DRG® PSA Total (CLA-4657)

Revised 14 Feb. 2011 rm (Vers. 3.1) **RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

For determination of total prostate specific antigen (tPSA) in human serum by a chemiluminescence immunoassay (CLIA).

2 PRINCIPLE OF THE TEST

The principle of this chemiluminescence immunoassay (CLIA) test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for total PSA is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of PSA is conjugated to horse radish peroxidase (HRP). Total PSA from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the luminescence substrate is added and the relative luminescence units (RLUs) are measured in a microwell plate luminometer. The RLUs formed by the enzymatic reaction are directly proportional to the concentration of total PSA in the samples.

A set of standards is used to plot a standard curve from which the concentration of tPSA in samples are 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. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.
11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

12. 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.
13. 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 determination of tPSA in human serum. The kit is not calibrated for the determination of tPSA in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any samples from subjects who have received preparation of mouse antibodies should be interpreted with caution.

5 SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

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 0.2 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer.

Store 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

This assay is a direct system; no specimen pretreatment is necessary.

8 REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100 and 300 µL

2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate luminometer

9 REAGENTS PROVIDED AND PREPARATION

1. Mouse Anti-PSA Monoclonal Antibody Coated on Microwell Plate-Break Apart Wells - Ready To Use.
Contents: One 96 well (12x8) monoclonal 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. Mouse Anti-PSA Monoclonal Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.
Contents: Anti-PSA monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.3 mL/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation of conjugate working solution: : Dilute conjugate concentrate 1:100 in assay buffer before use (eg. 20 µl of Conjugate Concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µl of Conjugate Concentrate in 12 ml of assay buffer. Discard any that is left over.

3. PSA Calibrators - Ready To Use.
Contents: Eight vials containing PSA in a protein-base buffer with a non-mercury preservative. Prepared by spiking buffer with an exact quantity of PSA. Calibrated against World Health Organization (WHO) 1st IS 96/670 (90:10).
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 ng/ml	2.0 ml
Calibrator B	0.005 ng/ml	0.5 ml
Calibrator C	0.04 ng/ml	0.5 ml
Calibrator D	0.2 ng/ml	0.5 ml
Calibrator E	1 ng/ml	0.5 ml
Calibrator F	4 ng/ml	0.5 ml
Calibrator G	20 ng/ml	0.5 ml
Calibrator H	100 ng/ml	0.5 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.

4. Controls– Ready To Use.
Contents: Two vials containing PSA in a protein-base buffer with a non-mercury preservative. Prepared by spiking buffer with an exact quantity of PSA. Refer to vial labels for expected value and acceptable range.
Volume: 0.5 ml/vial Storage: Refrigerate at 2-8 °C
Stability: 12 months in unopened vial or as indicated on label. Once opened, the control serum should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. 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.

6. Assay Buffer - Ready To Use.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 30 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

7. Chemiluminescence Substrate Reagent A - Requires Preparation.
Contents: One bottle containing luminol enhancer.
Volume: 1.0 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See below Preparation of Working Substrate Solution

10. Chemiluminescence Substrate Reagent B - Requires Preparation.
Contents: One vial containing peroxide solution.
Volume: 1.0 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See below Preparation of Working Substrate Solution

11. Chemiluminescence Substrate Reagent C - Requires Preparation.
Contents: One vial containing buffer with a non-mercury preservative.
Volume: 16 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See below Preparation of Working Substrate Solution

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 0.5 ml of reagent A with 0.5 ml of reagent B and 10 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:

1. Prepare working solutions of the conjugate, wash buffer and LIA substrate (refer to reagents provided and preparation section).
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µl of assay buffer into each well (We recommend using a multichannel pipette).
5. Cover the plate and incubate for 60 minutes on a plate shaker (approximately 200 rpm) at room temperature.
6. 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).
7. Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
8. Cover the plate and incubate for 30 minutes on a plate shaker (approximately 200 rpm) at room temperature.
9. Wash the wells again in the same manner as step 6.
10. Pipette 100 µl of chemiluminescence working substrate solution into each well (We recommend using a multichannel pipette).
11. Measure the RLUs in each well on a microplate luminometer within 10 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 100 ng/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.

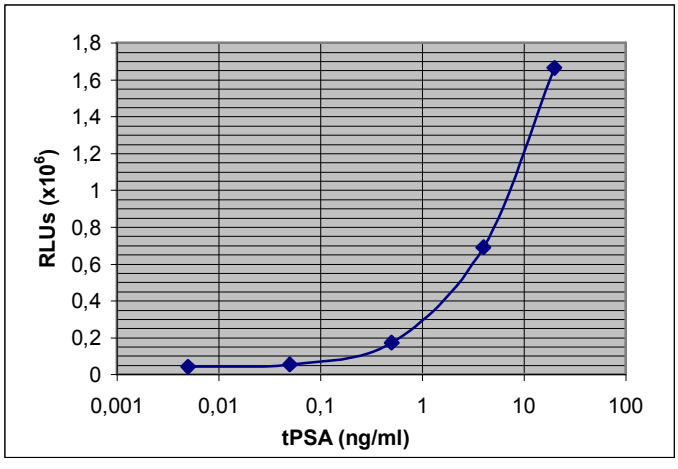
TYPICAL TABULATED DATA**

Calibrator	RLU 1	RLU 2	Mean RLU	RLU/RLU_{MAX} (%)
A, 0 ng/ml	30090	28090	29090	0.29
B, 0.005ng/ml	37310	38170	37740	0.37
C, 0.04 ng/ml	51060	56610	53835	0.53
D, 0.2 ng/ml	115140	121810	118475	1.17
E, 1 ng/ml	364800	326390	345595	3.41
F, 4 ng/ml	1161070	1186690	1173880	11.60
G, 20 ng/mL	4521690	4636920	4579305	45.25
H, 100 ng/ml	9743630	10496220	10119925	100

**- 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 quite different RLU values, however, the RLU/RLU_{MAX} values remain consistent.

TYPICAL CALIBRATOR CURVE

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



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