



Free TESTOSTERONE ELISA



BL-39-E

IN VITRO DIAGNOSTIC USE

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1 INTRODUCTION

Free Testosterone (antigen) in the sample competes with horseradish peroxidase testosterone (enzyme-labeled antigen) for binding the limited number of anti-testosterone (antibody) sites on the microplates (solid phase).

After incubation the bound/free separation is performed by a simple solid-phase washing procedure.

The enzyme conjugate (H_2O_2) and the TMB Substrate are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined. Free Testosterone concentration in the sample is calculated based on a series of standards.

The color intensity is inversely proportional to the Free Testosterone concentration in the sample.

Approximately 60% of blood testosterone is normally bound with high affinity to sex hormone-binding globulin SHBG; of the remainder 1-2% is bound to albumine. Thus, the determination of Free Testosterone permits the estimation of the biological active hormone.

2 PRINCIPLE OF THE TEST

The **Bio-Line free Testosterone ELISA KIT** procedure is an enzyme immunoassay, which is based on the principle of competitive binding. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Free Testosterone molecule. An aliquot of patient sample containing endogenous Free Testosterone is incubated in the coated well with enzyme conjugate, which is an anti-Free Testosterone antiserum conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off with aqua dest. The amount of bound peroxidase is proportional to the concentration of Free Testosterone in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Free Testosterone in the patient sample.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.3M H_2SO_4 . It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request.
The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1.

TLT

 12x8 strips, 96 wells
Wells coated with polyclonal anti-Testosterone IgG
2.

CAL	N
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 N=1 to 5
4 vials, 1 ml
See exact values on the vial labels
3.

CAL	0
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 1 vial
1 ml
4.

Ag	HRP
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 1 vial, 6 ml
Testosterone HRP-Conjugate
5.

CHROM	TMB
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 1 vial, 12 ml, ready to use
 H_2O_2 /TMB 0.25g/l
6.

STOP	SOLN
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 1 vial, 12 ml, ready to use
 H_2SO_4 0.3 mol/l (Attention: corrosive: avoid contact with skin)
Avoid contact with the stop solution. It may cause skin irritations and burns.

4.2 Equipment and material required but not provided

1. A microtiterplate calibrated reader (450±10 nm).
2. Calibrated variable precision micropipettes (Varipette Eppendorf), Multipette Eppendorf or similar products.
3. Absorbent paper.
4. Aqua dest.

4.3 Storage and stability of the Kit

- When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Enzyme-Conjugate, Substrate Solution, Calibrators and Zero Calibrator must be stored at 2° to 8°C.
- Microtiter wells must be stored at 2° to 8°C. Once the foilbag has been open care should be taken to close it tightly again.

4.4 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Calibrators: Before use, mix for 5 minutes with rotating mixer. After opening the calibrators are stable for six months at 4°C. For exact concentration see the labels of the calibrator vials.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, Bio-Line Europe have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN

5.1 Specimen collection

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not use haemolytic, icteric or lipaemic serum. Testosterone can be determined in plasma as well as in serum of patients who have been fasting. The clinical significance of the determination of Free Testosterone can be invalidated if the patient was treated with cortisone or natural or synthetic steroids

5.2 Specimen storage

Specimens which are not used at the same day of collection have to be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen dilution

Samples with expected values greater than the highest calibrator should be diluted with Sample Buffer before assaying.)

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipet tips for each calibrator, control of sample in order to avoid crosscontamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

6.2 Procedural Notes

- All calibrators, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

6.3 Assay Procedure

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **50 µl** Free Testosterone Calibrators, controls and samples **with new disposable tips** into appropriate wells.
3. Dispense **50 µl** Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **1 hour** at 37°C.
6. Briskly shake out the contents of the wells.
Rinse the wells 5 times with Aqua distilled water (300 µl per well). Strike the wells sharply on absorbent paper to remove residual water droplets.
Important note:
The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **100 µl** of Substrate Solution to each well.
8. Incubate for **15 minutes** at room temperature in the dark.
9. Stop the enzymatic reaction by adding **100 µl** of Stop Solution to each well.
10. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each calibrator against its concentration in IU/ml 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 of Free Testosterone from the calibrator curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this calibrator curve. Samples with Free Testosterone concentration higher than the concentration of the highest calibrator have to be diluted with zero calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

7 ASSAY CHARACTERISTICS

7.1 Expected values

The serum or plasma Free Testosterone values are comprised in the following intervals.

	N	MEDIAN	MEAN \pm 1 SD	Abs. Range
Healthy Male, age 20-50:	35	16	15 \pm 7	5.5 – 42
Female (ovulating):	29	1.3	1.4 \pm 0.9	ND – 4.1
(Taking oral contraceptives)	12	0.9	1.1 \pm 0.6	0.3 – 2.0
Postmenopause	22	0.8	0.9 \pm 0.5	0.1 – 1.7

7.2 Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Testosterone	100.0	%
DHT	0.006	%
Androstenedione	0.005	%
Androsterone	0.0	%
DHEA-S	0.0	%
Cortisol	0.0	%
Cortisone	0.0	%
17 α Estradiol	0.0	%
Estrone	0.0	%
Prednisone	0.0	%

7.3 Sensitivity

The sensitivity of this method is 0.10 pg/ml, calculated as two times the S.D. from B₀ is 0.10 pg/ml when the value of (B/B₀)% is approx. 90%.

7.4 Accuracy

Quality Control

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 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 the established 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.

7.5 Precision

The Inter- and Intra-run precision had a coefficient of variation of 3.9% and 6.2% respectively.

7.6 Method Comparison

Correlation with RIA and conventional equilibrium dialysis method were performed on the same samples (16 males and 19 females).

Linear regression analysis of the data yielded the following statistics:

Free Testosterone ELISA = 0.86 (Free Testosterone RIA) + 0.18 pg/ml.

r = 0.97

n = 35

Free Testosterone (% of Total Testosterone)

	N	MEAN	MEAN \pm 1SD	Abs. Range
Healthy Male (20-50):	35	0.45	0.5 \pm 0.12	0.15 – 0.8
Female (ovulating):	29	0.5	0.55 \pm 0.1	0.05 – 3.2
Female (Taking oral contraceptives):	12	0.5	0.6 \pm 0.1	0.01 – 1.1
Postmenopause:	22	0.4	0.45 \pm 0.1	0.05 – 1.2

8 LIMITATIONS OF USE

Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters 'Specimen - Collection'.

9 LEGAL ASPECTS

9.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to 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 within the given assay specifications.

9.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

9.3 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 subject to point 9.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

10 REFERENCES

1. McCann D, Kirkish L. Evaluation of Free Testosterone in serum. J.Clin. Immunoassay 1985; 8:234-236.
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4. Odland V. et al. Plasma androgenic activity in women with acne vulgaris and in healthy girls before, during and after puberty. Clin.Endocrinology 1982; 16:243-249.
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