Testosterone

ELISA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55105-96

www.zellx.de

Sample Types Validated for:

Urine, Dried Fecal Extracts, Extracted Serum/Plasma, and Tissue Culture Media

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

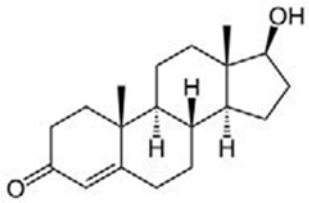
Introduction

Background

Testosterone ($C_{19}H_{28}O_2$) belongs to a class of steroid hormones called androgen and is found in mammals, reptiles, birds, and other vertebrates. In mammals, Testosterone is primarily secreted in the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid.

In men, Testosterone plays a critical 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, bone mass, and the growth of body hair. In the absence of Testosterone stimulation, spermatogenesis does not proceed beyond the meiosis stage.

In addition, Testosterone is essential for health and well-being as well as the prevention of osteoporosis. On average, an adult human male body produces about ten times more Testosterone than an adult human female body, however, females are more sensitive to the hormone. Testosterone plays a significant role in glucose homeostasis and lipid metabolism. A number of studies have shown an inverse relationship between the plasma Testosterone and severity of the metabolic syndrome, which is a clustering of risk factors predisposing to type 2 diabetes (T2D), atherosclerosis and cardiovascular diseases.



Assay principle

The ZellX® Testosterone Immunoassay kit is designed to quantitatively measure Testosterone present in urine, extracted dried fecal samples, extracted serum and plasma, and tissue culture media. This kit is not recommended for serum, plasma, or saliva samples without extraction as mentioned below. A Testosterone stock solution is provided to generate a standard curve for the assay, and all samples should be read off the standard curve.

The kit includes a 96-well plate that is pre-coated with a secondary anti-rabbit antibody. The function of this antibody is to capture the mouse anti-Testosterone antibody bound to Testosterone conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The Testosterone-conjugate (labeled) and the sample Testosterone (unlabeled) compete for binding to the rabbit antibody. After 2 hours of incubation, the substrate is added to react with the peroxidase-labeled antibody-antigen conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The lower the amount of Testosterone in the sample, the stronger the signal due to more labeled Testosterone bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
Testosterone Standard (200 ng/mL)	70 µL
Testosterone Antibody	2.6 mL
Testosterone Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

Phosphate Buffer Saline (PBS)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Microplate shaker, Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

For serum and plasma Sample:

Diethyl ether or ethyl acetate

A Speedvac/centrifugal concentrator or N_2 gas and gas manifold for evaporation

For Dried Fecal Sample:

ACS Grade Ethanol

Glass test tubes

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

The testosterone standard used for this kit is an anabolic steroid and may have a number of known and unknown biological actions. Handling this material needs appropriate precaution.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The antibody-coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers, containing sodium azide will inhibit color production by the enzyme. Make sure all buffers used for samples are azide-free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Concentrate with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Assay Buffer can be stored at room temperature for up to 3 months.

Sample preparation

This assay has been validated for dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to use. Testosterone can be assayed in other sample types; for inquiries about sample preparation methods, please contact us at technical@zellx.de.

Since Testosterone is identical across all species, it is expected that this kit can measure Testosterone in human and other species.

Samples containing visible particulate should be centrifuged prior to conducting the assay.

All samples and standards must be used within 2 hours of preparation.

I. **Serum and Plasma Sample:**

- Add diethyl ether to serum or plasma samples at a 5:1 (v/v) (5 volumes diethyl ether to 1 volume sample)
- Vortex for 2 minutes and leave it stable for 5 minutes (It allows the ether layer to separate).
- Freeze samples in a dry ice/ethanol bath and pipette off the ether solution from the top of the sample into a clean tube.
- Repeat all the previous steps for maximum extraction efficiency, and combine top layer of ether solutions.
- Dry pooled ether samples down in a speedvac for 2-3 hrs. The sample should be stored at -20°C for later use.
- Re-dissolve samples at room temperature in the Assay Buffer. A minimum of 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

