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CALBIOTECH

Mouse/Rat Testosterone ELISA

Cat. # TE187S-100 (96 Tests)

INTENDED USE

The Calbiotech Mouse/Rat Testosterone ELISA is for the quantitative determination of Testosterone concentration in mouse/rat serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Testosterone (17b-hydroxyandrost-4-ene-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 b position at C-17. This product detects testosterone in rodent serum, plasma, or tissue extracts. Useful for preclinical research on steroidogenesis, androgen receptor signaling, reproductive physiology, and endocrine disruption. Supports studies on diurnal variation, age-related changes, and hormonal feedback regulation in experimental animal models.

MATERIALS PROVIDED	96 TESTS
1. Microwell coated with Goat Anti-Rabbit IgG	12x8x1
2. Standard: 6 vials (ready to use)	0.5 mL
3. Control: 2 vials (ready to use)	0.5 mL
4. Rabbit Anti-Testosterone Reagent (ready to use)	7 mL
5. Assay Diluent: 1 bottle (Ready to Use)	12 mL
6. Enzyme Conjugate Conc. (20X): 1 Vial	0.7 mL
7. TMB Substrate: 1 bottle (ready to use)	12 mL
8. Stop Solution: 1 bottle (Ready to use)	12 mL
9. Wash Buffer (20X): 1 bottle	25 mL

MATERIALS NOT PROVIDED

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 AND STABILITY

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.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

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

SPECIMEN COLLECTION HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION

1. **20X Enzyme conjugate**: Prepare 1X 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. **20X Wash Buffer** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25 °C).

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 25µl of standards, specimens and controls into appropriate wells.
3. Dispense 100µl of working dilution of Testosterone-HRP Conj. Reagent into each well.
4. Dispense 50µl 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 1x 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 50µl 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 OF RESULTS

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.

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

LIMITATIONS OF THE PROCEDURE

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