



## **Mouse/Rat Testosterone ELISA**

For the quantitative determination of testosterone in mouse and rat serum and EDTA plasma

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 55-TESMS-E01

Size: 96 wells

Version: 8-01/23 - ALPCO 2.0

## 1 INTRODUCTION

### 1.1 INTENDED USE

The Mouse/Rat Testosterone ELISA is a competitive immunoassay for the quantitative measurement of testosterone in rat and mouse serum or EDTA plasma. **For research use only.** Not for use in diagnostic procedures.

### 1.2 SUMMARY AND EXPLANATION

Testosterone is a steroid hormone from the androgen group synthesized by the Leydig cells in the testes in males, the ovaries in females, and adrenal glands in both sexes. It exerts a wide-ranging influence over sexual behavior, muscle mass, strength, energy, cardiovascular health, and bone integrity.

Testosterone biosynthesis coincides with the spermatogenesis and fetal Leydig cell differentiation in the male rat. Several *in vivo* models including hormone-suppression, hormone-restoration and hypophysectomy were established for the study of the hormonal regulation of spermatogenesis by testosterone (1-3). In the Brown Norway rat, serum testosterone levels decrease with aging, accompanied by increases in serum FSH. The capacity of Leydig cells to produce testosterone is higher in young than in old rats (4). Testosterone secreted during late gestational and neonatal periods causes significant brain sexual dimorphism in the rat. This results in both sex-specific behavior and endocrinology in adults (5).

Analyses concerning the regulation of synthesis reveal that testosterone is able to regulate its own synthesis and indicate that this autoregulation is the result of rapid, specific inhibition by testosterone of 17-alpha-hydroxylase activity (6).

## 2 PRINCIPLE

The Mouse/Rat Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. An unknown amount of testosterone present in the sample and a defined amount of testosterone conjugated to horseradish peroxidase compete for the binding sites of testosterone antiserum coated to the wells of a microplate. After incubation for one hour the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of testosterone in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of testosterone in the sample. The enzymatic reaction is stopped by addition of Stop Solution (change from blue to yellow) and the optical density (OD) is measured. A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve..

## 3 WARNINGS AND PRECAUTIONS

1. For Research Use Only.
2. All blood components and biological materials should be handled as potentially hazardous during use and disposal. Follow universal precautions when handling and disposing of potentially-infectious agents.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.

6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. All reagents should be at room temperature (18-25°C) before use. Temperature will affect the absorbance readings of the assay.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may yield slightly different results.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, CMIT and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. Chemicals and prepared or used reagents must be treated as hazardous waste according to the national biohazard safety guideline or regulations.
20. For additional information please refer to Safety Data Sheets. Safety Data Sheets for this product are available on ALCO's website or upon request.

## 4 REAGENTS

### 4.1 REAGENTS PROVIDED

1. **Microtiter plate**, 12 x 8 (break apart) strips with 96 wells; wells coated with rabbit polyclonal anti-testosterone antibody.
2. **Calibrator 0**, 1 vial, 0.3 mL, ready to use
3. **Calibrator (Calibrator 1-5)**, 5 vials, 0.3 mL each, ready to use; concentrations: 0.1, 0.4, 1.5, 6.0, 25.0 ng/mL
4. **Incubation Buffer**, 1 vial 11 mL, ready to use
5. **Enzyme Conjugate**, 1 vial, 7 mL, ready to use; Testosterone conjugated to horseradish peroxidase
6. **Substrate Solution**, 1 vial, 22 mL, ready to use; contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix
7. **Stop Solution**, 1 vial, 7 mL, ready to use; contains 2 N Hydrochloric Acid solution

**Avoid contact with the Stop Solution. It may cause skin irritation and burns.**

8. **Wash Solution**, 1 vial, 50 mL (10X concentrated); see “Reagent Preparation”

**Note:** Additional Calibrator 0 for sample dilution is available for purchase upon request.

#### **4.2 MATERIALS REQUIRED BUT NOT PROVIDED**

- Centrifuge
- A microtiter plate reader capable of endpoint measurement at 450nm
- Microplate shaker operating at 900 rpm
- Vortex mixer
- Calibrated variable precision micropipettes and multichannel pipettes with disposable tips.
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi-logarithmic graph paper or software for data reduction

#### **4.3 REAGENT PREPARATION**

All reagents should be at room temperature (18-25°C) before use.

