ab178655 –
Testosterone ELISA Kit

Instructions for Use

A competitive immunoenzymatic assay for the quantitative measurement of Testosterone in saliva.

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

## INTRODUCTION
1. BACKGROUND 2
2. ASSAY SUMMARY 4

## GENERAL INFORMATION
3. PRECAUTIONS 5
4. STORAGE AND STABILITY 5
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 6
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

## ASSAY PREPARATION
9. REAGENT PREPARATION 8
10. SAMPLE COLLECTION AND STORAGE 9
11. PLATE PREPARATION 10

## ASSAY PROCEDURE
12. ASSAY PROCEDURE 11

## DATA ANALYSIS
13. CALCULATIONS 13
14. TYPICAL SAMPLE VALUES 14
15. ASSAY SPECIFICITY 15

## RESOURCES
16. TROUBLESHOOTING 16
17. NOTES 18
INTRODUCTION

1. BACKGROUND

Abcam’s Testosterone in vitro competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Testosterone in saliva.

A 96-well plate has been precoated with anti-Testosterone antibodies (solid-phase). Samples and the Testosterone-HRP conjugate are added to the wells, where Testosterone in the sample competes with the added Testosterone-HRP conjugate for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Testosterone in the sample and the intensity is measured at 450 nm.

Testosterone (17β-Hydroxy-4-androstene-3-one) is a steroid hormone from the androgen group.

In postpubertal males, testosterone is secreted primarily by the testes with only a small amount derived from peripheral conversion of androstenedione. In adult women over 50% of serum testosterone is derived from peripheral conversion of androstenedione secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands.

The level of testosterone in saliva (pg/mL) is significantly lower than levels in the general circulation (ng/mL).

Testosterone effects can be classified as virilizing and anabolic effects, although the distinction is somewhat artificial, as many of the effects can be considered both. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Virilizing effects include maturation of the sex organs and after birth (usually at puberty) a deepening of the voice, growth of the beard and axillary hair (male secondary sex characteristics).

Testosterone levels decline gradually with age in men (andropause). The signs and symptoms are non-specific, and are generally
INTRODUCTION

associated with aging such as loss of muscle mass and bone density, decreased physical endurance, decreased memory ability and loss of libido.

In females of all ages, elevated testosterone levels can be associated with a variety of virilizing conditions, including adrenal tumors and polycystic ovarian disease.
2. **ASSAY SUMMARY**

Prepare all reagents, samples, controls and standards as instructed.

Add samples, standards and controls to wells used.

Add prepared labeled HRP-Conjugate to each well. Incubate at 37°C.

After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at 2-8°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Testosterone IgG Coated Microplate (12 x 8 wells)</td>
<td>96 Wells</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>15 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Testosterone HRP Conjugate</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>TMB Substrate Solution</td>
<td>15 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Incubation Buffer</td>
<td>30 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Wash Solution 10X</td>
<td>50 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Testosterone Standard 0 - 0 pg/mL</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Testosterone Standard 1 - 10 pg/mL</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Testosterone Standard 2 - 50 pg/mL</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Testosterone Standard 3 - 250 pg/mL</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Testosterone Standard 4 - 1,000 pg/mL</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Strip Holder</td>
<td>1 Unit</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Cover Foil</td>
<td>1 Unit</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Control A</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Control B</td>
<td>1 mL</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620-630 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells
- Rotating mixer
- Centrifuge for 3,000 rpm
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

7. **LIMITATIONS**

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
To avoid cross-contamination and falsely elevated results, pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells.

8. **TECHNICAL HINTS**

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross-contamination of samples or reagents by changing tips between sample, standard, and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control, or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. **REAGENT PREPARATION**

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **Wash Solution 1X**

Prepare 1X Washing Solution by diluting Wash Solution 10X with deionized water. To make 500 mL 1X Wash Solution combine 50 mL Wash Solution 10X with 450 mL deionized water. Mix thoroughly and gently. The Wash Solution 1X is stable for 30 days at 2-8°C. If crystals are present in Wash Solution 10X, mix at room temperature until completely dissolved.

9.2 **1X Testosterone HRP Conjugate**

Prepare immediately before use. Add 10 µL of Testosterone HRP Conjugate to 1 mL Incubation Buffer. Mix gently for 5 minutes on a rotating mixer. Diluted solution is stable for 3 hours at room temperature.

