Importance of Testosterone Determination

Testosterone is one of the most important androgens secreted into the bloodstream and is synthesized from pregnolone which is itself formed from cholesterol. In adult humans approximately 5 mg of testosterone are synthesized per day and circulate in plasma predominately bound to proteins, including specific sex hormone binding globulin (SHBG) and nonspecific proteins such as albumin. It is believed that the bioavailable testosterone includes the free steroid and the albumin bound steroid and these equal about 35% of the total testosterone. Testosterone is the main androgen secreted by the Leydig cells of the testes and effects both primary and secondary sexual development such as muscle mass and sex drive. Steroid hormones such as Testosterone can be found in some environmental samples such as groundwater and near sewage plants, thus requiring cautious monitoring.

The Testosterone ELISA allows the determination of 40 samples in duplicate determination. Only a few mL of sample are required. The test can be performed in about 3 hours.

Performance Data

Test range: The range of detection for Testosterone in water samples is 7.81-2,000 pg/mL. The middle of the test (50% B/B_0) is at about 400 pg/mL. Determinations close to the middle of the tests give the most accurate results.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Cross-reactivities:

Testosterone	100% (per definition)
19-hydroxytestosterone	14.6%
Androstendione	7.2%
Dehydroepiandrosterone	0.72%
Estradiol	0.40%
Dihydrotestosterone	<0.001%
Estriol	<0.001%
Estrone	<0.001%
Progesterone	<0.001%

Cross-reactivities with other hormone classes have not been observed.

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ABRAXIS

Testosterone ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Testosterone in Water Samples

Product No. 900TES

1. General Description

The Testosterone ELISA is an immunoassay for the quantitative and sensitive detection of Testosterone, a steroid hormone. This test is suitable for the quantitative and/or qualitative detection of Testosterone in water samples.

2. Safety Instructions

The standard stock supplied with the test kit contains the steroid Testosterone. Avoid contact of solutions with skin and mucous membranes. If these reagents come in contact with the skin, wash with water. Consult state, local and federal regulations for proper disposal of all reagents.

3. Storage and Stability

The Testosterone ELISA should to be stored in the refrigerator (4–8°C). The solutions have to be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is based on the recognition of Testosterone by a specific monoclonal antibody. Testosterone present in the sample and a testosterone-enzyme-conjugate compete for the binding sites of the antibodies. After a washing step and addition of the substrate solution a color signal is produced. The intensity of the yellow color produced is inversely proportional to the concentration of the Testosterone present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader.

5. Limitations of the Testosterone ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded. Mistakes in handling the test also can cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures during the test performance (lower than 10°C or higher than 30°C).

The Abraxis Testosterone ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive samples requiring some action should be confirmed by an alternative method.

Working Instructions

A. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the enzyme conjugate, the substrate solution and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum foil. The remaining strips are stored back in the aluminum foil and closed again using the white plastic clip. Store the remaining kit in the refrigerator (4-8°C).
3. Prepare standard solutions as follows: Allow the 50,000 pg/mL Testosterone standard solution to warm to room temperature. Label five 12 X 75 mm glass tubes with std. 1-5. Pipette 1 mL of standard diluent into tube # 5. Pipette 750 uL of standard diluent into tubes 2-4. Remove 40 uL of diluent from tube # 5. Add 40 uL of the 50,000 standard stock to tube # 5. Vortex thoroughly. Add 250 uL of tube # 5 to tube # 4 and vortex thoroughly. Add 250 uL of tube # # 4 to tube # 3 and vortex thoroughly. Add 250 uL of tube # 3 to tube # 2 and vortex thoroughly. Add 250 uL of # 2 to tube # 1 and vortex thoroughly.
4. The antibody, enzyme conjugate, substrate and stop solution are ready to use and do not require any further dilutions.
5. Dilute the wash buffer at a ratio of 1:10. If using 30 mL of concentrate then add to 270 mL of deionized or distilled water.

B. Assay Procedure

1. Add 100 µL of the each standard solution (0, 7.81, 31.25, 125, 500, 2,000 pg/mL), and samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of enzyme conjugate (blue) solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution (yellow) solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
4. Cover strips with sealer provided and ncubate the strips for 120 min at room temperature (use an orbital shaker).
5. Wash the strips five (5) times using the diluted washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stock of paper.
6. Add 100 µL of substrate solution to the wells. The strips are sealed and incubated for 60 min at room temperature on a shaker.
7. Add 50 µL of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at **405 nm** using a microplate ELISA photometer.

C. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (Logit/Log or 4-Parameter). For a manual evaluation calculate the mean absorbance value for each of the standards. Calculate the %B/B_0 for each standard by dividing the mean absorbance value for the Zero Standard (Standard 0). Construct a standard curve by plotting the %B/B_0 for each standard on a vertical linear (y) axis versus the corresponding Testosterone concentration on horizontal logarithmic (x) axis on graph paper. %B/B_0 for controls and samples will then yield levels in ppb of Testosterone by interpolation using the standard curve.

The concentrations of the samples are determined using this standard curve. Samples showing a lower concentrations of Testosterone compared to standard 1 (7.81 pg/mL) are considered as negative. Samples showing a higher concentration than standard 5 (2,000 pg/mL) must be diluted further to obtain more accurate results. The concentration of the negative and positive controls should be in the range given in the test instructions (±20%).

D. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (10-100 µL, 100-1000 µL)
2. Multi-channel pipette (10-200 µL) or stepper pipette with plastic tips (10-200 µL)
3. Microtiter plate washer
4. Microtiter plate reader (wave length **405 nm**)
5. Shaker for microtiter plates.

E. Working Scheme

The microtiter plate consists of 6 double strips, which can be used individually for the test. The standards have to be run with each test. Never use the values of standards, which have been determined in a test performed previously.

St0-St6: Standards
(0, 7.81, 31.25, 125, 500, 2,000 pg/mL)

Sa1, Sa2, Sa3, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	St 0	St 0	Sa1	Sa1	etc.	etc.						
B	St 1	St 1	Sa2	Sa2								
C	St 2	St 2	Sa3	Sa3								
D	St 3	St 3	Sa4	Sa4								
E	St 4	St 4	Sa5	Sa5								
F	St 5	St 5	Sa6	Sa6								
G	NC	NC	Sa7	Sa7								
H	PC	PC	Sa8	Sa8								