II. **Dried Fecal Sample:**

- Ensure that the sample is completely dry, and powder the sample to improve extraction recovery. Remove any large particles if possible.
- Weigh out ≥ 0.2 gram of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60 °C), or freeze-drying (lyophilization).
- Add 1 mL of ethanol per 0.1 gram of solid fecal sample (100 mg/mL) and seal.
- Shake strongly for at least 30 minutes.
- Centrifuge at 5000 rpm at 4°C for 15 minutes and collect supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.
  - **Note:** Samples containing low levels of analyte can be concentrated by drying down the extract and resuspension in a reduced volume of Assay Buffer.
- Supernatant should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer.
- Vortex well and allow to rest 5 minutes at room temperature
- Repeat the vortex step 2 more times to ensure complete steroid solubility.
- The final concentration of ethanol in the sample to be added to the wells should be ≤ 5%. **(≥ 1:4 dilution with Assay Buffer is needed.)**

III. **Urine:**

- Urine should be diluted ≥ 1:4 by taking one part of sample and adding 3 or more parts of Assay Buffer prior to conducting assay.
- **Normalize the sample value based on creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in random urine specimen.**

IV. **Tissue Culture Media:**

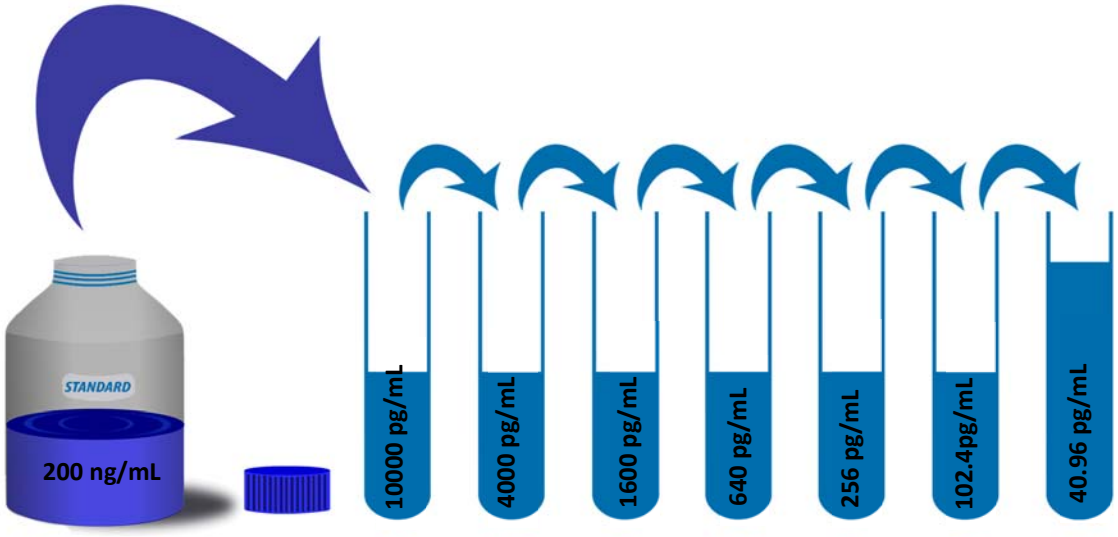
- For measuring Testosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

All the samples must be used within 2 hours of preparation.

Standard preparation

- Prepare a 1:20 dilution of Testosterone Standard with Assay Buffer (mix 25 µL of standard with 475 µL of Assay Buffer), and label as the Standard No.7 (10000 pg/mL).
- The Testosterone Standard contains an organic solvent. Prerinse the pipette tip several times to ensure accurate delivery
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.7	10000 pg/mL	25 µL Testosterone Standard + 475 µL Assay Buffer
Standard No.6	4000 pg/mL	200 µL Standard No.7 + 300 µL Assay Buffer
Standard No.5	1600 pg/mL	200 µL Standard No.6 + 300 µL Assay Buffer
Standard No.4	640 pg/mL	200 µL Standard No.5 + 300 µL Assay Buffer
Standard No.3	256 pg/mL	200 µL Standard No.4 + 300 µL Assay Buffer
Standard No.2	102.4 pg/mL	200 µL Standard No.3 + 300 µL Assay Buffer
Standard No.1	40.96 pg/mL	200 µL Standard No.2 + 300 µL Assay Buffer
Standard No.0	0 pg/mL	300 µL Assay Buffer



