Mouse/Rat Testosterone ELISA Kit

Enzyme Immunoassay for the quantification of Mouse/Rat Testosterone in Mouse or Rat serum and plasma.

Catalog number: ARG80662

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

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

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antibody specific for Testosterone has been pre-coated onto a microtiter plate. Endogenous Testosterone of a patient sample competes with a Testosterone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation 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 patient sample. Testosterone concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Standard 0	0.3 ml (ready to use)	4°C
Standard 1-5 (0.1, 0.4, 1.5, 6, 25 ng/ml)	5 x 0.3 ml (ready to use)	4°C
Incubation Buffer	11 ml (ready to use)	4°C
HRP-conjugated Testosterone	7 ml (ready to use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	22 ml (ready to use)	4°C (Protect from light)
STOP solution	7 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (~600rpm)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- Opened reagents must be stored at 2°-8°C. After first opening the reagents are stable for 30 days if used and stored properly.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- All kit reagents and specimens should be brought to room temperature (21-26°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Serum samples are suggested to be used to get better precision.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 10X Wash buffer + 450 ml of distilled water) The diluted Wash buffer is stable for at least 3 months at room temperature.
- Samples: If the results of samples are out of the measuring range, or the samples are expected to contain Testosterone higher than the highest standard (25 ng/ml), the samples can be diluted with Standard 0 and re-assay the samples. The dilution factor should be taken into account for the result calculation.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **10 µl** of **standards and samples** in duplicate into sample wells.
3. Add **100 µl** of **Incubation Buffer** into each well.
4. Add **50 µl** of **HRP-conjugated Testosterone** into each well. Incubate for **1 hour at RT** on a microplate shaker (~600rpm)
5. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **200 µl** of **TMB substrate** to each well. Incubate for **30 minutes at room temperature** in dark (without shaking).
7. Add **50 µl** of **Stop Solution** to each well.
8. Read the OD with a microplate reader at **450 nm** immediately. It is recommended to read the wells within 15 minutes after adding Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-logarithmic or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard 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 method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted with zero standard. For the calculation of the concentrations, this dilution factor has to be taken into account.

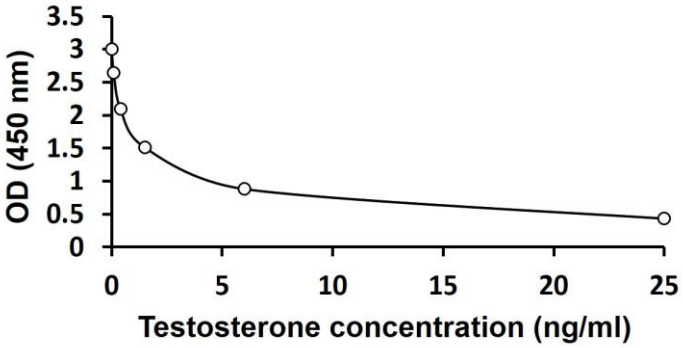
7. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	0	1	2	3	4	5
Testosterone (ng/ml)	0	0.1	0.4	1.5	6	25
Testosterone (nmol/L)	0	0.347	1.388	5.205	20.82	86.75
Conversion	Testosterone (ng/ml) x 3.47 = Testosterone (nmol/L)					

** Conversion: 1 ng/ml corresponds to 3.47 nmol/L.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



EXPECTED NORMAL VALUES

In order to determine the normal range of serum testosterone in rat, samples from 35 male rats and 20 female rats were collected and analyzed using the Mouse/Rat Testosterone ELISA Kit. The following ranges are calculated with the results of this study.

	Range (ng/ml) Morning	Mean (ng/ml)
Male	0.66-5.4	3.06
Female	0.11-0.31	0.21

In further studies serum samples of 10 mice were collected between 11.00 am and 3.00 pm and analyzed in a similar manner.

	Range (ng/ml)	Mean (ng/ml)
Male	1.7-14.4	6.78

It is recommended that each laboratory establish its own normal range since testosterone levels can vary due to handling and sampling techniques.

QUALITY ASSURANCE

Specificity

Steroid	Crossreactivity (%)
Dihydrotestoterone	69.6
Androstenedione	< 0.1
Androsterone	< 0.1
Epiandrosterone	< 0.1
Dihydroandrosterone	< 0.1
Dihydroxyandrosterone	7.4
Estron	< 0.1
Estradiol	< 0.1
Estriol	< 0.1
Cortisol	< 0.1
11-Deoxycortisol	< 0.1
Progesterone	< 0.1
17OH-Progesterone	< 0.1

Sensitivity

The lowest analytical detectable level of testosterone that can be distinguished from the Zero Calibrator is 0.066 ng/ml at the 2SD confidence limit.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 8.54% and inter-assay precision was 9.97%.

Recovery

86-118%

Linearity

81-118%