Tribioscience

Human Testosterone Fast ELISA (Catalog Number: TBS3257)

For determination of testosterone concentrations in serum, plasma, urea, and cell culture supernatant.

Introduction

Testosterone is an androgenic steroid hormone, the primary male sex hormone, and an anabolic steroid. It is secreted mainly by the testes in males and the ovaries in females, with smaller amounts also produced by the adrenal glands. Testosterone plays several vital roles, including driving the development of male reproductive organs, supporting sperm production, influencing muscle mass and strength, and maintaining bone density.

Synonyms: 17b-Hydroxyandrost-4-ene-3-one; 17b-Testosterone

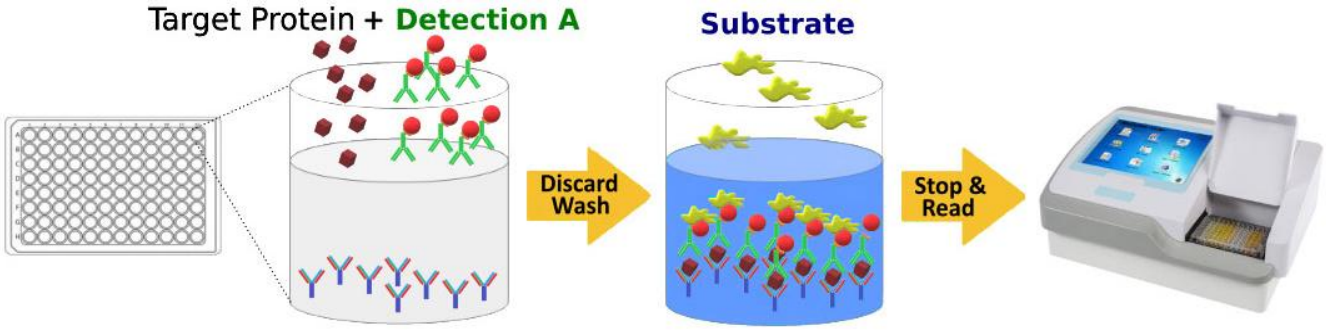
Intended Use

Tribioscience's Testosterone ELISA Kit utilizes competitive ELISA technologies for the quantitative analysis of testosterone in serum, plasma, cell culture supernatant and other biological samples. The limit of detection (LOD) of testosterone in ELISA Kit is 0.6 ng/ml.

Assay Principle

Tribioscience's Testosterone ELISA Kit is a competitive enzyme-labeled immunoassay (Fig. 1). The 96- well microtiter plate is pre-coated with an anti-testosterone antibody. During the assay, testosterone standard solution or samples are added to each well, followed by adding horse radish peroxidase (HRP) -testosterone conjugate, which will compete with testosterone in standard or sample for binding to antibody during the incubation. After plate wash, an ultra-sensitive HRP substrate is added to the wells leading to a colored product only in the presence of HRP, and optical density is inversely related to testosterone concentrations in the samples. The accurate concentration of testosterone can then be determined by interpolation using the standard curve constructed in the same run.

Fig. 1. Simple Procedures



Kit content and storage conditions

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Pre-coated Microplate	TBS3257A	96 well microplate (12 strips of 8 wells) coated with an antibody specific for testosterone.	Return unused wells to the foil pouch. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Testosterone Standard	TBS3257B	80 µl of testosterone (400 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3257C	50 µl of HRP-testosterone conjugate (50x).	May be stored for up to 6 months at 2-8 °C.
Assay Diluent	TBS3000D	25 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 1 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

Sample preparation for ELISA assay

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8°C before centrifugation for 20 min at 1000×g at 2-8°C. Collect the supernatant to carry out the assay.
Plasma: Collect plasma using EDTA or heparin (EDTA-Na2 is most recommended) as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8°C within 30 min of collection. Collect the supernatant to carry out the assay.

ELISA Procedures

Bring all reagents to room temperature before use.

