DRG® Testosterone (Rat) ELISA (EIA-5179)

Revised of 30 Jan. 2011 rm (Vers. 2.1) **RUO**

THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

THIS KIT IS NOT INTENDED FOR DIAGNOSTIC PROCEDURES.

1 INTRODUCTION

1.1 Intended Use

The **Testosterone Rat/Mouse ELISA** is a competitive immunoassay for measurement of testosterone in rat and mouse serum or plasma.

For research use only.

2 PRINCIPLE

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

An unknown amount of testosterone present in the sample and a defined amount of testosterone conjugated to horseradish peroxidase compete for the binding sites of testosterone antiserum coated to the wells of a microplate. After one-hour incubation on a shaker the microplate is washed four times. After addition of the substrate solution the concentration of testosterone is inversely proportional to the optical density measured.

3 WARNINGS AND PRECAUTIONS

1. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. Do not mix reagents of different lots. Do not use expired reagents.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.
5. Avoid contact with Stop Solution. It may cause skin irritation and burns.
6. Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.
7. Change pipette tips between samples, controls and reagents to avoid carry over contamination.
8. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
9. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
10. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
11. Assay reagents contain Proclin against microbial growth. In case of contact with eyes or skin, flush immediately with water.
12. All reagents should be at room temperature (21-26°C) before use. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.

13. TMB substrate has an irritant effect on skin and mucosa. In case of contact with skin or eyes, wash thoroughly with water. Please note that extreme temperature changes may cause spontaneous decay of the peroxide.

4 REAGENTS

4.1 Reagents provided

Microtiterplate, 12 x 8 (break apart) strips with 96 wells;
Wells coated with anti-testosterone antibody.

Calibrator 0, 1 vial, 0.3 ml, ready to use

Calibrator (Calibrator 1-5), 5 vials, 0.3 ml each, ready to use;
Concentrations: 0.1 – 0.4 – 1.5 – 6.0 – 25.0 ng/ml

Incubation Buffer, 1 vial 11 ml, ready to use;

Enzyme Conjugate, 1 vial, 7 ml, ready to use;
Testosterone conjugated to horseradish peroxidise.

Substrate Solution, 2 vials, 11 ml each, ready to use;
contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

Stop Solution, 1 vial, 7 ml, ready to use;
contains 2 N Hydrochloric Acid solution.

Wash Solution, 1 vial, 50 ml (10X concentrated);
see „Preparation of Reagents“.

Note: Additional Calibrator 0 for sample dilution is available upon request.

4.2 Materials required but not provided

Centrifuge
A microtiter plate reader capable for endpoint measurement at 450nm
Microplate mixer operating more than 600 rpm
Vortex mixer
Calibrated variable precision micropipettes (10 µl, 50 µl, 100 µl, 200 µl).
Absorbent paper
Distilled or deionized water
Timer
Semi logarithmic graph paper or software for data reduction

4.3 Reagent preparation

All reagents should be at room temperature before use.

Wash Solution:

Dilute 50 ml of 10X concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml.
The diluted Wash Solution is stable for at least 3 months at room temperature.

4.4 Storage conditions

When stored at 2°C to 8°C unopened reagents will be stable 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. Take care that the foil bag is sealed tightly.

5 SPECIMEN

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.
Avoid repeated freeze-thaw cycles.

Plasma

Collect plasma using citrate, EDTA, or heparin 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.
Avoid repeated freeze-thaw cycles.

Please note: The use of plasma as specimen can result in a diminished precision of this assay.

6 ASSAY 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 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.

6.2 Assay Procedure

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Dispense **10 µl** of each **Calibrator and Sample** with new disposable tips into appropriate wells.
3. Dispense **100 µl** of **Incubation Buffer** into each well.
4. Add **50 µl Enzyme Conjugate** into each well.
5. Incubate for **60 minutes** at room temperature on a Microplate mixer.
   Important Note:
   Optimal reaction in this assay is markedly dependent on shaking of the microplate!
6. Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
7. Add **200 µl** of **Substrate Solution** to each well.
8. Incubate without shaking for **30 minutes** in the dark.
9. Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
10. Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

6.3 Calculation of results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Using 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 calibration curve.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log are recommended.

5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

Conversion to SI units:
Testosterone (pg/mL) x 3.47 = pmol/L

7 REFERENCES / LITERATURE

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