BioVendor Group

ENG

Product Data Sheet:

HUMAN TESTOSTERONE (TOTAL) ELISA

Catalogue number:

RCD027R

For research use only!

BioVendor R&D

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CONTENTS

1. INTENDED USE

For the direct quantitative determination of Testosterone by enzyme immunoassay in human serum.

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2. PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of Testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of Testosterone in patient samples and controls can be directly read.

3. CLINICAL APPLICATIONS

Testosterone is the most important male sex hormone, it is responsible for genital development, beard growth, muscle development and general male characteristics. The measurement of serum or plasma levels is an index of leydig cell function and high or low values correlate well with hypo or hyper gonadism.

In females small amounts of Testosterone are produced by the adrenals and ovaries. High levels of Testosterone in females indicates excessive androgen production and are found in progressive hirsutism and virilization, Cushing's syndrome and a deficiency in one or more of the specific enzymes required for normal steroid biosynthesis.

4. PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

5. LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of Testosterone in human serum. The kit is not calibrated for the determination of Testosterone in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. 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 the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

6. SAFETY CAUTIONS AND WARNINGS

6.1 Potential biohazardous material

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

7. CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

8. SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4ºC for up to 24 hours or at -10ºC or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

9. SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

10. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

11. REAGENTS PROVIDED

11.1 Rabbit Anti-Testosterone Antibody Coated Microwell Plate-Break Apart Wells

Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2°C-8°C

Stability: 12 months or as indicated on label.

11.2 Testosterone-Horseradish Peroxidase (HRP) Conjugate Concentrate

Requires Preparation.

Contents: Testosterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 µl/vial

Storage: Refrigerate at 2°C-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of HRP in 12ml of assay buffer. Discard any that is left over.

11.3 Testosterone Calibrators

Ready To Use.

Contents: Six vials containing Testosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of Testosterone.

*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 ng/ml	1.0 ml
Calibrator B	0.08 ng/ml	0.5 ml
Calibrator C	0.42ng/ml	0.5 ml
Calibrator D	1.67 ng/ml	0.5 ml
Calibrator E	5.0 ng/ml	0.5 ml
Calibrator F	16.7 ng/ml	0.5 ml

Storage: Refrigerate at 2°C-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

11.4 Controls

Ready To Use.

Contents: Two vials containing Testosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of Testosterone. Refer to vial labels for the acceptable range.

Volume: 0.5 ml/vial

Storage: Refrigerate at 2°C-8°C

Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

11.5 Wash Buffer Concentrate

Requires Preparation.

Contents: One bottle containing buffer with a

non-ionic detergent and a non-mercury preservative.

Volume: 50 ml/bottle

Storage: Refrigerate at 2°C-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

11.6 Assay Buffer

Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 15 ml/vial

Storage: Refrigerate at 2°C-8°C

Stability: 12 months or as indicated on label.

11.7 TMB Substrate

Ready To Use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 ml/bottle

Storage: Refrigerate at 2°C-8°C

Stability: 12 months or as indicated on label.

11.8 Stopping Solution

Ready To Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 ml/vial

Storage: Refrigerate at 2°C-8°C

Stability: 12 months or as indicated on label.

12. ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the Testosterone-HRP conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 150 µl of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
9. Pipette 50 µl of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microwell plate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