##### **Wash Solution:**

Dilute 50 mL of 10X concentrated *Wash Solution* with 450 mL deionized water to a final volume of 500 mL. The 1X working Wash Solution is stable for at least 12 weeks at room temperature. Precipitates may form when stored at 2-8° C, which should dissolve again by swirling at room temperature (18-25° C). The Wash Solution should only be used when the precipitates have completely dissolved.

#### **4.4 STORAGE CONDITIONS**

When stored at 2°C to 8°C unopened reagents will be stable until expiration date.

Do not use reagents beyond this date. Opened reagents must be stored at 2°C to 8°C. After first opening, the reagents are stable for 30 days if used and stored properly. Keep away from heat and direct sunlight.

Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly. Protect TMB-Substrate Solution from light.

#### **4.5 DISPOSAL OF THE KITS**

Dispose of kits according to local and national regulations. Special information for this product is given in the Safety Data Sheet.

#### **4.6 DAMAGED TEST KITS**

In case of any severe damage of the test kit or components, ALPCO must be informed in writing no more than 1 week after receiving the kit. Severely damaged single components should not be used for a test run. They must be stored until a final solution has been found. After this, they should be disposed of according to official regulations.

## 5 SAMPLE COLLECTION AND PREPARATION

For determination of testosterone, rat/mouse **serum** and EDTA **plasma** can be used. The procedure calls for 10  $\mu\text{L}$  of matrix per well. The samples should be assayed immediately or aliquoted and stored at  $-20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Samples expected to contain rat/mouse testosterone concentrations higher than the highest calibrator (25 ng/mL) should be diluted with the zero calibrator before testing. The additional dilution step must be taken into account during the calculation of the results.

### Please note:

Samples containing sodium azide should not be used in the assay. This can cause false results. Furthermore do not use hemolytic, icteric, or lipemic samples..

## 6 ASSAY PROCEDURE

### 6.1 GENERAL REMARKS

- All reagents and samples must be allowed to come to room temperature ( $18-25^{\circ}$  before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposable plastic pipette tips for each standard, control, or sample to avoid cross contamination.
- Optical Density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule, the enzymatic reaction is linearly proportional to time and temperature.
- Follow the incubation times as stated in the instructions for use
- Calibrators, controls, and samples should be tested in duplicate
- Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or a multi-stepper, respectively, or an automatic microtiter plate washing system. Do not allow wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Solution, and that there are no residues in the wells
- For internal quality control, it is suggested to use the Rat Control Set catalog number 55-DEV99RC. Please contact ALPCO for more information.

### 6.2 ASSAY PROCEDURE

Each run must include a standard curve.

1. Prepare enough microplate wells to accommodate calibrators and samples in duplicate.
2. Dispense **10  $\mu\text{L}$**  of each **Calibrator, Sample, and Control** with new disposable tips into appropriate wells.
3. Dispense **100  $\mu\text{L}$**  of **Incubation Buffer** into each well.
4. Add **50  $\mu\text{L}$**  **Enzyme Conjugate** into each well.
5. Incubate for **60 minutes** at room temperature ( $18-25^{\circ}\text{C}$ ) on a microplate shaker (900 rpm).

#### Important Note:

Optimal reaction in this assay is markedly dependent on shaking the microplate!

