（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !）

T(Testosterone) ELISA Kit

Catalog No: E-EL-0072

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label on the side of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to the in vitro quantitative determination of T concentrations in serum, plasma and other biological fluids.

Specification

- Sensitivity: The minimum detectable dose of T is 0.1ng/mL (The sensitivity of this assay, or lowest detectable limit (LDL) was defined as the lowest protein concentration that could be differentiated from zero).
- Detection Range: 0.1-20ng/mL.
- Specificity: No significant cross-reactivity or interference with progesterone, dihydrotestosterone, estradiol.
- Repeatability: Coefficient of intra-CV and inter-CV are ≤ 15%.

Test principle

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Goat Anti-Rabbit IgG, make solid-phase secondary antibody. And then add samples, horseradish peroxidase-labeled Testosterone and anti- Testosterone antibody, so as to form a coated secondary antibody - anti- Testosterone antibody - HRP-labeled Testosterone complex. The amount of bound labeled Testosterone is inversely proportional to that of Testosterone in the samples. The TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Testosterone in the samples is then determined by comparing the OD of the samples to the standard curve.

Kit Components：

Item	Specifications	Storage
Micro ELISA Plate(Dismountable)	8 wells ×12 strips	4℃
Reference Standard (6 tubes)	0.5mL /tube	4℃
Detection Ab	1vial 6mL	4℃
HRP-labeled Testosterone	1vial 6mL	4℃(shading light)
Concentrated Wash Buffer (20×)	1vial 15mL	4℃
Substrate Reagent A	1vial 7mL	4℃(shading light)
Substrate Reagent B	1vial 7mL	4℃(shading light)
Stop Solution	1vial 7mL	4℃
Plate Sealer	5pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

#: A set of Standard concentrations is 0 ng/mL, 0.1 ng/mL, 0.4 ng/mL, 1.6 ng/mL, 5 ng/mL, 20 ng/mL.

The volume of reagents in partial shipments is a little more than the volume marked on the label, please use in measuring instead of directly pouring.

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. Please wear lab coats and latex gloves for protection. Please perform the experiment following the national security columns of biological laboratories, especially detecting samples of blood or other body fluid.
2. The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the diluted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solution should be stored back according to the storage condition in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wavelength at 450±10 nm. The optical density should be within 0~3.5.
5. Do not mix or use components from other lots (except for washing buffer and stop solution).
6. Change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable, non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysis samples are not suitable for ELISA assay!

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells by trypsin. Collect the cell suspension into the centrifugal tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells for 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 μL of pre-cooled PBS to keep the cells resuspended. Repeat the freeze-thaw process for several times until the cells are lysed fully. Centrifuge for 10min at 1500×g at 4℃. Remove the cell fragments, collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from other literatures before detecting different tissue types. For general information, hemolysis blood may affect the result, so the tissues should be minced to small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000×g to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay.

Note for sample:

1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
4. If lysis buffer is used to prepare tissue homogenate or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
5. Some recombinant protein may cannot be detected due to the mismatching with coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Preheat the Microplate reader for 15 min before OD measurement.
2. **Wash Buffer** - Dilute the concentrated wash solution to the working concentration using double distilled water (1:20), mix up. Put unused solution back at 4℃. Note: if crystals have formed in the concentrate, warm it in 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard –**Centrifuge at 1000×g for 1 minute, mix it thoroughly with a pipette.

Washing Procedure:

1. **Automated Washer:** Add 350μL wash buffer into each well, the interval between injection and suction should be set about 60s.
2. **Manual wash:** Add 350μLWash Buffer into each well, soak it for 1~2minutes. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean and toweling absorbent paper on a hard surface.

Assay procedure (A brief assay procedure is on the 8th page**)**

1. **Add Sample:** Take out pre-coated plates. Set a Blank well, do not add any liquid; Each Standard point set two wells, add 50μl of corresponding Standard per well; 50μl of Sample is added to the rest of each Sample well.
2. Immediately add 50 μl of HRP-labeled Testosterone to each well. Then add 50 μl of Detection Ab to each well. The adding order of Detection Ab should be as the same as that of the HRP-labeled Testosterone. Thorough mixing, cover with the Plate sealer provided in the kit. Incubate for 1 hour at 37℃. Note: solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible.
3. **Wash:** Aspirate or decant the solution from each well，add 350μL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times in total. Note: a microplate washer can be used in this step and other wash steps**.**
4. **Substrate:** Add 50μL of Substrate A and Substrate B to each well, mix fully. Incubate for about 15 min at 37℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
5. **Stop:** Add 50μL of **Stop Solution** to each well. Note: the order to add stop solution should be the same as the substrate solution.
6. **OD Measurement:** Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm.

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the OD of blank well. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the lower limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix.
	Wells not completely aspirated	Completely aspirate wells between steps.
Low signal	Too brief incubation times	Ensure sufficient incubation time;
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid colouring.
Deep color but low value	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of detector too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	Stop solution not added	Stop solution should be added to each well before measurement

SUMMARY

1. Add 50μL of standard or sample to each well. Immediately add 50μL of HRP-labeled Testosterone to each well. Then add 50μL of Detection Ab to each well. Incubate for 1 hour at 37℃

2. Aspirate and wash 3 times

3. Add 50μL of Substrate A and Substrate B to each well. Incubate for about 15 min at 37℃ with shading light.

4. Add 50μL Stop Solution. Read at 450nm immediately.

5. Calculation of results.

Declaration

1. Limited by current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operation skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions in the description!
4. Incorrect results may occur because of wrong operations during the reagents preparation and loading, as well as incorrect parameter setting of Micro-plate reader. Please read the instruction carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some unexpected reasons such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from above reasons, too.