

abia Testosterone



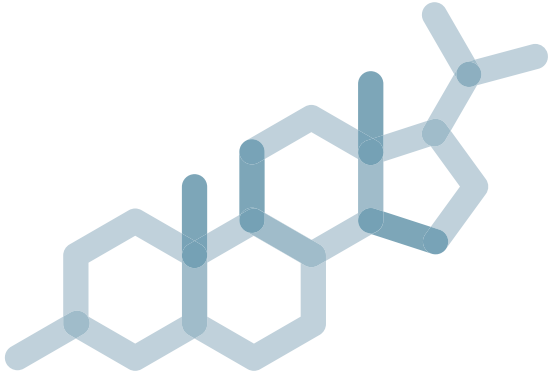
REF DK.040.01.3

IVD



Note: Changes highlighted ★

abia



Intended use

abia Testosterone enzyme immunoassay for the quantitative determination of testosterone concentration in human serum.

For professional use only.

Clinical value

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.

Principle of the test

abia Testosterone is a one-step immunoassay, based on the principle of the competitive method.

Testosterone present in the sample and the labeled enzyme-testosterone in the conjugate compete for binding to the capture antibody on the anti-testosterone coated microplate. The enzyme activity in the antibody-bound fraction is inversely proportional to the native testosterone concentration.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is inversely proportional to the concentration of the testosterone in the specimen and can be read at 450 nm.

Kit contents

	S	
Testosterone Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-testosterone antibodies
Conjugate	1 × 12 ml	ready to use; HRP-labeled testosterone; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 0.5 ml	human serum not containing testosterone; pale yellow liquid
Calibrator 1	1 × 0.5 ml	human serum containing testosterone in concentration approx. 0.5 nmol/l; pale yellow liquid
Calibrator 2	1 × 0.5 ml	human serum containing testosterone in concentration approx. 5 nmol/l; pale yellow liquid
Calibrator 3	1 × 0.5 ml	human serum containing testosterone in concentration approx. 10 nmol/l; pale yellow liquid
Calibrator 4	1 × 0.5 ml	human serum containing testosterone in concentration approx. 20 nmol/l; pale yellow liquid
Calibrator 5	1 × 0.5 ml	human serum containing testosterone in concentration approx. 40 nmol/l; pale yellow liquid
Control serum	1 × 0.5 ml	human serum based control containing testosterone; pale yellow liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colorless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H ₂ O ₂ ; colorless liquid
Stopping reagent 0.2M H ₂ SO ₄	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

Exact concentration levels for calibrators and control serum are given on the labels and certificates of analysis on a lot specific basis. For conventional units: nmol/l × 0.288 = ng/ml.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.

Once opened, the components should be used within 2 months. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- microplate shaker
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- human origin material used in the preparation of the calibrators and control serum has been tested and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2)
- as no known test method can offer complete assurance that infectious agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide, thiomersal
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures. In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- repeat 5 times
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion Stability: 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia Testosterone for the quantitative determination of testosterone concentration in human serum

- 1 Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.

- 2 Analyze each calibrator, control serum, and sample duplicate.
Add 25 μ l of calibrators 0 - 5 into appropriate wells.
Add 25 μ l of control serum into appropriate wells.
Add 25 μ l of samples to be tested in rest of the wells.
The total time should not exceed 10 min.

- 3 Add 100 μ l conjugate into each well. Cover the plate with protective film.

- 4 Incubate in microplate shaker (approximately 500-800 rpm) for 30 minutes at 37 ± 1 °C.

- 5 Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant).
Add not less than 300 μ l of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.

- 6 Add 100 μ l of TMB/substrate solution to all the wells. Keep the plates in a dark place for 25 ± 5 minutes at 20–25 °C.

- 7 Add 150 μ l of stopping reagent into each well. Mix gently for 5–10 sec.

- 8 Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative. For the instrumentation without established validated protocol follow section “Test procedure” and ensure all requirements described in section “Precautions” are followed. All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of control serum should be within established range.
The absorbance (OD) of calibrator 0 should be greater than 1.300.

Calculation procedure

- 1 Calculate the mean optical density of each calibrator duplicate.
- 2 Calculate the mean optical density of each sample duplicate.
- 3 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
- 4 Read the values of the unknowns directly off the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

Example	OD 1	OD 2	Mean OD	Value, nmol/l
Calibrator 0	2.758	2.751	2.755	0.00
Calibrator 1	2.504	2.495	2.500	0.50
Calibrator 2	1.554	1.593	1.574	5.00
Calibrator 3	1.062	1.093	1.078	10.00
Calibrator 4	0.705	0.727	0.716	20.00
Calibrator 5	0.419	0.455	0.437	40.00
Sample	1.416	1.444	1.430	5.90

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

Performance characteristics

Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 11 replicate analyses less $2 \times$ SD. Limit of detection defined at 0.2 nmol/l.

Specificity	Concentration, ng/ml	Cross reactivity, %
Progesterone	3.74	0.056
Cortisol	50.00	0.004
Estradiol (E2)	41.60	0.005
Dihydrotestosterone	0.04	4.80
Androstendione	0.06	3.60
Androsterone	4.37	0.048
Cortisone	53.70	0.004
Estriol	88.08	0.002
Estrone	30.84	0.007

Precision	Mean, nmol/l	SD	CV, %
Intra-assay, sample 1	4.00	0.128	3.2
Intra-assay, sample 2	20.60	0.371	1.8
Inter-assay, sample 1	4.00	0.096	2.4
Inter-assay, sample 2	20.60	0.236	1.2

Accuracy

The assay was compared with a chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 681. The values ranged from 0.40 to 51.60 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 $y = 1.03(x) + 0.46$ with correlation coefficient of 0.98.

Expected normal value	Range, nmol/l	
Males	6.40	31.80
Females	0.20	4.40

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of test

- the assay was validated only for the determination of testosterone in human serum
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient
- the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has to potential of causing interferences in immunological tests

References

1. Marcus, G.J., et al, A Simple Linked Immunoassay for Testosterone. *Steroids* 46:975, 1985.
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.

Key to symbols used



Manufacturer



For in vitro diagnostic use



Catalogue number



Batch code



Expiry date



Storage temperature limitation



Do not use if package is damaged



Do not reuse



Sufficient for [n] tests



Consult Instructions for use



Caution, consult documents



Changes highlighted

Hazard and precautionary statements for certain kit components

Stopping reagent



Warning

H315

Causes skin irritation.

H319

Causes serious eye irritation.

P264

Wash hands thoroughly after handling.

P280

Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352

IF ON SKIN: Wash with plenty of soap and water.

P305 + P351 +
P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Conjugate, calibrators 0 - 5, control serum



Warning

H317

May cause an allergic skin reaction.

P261

Avoid breathing dust/fume/gas/mist/vapours/spray.

P280

Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352

IF ON SKIN: Wash with plenty of soap and water.

P333 + P313

If skin irritation or rash occurs: Get medical advice/attention.

Attention!

For complete precautionary statements and detailed information see safety data sheets (SDS).



AB Diagnostic Systems GmbH
Sportfliegerstraße 4
12487 Berlin
Germany

☎ +49 30 208 987 160
☎ +49 30 208 987 199
✉ info@ab-ds.de
www.ab-ds.de