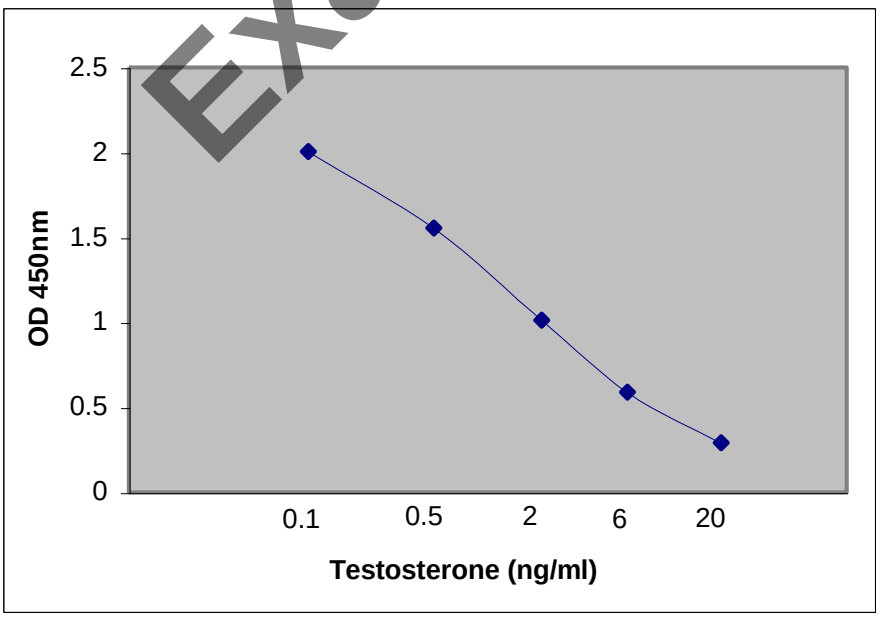
13. CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 20 ng/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

14. TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (ng/ml)
A	2.391	2.357	2.374	0
B	2.069	1.942	2.006	0.1
C	1.533	1.578	1.556	0.5
D	0.984	1.039	1.012	2
E	0.606	0.575	0.591	6
F	0.290	0.293	0.292	20
Unknown	1.266	1.238	1.252	1.1

15. TYPICAL CALIBRATOR CURVE



16. PERFORMANCE CHARACTERISTICS

16.1 Sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Testosterone ELISA kit is **0.022 ng/ml.**

16.2 Specifity (cross reactivity)

The following compounds were tested for cross-reactivity with the BioVendor Testosterone ELISA kit with Testosterone cross-reacting at 100%.

Steroid	%Cross Reactivity
Testosterone	100
5α-DHT	5.2
Androstenedione	1.4
Androstanediol	0.8
Progesterone	0.5
Androsterone	0.1

The following steroids were tested but cross-reacted at less than 0.1%:

Aldosterone, Andrenosterone, Cholesterol, Corticosterone, Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, Epiandrosterone, 17β-Estradiol, Estriol and Pregnenolone.

16.3 Intra assay precision

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV %
1	0.75	0.07	9.6
2	0.77	0.06	7.7
3	1.37	0.08	6.6

16.4 Inter assay precision

Three samples were assayed ten times over a period of four weeks. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV %
1	0.76	0.05	6.1
2	3.29	0.28	8.5
3	4.11	0.30	7.3

16.5 Recovery

Spiked samples were prepared by adding defined amounts of Testosterone to four patient serum samples. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery %
1 Unspiked	0.45	-	-
+6.67	5.73	7.12	80.5
2 Unspiked	0.67	-	-
+6.67	8.08	7.34	110.1
3 Unspiked	1.40	-	-
+6.67	7.13	8.07	88.4
4 Unspiked	2.01	-	-
+6.67	8.42	8.68	97.0

16.6 Linearity

Three patient serum samples were diluted with calibrator A. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery %
1	5.73	-	-
1:2	3.23	2.86	112.9
1:4	1.66	1.43	116.1
1:8	0.85	0.72	118.1
2	8.08	-	-
1:2	4.01	4.04	99.3
1:4	2.02	2.02	100.0
1:8	0.96	1.01	95.0
3	8.42	-	-
1:2	3.75	4.21	89.1
1:4	2.01	2.11	95.3
1:8	1.03	1.05	98.1

16.7 Comparative studies

The Testosterone ELISA kit (x) was compared with a competitors Testosterone ELISA kit (y). The comparison of 40 serum samples yielded the following linear regression results:

y = 1.4171x - 0.0941, r = 0.96

17. EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The results of an expected range study with apparently normal healthy subjects yielded the following results (all values are reported in ng/ml):

Group	N	Mean (ng/ml)	Central 95% (ng/ml)
Prepubertal infants	10	0.12	0.05-0.25
Puberty and Males adults	40	4.7	3.0-12.0
Females	40	0.5	0.2-1.0

18. REFERENCES

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