DRG

DRG® Testosterone (Saliva) (CLA-4661)

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1 INTENDED USE

For determination of Testosterone in human saliva by chemiluminescence immunoassay (CLIA). **In the United States, this kit is intended for Research Use Only.**

2 PRINCIPLE OF THE TEST

The principle of the following chemiluminescence immunoassay (CLIA or LIA) test follows a two-step competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and donor samples) and a biotin-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. After washing the streptavidin-horseradish peroxidase conjugate is incubated and bound to any bound biotinylated testosterone. The washing and decanting procedures remove unbound materials. After the second washing step, the luminescence substrate solution is added. The relative luminescence units (RLUs) are measured on a microtiter plate luminometer. The RLU values are inversely proportional to the concentration of testosterone in the sample. A set of calibrators are used to plot a standard curve from which the amount of testosterone in donor samples and controls can be directly read.

3 PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The kit control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
10. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
11. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

4 LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of testosterone in human saliva. The kit is not calibrated for the determination of testosterone in serum, plasma or other specimens of human or animal origin.
2. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
3. Only calibrator A may be used to dilute any high saliva samples. The use of any other reagent may lead to false results.

5 SAFETY CAUTIONS AND WARNINGS

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid direct contact with reagents. In case of contact, wash with plenty of water.

6 SPECIMEN COLLECTION AND STORAGE

Approximately 1 ml of saliva is required per duplicate determination.

Collect 2-3 ml of saliva into a clean glass tube without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens.

Store samples at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

7 SPECIMEN PRETREATMENT

Specimen samples are to be centrifuged. The supernatants are to be transferred into clean tubes.

The tubes containing the supernatant are to be places in a water bath and heated at 60-70º for 1 hour.

Allow heated samples to reach room temperature before assaying.

8 REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 100. 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate luminometer
6. Water bath

9 REAGENTS PROVIDED AND PREPARATION

1. Rabbit Anti-Testosterone Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

2. Biotin-Testosterone Conjugate Concentrate - Requires Preparation.

Contents: Testosterone-Biotin conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.2 ml
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: for conjugate working solution: Dilute biotin conjugate concentration 1:100 in biotin conjugate buffer before use (example: 20 µL of Biotin Conjugate concentrate in 2 mL of Biotin Conjugate Buffer). If the whole plate is to be used dilute 120 µL of biotin conjugate concentrate in 12 mL of biotin buffer. Discard any that is left over.

3. Streptavidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.

Contents: Streptavidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 300 µL
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

Preparation: for conjugate working solution: Dilute HRP conjugate concentration 1:100 in HRP conjugate buffer before use (example: 20 µL of HRP Conjugate concentrate in 2 mL of HRP Conjugate Buffer). If the whole plate is to be used dilute 180 µL of biotin conjugate concentrate in 18 mL of HRP buffer. Discard any that is left over.

4. Testosterone Saliva Calibrators - Ready To Use.

Contents: Six vials containing testosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of testosterone.

*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 pg/ml	4.0 ml
Calibrator B	2 pg/ml	1.0 ml
Calibrator C	10 pg/ml	1.0 ml
Calibrator D	50 pg/ml	1.0 ml
Calibrator E	200 pg/ml	1.0 ml
Calibrator F	800 pg/ml	1.0 ml

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Control - Ready To Use.

Contents: One vial containing testosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of testosterone.
Refer to vial label for expected value and acceptable range.

Volume: 1.0 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

6. Biotin Conjugate Buffer - Ready To Use.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 13 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

7. HRP Conjugate Buffer - Ready To Use.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 20 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

8. Wash Buffer Concentrate - Requires Preparation.
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

9. LIA Substrate Reagent A - Requires Preparation.
Contents: One bottle containing luminol enhancer.
Volume: 0.8 ml
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See below.

10. LIA Substrate Reagent B - Requires Preparation.
Contents: One vial containing peroxide solution.
Volume: 1.6 ml

Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See below.

11. LIA Substrate Reagent C - Requires Preparation.
Contents: One vial containing buffer with a non-mercury preservative.
Volume: 15 ml
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See below.

Preparation of Working Substrate Solution:
In a clean plastic container (glass is not suitable) mix **1 part** of the **substrate reagent A** with **1 part of reagent B** and **20 parts of substrate reagent C**. This gives the ready to use substrate solution.
If the whole plate is to be used prepare working substrate solution as follows:
Combine 0.7ml of reagent A with 1.4 ml of reagent B and 14 ml of reagent C.
It is suggested to wait at least 2 minutes prior to use after preparation of the working substrate solution.
The working substrate solution is stable for up to 2 hours at room temperature. Discard the leftovers.

10 ASSAY PROCEDURE

Important Notes:
1. All reagents must reach room temperature before use.
2. Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
3. The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.

Procedure:
Prepare working solutions of both conjugates, wash buffer and LIA substrate (refer to reagents provided and preparation section)
1. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.

2. Pipette 100 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
3. Pipette 100 µL of the testosterone-biotin conjugate working solution into each well (We recommend using a multichannel pipette).
4. Cover the plate and incubate for 60 minutes on a plate shaker at room temperature. (apx. 200 rpm) at room temperature
5. Wash the wells 5 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
6. Pipette 150 µL of the streptavidin-HRP conjugate working solution into each well (We recommend using a multichannel pipette).
7. Cover the plate and incubate for 30 minutes on a plate shaker at room temperature.
8. Wash the wells again in the same manner as step 5.
9. Pipette 150 µL of LIA working substrate solution into each well (We recommend using a multichannel pipette).
10. Measure the RLU/second in each well on a microplate luminometer within 5-15 minutes after addition of the substrate.

11 CALCULATIONS

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 800 pg/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

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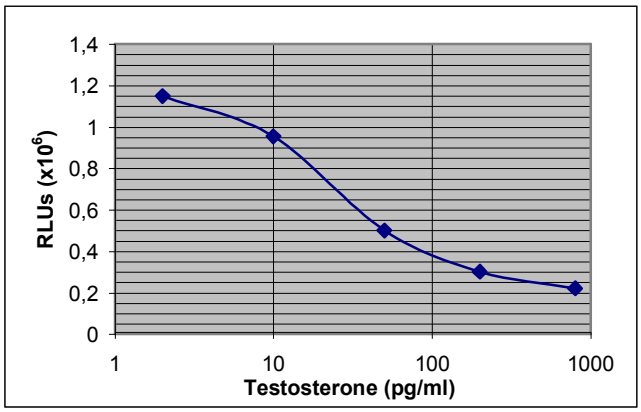
TYPICAL TABULATED DATA**

Calibrator	RLU 1	RLU 2	Mean RLU	RLU/RLUMAX (%)
A, 0 pg/ml	2083570	2021850	2052710	100
B, 2 pg/ml	1880710	1818140	1849425	90
C, 10 pg/ml	1103890	1098340	1101115	54
D, 50 pg/ml	336670	345240	340955	17
E, 200 pg/ml	117500	106900	112200	5
F, 800 pg/ml	45430	45580	45505	2

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

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



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