Testosterone Saliva ELISA Assay Kit

Catalog Number: TSS32-K01

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

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INTENDED USE

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

INTRODUCTION

Testosterone is a C-19 steroid secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovaries in women. Testosterone is also produced by peripheral tissues from androstenedione, which is of little physiological significance in men. However, in women about half of circulating testosterone is derived from this origin. The action of testosterone is both androgenic or anabolic. Testosterone measurements are used mainly for clinical evaluation of hypogonadism in males and hyperandrogenic states in females. Most of the circulating testosterone is bound to three proteins: sex hormone binding globulin (44–78%), albumin (20– 54%) and cortisol binding globulin (small amount). Only about 2–3% of the total circulating testosterone remains unbound or in the free form. Only the free portion (or the non-SHBG bound fraction) of the circulating testosterone is thought to be available to tissues where it exerts its biological actions. The salivary hormone assays are advocated for their noninvasive, easy sample collection method. Salivary testosterone is of great clinical value for it represents a filtered fraction of plasma testosterone and is independent of flow rate. Many studies have suggested that salivary testosterone correlates well with either free or non-SHBG bound testosterone.

PRINCIPLE OF THE ASSAY

The Testosterone Saliva ELISA is a competitive immunoassay. Competition occurs between testosterone present in calibrators, controls, specimen samples and an enzyme-labeled antigen (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 a blue to a yellow color. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of testosterone in specimen samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use 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 eyes 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 this 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 must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of 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 saliva 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 reagent storage.
13. When dispensing the substrate and stopping solutions, 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. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use blood contaminated saliva samples.
16. Samples or controls containing azide 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 >1000 pg/mL. If further dilution and retesting is required, only Calibrator A may be used to dilute saliva samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, 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 local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M 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 disposable 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 shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse 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. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
30. 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

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens, All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (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, AND PRE-TREATMENT

Specimen Collection & Storage Avoid sample collection within 1 hour after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. Do not use blood-contaminated specimens.

Approximately 0.25 mL of saliva is required per duplicate determination. Rinse mouth thoroughly with water 10 minutes before the sample is collected. Collect 1-2 mL of saliva into a clean polypropylene tube without force or inducement. Saliva samples may be stored at 2-8°C for up to 24 hours or at -20°C 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.

Specimen Pre-Treatment & Storage

Following collection, the sample must be pretreated according to the following procedure:

1. Freeze the sample for a minimum of 2 hours.
2. Thaw the sample.
3. Vortex to mix and centrifuge the sample at 2000x g for 10 minutes.
4. Carefully remove the supernatant and transfer to a new labeled tube. The supernatant will be used in the assay procedure of the test.

DO NOT pretreat the calibrators and controls; they are provided in a ready to use format.

Store pre-treated saliva samples at 2-8°C for up to 24 hours or at -20°C or lower if the analyses are to be done at a later date. Samples that have been stored should be inspected to ensure they are free from precipitates before being used in the assay. If there are precipitates present, follow steps 3-4 in section Specimen Pretreatment & Storage. Consider all human specimens as possible biohazardous materials.

MATERIALS NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 100 µL.
2. Calibrated multi-channel pipette to dispense 50 µL, 100 µL, and 150 µL.
3. Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker:
    a. Orbital shaker (3 mm diameter) set to 600 rpm
    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 filter and an upper OD limit of 3.0 or greater.
9. Polypropylene

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 expiry date printed on the label. After Opening: Stable for three weeks

2. HRP Conjugate

Contents	One bottle containing Testosterone-Horse Radish Peroxidase (HRP) conjugate in 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 expiry date printed on the label. After Opening: Stable for three weeks

3. Calibrator A – F

Contents	Six bottles of calibrator containing specified testosterone concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of testosterone. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 10, 40, 120, 360, 1000 pg/mL
Format	Ready to Use
Volume	Calibrator A: 4.0 mL/bottle Calibrator B-F: 1.0 mL/bottle
Storage	2-8°C
Stability	Unopened: Stable until expiry date printed on the label. After Opening: Stable for three weeks

