# Testosterone

**Testosterone**

cobas®

**11776061** 122 **100 tests**

• Indicates analyzers on which the kit can be used

| Elecsys 1010 | Elecsys 2010 | MODULAR ANALYTICS E170 | **cobas e** 411 | **cobas e** 601 |
|---|---|---|---|---|
| • | • | • | • | • |

***English***

**Intended use**

Immunoassay for the in vitro quantitative determination of testosterone in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

**Summary**[1,2,3]

The androgen testosterone (17β-hydroxyandrostenone) has a molecular weight of 288 daltons. In men, testosterone is synthesized almost exclusively by the Leydig cells of the testes. The secretion of testosterone is regulated by luteinizing hormone (LH), and is subject to negative feedback via the pituitary and hypothalamus.

Testosterone promotes the development of the secondary sex characteristics in men and serves to maintain the function of the prostate and seminal vesicles. Most of the circulating testosterone is bound to carrier proteins (SHBG = sex hormone-binding globulin).

In women, small quantities of testosterone are formed in the ovaries. In physiological concentrations, androgens have no specific effects in women. Increased production of testosterone in women can cause virilization (depending on the increase).

The determination of testosterone in women is helpful in the diagnosis of androgenic syndrome (AGS), polycystic ovaries (Stein-Leventhal syndrome) and when an ovarian tumor, adrenal tumor, adrenal hyperplasia or ovarian insufficiency is suspected.

Testosterone is determined in men when reduced testosterone production is suspected, e.g. in hypogonadism, estrogen therapy, chromosome aberrations (as in the Klinefelter's syndrome) and liver cirrhosis.

The Elecsys Testosterone assay is based on a competitive test principle using a monoclonal antibody specifically directed against testosterone. Endogenous testosterone released from the sample by ANS (8-anilino-1-naphthalene sulfonic acid) and norgestrel competes with the added testosterone derivative labeled with ruthenium complex[a] for the binding sites on the biotinylated antibody.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

**Test principle**

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample is incubated with a testosterone-specific biotinylated antibody and a testosterone derivative labeled with a ruthenium complex. The binding sites of the labeled antibody become occupied partly by the sample analyte (depending on its concentration) and partly by the ruthenium-labeled hapten to form the respective immunocomplexes.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

**Reagents - working solutions**

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL, preservative.

R1 Anti-testosterone-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-testosterone antibody (mouse) 55 ng/mL; phosphate buffer 40 mmol/L, pH 7.0; preservative.

R2 Testosterone-peptide~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL: Testosterone derivative, labeled with ruthenium complex 3 ng/mL; releasing reagent ANS/Norgestrel; phosphate buffer 40 mmol/L, pH 7.0; preservative.

**Precautions and warnings**

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

**Reagent handling**

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

**Storage and stability**

Store at 2-8 °C.

Store the Elecsys Testosterone reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

| | |
|---|---|
| unopened at 2-8 °C | up to the stated expiration date |
| after opening at 2-8 °C | 12 weeks |
| on MODULAR ANALYTICS E170 and **cobas e** 601 | 8 weeks |
| on Elecsys 2010 and **cobas e** 411 | 8 weeks |
| on Elecsys 1010 | 4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25 °C; up to 20 hours opened in total) |

**Specimen collection and preparation**

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-, Na-, $NH_4^+$-heparin, $K_3$-EDTA, and sodium fluoride/potassium oxalate plasma. When sodium citrate is used, the results must be corrected by + 10 %.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 1 week at 2-8 °C, 6 months at -20 °C. Freeze only once.[4]

Stability of serum obtained with tubes containing separating gel: 48 hours at 2-8 °C (note the data provided by the tube manufacturer).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

**Materials provided**

See "Reagents - working solutions" section for reagents.