6. Discard the content of the wells and rinse the wells **4 times** with 1X working **Wash Solution** (300  $\mu$ L per well). Remove as much Wash Solution as possible by tapping the microplate on absorbent paper.
7. Add **200  $\mu$ L** of **Substrate Solution** to each well.
8. Incubate without shaking for **30 minutes** in the dark at room temperature (18-25°C).
9. Stop the reaction by adding **50  $\mu$ L** of **Stop Solution** to each well.  
Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

### 6.3 CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of calibrators, controls, and samples.
2. The obtained OD values of the Calibrators (y-axis, linear) are plotted against their corresponding concentrations (x-axis, logarithmic) either on semi-logarithmic paper or using an automated method.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the calibration curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4-PL (4-Parameter Logistics) curve fit. 4-Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator must be further diluted. For the calculation of the concentrations, this dilution factor must be taken into account.

#### Conversion to SI units:

Testosterone (pg/mL) x 3.47 = pmol/L

#### 6.3.1 Example of a Typical Calibrator Curve

The following data are for illustration only and should not be used to calculate results from another run.

Standard	Absorbance Units
Calibrator 0 (0 ng/mL)	2.865
Calibrator 1 (0.1 ng/mL)	2.611
Calibrator 2 (0.4 ng/mL)	2.141
Calibrator 3 (1.5 ng/mL)	1.278
Calibrator 4 (6.0 ng/mL)	0.615
Calibrator 5 (25.0 ng/mL)	0.221

## 7 EXPECTED NORMAL VALUES

To determine the normal range of testosterone, samples from apparently healthy and untreated Sprague-Dawley rats and BL6N and CD1 mice were analyzed using the Mouse/Rat Testosterone ELISA kit. The following ranges were calculated with the results of this study.

Population	gender	n	Range (ng/ml)	Mean (ng/ml)	Median (ng/ml)	2.5 - 97.5. percentile (ng/ml)
Rat Serum	male	10	1.81 - 11.59	5.63	5.96	1.94 - 10.66
	female	10	0.57 - 1.42	1.16	1.23	0.64 - 1.42
Rat EDTA Plasma	male	5	1.51 - 6.01	3.22	3.04	1.53 - 5.79
	female	5	0.44 - 0.96	0.81	0.93	0.47 - 0.95
Mouse Serum	male	12	0.44 - 21.36	5.20	1.21	0.49 - 20.70
	female	10	0.09 - 0.47	0.30	0.33	0.11 - 0.46

On average, rat EDTA plasma results were lower compared to rat serum samples. It is recommended that each laboratory establish its own normal range, as testosterone levels can vary due to handling and sampling techniques.

## 8 PERFORMANCE CHARACTERISTICS

### 8.1 ANALYTICAL SENSITIVITY

The lowest analytically detectable level of testosterone that can be distinguished from the Zero Calibrator is 0.024 ng/mL at the 2SD confidence limit.

### 8.2 SPECIFICITY

The following materials have been evaluated for cross-reactivity. The percentage indicates cross-reactivity at 50% displacement compared with Testosterone.

Steroid	%Cross-reactivity
5 $\alpha$ Dihydrotestosterone	56.7
Androstenedione	3.4
Androsterone	0.7
Dihydroandrosterone	4.6
Estrone	< 0.1
Estradiol	< 0.1
Estriol	< 0.1
Cortisol	< 0.1
11-Deoxycortisol	< 0.1
Progesterone	< 0.1
17-OH-Progesterone	< 0.1

### 8.3 REPRODUCIBILITY

#### 8.3.1 Intra-Assay

The intra-assay (within-run) variation was determined by 20 replicate measurements of 3 different samples within one run. The intra-assay variability is shown below:

<b>Mean (ng/mL)</b>	1.06	3.30	9.00
<b>SD</b>	0.04	0.13	0.34

<b>CV (%)</b>	3.6	4.0	3.8
<b>n</b>	20	20	20

### 8.3.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of three different samples in 10 different runs. The inter-assay variability is shown below:

<b>Mean (ng/mL)</b>	1.08	3.22	8.38
<b>SD</b>	0.04	0.13	0.38
<b>CV (%)</b>	3.3	4.0	4.6
<b>n</b>	10	10	10

### 8.4 RECOVERY

Recovery was determined by adding increasing amounts of the analyte to three different samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was measured by the Mouse/Rat Testosterone ELISA. The percentage recoveries were determined by comparing expected and observed results of the samples.

