Instructions for Use

Testosterone Saliva ELISA

Enzyme immunoassay for the quantitative determination of free testosterone in human saliva.

REF **RE52631**

Σ **96**

EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the quantitative determination of free testosterone in human saliva. Measurement of testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, impotence in males and in females hirsutism (excessive hair) and virilisation (masculinisation) due to tumors, polycystic ovaries and adrenogenital syndromes.

2. SUMMARY AND EXPLANATION

Testosterone, a C19-Steroid, is the most effective natural hormone in the family of androgens. In males, it is mainly produced in the Leydig cells of the testes, only a small amount is produced in the adrenal cortex. On the whole, adult males have 10 to 20 fold higher testosterone plasma concentrations than females. In the circulation, the main part of testosterone is bound to plasma proteins like sex hormone binding globuline (SHBG) and albumine. Only 1 – 2 % of the testosterone is unbound and therefore biologically active.
The free testosterone is released via the salivary glands. In their cells a great part of the hormone is transformed to 5a-dihydrotestosterone. Nevertheless, the concentration of testosterone in saliva reflects the level of free testosterone in plasma.
Testosterone levels in females should be measured in patients having clinical symptoms of virilism caused by the adrenogenital syndrome, the polycystic ovary syndrome or neoplasms of the adrenal cortex or the ovaries. Due to the fluctuations, repeated measurements are recommended. A therapy with androgen suppressive drugs can be followed up by taking controls.
In men with signs of primary or secondary hypogonadism, confirmatory testosterone levels can be determined.
Testosterone together with cortisol levels represents a useful parameter in stress research and sports medicine. Due to the many factors that may influence the testosterone levels, it is in some situations advisable to take a profile. Therefore measuring the free testosterone in saliva is a convenient method, due to the easier sample collection without repeated venipunctures.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Some reagents contain ProClin 300 as preservative. In case of contact with eyes or skin, flush immediately with water. When disposing reagents, flush with a large volume of water to avoid built-up.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is broken. Make sure that the broken bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Saliva

The patient should not eat, drink, chew gums or brush teeth for 30 min before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

Saliva can be collected in a suitable sampling device. A minimum of 0.5 mL liquid should be collected. Saliva flow can be stimulated by chewing on a piece of Parafilm®. It is recommended to freeze samples at –20°C prior to laboratory testing. After thawing, mix and centrifuge 10 min at 2000 – 3000 x g to remove particulate material.

Take care that the saliva samples are visually okay.
(Reddish color indicating blood contamination)

Storage:	37°C	18-25°C	2-8°C	≤ -20°C (Aliquots)
Stability:	1 week	> 2 weeks	> 4 weeks	≥ 6 months

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	MTP	**Microtiter Plate** Break apart strips. Coated with rabbit anti-mouse antibody.
1 x 0.15 mL	ENZCONJ CONC	**Enzyme Conjugate** Concentrate (101x) Contains: Testosterone conjugated to HRP, stabilizers.
1 x 7 x 1.0 mL	CAL A-G	**Standard A-G** 0; 6.4; 16; 40; 100; 250; 760 pg/mL Ready to use. Contains: Testosterone, Buffer, ProClin 300.
1 x 2 x 1.0 mL	CONTROL 1+2	**Control 1+2** Ready to use. Contains: Testosterone, low and high, Buffer, ProClin 300. Concentrations / acceptable ranges see QC certificate.
1 x 9 mL	ANTISERUM	**Testosterone Antiserum** Red colored. Ready to use. Contains: mouse anti-testosterone antibody, ProClin 300
1 x 10 mL	ASSAYBUF	**Assay Buffer** Ready to use. Contains: Tris buffer, BSA, ProClin 300
1 x 100 mL	WASHBUF CONC	**Wash Buffer** Concentrate (10x) Contains: phosphate buffer, Tween, NaCl.
1 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4
3 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 20; 50; 100; 1000 µL
2. A suitable sampling device should be used.
3. Orbital shaker (400-600 rpm)
4. Vortex mixer
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
8. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

⚠	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 4 strips (32 determinations).**

10.1. Preparation of lyophilized or concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
20 mL	**WASHBUF CONC**	ad 200 mL	bidist. water	1:10	Mix vigorously. A yellowish-brown color may occur without influence of test results.	2-8°C	4 weeks
20 µL	**ENZCONJ CONC**	with 2 mL	**ASSAYBUF**	1:101	Mix without foaming.	Prepare freshly and use only once.	

10.2. Dilution of Samples

Samples suspected to contain concentrations higher than the highest standard have to be diluted with Sample Diluent. (available from IBL REF KLZZ731). Dilution has to be made in glass tubes. Measured results must be multiplied with the dilution factor to obtain corrected results.

11. TEST PROCEDURE

1.	Pipette **50 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate.
2.	Pipette **50 µL** of freshly prepared **Enzyme Conjugate** into each well.
3.	Pipette **50 µL** of **Testosterone Antiserum** into each well. Cover plate with adhesive foil.
4.	**Incubate 2 h** at **RT (18-25°C)** on an orbital shaker (400-600 rpm).
5.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **250 µL** of **diluted Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
7.	**Incubate 30 min** at **RT (18-25°C)** on an orbital shaker (400-600 rpm).
8.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Shake plate briefly. Color changes from blue to yellow.
9.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600–650 nm) **within 15 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Saliva samples with remarkably elevated values should be reviewed for blood contamination.