All standard must be used within 2 hours of preparation

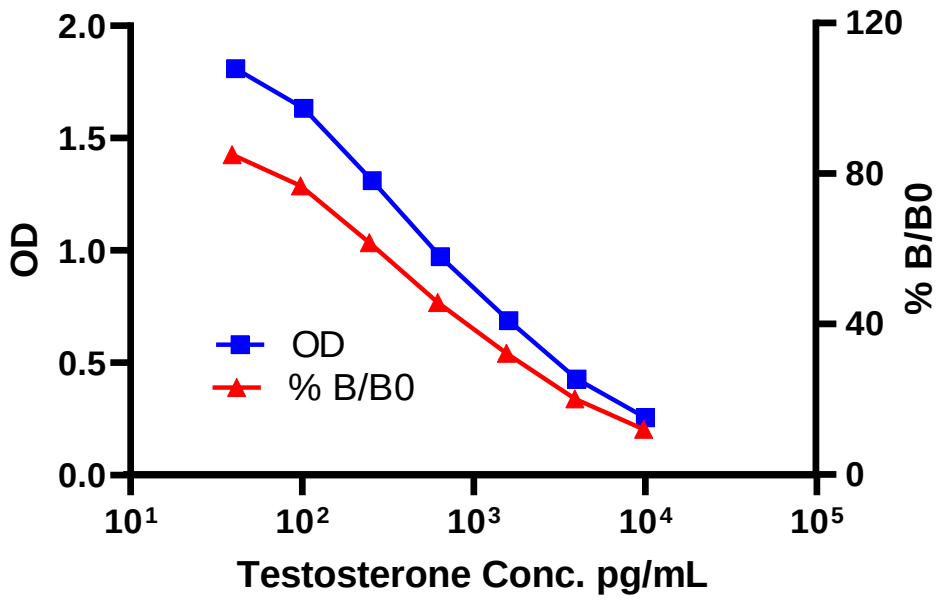
Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 75 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of Testosterone Conjugate to each well, using a repeater pipette.
5. Add 25 µL of Testosterone Antibody to each well except the NSB wells, using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Cover the plate with the plate sealer and shake for 2 hours at room temperature. If the plate is not shaken, signals will be approximately 20 % lower.
8. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
9. Tap the plate on clean absorbent towels to dry.
10. Add 100 µL of TMB Substrate to each well using a multichannel/repeater pipette.
11. Incubate at room temperature for 30 minutes without shaking.
12. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
13. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in competitive ELISA. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 100 pg/mL of Testosterone is equivalent to 346.7 pM



A typical standard curve of ZellX® Testosterone ELISA Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® Testosterone ELISA assay was determined as 30.6 pg/mL.

Sensitivity

The sensitivity of the ZellX® Testosterone ELISA assay was determined as 9.92 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 human samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human samples were tested in duplicate on 15 different assays over multiple days.