- All other solutions are supplied ready to use
10. SAMPLE COLLECTION AND STORAGE

- The determination of Testosterone can be performed in saliva. It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw. Do not use commercially available sample collectors. Other equipment of sample collection commercially available has not been tested.

- If no specific instructions have been given, saliva samples may be collected at any time, paying attention to the following indications:
  - Let the saliva flow down through the straw into the centrifuge glass tube
  - Centrifuge the sample for 15 minutes at 3,000 rpm
  - Store at -20°C for at least 1 hour
  - Defrost samples
  - Centrifuge again for 15 minutes at 3,000 rpm
  - The saliva sample is now ready to be tested

- The specimen should be kept at 2-8°C for up to one week; otherwise it should be aliquoted and stored deep-frozen (-20°C).

**Notes:** If saliva collection has to be carried out in the morning ensure that this is carried out prior to brushing teeth.

During the day saliva should be collected at least 1 hour after any food or drink, oral intake of pharmaceutical drugs or tooth cleaning.

It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other such extraneous materials.
11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).
12. **ASSAY PROCEDURE**

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.
- Assay all standards, controls and samples in duplicate.

12.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3. Add 100 µL standard, control or sample into their respective wells. Add 100 µL Testosterone-HRP Conjugate to each well. Leave a blank well for substrate blank.

12.4. Cover wells with the foil supplied in the kit.

12.5. Incubate for 1 hour at 37°C.

12.6. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL Wash Solution 1X. Avoid overflows from the reaction wells. The soak time between each wash cycle should be > 5 seconds. During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. Automatic washer: In case you use an automatic washer, it is advised to do six washing steps.

Note: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.

12.7. Add 100 µL TMB Substrate Solution into all wells.
12.8. Incubate for exactly 15 minutes at room temperature in the dark.

12.9. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

12.10. Read the absorbance at 450 nm against a reference wavelength of 620-630 nm or against blank within 5 minutes of addition of the Stop Solution.
13. **CALCULATIONS**

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e.g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.
14. **TYPICAL SAMPLE VALUES**

**TYPICAL STANDARD CURVE** -

- **REFERENCE VALUES** -

As the values of salivary Testosterone have a circadian pattern we suggest collecting the samples at the same hour (8 AM):

The following values can be used as preliminary guideline until each laboratory has established its own normal range.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women Normal</td>
<td>10 – 55</td>
</tr>
<tr>
<td>Women Hirsutism</td>
<td>25 – 85</td>
</tr>
<tr>
<td>Women (after treatment)</td>
<td>16 – 40</td>
</tr>
<tr>
<td>Women PCO</td>
<td>16 – 50</td>
</tr>
<tr>
<td>Children</td>
<td>35 – 160</td>
</tr>
<tr>
<td>Men</td>
<td>50 – 210</td>
</tr>
<tr>
<td>Men Hypogonadism</td>
<td>10 – 80</td>
</tr>
</tbody>
</table>
DATA ANALYSIS

SENSITIVITY –
The lowest detectable concentration of Testosterone that can be distinguished from standard 0 is 2.96 pg/mL at the 95% confidence limit.

PRECISION –

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>%CV</td>
<td>8%</td>
<td>13.2%</td>
</tr>
</tbody>
</table>

RECOVERY –
The recovery of 37.5 – 100 – 500 pg/mL Testosterone added to saliva sample gave an average value (±SD) of 98.97% ± 10.92% with reference to the original concentrations.

15. ASSAY SPECIFICITY
The cross reaction of the antibody calculated at 50% is:

<table>
<thead>
<tr>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosteron</td>
<td>100.0 %</td>
</tr>
<tr>
<td>DHT</td>
<td>16.0 %</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.8 %</td>
</tr>
<tr>
<td>Androsterone</td>
<td>0.0 %</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Cortisone</td>
<td>0.0 %</td>
</tr>
<tr>
<td>17 α Estradiole</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Prednisone</td>
<td>0.0 %</td>
</tr>
</tbody>
</table>
# Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes &amp; ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------------</td>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate</td>
<td>Read plate immediately after adding stop solution</td>
</tr>
<tr>
<td></td>
<td>after adding stop solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type</td>
<td>Detection may be reduced or absent in untested sample</td>
</tr>
<tr>
<td></td>
<td>(e.g. Serum vs. cell extract)</td>
<td>types</td>
</tr>
</tbody>
</table>
17. NOTES
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp

Copyright © 2017 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.
All information / detail is correct at time of going to print.