Catalog #80552

V.2

Crystal Chem
High Performance Assays

Mouse Testosterone ELISA Kit Instructions

For the quantitative determination of testosterone in mouse serum and plasma

Catalog #80552
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Mouse Testosterone ELISA kit is for the quantitative determination of testosterone in mouse serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Testosterone is a steroid hormone secreted from the Leydig cells of the testes in the male. The dihydro derivative of Testosterone exerts a potent anabolic action responsible for the post pubescent growth rate and subsequent muscle and bone tissue maintenance of adult males. Testosterone levels in serum may be raised by certain drugs.

C. Principle of the Assay

The Mouse Testosterone ELISA kit is a solid phase enzyme-linked immunosorbent assay 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 on a shaker, the microplate is washed four times. After addition of the substrate solution the concentration of testosterone is inversely proportional to the optical density measured.

D. Kit Storage

1. Upon receipt of the Mouse Testosterone ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD0-5	Standards	6 x 0.3 mL
INC	Incubation Buffer	1 x 11 mL
ENZ	Enzyme Conjugate	1 x 7 mL
WASH	Wash Buffer (10X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 22 mL
STOP	Stop Solution	1 x 7 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Volumetric flasks
Microplate shaker (600 rpm)
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (10 µL), pipetting should be done as carefully as possible. A high quality 50 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Plasma: Collect blood into a tube containing an anticoagulant such as heparin (final concentration: 1 unit/mL), EDTA (final concentration: 0.1%), or sodium citrate (final concentration: 0.76%), and centrifuge for 20 min at 2,000 x g.

Serum: Collect blood, allow to clot, and centrifuge for 20 min at 2,000 x g.

The samples should be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Lipemic and hemolytic samples should not be used.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standards 0-5
   Provided as ready to use. 0, 0.1, 0.4, 1.5, 6.0, and 25 ng/mL.
3. Incubation Buffer
   Provided as ready to use.
4. Enzyme Conjugate
   Provided as ready to use.
5. Wash Buffer (10X Concentrated)
   The wash buffer has to be diluted 1:10 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 450 mL of distilled or deionized water. Wash buffer is stable for at least 3 months after dilution.
6. Substrate Solution
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use. Stable up to 2 months after opening.

I.2. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 10 µL of sample, standard, or control.
   Note: *Optional control is available separately (Cat# 80553).*
2. In each well, add 100 µL of Incubation Buffer and mix well by repeated pipetting.
3. In each well, add 50 µL of Enzyme Conjugate and mix well by repeated pipetting.
4. Incubate the plate for 1 hour at room temperature on a microplate shaker (shake at 600 rpm).
   Note: *For optimal results, microplate shaking is critical.*
5. Aspirate well contents and wash four times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
6. Add 200 µL of the Substrate Solution in each well.
7. Incubate plate for 30 mins in dark room at room temperature without shaking.
8. Stop the reaction by adding 50 µL of Stop Solution.
9. Measure absorbance within 15 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.3. Determining the testosterone concentration

1. Using computer software, construct the testosterone calibration curve by plotting the mean change in absorbance value for each calibrator on the Y axis versus the corresponding testosterone concentration on the X axis. A semi-log, four parametric logistic (4-PL) curve fit, or Logit-Log curve are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Mouse testosterone concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The testosterone concentration is expressed in ng/mL.
   Note: *Samples with high mouse testosterone concentrations (ie. fall above 25 ng/mL) should be further diluted with the zero standard and rerun.*

J. Performance characteristics

J.1. Assay range

The Mouse Testosterone ELISA Kit has an assay range from 0.1 – 25 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

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