<b>Sample</b>	<b>Spiking Solution</b>	<b>Observed (ng/mL)</b>	<b>Expected (ng/mL)</b>	<b>Recovery</b>
1	native	0.90	-	-
	+ 0.5 ng/ml	1.31	1.40	93%
	+ 2.5 ng/ml	3.21	3.40	94%
	+ 10.0 ng/ml	9.17	10.90	84%
2	native	3.40	-	-
	+ 0.5 ng/ml	3.79	3.90	97%
	+ 2.5 ng/ml	5.80	5.90	98%
	+ 10.0 ng/ml	13.41	13.40	100%
3	native	1.86	-	-
	+ 0.5 ng/ml	2.25	2.36	95%
	+ 2.5 ng/ml	3.52	4.36	81%
	+ 10.0 ng/ml	11.12	11.86	94%

### 8.5 LINEARITY

Four samples containing different amounts of analyte were serially diluted with Calibrator 0 and assayed with the Mouse/Rat Testosterone ELISA. The percent linearity was calculated by comparing the expected and observed values of the samples..

<b>Sample</b>	<b>Dilution</b>	<b>Observed (O) (ng/mL)</b>	<b>Expected (E) (ng/mL)</b>	<b>O/E %</b>
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1	native	3.55	-	-
	1 : 2	1.97	1.78	111%
	1 : 4	0.98	0.89	110%
	1 : 8	0.50	0.44	113%
2	native	2.31	-	-
	1 : 2	1.32	1.16	114%
	1 : 4	0.73	0.58	126%
	1 : 8	0.36	0.29	125%
3	native	11.96	-	-
	1 : 2	5.46	5.98	91%
	1 : 4	2.99	2.99	100%
	1 : 8	1.56	1.49	104%
4	native	3.96	-	-
	1 : 2	2.11	1.98	106%
	1 : 4	1.10	0.99	111%
	1 : 8	0.53	0.50	107%

## 9 LIMITATIONS OF PROCEDURE

Reliable and reproducible results are obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

### 9.1 INTERFERING SUBSTANCES

- Do not use any hemolytic, icteric, or lipemic samples to avoid any interferences.
- Samples containing sodium azide should not be used in the assay.
- Non-specific interferences with this in vitro immunoassay cannot be excluded. If unpalusible results are suspected, they should be considered invalid and verified by further testing.
- Up to a tested concentration of 1000 ng/ml Testosterone no High Dose Hook Effect could be observed for the Mouse/Rat Testosterone ELISA.

### 9.2 DRUG INTERFERENCES

Until now no substances (drugs) are known to influence the measurement of rat or mouse testosterone in serum and plasma. The determination of testosterone can be invalidated if the subject was treated with natural or synthetic steroids. Any medication should be considered when assessing results..

## **10 LEGAL ASPECTS**

### **10.1 RELIABILITY OF RESULTS**

The assay must be performed exactly per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of Good Laboratory Practice (GLP) or other applicable national standards and/or laws. This is especially relevant for the use of controls reagents. It is important to always include within the test procedure a sufficient number of controls for validating the accuracy and precision of the assay. The assay results are valid only if all controls are within the specified ranges and if all other assay parameters are also within the given assay specifications. In case of any doubt or concern please contact ALPCO.

### **10.2 LIABILITY**

Any modification of the assay and/or exchange of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Regardless, in the event of any claim, ALPCO's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of ALPCO.

## 11 REFERENCES

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