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Product Information

Testosterone ELISA Kit

Catalog Number: EA101008
Storage Temperature: 2 – 8°C

Instruction for Use

THIS KIT IS INTENDED FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1 INTRODUCTION

1.1 Intended Use

The **ORIGENE Testosterone ELISA** is an enzyme immunoassay for the measurement of testosterone in serum or plasma (EDTA, lithium heparin or citrate plasma).

2 PRINCIPLE OF THE TEST

The ORIGENE Testosterone ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the principle of competitive binding.
The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site of the testosterone molecule.
During the first incubation, the testosterone in the added sample competes with the added enzyme conjugate, which is testosterone conjugated to horseradish peroxidase, for binding to the coated antibody.
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is inversely proportional to the concentration of the analyte in the sample.
A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3 REAGENTS

3.1 Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-testosterone antibody (monoclonal).
2. ***Standard (Standard 0 - 6)***, 7 vials, 1 mL each, ready to use;
   Concentrations: 0 – 0.2 – 0.5 – 1.0 – 2.0 – 6.0 - 16.0 ng/mL
   Conversion: 1 ng/mL = 3.467 nmol/L
   Contain non-mercury preservative.
3. ***Enzyme Conjugate***, 1 vial, 25 mL, ready to use;
   Testosterone conjugated with horseradish peroxidase;
   Contains non-mercury preservative.
4. ***Substrate Solution***, 1 vial, 25 mL, ready to use;
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   Contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   See "Reagent Preparation"

3.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction

3.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 8 weeks if stored as described above.

3.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Wash Solution

Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL distilled water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

4 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA, lithium heparin or citrate plasma) can be used in this assay.

Note: Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use haemolytic, icteric, or lipaemic specimens. For further information refer to chapter "*Interfering Substances*".

4.1 Specimen Collection

Serum:

Collect blood by venipuncture, Allow to clot and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy mayrequire increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anticoagulant and centrifuged immediately after collection.

4.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying.
Specimens stored for a longer time (up to 12 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

4.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more analyte than the highest standard, the specimen can be diluted with *Standard 0* and re-assayed as described in "Assay Procedure".
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL sample + 90 µL *Standard 0* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

5 ASSAY PROCEDURE

5.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixedwithout foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended thatall reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

5.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each ***Standard,*** control and **sample** with new disposable tips into appropriate wells.
3. Dispense **200 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature.
5. Rinse the wells **3 times** with **400 µL** diluted *Wash Solution* per well, if a plate washer is used.
   - OR -
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **300 µL** diluted *Wash Solution* per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **200 µL** of ***Substrate Solution*** to each well.
7. Incubate for **15 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
9. Determine the optical density of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm(background subtraction, recommended)** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

5.3 Calculation of Results

1. Calculate the average optical density (OD) values for each set of standards, controls and patient samples.
2. Using graph paper, construct a standard curve by plotting the mean OD obtained from each standard against itsconcentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-

Parametercurve fit. (4-Parameter Rodbard or 4-Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.

5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higherthan that of the highest standard have to be further diluted or reported as > 16.0 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Density (450 nm)
Standard 0 (0 ng/mL)	2.1
Standard 1 (0.2 ng/mL)	1.71
Standard 2 (0.5 ng/mL)	1.44
Standard 3 (1.0 ng/mL)	1.18
Standard 4 (2.0 ng/mL)	0.89
Standard 5 (6.0 ng/mL)	0.46
Standard 6 (16.0 ng/mL)	0.24

6 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to theestablished acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or OriGene directly.

7 ASSAY CHARACTERISTICS

7.1 Assay Dynamic Range

The range of the assay is between 0.083 ng/mL – 16 ng/mL.

7.2 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay:

Analyte	Cross-Reactivity (%)
Testosterone	100.0
DHT	129
5α-Dihydrotestosterone	0.8
Androstenedione	0.9
11β-Hydroxyestosterone	3.3
17a-Methyltestosterone	0.1
19-nortestosterone	3.3
DHEA	0.3
DHEA-S	< 0.1
Epitestosterone	< 0.1
Progesterone	< 0.1
Cortisol	< 0.1
Estrone	< 0.1
Estradiol	< 0.1
Estriol	< 0.1
Danazol	< 0.1

7 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understandingof the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

7.1 Interfering Substances

Hemoglobin (up to 4 mg/mL), bilirubin (up to 0.25 mg/mL) and triglyceride (up to 7.5 mg/mL) have no influence on theassay results.

7.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of testosterone in a sample.

7.3 High-Dose-Hook Effect

A High-Dose-Hook Effect is not known for competitive assays.

8 LEGAL ASPECTS

8.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhereto the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also withinthe given assay specifications. In case of any doubt or concern please contact OriGene.

8.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of

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