Testosterone ELISA Assay Kit

Catalog Number:

TST31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 9.1 (10 MAY 24)

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INTENDED USE

The Eagle Biosciences Testosterone ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the direct quantitative determination of Testosterone in human serum. The Eagle Biosciences Testosterone ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

LIMITATIONS RELATED TO INTENDED PURPOSE AND USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of testosterone in human serum. The kit is not calibrated for the determination of testosterone in other specimens of human or animal origin.
4. The results obtained with this kit shall never be used for a clinical diagnosis.
5. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as durgs and the occurene of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.

INTRODUCTION

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 hypergonadism. 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.

PRINCIPLE OF THE ASSAY

The Testosterone ELISA is a competitive immunoassay. Competition occurs between testosterone present in calibrators, controls, specimen samples and an enzyme labelled antige (HRP conjugate) for a limited number of anti-testosterone antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of testosterone present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators used to plot a calibrator curve from which the amount of testosterone in specimens samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for used by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a Do not pipette by mouth
    b Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c Wear protective clothing and disposable gloves.
    d Wash hands thoroughly after performing the test.
    e Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush with water immediately and contact a doctor.

3. 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.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. 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.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB substrate is sensitive to light and should remain colorless if properly stored. Insability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do no use grossly hemoluyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing axide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >16.7 ng/mL. If further dilution and retesteing is required only calibrator A may be used to dilute dserum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
22. Consumables used with the kit that are potentially biohazardouse (e.g., pipette tips, bottles or containers containing huan materials) must be handled according to biosafey practices to mimize riesk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and dispoable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.

25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader is required.
26. If a microplate reader is required for the assay procedure, the type and speed of shaker is required is stated in the REAGENTS AND EQUPMNT NEEDED BUT NOT PROVIDED section. Both the type and speed of the shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not resuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

The reagents should be considered a potential biohazard and handled with the same precaution applied to blood specimens. All human specimens should be considered potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices. The calibrators and controls provided with the kit contain processed human serum/plasma that has been tested by approved methods and found to be negative for presence of Hepatitis B surface antigen (HBsAg) and antibodies to HCV, HIV ½ and HIV NAT. However, not test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, & PRE-TREATMENT

Specimen Collection & Storage

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower for up to 1 month. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Specimen Pre-Treatment

Specimen pre-treatment is not required

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 50 µL.
2. Calibrated multi-channel pipette to dispense 50 µL, 100 µL, and 150 µL
3. Calibrated multi-channel pipettes to dispense 300 µL (if washing manually)

4. Automatic microplate washer (recommended)
5. Microplate shaker:
    a Orbital shaker (3 mm diameter) set to 600 rpm or
    b Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute
6. Disposable pipette tips
7. Distilled or deionized water
8. Calibrated absorbance microplate reader with a 450 nm and an upper OD limit of 3.0 or greater

REAGENTS PROVIDED

1. **Microplate**

Contents:	One anti-testosterone polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

2. **HRP Conjugate Concentrate**

Contents:	One bottle containing Testosterone-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	0.3 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
Preparation of HRP Conjugate Working Solution:	Dilute 1:51 in assay buffer before use (e.g., 40 µL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. **Calibrator A-F**

Contents:	Six bottles of calibrator containing specified testosterone concentrations. Human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of testosterone. Listed below are approximate concentration, please refer to vial labels for exact concentrations. Concentrations: 0, 0.08, 0.42, 1.67, 5.0, 16.7 ng/mL.
Format:	Ready to Use
Volume:	Calibrator A: 1.0 mL/bottle Calibrator B-F: 0.5 mL/bottle

Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

4. **Controls 1-2**
   Contents: Two bottles of control containing different testosterone concentrations. Human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of testosterone.
   Refer to the QC certificate for the target values and acceptable ranges.
   Format: Ready to Use
   Volume: 0.5 mL/bottle
   Storage: 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

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5. **Assay Buffer**
   Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   Format: Ready to Use
   Volume: 15 mL/bottle
   Storage: 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

6. **TMB Substrate** – Ready to Use
   Contents: One bottle containing a tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   Format: Ready to Use
   Volume: 16 mL/bottle
   Storage: 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

7. **Stopping Solution** – Ready to Use
   Contents: One bottle containing 1M sulfuric acid.
   Format: Ready to Use
   Volume: 6 mL/bottle
   Storage: 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
   Safety: Refer to product SDS

8. **Wash Buffer Concentrate**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative
Format:	Concentrate; Requires preparation
Volume:	50 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.
Preparation of Wash Buffer Working Solution:	Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

