Testosterone ELISA Kit

Cat#: orb1173301 (Manual)

Testosterone ELISA Kit	
Cat#: orb1173301	**Lot #:** Refer to product label
Detection range: 0.1 ng/mL-20 ng/mL	**Sensitivity:** 0.1 ng/mL
Precision: Intra-assay Precision: The CV (%)＜15%.Inter-assay Precision :The CV (%)＜15%	**Recovery:** The recovery ranged from 85%to 115%with an overall mean recovery of 100%.
Specificity: Testosterone ELISA Kit has high sensitivity and excellent specificity for detection of Testosterone. No significant cross-reactivity or interference between Testosterone and analogues was observed.	
Applicable samples: Serum, Plasma	
Storage: The unopened kit should be stored at 4℃for 12 months.	

Assay Principle

Testosterone is the primary male sex hormone and an anabolic steroid. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair. In addition, testosterone is involved in health and well-being, and the prevention of osteoporosis. Insufficient levels of testosterone in men may lead to abnormalities including frailty and bone loss.

Testosterone ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for testosterone and Horseradish Peroxidase (HRP) conjugated testosterone. The competitive inhibition reaction is launched between with HRP labeled testosterone and unlabeled testosterone with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of testosterone in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	**Size**		**Storage conditions**
48T		**96 T**	
Testosterone standard	0.25mL×6	0.5mL×6	4℃
HRP conjugated testosterone	3mL	6 mL	4℃
Testosterone detect antibody	3mL	6 mL	4℃

HRP substrate A	3.5mL	7mL	4℃, protected from light
HRP substrate B	3.5mL	7mL	4℃, protected from light
Stop solution	3.5mL	7mL	4℃
Wash buffer (20×)	7.5 mL	15 mL	4℃
Testosterone microplate	48 wells	96 wells	4℃
Plate covers	1	2	RT

Note: Std1: 0 ng/mL; Std2: 0.1 ng/mL; Std3: 0.4 ng/mL; Std4: 1.6 ng/mL; Std5: 5 ng/mL; Std6: 20 ng/mL.

Materials Required but Not Supplied

·Microplate Reader capable of measuring absorbance at 450 nm
·Multi channel pipetteor automated microplate washer
·Incubator,Refrigerated Centrifuge
·Precision Pipettes, Disposable Pipette Tips
·Deionized Water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.

1×Wash buffer: Wash buffer (20×) dilute with deionized water 1:20to obtain the1×Wash Buffer. Store at 4℃. If crystals have formed in the Wash buffer concentrates, warm them gently until they completely dissolved. Store at room temperature. Please note that 1×Wash buffer is stable for 30 days.

Sample Preparation

1. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at-20℃. Avoid repeated freeze-thaw cycles.
2. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at-20℃. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 hours, they may be stored at 2 to 8℃. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

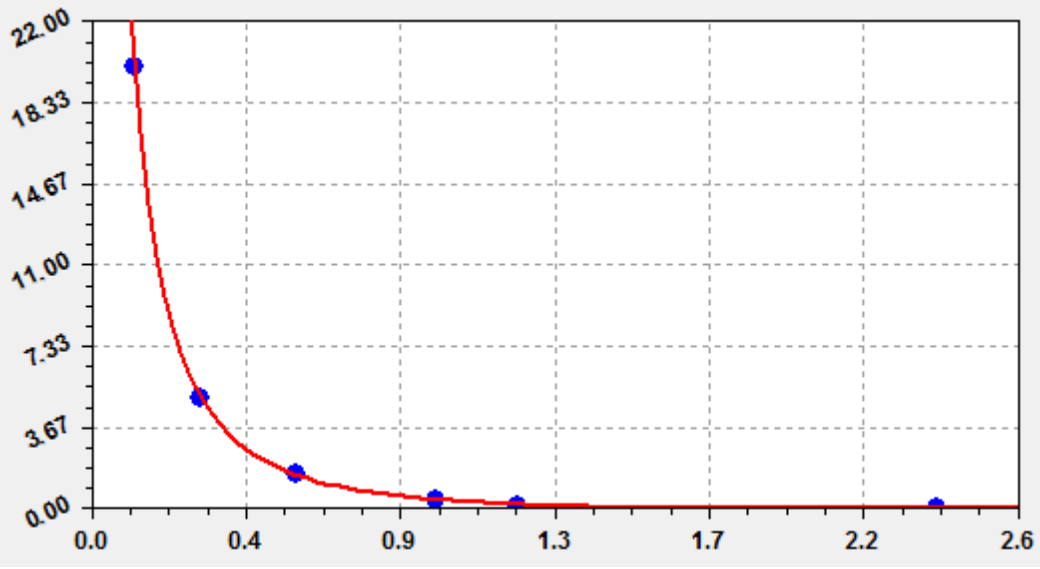
2. Add 50µL of Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.
3.Add 50µLof HRP-conjugate testosteroneto each well (not to Blank well), then add 50µLof detect antibody to each well in the same order. Mix well, cover with the plate cover provided and then incubate for 1 h at 37℃.
4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250µL) using a Multi-channel pipetteor automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.
5. Add 50µLof Substrate A and 50µLof Substrate B to each well, mix well and cover with the plate cover provided. Incubate for 15 min at 37℃. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50 µL of Stop solution to each well. Stop Solution should be added to the plate in the same order as HRP substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1. Average the duplicate readings for each standard and sample.
2. Drawing of standard curve: With the standard solution concentration as the y-axis and the mean absorbance for each standard as the x-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

Typical standard curve （R2≥0.99）



O.D. Value

Figure1. Standard Curve of Testosterone in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. Do not mix or substitute reagents with those from other lots or sources.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.
4. Stop Solution has certain Corrosive. Please take protective measures when operating.