4. Control 1 – 2

Contents	Two bottles of control containing different testosterone concentrations. Protein-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	1.0 mL/bottle
Storage	2-8°C
Stability	Unopened: Stable until expiry date printed on the label. After Opening: Stable for three weeks

5. TMB Substrate

Contents	One bottle containing 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 expiry date printed on the label. After Opening: Stable for three weeks

6. Stop Solution

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

7. Wash Buffer Concentrate

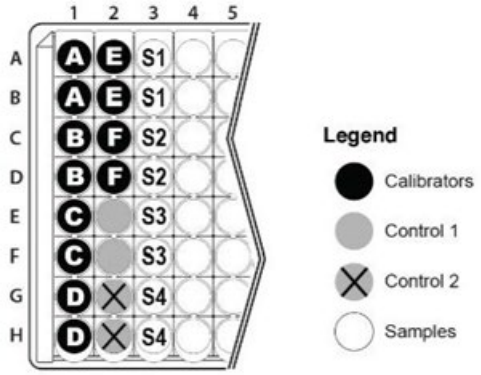
Contents	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format	Concentrated; Requires Preparation
Volume	50 mL/bottle
Storage	2-8°C
Stability	Unopened: Stable until expiry date printed on the label. After Opening: Stable for three weeks. Following Preparation: 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 (X10)	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.

8. Microplate Film

Contents	Microplate Adhesive Film
Format	Ready to Use
Quantity	2
Stability	Unopened: Stable until expiry date printed on the label.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Specimen Pretreatment: All specimens that will be tested must be pre-treated before being tested (see section 7.2. Specimen Pre-Treatment & Storage). Do not pre-treat the calibrators and it controls as they are provided ready to use.

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 Wash Buffer Working Solution (See section Reagents Provided, Wash Buffer Concentrate).
3. **Prepare** all specimen samples that will be tested. Refer to section Specimen Pre-Treatment & Storage.
4. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section 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.

5. **Pipette 100 µL** of each calibrator, control, and pre-treated specimen sample into assigned wells.
6. **Pipette 100 µL** of the HRP conjugate into each well (the use of a multi-channel pipette is recommended). **Cover** with Provided Microplate Film
7. **Incubate** the microplate on a microplate shaker** for 60 minutes at room temperature.
8. **Remove** the microplate sealing film and 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 350 µL /well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 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: For manual washing, perform a 3-cycle wash using 350 µL /well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waster container, then pipetting 350 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.
9. **Pipette 150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
10. **Incubate** the microplate on a microplate shaker** for 30 minutes at room temperature. Do not cover the microplate.
11. **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 the addition of TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
12. **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.

If shaking required: **See Section Reagents and Equipment Needed But Not Provided for microplate shaker options

CALCULATION

1. Calculate the mean optical density for 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 1000 pg/mL and needs to be diluted and retested, then dilute it with calibrator A at a dilution of no more than 1:4. The result obtained must 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 within the acceptable ranges as stated in the QC Certificate.
4. The results of any external controls that were used meet the acceptable ranges.

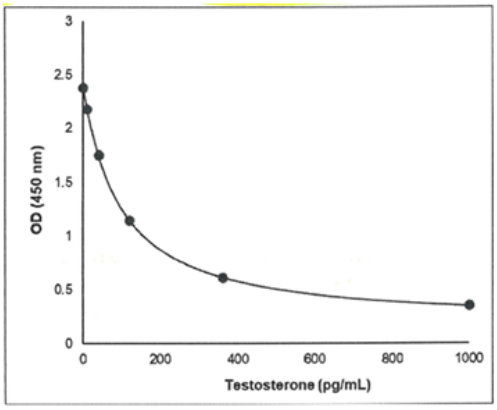
TYPICAL DATA

TYPICAL TABULATED DATA

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.383	100	0
B	2.178	91	10
C	1.751	73	40
D	1.148	48	120
E	0.612	26	360
F	0.353	15	1000
Unknown	1.867	-	30

TYPICAL CALIBRATOR CURVE

Sample curve only. DO NOT use to calculate results



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.

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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.