



**Diagnostics Biochem Canada Inc.**

Manufacturer of In Vitro Diagnostic Test Kits Since 1973

**Direct ELISA Kit...The EiAsy™ Way  
TOTAL PROSTATE SPECIFIC ANTIGEN  
(PSA)**

Cat. No.: CAN-tPSA-4300

Version: 6.0

Effective: May 25, 2004

**INTENDED USE**

For the direct quantitative determination of Total Prostate Specific Antigen by enzyme immunoassay in human serum. For *in vitro* use only.

**PRINCIPLE OF THE TEST**

The principle of the following enzyme immunoassay test follows a typical two-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 enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of total PSA in the sample. A set of standards is used to plot a standard curve from which the amount of total PSA in patient samples and controls can be directly read.

**CLINICAL APPLICATIONS**

Prostate specific antigen (PSA) is a 33-kDa glycoprotein secreted by epithelial cells of the prostate gland. In human serum, PSA is primarily complexed with  $\alpha_1$ -antichymotrypsin, and to a lesser extent with other serum proteins. Only a small portion of PSA is present as the free form (free PSA). The expected normal level of total PSA in male serum is lower than 4 ng/ml. A rise in the concentration of PSA indicates prostate pathology, including benign prostatic hyperplasia and prostate cancer.

**PROCEDURAL CAUTIONS AND WARNINGS**

- 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.
- Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
- 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.
- A calibrator curve must be established for every run.
- The control should be included in every run and fall within established confidence limits.

- 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.
- When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a green colour, in which case it should not be used.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- 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.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

**LIMITATIONS**

- All the reagents within the kit are calibrated for the direct determination of PSA in human serum. The kit is not calibrated for the determination of PSA in saliva, plasma or other specimens of human or animal origin.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
- Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.
- Serum PSA levels should not be interpreted as absolute evidence for the presence or absence of malignant disease.
- PSA expression may be altered due to hormonal prostate treatment. As a result, a low PSA value following this kind of treatment may not adequately reflect the presence of residual or recurrent disease.

**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 contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

**SPECIMEN COLLECTION AND STORAGE**

Approximately 0.1 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.

**SPECIMEN PRETREATMENT**

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

**REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

- Precision pipettes to dispense 25, 50, 100 and 300 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microwell plate reader with a filter set at 415nm and an upper OD limit of 3.0 or greater\* (see assay procedure step 14).

**REAGENTS PROVIDED**

**1. Mouse Anti-PSA Antibody Coated 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 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: 300 µl/vial  
Storage: Refrigerate at 2-8°C  
Stability: 12 months or as indicated on label.  
Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of HRP in 12 ml of assay buffer. Discard any that is left over.

**3. PSA Calibrators** - Ready To Use.

Contents: Six vials containing PSA in a protein-base buffer with a non-mercury preservative. Prepared by spiking buffer with a defined 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 | 0.5 ng/ml     | 0.5 ml |
| Calibrator C | 2 ng/ml       | 0.5 ml |
| Calibrator D | 10 ng/ml      | 0.5 ml |
| Calibrator E | 40 ng/ml      | 0.5 ml |
| Calibrator F | 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. Control** – Ready To Use.

Contents: Two vials containing PSA in a protein-base buffer with a non-mercury preservative. Prepared by spiking bufer with a defined quantity of PSA. Refer to vials label for expected values and acceptable ranges.  
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. TMB Substrate** - Ready To Use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.  
Volume: 16 ml/bottle  
Storage: Refrigerate at 2-8°C  
Stability: 12 months or as indicated on label.

**8. Stopping Solution** - Ready To Use.

Contents: One vial containing 1M sulfuric acid.  
Volume: 6 ml/vial  
Storage: Refrigerate at 2-8°C  
Stability: 12 months or as indicated on label.

**ASSAY PROCEDURE**

Specimen Pretreatment:

**None.**

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-PSA-HRP conjugate and wash buffer.
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. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 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. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
9. Wash the wells again in the same manner as step 6.
10. Pipette 100 µl of TMB substrate into each well at timed intervals.
11. Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator F attains dark green colour for desired OD).
12. Pipette 50 µl of stopping solution into each well at the same timed intervals as in step 10.
13. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

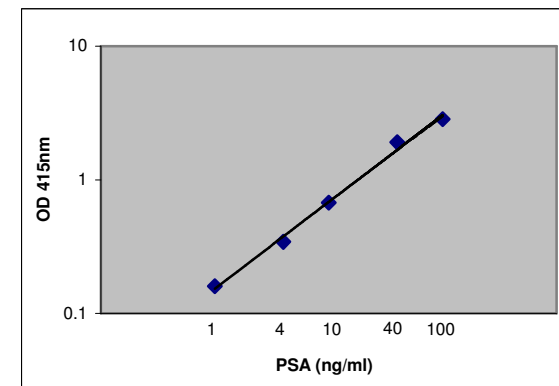
\* If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