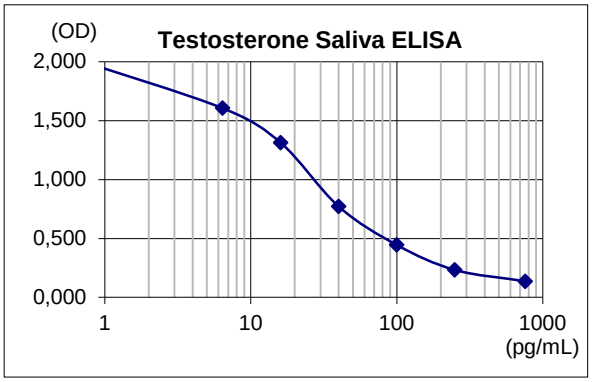
Conversion:
Testosterone (pg/mL) x 3.47 = pmol/L

Reportable Ranges:
Saliva: 2 – 760 pg/mL Testosterone

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Testosterone (pg/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0	2.066	100.0
B	6.4	1.606	77.7
C	16	1.313	63.6
D	40	0.771	37.3
E	100	0.445	21.5
F	250	0.233	11.3
G	760	0.137	6.6



14. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values:

Specimen	Age (years)	Testosterone (pg/mL)					
		♀			♂		
		Median	Range (5-95%)	N	Median	Range (5-95%)	N
Saliva	20–29	19.0	5.5–49.0	40	78.8	41.4–142.5	55
	30–39	17.3	5.2–49.0	39	58.8	31.8–100.4	35
	40–49	13.8	4.5–49.0	47	54.4	30.1–97.8	48
	50–59	13.2	3.6–49.0	53	54.8	30.0–92.0	64
	60–69	15.8	2.9–38.8	33	42.9	23.2–86.9	63

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.
For cross-reactivities, see PERFORMANCE.
The following substances do not have a significant effect on the test results up to the concentration stated below:

	Saliva	
	Conc.	**Testosterone (pg/mL)**
Blood	0.1%	25.1–116.0
Thimerosal	0.1%	18.2–283.1
NaN_3	0.03%	24.9–231.4

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%) Buffer		Cross-reactivity of other substances tested ≤ 0.01%
	Testosterone	100		
	11β-OH-Testosterone	4.22		
	11α-OH-Testosterone	3.59		
	Dihydrotestosterone	2.52		
	Androstenedione	0.85		
	Methyl- Testosterone	1.02		
	DHEA-S	0.05		
	Testosterone Sulfate	0.05		
	Progesterone	0.04		
	Androsterone Sulfate	0.06		
	Androsterone	0.03		
Analytical Sensitivity (Limit of Detection)	Saliva	4.7 pg/mL	Mean signal (Zero-Standard) - 2SD	
Functional Sensitivity	Saliva	6.1 pg/mL	Mean conc. < 20% CV	

Precision	Saliva (pg/mL)			
	Mean	SD	CV (%)	N
Intra-Assay	10.9	0.76	7.0	24
	52.3	4.08	7.8	24
	620.3	55.34	8.9	24
Inter-Assay	19.01	2.52	13.3	20
	50.35	3.88	7.7	20
	132.36	18.43	13.9	20
	230.68	24.45	10.6	20
	350.60	44.37	12.7	20

Linearity	Saliva (pg/mL)		
	Dilution	Measured	Recovery (%)
	-	75.9	100
	1:2	36.6	97
	1:4	20.8	109
	-	227.8	100
	1:2	125.7	110
	1:4	53.6	94
	1:8	24.1	85
	1:16	14.2	99
	-	744.4	100
	1:2	381.4	103
	1:4	206.6	111
	1:8	102.3	110
	1:16	51.3	110

	Conc. (pg/mL)	Added (pg/mL)	Measured (pg/mL)	Recovery (%)
Recovery	Saliva 1 (7.4)	6.4	12.4	90
		16	22.0	94
		40	50.5	107
		100	101.5	95
		250	271.8	106
		380	443.9	115
	Saliva 2 (24.7)	6.4	30.0	97
		16	40.8	100
		40	62.3	96
		100	125.4	100
		250	305.7	111
		760	811.6	103
Method Comparison	Saliva	IBL-ELISA = 0.96 x IBL-Luminescence IA + 2.9		r = 0.995; n = 154

17. PRODUCT LITERATURE REFERENCES

1. Lewis, J.G.: Steroid Analysis in Saliva: An overview. Clin Biochem Rev 2006, Volume 27:139-146
2. Martin A., Saathoff M., Kuhn F. et al.: A Functional ABCC11 Allele Is Essential in the Biochemical Formation of Human Axillary Odor, Journal of Investigative Dermatology 2010, Volume 130:529–540
3. Schulz K., Korz V.: Hippocampal testosterone relates to reference memory performance and synaptic plasticity in male rats, Frontiers in Behavioral Neuroscience, 2010, Volume 4:Article 187

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.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