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Instructions for use

Testosterone rat/mouse ELISA

REF AR E-8000

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Testosterone rat / mouse ELISA

1. INTRODUCTION

1.1 INTENDED USE

The **Testosterone rat / mouse ELISA** is a competitive immunoassay for the measurement of testosterone in rat and mouse serum or 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 behaviour, 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 (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 behaviour 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 **Testosterone rat / mouse 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 one-hour 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.

3. WARNINGS AND PRECAUTIONS

1. This kit is strictly intended for research use only. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.
2. 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.
3. 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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 reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21 - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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 result slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation and burns.

17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from manufacturer.

4. REAGENTS

4.1 Reagents provided

AR E-8031 96 **Microtiter Plate**, 12 x 8 (break apart) strips with 96 wells;
Wells coated with anti-testosterone antibody.

Standard - ready to use.
Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Cat. no.	Symbol	Standard	Concentration	Volume / Vial
AR E-8001	STANDARD A	**Standard A**	0 ng/ml	0.3 ml
AR E-8002	STANDARD B	**Standard B**	0.1 ng/ml	0.3 ml
AR E-8003	STANDARD C	**Standard C**	0.4 ng/ml	0.3 ml
AR E-8004	STANDARD D	**Standard D**	1.5 ng/ml	0.3 ml
AR E-8005	STANDARD E	**Standard E**	6.0 ng/ml	0.3 ml
AR E-8006	STANDARD F	**Standard F**	25 ng/ml	0.3 ml

AR E-8013 INC-BUFF **Incubation Buffer,** 1 vial, 11 ml, ready to use;

AR E-8040 CONJUGATE **Enzyme Conjugate**, 1 vial, 7 ml, ready to use;
Testosterone conjugated to horseradish peroxidise.

AR E-0055 SUBSTRATE **Substrate Solution**, 1 vial, 22 ml, ready to use;
contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

AR E-0080 STOP-SOLN **Stop Solution**, 1 vial, 7 ml, ready to use;
contains 2 N Hydrochloric Acid solution.

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H335 May cause respiratory irritation.

AR E-0030 WASH-CONC 10x **Wash Solution**, 1 vial, 50 ml (10x concentrated);
see „Preparation of Reagents".

Note: Additional Standard A for sample dilution is available upon request.

4.2 Materials required but not provided

- Centrifuge
- A microtiter plate reader capable for endpoint measurement at 450 nm
- Microplate mixer operating more than 600 rpm
- Vortex mixer
- Calibrated variable precision micropipettes (10 µl, 50 µl, 100 µl, 200 µl).
- 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 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 diluted Wash Solution is stable for at least 3 months at room temperature.

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 - 8 °C. After first opening the reagents are stable for 30 days if used and stored properly.
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

The disposal of the kit must be made according to the 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, the manufacturer have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SAMPLE

For determination of Testosterone rat/mouse **serum** and **plasma** can be used. The procedure calls for 10 µl matrix per well. The samples should assay immediately or aliquot and stored at -20 °C. Avoid repeated freeze-thaw cycles. Samples expected to contain rat/mouse Testosterone concentrations higher than the highest standard (25 ng/ml) should be diluted with Standard A before assay. The additional dilution step has to be taken into account for the calculation of the results.

Please note: The use of plasma as sample can result in a diminished precision of this assay.

6. ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance 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.
- For internal quality control we suggest to use **Rat Control Set coded AR K-8000. For more information please contact the manufacturer directly.**

6.2 Assay Procedure

Each run must include a standard curve.

1.	Prepare a sufficient number of microplate wells to accommodate Standards and Samples in duplicates
2.	Dispense **10 µl** of each **Standard, Control** and **Sample** with new disposable tips into appropriate wells.
3.	Dispense **100 µl** of **Incubation Buffer** into each well.
4.	Add **50 µl Enzyme Conjugate** into each well.
5.	**Incubate** for **60 minutes** at room temperature on a Microplate mixer. *Important Note:* Optimal reaction in this assay is markedly dependent on shaking of the microplate!
6.	Discard the content of the wells and rinse the wells **4 times** with **diluted Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
7.	Add **200 µl** of **Substrate Solution** to each well.
8.	Incubate without shaking for **30 minutes** in the dark.
9.	Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
10.	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 standards, controls and samples.
2. Using semi logarithmic 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 calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

Conversion to SI units:
Testosterone (pg/ml) x 3.47 = pmol/l

6.3.1 Example of Typical Standard Curve

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

Standard	Absorbance Units
Standard A (0 ng/ml)	2.478
Standard B (0.1 ng/ml)	2.078
Standard C (0.4 ng/ml)	1.668
Standard D (1.5 ng/ml)	1.170
Standard E (6.0 ng/ml)	0.645
Standard F (25.0 ng/ml)	0.330

7. 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 Testosterone rat/mouse ELISA kit. The following ranges are calculated with the results of this study.