**CALCULATIONS**

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, control and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. 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 | OD 1  | OD 2  | Mean OD | Value (ng/ml) |
|------------|-------|-------|---------|---------------|
| A          | 0.095 | 0.090 | 0.093   | 0             |
| B          | 0.164 | 0.156 | 0.160   | 1             |
| C          | 0.343 | 0.345 | 0.344   | 4             |
| D          | 0.658 | 0.688 | 0.673   | 10            |
| E          | 1.958 | 1.857 | 1.908   | 40            |
| F          | 2.869 | 2.790 | 2.830   | 100           |
| Unknown    | 0.295 | 0.289 | 0.292   | 3.3           |

**TYPICAL CALIBRATOR CURVE**Sample curve only. **Do not** use to calculate results.**PERFORMANCE CHARACTERISTICS****SENSITIVITY**

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the dbc Direct Total PSA ELISA kit is **0.1 ng/ml**.

**INTRA-ASSAY PRECISION**

Two samples were assayed ten times each on the same calibrator curve. The results (in ng/ml) are tabulated below:

| Sample | Mean  | SD   | CV% |
|--------|-------|------|-----|
| 1      | 5.59  | 0.02 | 6.1 |
| 2      | 22.89 | 0.06 | 4.6 |

**INTER-ASSAY PRECISION**

Two samples were assayed ten times over a period of four weeks. The results (in ng/ml) are tabulated below:

| Sample | Mean | SD   | CV% |
|--------|------|------|-----|
| 1      | 0.92 | 0.09 | 9.5 |
| 2      | 3.64 | 0.14 | 3.8 |

**RECOVERY**

Spiked samples were prepared by adding defined amounts of PSA to three patient serum samples. The results (in ng/ml) are tabulated below:

| Sample     | Obs.Result | Exp.Result | Recovery% |
|------------|------------|------------|-----------|
| 1 Unspiked | 7.00       | -          | -         |
| +0.86      | 7.79       | 7.86       | 99.1      |
| +9.43      | 16.42      | 16.43      | 99.9      |
| +26.57     | 39.66      | 33.57      | 118.1     |
| 2 Unspiked | 2.72       | -          | -         |
| +2.08      | 4.65       | 4.80       | 96.8      |
| +10.65     | 14.97      | 13.37      | 111.9     |
| +27.8      | 34.92      | 30.52      | 114.4     |
| 3 Unspiked | 14.26      | -          | -         |
| +7.35      | 20.32      | 21.61      | 94.0      |
| +24.49     | 39.85      | 38.75      | 102.8     |
| +32.15     | 47.49      | 46.41      | 102.3     |

**LINEARITY**

Three patient serum samples were diluted with calibrator A. The results (in ng/ml) are tabulated below:

| Sample | Obs.Result | Exp.Result | Recovery% |
|--------|------------|------------|-----------|
| 1      | 39.66      | -          | -         |
| 1:2    | 18.33      | 19.83      | 92.4      |
| 1:4    | 8.93       | 9.92       | 90.0      |
| 1:8    | 4.56       | 4.96       | 91.9      |
| 2      | 34.92      | -          | -         |
| 1:2    | 17.80      | 17.46      | 101.9     |
| 1:4    | 8.16       | 8.73       | 93.5      |
| 1:8    | 4.08       | 4.36       | 93.6      |
| 3      | 47.49      | -          | -         |
| 1:2    | 23.24      | 23.75      | 97.9      |
| 1:4    | 12.90      | 11.87      | 108.7     |
| 1:8    | 6.67       | 5.94       | 112.3     |

**COMPARATIVE STUDIES**

The dbc Direct Total PSA ELISA kit (y) was compared with a competitors Total PSA ELISA kit (x). The comparison of 168 serum samples yielded the following linear regression results:  
 $y=0.7972x+0.8551$   
 $r=0.9837$

**HIGH DOSE HOOK EFFECT**

The Direct Total PSA ELISA kit did not experience a high dose hook effect when it was tested up to a PSA concentration of 1000 ng/ml.

**EXPECTED NORMAL VALUES**

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. Serum samples from a group (n=96) of apparently healthy males and females aged from 3-70 years old were assayed with the dbc Direct PSA ELISA Kit. Results are as follows

| Group                              | Range (ng/ml) |
|------------------------------------|---------------|
| Males and Females (3-70 years old) | All <4.0      |

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**ISO 9001:2000 and ISO 13485 Certified**

**OTHER RELATED dbc ELISA KITS**

Also available from stock are the following DBC ELISA kits:

➤ dbc Direct Total Free PSA ELISA Kit,  
 Cat. No.: CAN-fPSA-4400

Please contact us if you require any further information.