Version 26-04/24

User's Manual

Testosterone Rat/Mouse ELISA Kit

REF **DEIA2183**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Mouse/Rat Testosterone ELISA is for the quantitative determination of Testosterone concentration in mouse/rat serum or plasma.

Principles of Testing

The Mouse/Rat Testosterone ELISA is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25ml of Testosterone standards, controls, patient samples, 100 ml Testosterone-HRP conjugate reagent and 50ml rabbit anti-Testosterone reagent at room temperature 60 minutes. During the incubation, a fixed amount of HRP-labeled Testosterone competes with the endogenous Testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific Testosterone antibody. Thus, the amount of Testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Testosterone in the specimen increases. Unbound Testosterone peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is then added and incubated at room temperature for 15 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm.

Reagents And Materials Provided

1. Microwell coated with Goat Anti-Rabbit IgG, 12×8×1
2. Standard: 6 vials (ready to use), 0.5ml
3. Rabbit Anti-Testosterone Reagent (ready to use), 7ml
4. Assay Diluent: 1 bottle (Ready to Use), 12ml
5. Enzyme Conjugate Conc. (20×): 1 Vial, 0.7ml
6. TMB Substrate: 1 bottle (ready to use), 12ml
7. Stop Solution: 1 bottle (Ready to use), 12ml
8. Wash Buffer (20×): 1 bottle, 25ml

Materials Required But Not Supplied

1. Distilled or deionized water. Precision pipettes. Disposable pipette tips
2. Micortiter well reader capable of reading absorbance at 450nm
3. Absorbance paper
4. Paper towel
5. Graph paper

Storage

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents to heat, sun, or strong light.
5. Product is stable for 24 months from the date of manufacturing.

Specimen Collection And Preparation

1. Collect blood specimens and separate the serum immediately.
2. Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed.
5. Do not use grossly lipemic specimens.

Reagent Preparation

1. **20× Enzyme conjugate:** Prepare 1× working solution at 1:20 with assay diluent (e.g. Add 0.1ml of the Testosterone enzyme conjugate concentrate to 1.9ml of assay diluent)
2. **20× Wash Buffer:** Prepare 1× Wash buffer by adding the contents of the bottle (25 ml, 20×) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µl of standards, specimens and controls into appropriate wells.
3. Dispense 100 µl of working dilution of Testosterone-HRP Conjugate Reagent into each well.
4. Dispense 50 µl of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature 60 minutes.
6. Rinse and flick the microwells 3 times with 1× wash buffer water.
7. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
8. Incubate at room temperature (20-25°C) for 15 minutes.
9. Stop the reaction by adding 50 µl of Stop Solution to each well.
10. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

Calculation

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Testosterone in ng/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Typical Standard Curve

EXAMPLE OF THE STANDARD CURVE

Testosterone (ng/ml)	Absorbance (450nm)
0	2.38
0.1	1.75
0.5	1.02
2.0	0.59
6.0	0.34
18.0	0.17

Detection Range

0.1-18 ng/ml

Sensitivity

0.1 ng/ml

Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:

   The kit contains animal and/or human source components. All the human components have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984. All the animal products, if any, have been derived from animals of US origin and processed in USDA licensed facilities.
4. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that serum samples be run in duplicate.
6. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

Limitations

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

References

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