Instructions for Use

Testosterone (Rat / Mouse) ELISA

RUO

REF EIA-5179

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(230104)

Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

Introduced modifications / Durchgeführte Änderungen / Modifiche introdotte / Modificaciones introducidas	
The following changes have been made in comparison to the previous version: Im Vergleich zur Vorgängerversion wurden folgende Änderungen vorgenommen: Rispetto alla versione precedente, sono state apportate le seguenti modifiche: Se han introducido los siguientes cambios en comparación con la versión anterior:	
Detailed editorial revision. Changed wording in several chapters. **Ausführliche redaktionelle Überarbeitung.** Geänderter Wortlaut in mehreren Kapiteln. **Revisione editoriale dettagliata.** Modificato il testo in diversi capitoli. **Revisión editorial detallada.** Se ha cambiado la redacción de algunos capítulos.	
1.1 Intended Use:	plasma specified as EDTA plasma
2 PRINCIPLE:	updated
3 WARNINGS AND PRECAUTIONS:	updated
4.1 Reagents provided:	for microtiter plate source of antibody added (rabbit polyclonal antibody)
4.2 Materials required but not provided:	updated (e.g. shaker needed at 900 rpm) (old: 600 rpm)
5 SAMPLES COLLECTION AND PREPARATION:	updated
6.1 General Remarks:	additions (e.g. notes on the washing procedure)
6.2 Assay Procedure:	shaking speed at 900 rpm (previously > 600 rpm)
7 EXPECTED NORMAL VALUES:	updated, more detailed values
8 QUALITY CONTROL:	added
9 PERFORMANCE CHARACTERISTICS:	Changed data
10.1 Interfering Substances:	added
10.2 Drug Interferences:	updated

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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 (EDTA).

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 testosterone conjugated to horseradish peroxidase compete for the binding sites of testosterone antibodies 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. 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. All blood components and biological materials should be handled as potentially hazardous in use and for disposal. Follow universal precautions when handling and disposing of 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 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. If using reservoirs, use 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.
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. Allow the reagents to reach room temperature (18 °C - 25 °C) before starting the test. 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 be slightly different.
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 have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from DRG.
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

4 REAGENTS

4.1 Reagents provided

1. **SORB | MT Microtiter Plate**, 12 x 8 (break apart) strips with 96 wells;
   Wells coated with rabbit polyclonal anti-testosterone antibody.
2. **CAL | 0 Calibrator 0,** 1 vial, 0.3 ml, ready to use
3. **CAL | 1-5 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. **INC | BUF Incubation Buffer,** 1 vial 11 ml, ready to use;
5. **ENZ | CONJ Enzyme Conjugate**, 1 vial, 7 ml, ready to use;
   Testosterone conjugated to horseradish peroxidase.
6. **SUB | TMB Substrate Solution**, 1 vial, 22 ml, ready to use;
   contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
7. **STOP | SOLN 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 irritations and burns.
8. **WASH | SOLN | 10x Wash Solution**, 1 vial, 50 ml (10X concentrated);
   see „Preparation of Reagents“.

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

4.2 Materials required but not provided

- Microtiter plate reader capable for endpoint measurement at 450nm
- Microtiter plate shaker operating at 900 rpm
- Vortex mixer
- Calibrated variable precision micropipettes and multichannel pipettes with disposable pipette tips
- Manual or automatic equipment for microtiter plate washing
- Absorbent paper
- Deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 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.

4.4 Reagent preparation

Allow the reagents and the required number of wells to reach room temperature (18 °C to 25 °C) before starting the test.

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 12 months at room temperature (18 °C to 25 °C).

Precipitates may form when stored at 2 °C to 8 °C, which should dissolve again by swirling at room temperature (18 °C to 25 °C). The Wash Solution should only be used when the precipitates have completely dissolved.

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, DRG has to be informed in writing within 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 SAMPLES COLLECTION AND PREPARATION

For determination of Testosterone rat/mouse **serum** and **EDTA plasma** can be used.

The procedure calls for 10 µL matrix per well.

The samples should be assayed immediately or aliquoted and stored at -20 °C. Avoid repeated freeze-thaw cycles.

Samples expected to contain Testosterone concentrations higher than the highest calibrator (25 ng/mL) should be diluted with the Calibrator 0 before assayed. The additional dilution step has to be taken into account for the calculation of the results.

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°C) 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 Calibrator, control or sample in order 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.
- Respect the incubation times as stated in this instructions for use.
- Calibrators, Controls, and samples should at least be assayed in double determinations.
- 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.
- A Calibrator curve must be established for every run.
- For internal quality control we suggest to use Rat Control (Fertility / Pregnancy), REF CTL-5262. For more information please contact DRG.

6.2 Assay Procedure

1. Prepare a sufficient number of microtiter plate wells to accommodate Calibrators and samples in duplicates.
2. Dispense **10 µL** of each **Calibrator, Sample and Control** with new disposable tips into appropriate wells of the microtiter plate.
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 (18 °C – 25 °C) on a microplate shaker (900 rpm).
   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 at room temperature (18 °C – 25 °C).
9. Stop the reaction by adding **50 µL** of **Stop Solution** to each well.
10. Determine the optical density 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 Optical Density (OD) 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 OD 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 the highest Calibrator 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 (ng/mL) x 3.47 = nmol/L

6.3.1 Example of Typical Calibrator Curve

Following data are intended for illustration only and must 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

In order 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 DRG Testosterone (Rat/Mouse) ELISA kit. The following ranges are 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 seems to be lower compared to rat serum samples.

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

8 QUALITY CONTROL

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The use of control samples is advised to assure the day-to-day validity of results.

For internal quality control we suggest to use Rat Control (Fertility / Pregnancy), REF CTL-5262.

Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices, microtiter plate reader, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Analytical sensitivity

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

9.2 Specificity

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

Steroid	% Cross reaction
5α-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

9.3 Reproducibility

9.3.1 Intra-Assay

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

Mean (ng/mL)	1.06	3.30	9.00
SD	0.04	0.13	0.34
CV (%)	3.6	4.0	3.8
n =	20	20	20

9.3.2 Inter-Assay

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

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

9.4 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 Demeditec Testosterone rat/mouse ELISA. The percentage recoveries were determined by comparing expected and observed results of the samples.

Sample	Spiking Solution	Observed (ng/mL)	Expected (ng/mL)	Recovery
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%

9.5 Linearity

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

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Linearity
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%

10 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 GLP (Good Laboratory Practice). Any improper handling of samples or modification of this test might influence the results.

10.1 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 unplausible 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 Testosterone (Rat/Mouse) ELISA.

10.2 Drug Interferences

Until now no substances (drugs) are known influencing 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 taken into account when assessing the results.

11 LEGAL ASPECTS

11.1 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 DRG.

11.2 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 USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité