Tech Development Area,
Zhengzhou , China 450016

Tel: +86-371-67985313 Fax: +86-371-67985804

Web: www.autobio.com.cn