DRG

DRG® Free Testosterone (EIA-2924)

CE

USA: RUO

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1 INTRODUCTION

1.1 Intended Use

The **DRG Free Testosterone ELISA** is an enzyme immunoassay for measurement of Free Testosterone in serum and plasma. This kit is intended for Research Use Only. Not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The DRG Free Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with an antibody directed towards an antigenic site on the Testosterone molecule. Endogenous Free Testosterone of a sample competes with a Testosterone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is inversely proportional to the concentration of Free Testosterone in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Free Testosterone in the sample.

Testosterone in the blood is bound to SHBG (60 %) and in lower quantity to other protein. Only the measurement of Free Testosterone (< 1% of Total Testosterone) permits the estimating of the hormone biologically active.

3 PRECAUTIONS

- This kit is intended for Research Use Only. Not for use in diagnostic procedures. .
- 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 0.15 mol/L 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 and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of 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 directly from DRG.
  The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-Testosterone IgG antibody.
2. ***Standard (Standard 0-5)*****,** 6 vials, 1 mL, ready to use;
   Concentrations: 0 - 0.2 - 1.0 - 4.0 - 20.0 - 100.0 pg/mL
3. ***Enzyme Conjugate***, 1 vial, 15 mL, ready to use;
   Testosterone conjugated to horseradish peroxidase;
4. ***TMB Substrate Solution***, 1 vial, 15 mL, ready to use;
   H_2O_2-TMB, 0.25 g/L. Avoid any skin contact.
5. ***Stop Solution***, 1 vial, 15 mL, ready to use;
   contains 0.15M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 50 mL (10X concentrated),
   Phosphate buffer 0.2M, Proclin < 0.002%
   see „Preparation of Reagents“.

4.1.1 Equipment and material required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm), (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.
- Incubator 37°C

4.2 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

4.3 Preparation of Reagents

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

Standards

Before use, mix for 5 min. with rotating mixer

Note: *The opened standards are stable for 6 months at 2-8°C.*

Wash Solution

Add deionized water to the 10X concentrated Wash Solution.
Dilute 50 mL of concentrated *Wash Solution* with 450 mL deionized water to a final volume of 500 mL.

The diluted Wash Solution is stable for 30 days at 2-8°C.

In concentrated wash solution it is possible to observe the presence of crystals. In this case mix at room temperature until complete dissolution of crystals. For greater accuracy dilute the whole bottle of concentrated wash solution to 500 mL taking care that all crystals have been transferred, then mix until crystals are completely dissolved.

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.5 Damaged Test Kits

In case of any severe damage of the test kit or components, DRG 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

Testosterone can be determined in plasma as well as in serum of persons who have been fasting.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

The clinical significance of the determination of Free Testosterone can be invalidated if the sample specimen was treated with cortisone or natural or synthetic steroids

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Persons receiving anticoagulant therapy may require increased clotting time.

Plasma:

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

(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001;
for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

5.2 Specimen Storage

Store specimen at -20°C if the determination is not performed on the same day of the sample collection. Freeze only once. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

Samples reading higher than 100 pg/mL should not be diluted. Dilution will alter the equilibrium between free testosterone and serum proteins.

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.
- Pipetting of samples should not extend beyond ten minutes to avoid assay drift.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all 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.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **20 µL** of each ***Standard, control*** and **samples** with new disposable tips into appropriate wells. Leave well A1 empty for substrate blank.
3. Dispense **100 µL** ***Enzyme Conjugate*** into each well, except for the blank well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at 37°C.
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted wash solution (300 µL per well). 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 **100 µL** of ***Substrate Solution*** to each well.
7. Incubate for **15 minutes** at room temperature (22°C – 28°C) in the dark.
8. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
9. Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader (against the blank). It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. 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: 4 Parameter Logistics is the preferred method. 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 higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

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7 REFERENCES

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