AB Diagnostic Systems

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CE

IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia Testosterone
Enzyme immunoassay for the quantitative determination of testosterone concentration in human serum

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 assays (one breakable wells) including controls; partial use of the kit is possible, can be used for manual protocol.

I. INTENDED USE

The abia Testosterone kit is intended for the quantitative determination of testosterone concentration in human serum by a microplate immunoenzymometric assay.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

Testosterone is a male sex hormone, secreted by Leydig or interstitial cells of the testes, regulated and controlled through negative feedback on the hypothalamus and pituitary hormone, luteinizing hormone (LH). In females, testosterone is mainly produced by peripheral conversion of prehormones. The increase of testosterone levels in males is gradual post puberty until it reaches the adult level. In females, testosterone levels are normally found to be much lower than those encountered in the healthy male.

Testosterone monitoring is used clinically to diagnose and differentiate endocrine disorders. In males, these include hypogonadonism, testicular failure, infertility, hypopituitarism and hyper-prolactinemia. In females, polycystic ovary syndrome, adrenal hyperplasia, infertility, hirsutism, amenorrhea, obesity and virilization can cause changes in serum testosterone levels.

III. PRINCIPLE OF THE TEST

The abia Testosterone is a one-step immunoasay to determine the presence of testosterone in human serum using competitive microplate enzyme immunoassay.

Plates are coated with anti-testosterone antibodies. Serum reference, patient specimen or control is first added to microplate well. Enzyme-Testosterone conjugate is added. Testosterone present in the sample competes with Enzyme-Testosterone conjugate for binding with anti-testosterone coated microplate to form an antigen-antibody complex.

Unbound conjugate is removed by washing. The enzyme activity in the antibody-bound fraction is inversely proportional to the native testosterone concentration. The enzyme activity is revealed by a color change in TMB-Substrate solution.

IV. CONTENT OF THE KIT abia Testosterone

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-Testosterone-coated microtiter wells	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal anti-Testosterone antibodies. Once opened, microtiter wells should be stored at 2-8 °C during until expiration date of the kit.	1 plate
Conjugate	Testosterone, conjugated to horseradish peroxidase. Pink transparent or opalescent liquid. Preserving agent: 0.1% ProClin 300, 0.004% gentamycin sulfate. Once opened, Conjugate should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 12.0 ml
Calibrator 0 Calibrator 1 Calibrator 2 Calibrator 3 Calibrator 4 Calibrator 5	Six vials of human serum based reference calibrators for Testosterone. The testosterone concentration levels in Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis.* Transparent or slightly opalescent liquids, pale yellow. Preserving agent: 0.1% ProClin 300, 0.1% phenol. Once opened, Calibrators should be used within two months. Store at 2-8 °C in tightly sealed vials.	6 vials 0.5 ml
Control Serum	Control, human serum based. The testosterone concentration level in Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent liquid, pale yellow. Preserving agent: 0.1% ProClin 300, 0.1% phenol. Once opened, Control Serum should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 0.5 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7). Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves completely at 35-39 °C and shaking. Once opened, Washing Solution should be stored at 2-8 ° C during shelf-life of the kit.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric acid buffer, containing H_2O_2 (0.01%). Transparent colorless liquid. Once opened, TMB-Substrate should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulfuric acid solution. Transparent colorless liquid. Once opened, Stopping Reagent should be stored at 2-8 °C during shelf-life of the kit.	1 vial 25.0 ml

* Nominal values of Calibrators are traceable to a collection of serum samples certified using a chemiluminescence immunoassay in accordance with EN ISO 17511:2021 In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples.

Additionally the following may be included in the delivery set:

- a lid for polystyrene 96-well plates or a protective film for EIA plates;
- disposable tips;
- a plastic dish for liquid reagents;
- polyethylene bag with a Zip-Lock.

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the laboratory should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. If labels are lost or labels/vials damage, vials should be disposed, and **kit cannot be used**.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various Conjugate or TMB-Substrate.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Do not let the wells dry once the assay has been started.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of Control Serum and Calibrators has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Certain reagents contain biological material of animal origin.

- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.
- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Calibrators 0-5, Control Serum contain ProClin 300.

H317: May cause an allergic skin reaction.
P261: Avoid breathing vapors.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention

Danger!

Stopping Reagent contains 0.2M sulfuric acid.

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Thermoshaker at (37.0 ± 1.0) °C, 500-800 rpm.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm filter.
- Open type automated analyzer with 450 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Blood samples should be collected according to the current practices. Serum only may be used. Separate serum as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Store/transport the samples in accordance with the current regulatory documentation. If samples are to be stored/transported for a longer period of time, they must be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed bacterial growing, hemolysis, hyperlipidemia and which were preserved by sodium azide must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-Testosterone-coated microtiter wells.** Each 12-strips plate (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of strips/wells required for the assay. Place the unused strips/wells back into the foil-lined bag; reseal the foil-lined bag in a Zip-Lock plastic bag. Do not remove desiccant.
- **Calibrators 0-5;**
- **Control Serum;**
- **Conjugate;**
- **TMB-Substrate;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Working Washing Solution**. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial. Thoroughly mix the solution. The prepared Working Washing Solution is stable for 14 days at 18-24 °C or for 28 days at 2-8 °C.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature for 30 min.

1. To the wells add 25 µl of Calibrators and Control Serum in duplicate.

2. To the rest of the wells, add 25 µl of samples in duplicate. Pipetting of samples should not extend beyond ten (10) minutes.

3. Add 100 µl of Conjugate to all wells.

4. Cover the plate with a lid or a protective film and incubate on a thermoshaker (approximately 500-800 rpm) for 30 minutes at (37.0 ± 1.0) °C.

5. Aspirate the contents of the wells into the container with disinfecting solution. Wash the wells 5 times with 300 µl of Working Washing Solution per well and remove Working Washing Solution using a washer into the container with disinfecting solution. Tap the plate firmly against absorbent paper to ensure that it is dry – the residual volume must be lower than 10 µl (the use of a washer is recommended). Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.

6. Pipette 100 µl of TMB-Substrate into each well.

7. Incubate for 20-30 minutes at room temperature in the dark.

8. Add 150 µl of Stopping Reagent into each well. Gently mix for 5-10 seconds.

9. Read the absorbance on the microplate reader at 450 nm within 20 minutes after stopping reaction.

Scheme of the assay is represented in Annex.

Spectrophotometric verification of reagent pipetting

The presence of Conjugate + sample in the well can be verified by automatic reading at 540 (550) nm. Each well containing sample and Conjugate must have an OD higher than 0.500.

10. Automated analyzers

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XIV). For the instrumentation without established validated protocol follow the section “TEST PROCEDURE” and ensure all requirements described in the section “PRECAUTIONS” are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider “dead” volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. CALCULATION OF RESULTS

1. Calculate the mean absorbance value of each calibrator duplicate.
2. Draw a calibration curve on graph paper with the mean absorbance on Y axis and the calibrator concentration on the X axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean absorbance values for each specimen.
4. Read the value of Testosterone concentration in nmol/l in the unknowns directly off the calibration curve.

Typical tabulated data

Calibrator	OD 1	OD 2	Mean OD	Value (nmol/l)
0	2.758	2.751	2.755	0
1	2.504	2.495	2.500	0.5
2	1.554	1.593	1.574	5
3	1.062	1.093	1.078	10
4	0.705	0.727	0.716	20
5	0.419	0.455	0.437	40
unknown	1.416	1.444	1.430	5.9

This data is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. The absorbance (OD) of **Calibrator 0** should not be less than 1.3.
2. Calculated Value of **Control Serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia Testosterone

1. Assay Dynamic Range

The range of the assay is between 0-40 nmol/l.

2. Analytical sensitivity

The lower detection limit is 0.2 nmol/l. The sensitivity was calculated by determining the variability of the 0 nmol/l serum calibrator and using the 2 SD (95% certainty) statistics.

