DRG® Free Testosterone (EIA-2924)

USA: RUO

Revised 1 Dec. 2009 (Vers. 15.0)

1 INTRODUCTION

1.1 Intended Use

The **DRG Free Testosterone ELISA** is an enzyme immunoassay for the quantitative *in vitro* measurement of Free Testosterone in serum and plasma.

1.2 Summary and Explanation

Testosterone is a steroid hormone from the androgen group. Testosterone is primarily secreted in the testes of males and the ovaries of females although small amounts are secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. In both males and females, it plays key roles in health and well-being.

Only 1-2% of circulating testosterone exists as unbound or free testosterone. The majority, approximately 60%, is bound to SHBG with high affinity, while the remainder is loosely bound to albumin. Both the albumin-bound and free fractions may be biologically active, while SHBG effectively inhibits testosterone action.

Testosterone effects can be classified as virilizing and anabolic effects. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Virilizing effects include maturation of the sex organs.

Testosterone levels decline gradually with age in men.

Measurement of the free or unbound fraction of serum testosterone has been proposed as a means of estimating the physiologically bioactive hormone. Free testosterone levels are elevated in women with hyperandrogenism associated with hirsutism in the presence or absence of polycystic ovarian disease. In addition, free testosterone measurements may be more useful than total testosterone in situations where SHBG is increased or decreased (e.g. hypothyroidism and obesity).

2 PRINCIPLE OF THE TEST

The DRG Free Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with an antibody directed towards an antigenic site on the Testosterone molecule. Endogenous Free 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 Free Testosterone in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Free Testosterone in the patient sample.

Testosterone in the blood is bound to SHBG (60 %) and in lower quantity to other protein. Only the measurement of Free Testosterone (< 1% of Total Testosterone) permits the estimating of the hormone biologically active.

3 PRECAUTIONS

- This kit is for in vitro use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.

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- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with *Stop Solution* containing 0.15 mol/L H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DRG Instruments GmbH.
  The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-Testosterone IgG antibody.
2. ***Standard (Standard 0-5)*****,** 6 vials, 1 mL, ready to use;
   Concentrations: 0 - 0.2 - 1.0 - 4.0 - 20.0 - 100.0 pg/mL
3. ***Enzyme Conjugate***, 1 vial, 15 mL, ready to use;
   Testosterone conjugated to horseradish peroxidase;
4. ***TMB Substrate Solution***, 1 vial, 15 mL, ready to use;
   H_2O_2-TMB, 0.25 g/L. Avoid any skin contact.
5. ***Stop Solution***, 1 vial, 15 mL, ready to use;
   contains 0.15M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 50 mL (10X concentrated),
   Phosphate buffer 0.2M, Proclin < 0.002%
   see „Preparation of Reagents“.

4.1.1 Equipment and material required but not provided

– A microtiter plate calibrated reader (450 ± 10 nm), (e.g. the DRG Instruments Microtiter Plate Reader).

- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.
- Incubator 37°C

4.2 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

4.3 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Standards

Before use, mix for 5 min. with rotating mixer

Note: *The opened standards are stable for 6 months at 2-8°C.*

Wash Solution

Add deionized water to the 10X concentrated Wash Solution.
Dilute 50 mL of concentrated *Wash Solution* with 450 mL deionized water to a final volume of 500 mL.

The diluted Wash Solution is stable for 30 days at 2-8°C.

In concentrated wash solution it is possible to observe the presence of crystals. In this case mix at room temperature until complete dissolution of crystals. For greater accuracy dilute the whole bottle of concentrated wash solution to 500 mL taking care that all crystals have been transferred, then mix until crystals are completely dissolved.

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.5 Damaged Test Kits

In case of any severe damage of the test kit or components, DRG 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 SPECIMEN

Testosterone can be determined in plasma as well as in serum of patients who have been fasting.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

The clinical significance of the determination of Free Testosterone can be invalidated if the patient was treated with cortisone or natural or synthetic steroids

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001;
for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

5.2 Specimen Storage

Store specimen at -20°C if the determination is not performed on the same day of the sample collection. Freeze only once. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

Samples reading higher than 100 pg/mL should not be diluted. Dilution will alter the equilibrium between free testosterone and serum proteins.

6 TEST 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.
- Pipetting of samples should not extend beyond ten minutes to avoid assay drift.
- 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.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **20 µL** of each ***Standard, control*** and **samples** with new disposable tips into appropriate wells. Leave well A1 empty for substrate blank.
3. Dispense **100 µL** ***Enzyme Conjugate*** into each well, except for the blank well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at 37°C.
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted wash solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of ***Substrate Solution*** to each well.
7. Incubate for **15 minutes** at room temperature (22°C – 28°C) in the dark.
8. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
9. Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader (against the blank). It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. 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: 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read 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.

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

		Median	Mean ± 1SD pg/mL	Range pg/mL
Normal Male		14	13 ± 7	4.5 - 42
Female:	Ovulating	1.3	1.4 ± 0.9	ND - 4.1
	Oral contraceptives	0.9	1.1 ± 0.6	0.3 - 2.0
	Postmenopausal	0.8	0.9 ± 0.5	0.1 – 1.7

8 QUALITY CONTROL

Good laboratory practice requires that controls be 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.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, 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 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.06 pg/mL – 100 pg/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Analyte	% Cross reactivity
Testosterone	100
DHT	0,00008
Androstenedione	0,0043
Androsterone	0,00029
DHEA-S	0,00007
Cortisol	< 0,00001
Cortison	< 0,00001
17α Estradiol	0,00005
Estrone	< 0,00001
Prednisone	< 0,00001
17α Ethynilestradiol	< 0,00001
Norgestrel	0,00001

9.3 Analytical Sensitivity

The lowest detectable concentration of Free Testosterone that can be distinguished from the zero standard is 0.06 pg/ml.

9.4 Precision

9.4.1 Intra Assay Variation

Within run variation was determined by replicate determination (15x) of three different serum samples in the same assay. The within assay variability is <10%.

9.4.2 Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera and two serum samples in 10 different assays. The between assay variability is <10%.

9.5 Correlation with RIA

The Free Testosterone ELISA was compared to Free Testosterone RIA (DPC-Coat a Count) kit. Serum samples of 24 females and 17 males were analysed according in both test systems.

The linear regression curve was calculated
y = 0,957 x + 0,953
r = 0,9681

10 LIMITATIONS OF USE

Any improper handling of samples or modification of this test might influence the results.

The clinical significance of the determination Free Testosterone can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

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 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 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.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. 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.

12 REFERENCES

1. McCann D, Kirkish L. J. Clin. Immunoassay 8:234-6 (1985)
2. Ekins RP., J. Clin. Immunoassay 1984; 7(2): 163 – 80
3. Paulson JD, et al., Am. J Obst. Gynecol 1977;128:851-7

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4. Odlind V. et al., Clin. Endocrinology 1982;16:243-49
5. Green PJ., Clin Chem 1982;28:1237
6. Wu CH., Obstet Gynecol. 1982;60:188-94

Symbols used with DRG Assays

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις

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	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..