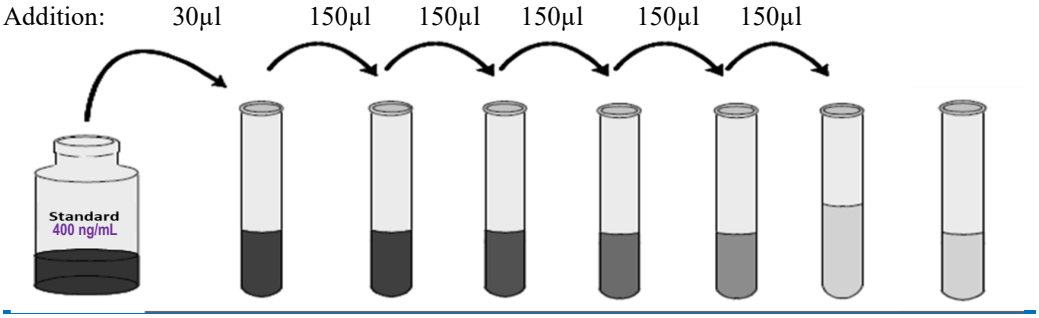
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer.

Detection A working solution: Dilute 40 µL Detection A Stock (50x) into 2 mL of Diluent Buffer to make working solution.

Testosterone Standard Preparation: Label test tubes as #1 through #7. Pipet 270 µL of 1x Assay Diluent into tube #1, and 150 µL into tubes #2 to #7 **as diagram below.**

1. Add 30 µL of the testosterone Standard stock solution (400 ng/mL) to tube #1 (40 ng/mL) and mix.
2. Make 2x serial dilutions of the standard using the Tube#1(40 ng/mL standard solution) from Tube #2 through #6 with sequential transfer of 150 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 6 will be 40, 20, 10, 5, 2.5, and 1.25 ng/mL. Tube# 7 is Standard 0.

Fig.2 Diagram for standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7
Assay Buffer(µL)	270	150	150	150	150	150	150
Addition	Stock	Std1	Std2	Std3	Std4	Std5	
Addition Vol. (µL)	30	150	150	150	150	150	0
Final Conc (ng/ml)	40	20	10	5	2.5	1.25	0

Assay Procedures:

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 30min**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 100 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Quantitative Calculation of Testosterone Concentration

a) Calculate B/B0

Dividing average absorbance of each standard and sample (B) by absorbance of the standard 0 ng/mL testosterone concentration, B0) to obtain percentage absorbance as below:

Percentage absorbance (%) = 100% x (B/B0)

B: Average absorbance of a standard or sample
B0: Average absorbance of 0 ng/mL standard

b) A standard curve Calculation: A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis), and sample concentration can be read from this standard curve. Alternatively, testosterone concentration in the samples can be calculated with regression equation correlating percentage absorbance to testosterone concentration.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

Sensitivity

The analytical sensitivity of the assay is 0.24 ng/mL Human Testosterone.

Precision:

Intra-assay CV: <6.63%
Inter-assay CV: <6.08%

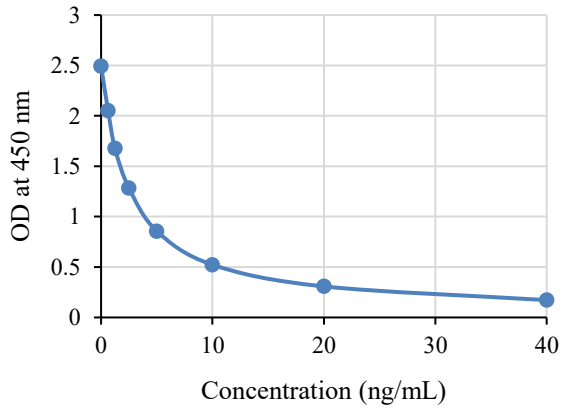
Cross reactivity:

No obvious cross reaction with other analogues.

Precautions

1. Assay kit should be stored at 2-8℃ and avoid freezing conditions; unused test strips should be sealed in reclosable bag. The substrate is sensitive to light so prolonged exposure to light needs to be avoided.
2. Reagents should be brought to room temperature (20-25℃) prior to use. A room temperature of lower than 20℃ or failure to equilibrate reagents or samples to room temperature could result in low OD readings for all samples. All reagents should be put back into 2-8℃storage immediately after use.
3. Reagents need to be thoroughly mixed to improve reproducibility.
4. During all incubation steps, avoid light and seal plate with sealer.

Fig3. Testosterone Standard Curve



Storage and Expiration Date

Storage: All components of the kit should be stored at 2-8℃. Expiration Date: This kit expires 6 months after receipt.

Technical Assistance

For ordering or technical assistance regarding this kit, or for additional information about TribioScience products, please email: support@tribioscience.com or call (408) 498-0197, or 833-697-8998 (Toll Free).

For research use only.