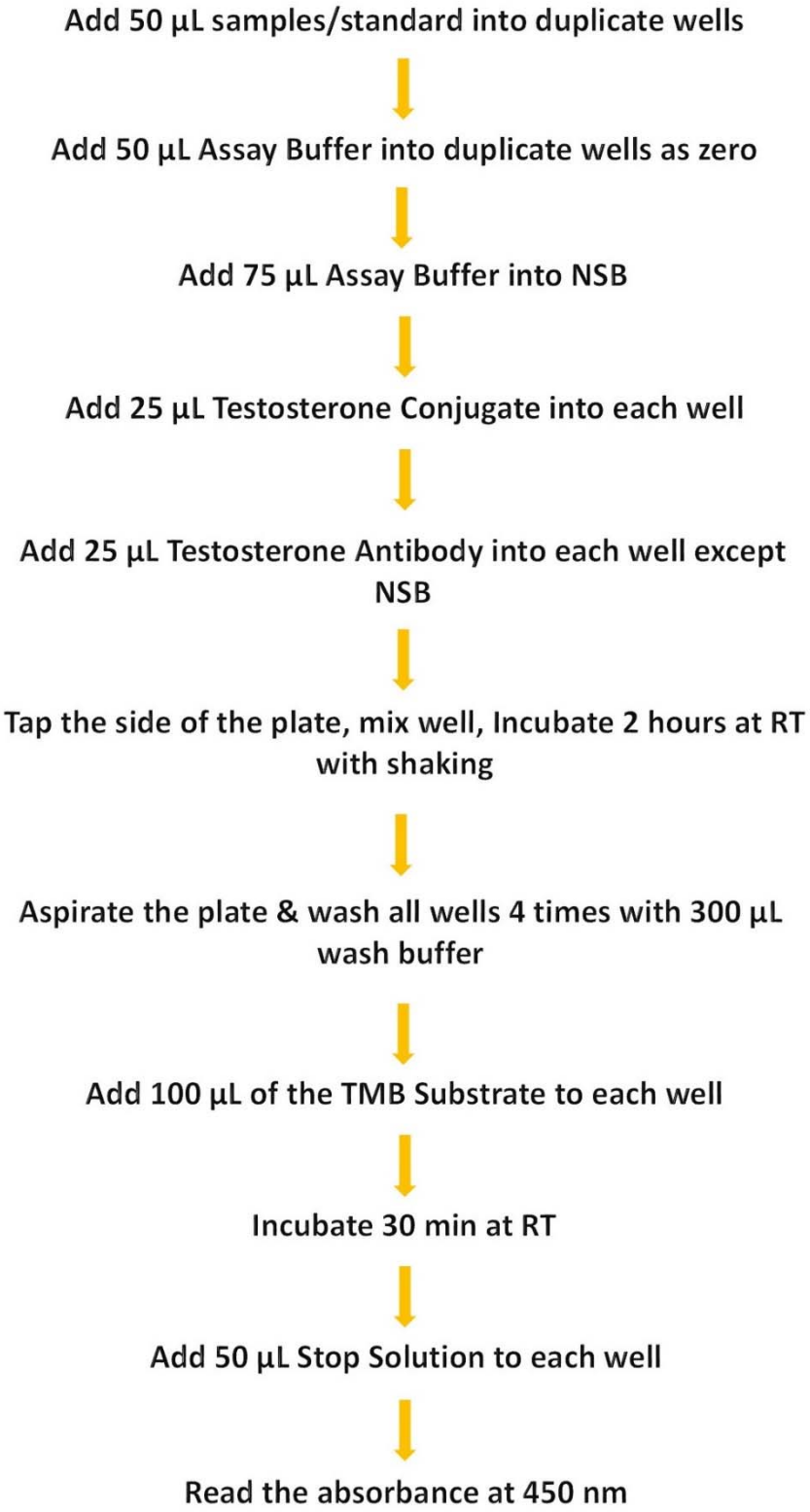
Item	*% CV*
Intra assay	15.8, 5.7, 11.1
Inter assay	10.5, 11.2, 6.2

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50 % binding point.

Steroid	*Cross Reactivity (%)*
Testosterone	100
5α-Dihydrotestosterone	56.8
Androstendione	0.27
Androsterone	0.04
DHEA	0.04
Cholesterol	0.03
17β-Estradiol	0.02
Progesterone	< 0.02
Pregnenolone	< 0.02
Hydrocortisone	< 0.02
Cholic Acid	< 0.02
Cholic Derivatives	< 0.02

Protocol summary



References

1. Cox RM, John-Alder HB., “Testosterone has opposite effects on male growth in lizards (Sceloporus spp.) with opposite patterns of sexual size dimorphism”. 2005, J. Exp. Biol. 208:4679–87.

2. Reed WL, et. al., “Physiological effects on demography: a long-term experimental study of testosterone’s effects on fitness”. 2006, Am. Nat. 167:667–83.

3. Mooradian AD, Morley JE, Korenman SG., “Biological actions of androgens”. 1987, Endocr. Rev. 8:1–28.

4. Bassil N, Alkaade S, Morley JE., “The benefits and risks of testosterone replacement therapy: a review”. 2009, Ther. Clin. Risk Manag. 5:427–48.5.

5. Tuck SP, Francis RM., “Testosterone, bone and osteoporosis”. 2009, Frnt. Horm. Res. 37:123–32.

6. Dabbs M, Dabbs JM., In: “Heroes, rogues, and lovers: testosterone and behavior.” 2000, New York: McGraw-Hill.

7. Nelson, RF., In: “An introduction to behavioral endocrinology.” 2005, Sunderland, Mass: Sinauer Associates. pp. 143.

8. De Loof A., “Ecdysteroids: the overlooked sex steroids of insects? Males: the black box”. 2006, Insect Sci., 13:325–338.

9. Mechoulam R, Brueggemeier RW, Denlinger DL, R.; Brueggemeier, R. W.; Denlinger, D. L., “Estrogens in insects”., 1984, J. Cell. and Mol. Life Sci., 40:942–944.