ASSAY PROCEDURE

Specimen Pretreatment: None

All kit components, controls and specimen samples must reach room temperature prior to 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. After all kit components have reached room temperature, **mix** gently by inversion.
2. **Prepare** the HRP Conjugate Working Solution and Wash Buffer Working Solution (see *Reagents Provided*)
3. **Plan** the microplate wells to bee used for calibrators, controls, and samples. (See *Recommended Assay Layout*). Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. **Pipette 50 µL** of each calibrator, control and specimen sample into assigned wells.
5. **Pipette 100 µL** of the HRP Conjugate Working solution into each well. (We recommend using a multichannel pipette.)
6. **Incubate** the microplate on a microplate shaker** for **60 minutes** at room temperature.
7. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.
    a **Automatic:** Using an automatic microplate washer, perform a **3-cycle** wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

b **Manually:** Using an automatic microplate washer, perform a **3-cycle** wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 300 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

8. **Pipette 150 µL** of TMB substrate into each well (the use of a multi-channel pipette is recommended).
9. **Incubate** the microplate on a microplate shaker** for **10–15** minutes at room temperature.
10. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
11. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

** See *Reagents and Equipment Needed But Not Provided* for microplate shaker options.

CALCULATIONS

1. Calculate the mean optical density of each calibrator, control, and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 16.7 ng/mL and needs to be diluted and retested, then dilute with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator / OD of calibrator A) x 100.
3. The values obtained for the kit controls are withing the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

TYPICAL TABULATED DATA

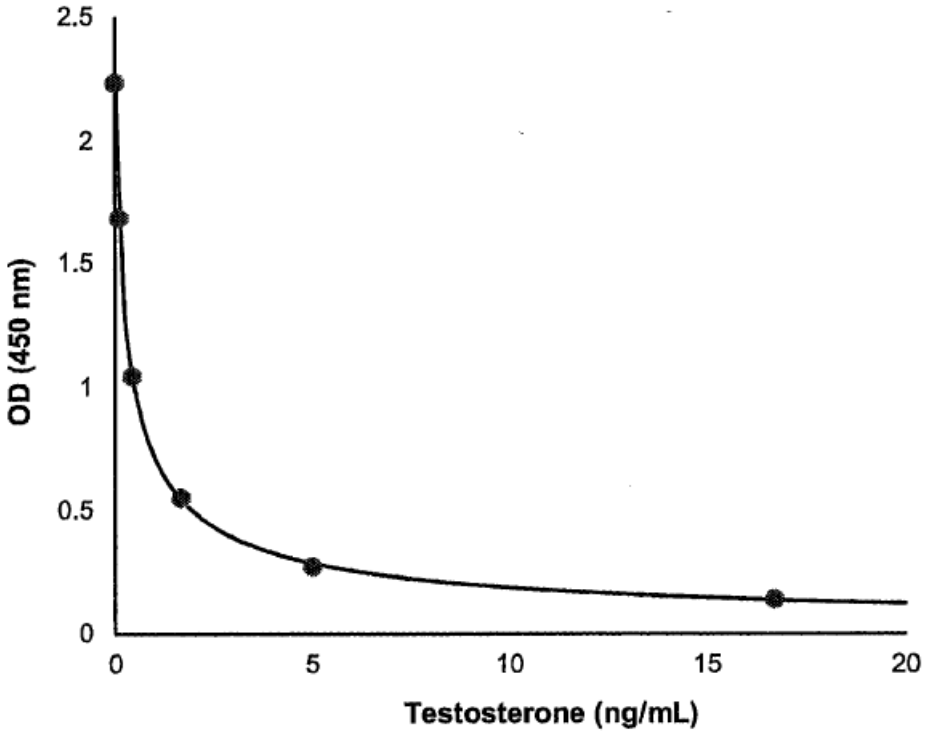
Sample data only. Do not use to calculate results.

Calibrator	Mean OD	% Binding	Value (ng/mL)
A	2.236	100	0
B	1.685	75	0.08
C	1.044	47	0.42
D	0.551	25	1.67

E	0.274	12	5.0
F	0.140	6	16.7
Unknown	0.681	-	1.08

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

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 Direct Testosterone ELISA kit is 0.022 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

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

Steroid	% Cross Reactivity
Testosterone	100
5a-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, Androsterone, Cholesterol, Corticosterone, Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, Epiandrosterone, 17β-Estradiol, Estriol and Pregnenolone.

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

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

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 + 6.67	0.45 5.73	- 7.12	- 80.5
2 Unspiked + 6.67	0.67 8.08	- 7.34	- 110.1
3 Unspiked + 6.67	1.40 7.13	- 8.07	- 88.4
4 Unspiked + 6.67	2.01 8.42	- 8.68	- 97.0

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

COMPARATIVE STUDIES

The Direct 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

REFERENCE RANGES

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

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Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.