ab285350 – Mouse/Rat Testosterone ELISA Kit

For quantitative protein detection, establishing normal range etc.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285350

Materials Supplied

Item	Quantity	Storage Condition
Goat Anti-Rabbit IgG pre-coated Microplate	8 wells ×12 strips	4°C
Standard	6 vials	4°C
Rabbit Anti-Testosterone Reagent	7 ml	4°C
20X Enzyme Conjugate	0.7 ml	4°C
20X Wash Buffer	25 ml	4°C
TMB Substrate	12 ml	4°C
Stop Solution	12 ml	4°C
Assay Diluent	12 ml	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

Storage conditions and Reagent Preparation

- Store kit at 2-8C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes.
- Reagents are stable until the expiration of the kit.
- Do not expose reagent to heat, sun, or strong light.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

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 (18-26° C).

Enzyme Conjugate: Dilute enzyme conjugate 1:20 with assay diluent in a suitable container. For example, add 100 µl of conjugate to 1.9 ml of assay diluent. We recommend preparing diluted solution just before use.

Sample Preparation and Storage

- Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 5 days. Store frozen at (-20°C) for up to one month.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen sera should be completely thawed and mixed well.
- Don't use grossly lipemic specimens. Samples containing sodium azide should not be used in the assay.

Assay Protocol

1. Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 25 µl of Standards, control, or samples into designated wells.
3. Add 100 µl of diluted enzyme conjugate to all wells.
4. Dispense 50 µl of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 sec.
5. Cover the plate and incubate for 60 min. at room temperature.
6. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells, mix & incubate for 15 min. at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution. It important to make sure that all the blue color changes to yellow color completely.
9. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stop solution

Calculation

Construct the standard curve; plot the absorbance for the Testosterone standards (vertical axis) versus Testosterone standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Example of Standard Curve

Standard	OD (450 nm)
Standard 1 (0 ng/mL)	2.38
Standard 2 (0.1 ng/mL)	1.75
Standard 3 (0.5 ng/mL)	1.02
Standard 4 (2.0 ng/mL)	0.59
Standard 5 (6.0 ng/mL)	0.34
Standard 6 (18.0 ng/mL)	0.17

Expected Values

Each laboratory should establish its own normal range

Technical Support

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