Testosterone

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Testosterone (ELISA)

REF: DS177714 Σ 96

Intended use
The DiaSino Testosterone assay is an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of testosterone in human serum. For professional use only.

Summary
Testosterone is a steroid from the androstane class containing a keto and a hydroxyl group at positions three and seventeen respectively. Testosterone is the primary sex hormone and anabolic steroid in males.[1] In male humans, testosterone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair.[2] In addition, testosterone in both sexes is involved in health and well-being, including moods, behaviour, and in the prevention of osteoporosis.[3,4,5] Insufficient levels of testosterone in men may lead to abnormalities including frailty and bone loss.

Test principle
One-step competitive principle. Total duration of assay: **90 minutes.**
- Sample, Testosterone derivant coated microwells and enzyme labeled Anti- Testosterone are combined.
- During the incubation, Testosterone derivant coated on microwells and Testosterone present in the sample compete for binding to the enzyme labeled antibodies.
- After washing, a complex is generated between the solid phase and enzymelinked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The color intensity is inversely proportional to the amount of Testosterone in the sample.

Reagents
Materials provided
- **Testosterone Coated Microplate - symbol** TESTO PLATE 8 x 12 strips, 96 wells, pre-coated with Testosterone derivant.
- **Testosterone Calibrators - symbols** TESTO CAL A-F 6 vials, 1 mL of each, ready to use; Concentrations: 0(A), 1.0(B), 2.5(C), 5.0(D), 10(E) and 15(F) ng/mL.
- **Testosterone Incubation Buffer - symbol** TESTO BUFFER 1 vial, 6.0 mL. Contains 0.2% ProClin300 preservative.
- **Testosterone Enzyme Conjugate - symbol** TESTO CONJ Ready to use, 1 vial, 6.0 mL of HRP (horseradish peroxidase) labeled sheep monoclonal Anti-Testosterone in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.2% ProClin300 preservative.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05%0 0.2% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

Storage and stability
- Store at 2-8℃.
- Seal and return unused reagents to 2-8℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Calibration
The DiaSino Testosterone ELISA has been standardized against the Elecsys Testosterone II assay, which is traceable via ID–GC/MS (isotope dilution gas chromatography/mass spectrometry).[6,7].
Recalibration is recommended when a new reagent lot is used, or the quality controls are out of specified range.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples, reagents (Conjugate, Incubation Buffer, Calibrators) are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.
- Use only the number of wells required and format the microplates wells for each calibrator and sample to be assayed.
- Add **50 µL** of **Calibrators** or **Samples** to each well.
- Add **50 µL** of **Incubation Buffer** to each well.
- **Shake** the **microplate** gently for **10 minutes** to mix.
- Add **50 µL** of **Enzyme Conjugate** to each well.
- Shake the microplate gently for 30 seconds to mix.
- Cover the plate with a plate lid and incubate at **37 ℃** for **60 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **Wash Solution,** decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µL** of **Substrate** to each well.
- Cover and **Incubate** at **ambient temperature** (18-25℃) in the dark for reaction for **20 minutes**. **Do not shake** the plate after substate addition.
- Add **50 µL** of **Stop Solution** to each well.
- Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader.
- The results should be read within 30 minutes of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in ng/mL for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. **Point- to-Point** method is suggested to generate a calibration curve.

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The following data and calibration curve is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (ng/mL)	Absorbance
Calibrator A	0	2.516
Calibrator B	1.0	1.723
Calibrator C	2.5	1.457
Calibrator D	5.0	0.987
Calibrator E	10	0.624
Calibrator F	15	0.318
Control 1	1.1	1.705
Control 2	4.9	1.006
Sample	3.66	1.238

Limitations - interference

- The assay is unaffected by icterus (bilirubin < 513 µmol/L or < 30 mg/dL), hemolysis (Hb < 0.372 mmol/L or < 0.600 g/dL), lipemia (Intralipid < 1000 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).
- Criterion: Recovery within ± 10 % of initial value (concentration range > 1–15 ng/mL), recovery within ± 15 % of initial value (concentration range > 0.5–1 ng/mL) and recovery of ± 0.075 ng/mL (concentration range of 0.150–0.500 ng/mL).
- Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
- No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.
- In isolated cases, elevated testosterone levels can be seen in samples from female patients with end stage renal disease (ESRD).
- Implausible elevated testosterone values in women should be verified by an extraction method or a validated LC–MS/MS tandem method.[8]
- In rare cases, interference due to extremely high titers of antibodies to analyte–specific antibodies, 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

0.05-15 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.05 ng/mL. Values above the measuring range are reported as > 15 ng/mL.

Lower detection limit

0.05 ng/mL

The detection limit represents the lowest 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, repeatability study, n=21).

Expected values

Male > 18 years	1.49-10.36 ng/mL
Female > 18 years	< 1.08 ng/mL

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

Measurement with the DiaSino Testosterone assay on 212 healthy male serum samples from test subjects in China yielded the above values (2.5th-97.5th percentile): the average level is around 8.50 ng/mL.

Specific performance data

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

Precision

Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

Sample	Mean ng/mL	Repeatability* SD ng/mL	Repeatability* CV %	Intermediate precision SD ng/mL	Intermediate precision CV %
Human Serum 1	0.451	0.04	8.17	0.04	9.26
Human Serum 2	3.615	0.23	6.25	0.26	7.14
Human Serum 3	6.257	0.43	6.87	0.39	6.28
PC Universal 1	1.115	0.08	7.23	0.09	7.84
PC Universal 2	4.828	0.25	5.16	0.30	6.21

*Repeatability = within-run precision

Method comparison

A comparison of the DiaSino Testosterone assay (y) with Elecsys Testosterone II (x) using 75 clinical samples gave the followng correlations:
Linear regression
y = 0.9915x + 0.0685
r = 0.9630
The sample concentrations were between approx. 0.12 and 10.33 ng/mL.

Analytical specificity

Substance	Concentration (ng/mL)	Cross-reactivity (%)
Estradiol	1000	< 0.160
Estrone	1000	< 0.004
Androstenedione	100	< 3.00
Progesterone	1000	n.d
DHEA-S	50000	< 0.003
Cortisol	1000	< 0.01
Cortisone	2000	n.d
Ethisterone	1000	< 2.40

Functional sensitivity

0.055 ng/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

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4. Crewther BT, Thomas AG, Stewart-Williams S, Kilduff LP, Cook CJ. Is salivary cortisol moderating the relationship between salivary testosterone and hand-grip strength in healthy men? Eur J Sport Sci. 2017;17(2):188–94.
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7. Thienpont LM, Franzini C, Kratochvila J, et al. Analytical quality specifications for reference methods and operating specifications for networks of reference laboratories. Recommendations of the European EQA-Organizers Working Group B. Eur J Clin Chem and Clin Biochem 1995;33:949-957.
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Symbols

IVD **In vitro diagnostic medical device**	8°C / 2°C **Temperature limit**	**Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	**Date of manufacture**	**Use-by date**	Σ **Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**	CE **European Conformity**	EC REP **Authorized representative in the European Community**

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