Version 10d, Last updated 1 March 2024

ab108666
Testosterone ELISA Kit

A competitive immunoenzymatic assay for the quantitative measurement of Testosterone in serum, plasma and urine.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

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

A 96-well plate has been precoated with anti-Testosterone antibodies. Samples and the Testosterone-HRP conjugate are added to the wells, where Testosterone in the sample competes with the added Testosterone-HRP 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 post pubertal 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 gland and ovaries, with the remainder from direct secretion of testosterone by these glands. The majority of circulating testosterone is bound by SHBG and a smaller portion is bound by albumin. Only a small percentage (<1%) exists in circulation as unbound or free testosterone.

Testosterone's effects can be classified as virilizing and anabolic, although the distinction is somewhat artificial, as many of the effects can fall into both categories. Anabolic effects include growth of muscle mass and strength, increase in 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 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 syndrome.

2. Protocol 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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Stop Solution	15 mL	4°C
Testosterone-HRP Conjugate	12 mL	4°C
TMB Substrate Solution	15 mL	4°C
Testosterone Control A	1 mL	4°C
Testosterone Control B	1 mL	4°C
Testosterone IgG Coated Microplate (12 x 8 wells)	96 wells	4°C
Testosterone Standard 0– 0.0 ng/mL	1 mL	4°C
Testosterone Standard 1 – 0.6 ng/mL	1 mL	4°C
Testosterone Standard 2 – 1.4 ng/mL	1 mL	4°C
Testosterone Standard 3 – 4.0 ng/mL	1 mL	4°C
Testosterone Standard 4 – 16.0 ng/mL	1 mL	4°C
10X Wash Buffer	50 mL	4°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm or 620 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
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

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
- When using automatic equipment, the user has the responsibility to make sure that the kit has been appropriately tested. To improve the performance of the kit on ELISA automatic systems, it is recommended to increase the number of washes.
- 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
- If crystals are present in the 10X Wash Buffer, mix gently at room temperature until they have dissolved.
- 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 to room temperature (18-25°C) prior to use for at least 30 minutes.
- At the end of the assay store the reagents immediately at 4°C; avoid long exposure to room temperature.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Wash Buffer

To prepare the 1X Wash Buffer, dilute the 10X wash buffer 1/10 with distilled water prior to use.

10. Sample Preparation

The determination of Testosterone can be performed in urine, and plasma as well as in serum. If the assay is performed on the same day of sample collection, the specimen should be kept at 4°C; otherwise it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 minutes before testing. Urine may be used undiluted in this assay.

ΔNote: Avoid repeated freezing and thawing.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

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 25 µL standards, control and samples 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 and incubate for 1 hour at 37°C.

12.5 Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Wash Buffer. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining fluid by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid. Automatic washer: in case you use an automatic washer, it is advised to do 6 washing steps.

ΔNote: Complete removal of liquid at each step is essential for good assay performance.

12.6 Add 100 µL TMB Substrate Solution into all wells.

12.7 Incubate for 15 minutes at 37°C in the dark. Incubation at lower temperature may require longer incubation timing.

12.8 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.

12.9 Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630 nm within 30 min after addition of the Stop Solution or against blank within 5 minutes.

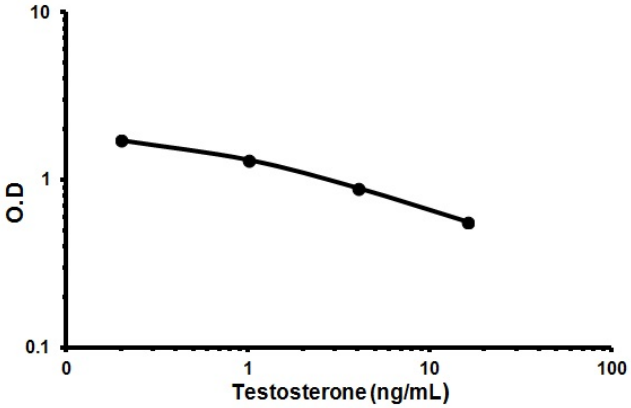
13. Calculations

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 on a log scale as in the example below. 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 ng/mL.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (ng/mL)	O.D
0.0	2.928
0.2	2.200
1.0	1.625
4.0	1.104
16.0	0.645

Figure 1. Example of Testosterone standard curve.

15. Typical Sample Values

REFERENCE VALUES –

Human serum Testosterone reference values are in the following ranges:

WOMEN: 0.2 - 1.2 ng/ml

CHILDREN: 0.1 - 0.4 ng/ml

MEN: 1.8 - 9.0 ng/ml

SENSITIVITY –

The lowest detectable concentration of Testosterone that can be distinguished from the zero standard is 0.10 ng/mL at the 95 % confidence limit.

PRECISION –

Intra-assay precision was determined by testing replicates of three plasma samples twenty times in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-assay Precision	Inter-Assay Precision
n =	16	9
CV (%)	$\leq$ 7.0	$\leq$ 8.3

RECOVERY –

The recovery of 0.4, 0.8, 4.0, 14.0 ng/mL of Testosterone added to the samples gave an average value of 98.9% with reference to the original concentrations. The dilution test performed on 3 samples diluted up to 4 times gave an average value of 99.7%.

16. Assay Specificity

The cross reaction of the antibody calculated at 50 % is:

Testosterone	100.0 %
DHT	2.03 %
Androstenedione	0.01 %
Androsterone	0.05 %
DHEA-S	0.0 %
Cortisol	0.01 %
Cortisone	0.0 %
17 α Estradiole	0.16 %
Estrone	0.0 %
Prednisone	0.01 %

Please contact our Technical Support team for more information.

17. Troubleshooting

Problem	Cause	Solution
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)