**Materials required (but not provided)**

- Cat. No. 03005658122, Testosterone CalSet II, for 4 x 1 mL
- Cat. No. 11731416122, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 or
  Cat. No. 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- Cat. No. 11731416160, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 (for USA)

# Testosterone

**Testosterone**

- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988122, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159001, Adapter for SysClean
- Cat. No. 11706829001, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or Cat. No. 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340190, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027190, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- Cat. No. 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150001, WasteLiner, waste bags
- Cat. No. 03027651001, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 11776860160, Elecsys Testosterone CalCheck, 3 concentration ranges

### Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:
PreClean M solution is necessary.
Resuspension of the microparticles takes place automatically before use.
Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

### Calibration

Traceability: This method has been standardized via ID-GC/MS ("Isotope Dilution Gas Chromatography Mass Spectrometry").[5]
Every Elecsys Testosterone reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Testosterone CalSet II.

*Calibration frequency:* Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25 °C)
- after 3 days (ambient temperature 25-32 °C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

### Quality control

For quality control, use Elecsys PreciControl Universal 1 and 2.
Other suitable control material can be used in addition.
Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements.
Values obtained should fall within the defined limits.
Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

### Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, ng/mL or ng/dL).

Conversion factors: nmol/L x 0.288 = ng/mL
ng/mL x 3.47 = nmol/L
ng/mL x 100 = ng/dL

### Limitations - interference

The assay is unaffected by icterus (bilirubin < 513 µmol/L or < 30 mg/dL), hemolysis (Hb < 1.1 mmol/L or < 1.8 g/dL), lipemia (triglycerides < 22.8 mmol/L or < 2000 mg/dL), and biotin < 123 nmol/L or < 30 ng/mL.
Criterion: Recovery within ± 10 % of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
In vitro tests were performed on 17 commonly used pharmaceuticals.
No interference with the assay was found.
In isolated cases, elevated testosterone levels were seen in samples from female dialysis patients.
In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies (such as HAMA), streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

### Measuring range

0.069-52.0 nmol/L or 0.020-15.0 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.069 nmol/L or < 0.020 ng/mL. Values above the measuring range are reported as > 52.0 nmol/L or > 15.0 ng/mL.

### Dilution

Dilution is unlikely due to the broad measuring range.
If necessary, samples with testosterone concentrations above the measuring range can be diluted using human serum with a low analyte concentration. The recommended dilution is 1:5. The concentration of the diluted samples must be > 10 nmol/L (> 3 ng/mL).

### Expected values

The following table shows the results obtained with the Elecsys Testosterone assay in the multicenter study "Fertility Hormones" of March 1997.

| Test subjects | N | Percentiles | | | |
|---|---|---|---|---|---|
| | | 50$^{th}$ | 5-95$^{th}$ | 50$^{th}$ | 5-95$^{th}$ |
| | | nmol/L | | ng/mL | |
| Men | 132 | 17.5 | 9.9-27.8 | 5.0 | 2.8-8.0 |
| Women | 956 | 1.2 | 0.22-2.9 | 0.35 | 0.06-0.82 |
| Boys | | | | | |
| • < 1 year | 22 | 0.42 | 0.42-0.72 | 0.12 | 0.12-0.21 |
| • 1-6 years | 29 | 0.42 | 0.10-1.12 | 0.12 | 0.03-0.32 |
| • 7-12 years | 31 | 0.63 | 0.10-2.37 | 0.18 | 0.03-0.68 |
| • 13-17 years | 16 | 12.6 | 0.98-38.5 | 3.6 | 0.28-11.1 |

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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### Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

### Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers

| | | | Within-run precision | | | Total precision | | |
|---|---|---|---|---|---|---|---|---|
| Sample | Mean | | SD | | CV | SD | | CV |
| | nmol/L | ng/mL | nmol/L | ng/mL | % | nmol/L | ng/mL | % |
| HS[b] 1 | 0.85 | 0.24 | 0.038 | 0.011 | 4.6 | 0.062 | 0.018 | 7.4 |
| HS 2 | 9.55 | 2.75 | 0.132 | 0.038 | 1.4 | 0.212 | 0.061 | 2.2 |
| HS 3 | 24.3 | 7.01 | 0.267 | 0.077 | 1.1 | 0.409 | 0.118 | 1.7 |
| PC U[c]1 | 21.5 | 6.20 | 0.201 | 0.058 | 0.9 | 0.337 | 0.097 | 1.6 |
| PC U2 | 6.75 | 1.95 | 0.115 | 0.033 | 1.7 | 0.174 | 0.050 | 2.6 |

b) HS = human serum
c) PC U = PreciControl Universal

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

| | Within-run precision | | | | | Total precision | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Mean | | SD | | CV | Mean | | SD | | CV |
| | nmol/L | ng/mL | nmol/L | ng/mL | % | nmol/L | ng/mL | nmol/L | ng/mL | % |
| HS 1 | 1.91 | 0.55 | 0.05 | 0.01 | 2.7 | 1.67 | 0.48 | 0.09 | 0.03 | 5.6 |
| HS 2 | 20.4 | 5.89 | 0.42 | 0.12 | 2.1 | 18.8 | 5.43 | 0.48 | 0.14 | 2.5 |
| HS 3 | 30.6 | 8.81 | 0.54 | 0.16 | 1.8 | 28.2 | 8.14 | 0.79 | 0.23 | 2.8 |
| PC U1 | 23.3 | 6.72 | 0.31 | 0.09 | 1.3 | 21.6 | 6.22 | 0.92 | 0.26 | 4.3 |
| PC U2 | 12.0 | 3.45 | 0.17 | 0.05 | 1.5 | 10.6 | 3.06 | 0.62 | 0.18 | 6.0 |

### Analytical sensitivity (lower detection limit)

0.069 nmol/L (0.020 ng/mL)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

### Method comparison

A comparison of the Elecsys Testosterone assay (y) with a commercially available RIA Testosterone test (x) using clinical samples gave the following correlations (ng/mL):
Number of samples measured: 71

| Passing/Bablok[6] | Linear regression |
|---|---|
| y = 1.02x - 0.11 | y = 0.96x + 0.05 |
| τ = 0.859 | r = 0.963 |

The sample concentrations were between approx. 0.7 and 44 nmol/L (approx. 0.2 and 12.7 ng/mL).

### Analytical specificity

For the antibody derivative used, the following cross-reactivities were found (in %):

| | |
|---|---|
| Androstendione | 0.91 |
| Danazol | n.d.[d] |
| DHEA-S | 0.01 |
| D-5-Androstene-3β,17β-diol | 0.30 |
| Estradiol | n.d. |
| Ethisterone | 0.02 |
| Norgestrel | n.d. |
| Testosterone propionate | 0.30 |
| 5-α-Androstane-3β,17β-diol | 0.51 |
| 5-α-Dihydro-testosterone | 1.89 |
| 11-β-Hydroxy-testosterone | 8.34 |
| 11-Keto-testosterone | 10.4 |

d) n.d. = not detectable

### Functional sensitivity

0.420 nmol/L (0.120 ng/mL)
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20 %.

### References


1. Runnebaum B, Rabe T. Gynäkologische Endokrinologie und Fortpflanzungsmedizin Springer Verlag 1994; Band 1:36-38,70,116 Band 1:39-40, 520-521, 593-594, 422-423. ISBN 3-540-57345-3, ISBN 3-540-57347-x.
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3. Wheeler MJ. The determination of bio-available testosterone. Ann Clin Biochem 1995;32:345-357.
4. Tietz NW. Clinical Guide To Laboratory Tests. 3rd ed. Philadelphia, Pa: WB Saunders Co, 1995:578.
5. Thienpont L, Verhseghe PG, Van Brussel KA, De Leenheer AP. Estradiol-17-β Quantified in Serum by Isotope Dilution-Gas Chromatography-Mass Spectrometry. ClinChem 1988(34);10:2066-2069.
6. Passing H, Bablok W, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.


For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.

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