	Range (ng/ml)	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 und analyzed in a similar manner.

	Range (ng/ml)	Mean (ng/ml)
Male mice ♂	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.

8. PERFORMANCE CHARACTERISTICS

8.1 Analytical sensitivity

The lowest analytical detectable level of testosterone that can be distinguished from the Standard A is 0.066 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 to Testosterone.

Steroid	% Cross reaction
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

8.3 Reproducibility

8.3.1 Intra-Assay

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

Mean (ng/ml)	3.23	1.44	0.84
SD	0.21	0.12	0.09
CV (%)	6.50	8.06	11.07
n =	20	20	20

8.3.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of 3 serum samples over 10 days.

Mean (ng/ml)	0.29	1.23	9.50
SD	0.03	0.11	0.88
CV (%)	11.3	9.3	9.3
n =	10	10	10

8.4 Recovery

Using the Standard Matrix three spiking solutions were prepared (A = 50 ng/ml, B = 100 ng/ml and C = 150 ng/ml). A 25 µl aliquot of each solution was spiked into 475 µl of six different rat sera with low testosterone concentrations for a spiking ratio of 1 to 20, leaving the serum matrix of the spiked samples relatively intact. All samples were then measured by the Testosterone rat/mouse ELISA procedure. To calculate expected values 95% of the unspiked values were added to 5% of the spiking solution concentrations (2,5, 5 and 7,5 ng/ml, respectively).

Serum	Spiking Solution	Observed (O)	Expected (E)	O/E %
1	-	0,31	-	-
	A	3,15	2,81	112%
	B	5,11	5,31	96%
	C	7,27	7,81	93%
2	-	0,40	-	-
	A	3,42	2,90	118%
	B	5,88	5,40	109%
	C	7,90	7,90	100%
3	-	0,36	-	-
	A	2,88	2,86	101%
	B	5,50	5,36	103%
	C	7,50	7,86	95%
4	-	0,25	-	-
	A	2,65	2,75	96%
	B	4,65	5,25	89%
	C	7,08	7,75	91%
5	-	0,38	-	-
	A	3,17	2,88	110%
	B	4,79	5,38	89%
	C	7,36	7,88	93%
6	-	0,28	-	-
	A	2,78	2,78	100%
	B	4,61	5,38	86%
	C	7,22	7,78	93%

8.5 Linearity

Five native serum samples were assayed undiluted and diluted with the standard matrix.

Serum	Dilution	Observed (O) (ng/ml)	Expected(E) (ng/ml)	O/E %
3	native	2.54	-	-
	1 in 2	1.22	1.27	96%
	1 in 4	0.61	0.64	95%
	1 in 8	0.34	0.32	106%
15	native	1.85	-	-
	1 in 2	0.86	0.93	92%
	1 in 4	0.43	0.46	93%
	1 in 8	0.21	0.23	91%
17	native	1.94	-	-
	1 in 2	1.04	0.97	107%
	1 in 4	0.52	0.49	106%
	1 in 8	0.26	0.24	108%
21	native	0.75	-	-
	1 in 2	0.45	0.38	118%
	1 in 4	0.18	0.19	95%
	1 in 8	0.09	0.09	100%
9	native	1.35	-	-
	1 in 2	0.75	0.68	110%
	1 in 4	0.30	0.34	88%
	1 in 8	0.13	0.16	81%

9. Limitations of Procedure

Reliable and reproducible results will be 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 Drug Interferences

Until now no substances (drugs) are known influencing the measurement of rat or mouse testosterone in serum and plasma. Lipemic and haemolysed samples can cause false results.

10. LEGAL ASPECTS

10.1 Reliability of results

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

10.2 Liability

Any modification of the test kit and/or exchange or mixture 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, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!