3. Specificity (cross reactivity)

The following substances were tested for cross reactivity of the assay:

Substance	Cross reactivity, %
Testosterone	100
Progesterone	0.056
Cortisol	0.004
Estradiol	0.005
Dihydrotestosterone	4.8
Androstenedione	3.6
Androsterone	0.048
Cortisone	0.004
Estriol	0.002
Estrone	0.007

4. Precision

Intra-Assay Precision

Two samples were assayed 8 times each on the same calibrator curve. The results (in nmol/l) are tabulated below:

Sample	Mean	SD	CV, %
1	4.0	0.128	3.2
2	20.6	0.371	1.8

Inter-Assay Precision

Two samples were assayed 4 times on the different calibrator curves. The results (in nmol/l) are tabulated below:

Sample	Mean	SD	CV, %
1	4.0	0.096	2.4
2	20.4	0.236	1.2

5. Accuracy

The abia Testosterone test system was compared with a Chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 681. The values ranged from 0.4 to 51.6 nmol/l. The least square regression equation and the correlation coefficient were computed for abia Testosterone in comparison with the reference method. The least square regression analysis was y= 1.03(x)+0.46. Correlation coefficient is 0.98.

6. Expected normal Value

A normal range 6.4-31.8 nmol/l was obtained by testing serum specimens from 96 males (21-45 years old). For 55 females normal range was 0.2-4.4 nmol/l. It is strongly recommended that each laboratory should determine its own normal range values.

Unit Conversion Calculator: nmol/l x 0.288 = ng/ml; ng/ml x 3.47 = nmol/l.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the determination of testosterone in human serum. This test is not calibrated for the Testosterone determination in saliva, plasma or other specimens of human or animal origin.
2. Highly lipemic, hemolyzed or grossly contaminated specimens should not be used.
3. It is important that the time of reaction in each well is held constant for reproducible results.
4. If more than 1 plate is used, it is recommended to repeat the dose response curve.
5. Do not touch the bottom of the wells.
6. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has to potential of causing interferences in immunological tests. For diagnostic purposes, results should be used in conjunction with other data; eg., symptoms, results of other tests, clinical impressions, etc.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging.

Keep in dark dry place at 2-8 °C. Freezing is prohibited.

Transportation should be done at 2-8 °C. Transportation at 9-20 °C is allowed not more than during ten (10) days.

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XV. REFERENCES

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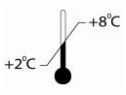
2. Joshi, U.M., et al., A Sensitive Specific Enzyme Immunoassay for Serum Testosterone. Steroids 34:35, 1979.

3. Ekins, R. Hirsutism: Free and Bound Testosterone. Ann. Clin. Biochem 27:91, 1990.

4. Manni, A., et al., Bioavailability of Albumin-Bound Testosterone. J. Clin. Endo. Metab. 61:705, 1985.

5. Ekins, R. The Science of Free Testosterone Measurement. Proc.UK NEQAS Meeting. 3:35-39, 1998.

XVI. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)		Storage temperature limitation
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM	IVD	For in vitro diagnostic use
	Expiry date CCYY-MM-DD		Sufficient for
LOT	Batch code		Symbol “exclamation mark”
REF	Catalog number	**Warning!**	Signal word
	Fragile, handle with care		Symbol “corrosion”
	Keep away from sunlight	**Danger!**	Signal word
	Keep dry		Top

Annex

Scheme of the assay

1	**Add**	25 µl of Calibrators, Control Serum in duplicates; 25 µl of samples in duplicates
2	**Add**	100 µl of Conjugate into all wells
3	**Incubate**	30 min, thermoshaker (500-800 rpm), at (37.0 ± 1.0) °C
4	**Wash the plate**	**Working Washing Solution, 300 µl, 5 times**
5	**Add**	100 µl of TMB-Substrate into all wells
6	**Incubate**	20-30 min, in a dark place
7	**Add**	150 µl of Stopping Reagent into all wells
8	**Mix**	5-10 seconds
9	**Read the optical density**	450 nm

nmol/l x 0.288 = ng/ml ng/ml x 3.47 = nmol/l