Human Testosterone ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Testosterone in serum and plasma

Catalog number: ARG80854

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Testosterone (17β-hydroxy-4-androstene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in C-3 and a hydroxyl group in the β position at C-17.

This steroid hormone has a molecular weight of 288.47. Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in females ca. 50% of circulating testosterone is derived from peripheral conversion of androstenedione, ca. 25% from the ovary and ca. 25% from the adrenal glands.

Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states. In women, high levels of testosterone are generally found in hirsutism and virilization, polycystic ovaries, ovarian tumors, adrenal tumors and adrenal hyperplasia. In men, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer. Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminization, Orchidectomy and Cryptorrchidism, enzymatic defects and some autoimmune diseases.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly specific Testosterone antibody has been pre-coated onto a microtiter plate. Testosterone containing samples or standards and a Testosterone-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and

free Testosterone compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Testosterone is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	**Quantity**	**Storage information**
Antibody-coated microplate	12 strips x 8-well	4°C
Standards 0-6 (0, 0.2, 0.5, 1, 2, 6, 16 ng/ml)	7 X 1 ml (ready to use)	4°C
HRP-Testosterone Conjugate	25 ml (ready to use)	4°C
40X Wash Buffer	30 ml	4°C
TMB substrate	25 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620-650 nm as reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- Briefly spin down the HRP-Testosterone conjugate and standards before use.
- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Once
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and

devices are prepared ready at the appropriate time.

- The standards should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. Thawed samples should be inverted several times prior to testing.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C for up to 1 year. The frozen sample can be thawed for only once. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C for up to 1 year. The frozen sample can be thawed for only once. Avoid repeated freeze-thaw cycles.

Note:

1. Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
2. Do not use haemolytic, icteric or lipaemic specimens.
3. Samples containing sodium azide should not be used in the assay.
4. Thawed samples should be inverted several times prior to testing

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **40X** Wash buffer into distilled water to yield 1X Wash buffer. Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.
- **Sample:** If in an initial assay, a specimen is found to contain more than the highest standard, the specimens 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 of diluted a) sample + 90 µl Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** of **Standards and samples** in duplicate into the appropriate wells.
3. Add **200 µl** of **HRP-Testosterone** into each well. Mix thoroughly for 10 sec and then **incubate for 60 minutes at RT**.
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1X Wash Buffer (300-400 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **200 µl** of **TMB subsrtate mixture** to each well. Incubate for **15 minutes at room temperature** in dark.
6. Add **100 µl** of **Stop Solution** to each well.

1. **Read** the OD with a microplate reader **at 450 nm** immediately. (optional: read at 620-650nm as reference wave length) It is recommended that the wells should be read within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear or semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than 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.

7. TEST VALIDITY: The following specifications must be met for each run to be valid:

O.D. value for the S0 value calibrator: ≥ 1.200

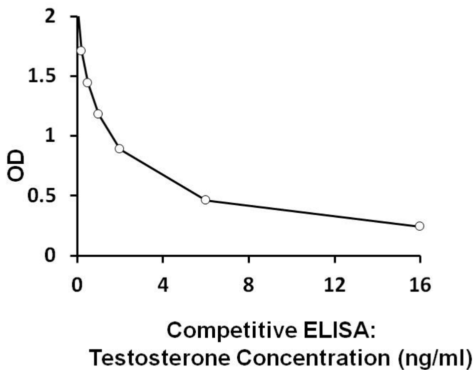
If the specifications is not met, the test run should be repeated.

6. Refer to the table below for molar conversion:

	Concentration of standards						
Standard	0	1	2	3	4	5	6
Testosterone (ng/ml)	0	0.2	0.5	1.0	2.0	6.0	16.0
Testosterone (nmol/L)	0	0.6934	1.7335	3.467	6.934	20.802	55.472
Conversion	Testosterone (ng/ml) x 3.467 = Testosterone (nmol/L)						

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.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 0.083 ng/ml.

Specificity

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

Steroid	Cross Reaction (%)
Testosterone	100
DHT	12.9
5α-Dihydrotestosterone	0.8
Androstenedione	0.9
11β-Hydroxyestosterone	3.3
17α-Methyltestosterone	0.1
19-Nortestosterone	3.3
DHEA	0.3
DHEA-S	< 0.1
Epitestosterone	< 0.1
Pregnenolone	< 0.1
Cortison	< 0.1
Estrone	< 0.1
Estradiol	< 0.1
Estriol	< 0.1
Danazol	< 0.1

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 3.6% and the CV value of inter-assay precision was 7.1%.

Interferences

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Recovery

86.9-110.7%

Linearity

86.1-110%