DRG® Testosterone (serum) CLIA (CLA-4662)

RUO in the USA

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1 INTRODUCTION

The **DRG Testosterone CLIA** is a chemiluminescence immunoassay for the measurement Testosterone in serum and plasma. In the United States, this kit is intended for Research Use Only.

2 PRINCIPLE OF THE TEST

The DRG Testosterone CLIA Kit is a chemiluminescence immunoassay (CLIA), based on the principle of competitive binding.
The microtiter wells are coated with a monoclonal [mouse] antibody directed towards an unique antigenic site on the Testosterone molecule. Endogenous Testosterone of a donor 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 reverse proportional to the concentration of Testosterone in the sample. After addition of the substrate solution, the intensity of emitted light is inversely proportional to the concentration of Testosterone in the donor sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro use only. For professional use only. In the United States, this kit is intended for Research Use Only.
2. 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.
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. 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.
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 (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the donor samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens 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 luminometer.

16. The luminescence substrate reagents (*Reagent A* and *Reagent B*) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
17. 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.
18. Some reagents contain Proclin 300, BND 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 on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

Reagents Provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a mouse monoclonal anti-Testosterone antibody.
2. ***Standard (Standard 0-6),*** 7 vials, 1 ml, ready to use
   Concentrations: 0 – 0.2 – 0.5 – 1 – 2 – 6 – 16 ng/mL
   Conversion: 1 ng/mL = 3.467 nmol/L
   contain 0.03% Proclin 300 + 0.005% gentamicin sulfate as a preservative.
3. ***Enzyme Conjugate***, 1 vial, 24 mL, ready to use
   Testosterone conjugated to horseradish peroxidase
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as a preservative.
4. ***Substrate Solution***,
   Reagent A, 1 vial, 4 mL, *Note: light sensitive!*
   Reagent B, 1 vial, 4 mL, *Note: light sensitive!*
   Reagent C, 1 vial, 5 mL
   see „ Reagent Preparation“.
5. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Reagent Preparation“.

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

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

Materials required but not provided

- A microtiter plate luminometer.
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

– Timer
– Semi logarithmic graph paper or software for data reduction

Storage Conditions

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.
Opened kits retain activity for 2 months if stored as described above.

Reagent Preparation

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

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Chemiluminescence Substrate Solution

Mix **1 part** of the chemiluminescence ***Reagent A*** with **1 parts** of ***Reagent B*** and dilute this mixture 1:1.5 with ***Reagent C***.
This gives the ready to use substrate solution.
The prepared substrate solution is stable for one hour. Prepare fresh before use.
If the whole plate is to be used prepare the substrate solution as follows:
Add 3.5 mL of each *Reagent A* and *Reagent B* into 3.5 mL *Reagent C*

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.

Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the 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 COLLECTION AND PREPARATION

Serum or plasma (EDTA-, Heparin plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

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. Donors 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)

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6 ASSAY PROCEDURE

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.
- Light intensity 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.

Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **20 µL** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Dispense **200 µL** *Enzyme Conjugate* into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature (without covering the plate).
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted Wash Solution (400 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 the freshly prepared Substrate Solution to each well. *(See "Reagent Preparation".)*
7. Incubate for **10 minutes** at room temperature.
8. Read the RLU with a microtiter plate luminometer **within 20 minutes** after incubation time of substrate.

Calculation of Results

1. Calculate the average Relative Light Units (RLU) values for each set of standards, controls and donor samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean RLU obtained from each standard against its concentration with RLU value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean RLU 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. 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 or reported as > 16 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.1.1 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.

Standard	RLU (x10^2)	RLU/RLU_{max} (%)
Standard 0 (0 ng/mL)	4860	100
Standard 1 (0.2 ng/mL)	3458	71.2
Standard 2 (0.5 ng/mL)	2535	52.2
Standard 3 (1 ng/mL)	1827	37.6
Standard 4 (2 ng/mL)	1075	22.1
Standard 5 (6 ng/mL)	447	9.2
Standard 6 (16 ng/mL)	216	4.5

** It is recommended to use the RLU/RLU_{max} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show different RLU values, however, the RLU/RLU_{max} values remain consistent.

7 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 donor 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.

8 PERFORMANCE CHARACTERISTICS

Assay Dynamic Range

The range of the assay is between 0.05 – 16 ng/mL.

Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Analyte	Cross Reactivity
Testosterone	100.0
5α-Dihydrotestosterone	0.8
Androstenedione	0.9
11β-Hydroxyestosterone	3.3
17α-Methyltestosterone	0.1
19-Nortestosterone	3.3
Epitestosterone	< 0.1
Oestradiol	< 0.1
Progesterone	< 0.1
Cortisol	< 0.1
Oestrone	< 0.1
Danazol	< 0.1

9 LIMITATIONS OF USE

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.

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.25 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Testosterone in a sample.

10 LEGAL ASPECTS

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.

11 REFERENCES

1. Tietz, N.W. Textbook of Clinical Chemistry. Saunders, 1986.