**FREE PROSTATE SPECIFIC ANTIGEN (PSA) ELISA**

**USA:** For Research Use Only. Not for Use in Diagnostic Procedures.

**FREE PROSTATE SPECIFIC ANTIGEN** by an enzyme immunoassay in human serum.

**PRINCIPLE OF THE TEST**

The principle of the following enzyme immunoassay test provides a typical one-step capture or "sandwich" type assay. The assay makes use of two highly specific monoclonal antibodies. A monoclonal antibody specific for PSA is immobilized onto the microplate and another monoclonal antibody specific for a different region of PSA is conjugated to horse radish peroxidase (HRP). PSA from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decantation steps remove unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured in a microtiter plate reader. The intensity of colour formed by the enzymatic reaction is directly proportional to the concentration of PSA in the sample. A set of standards is used to plot a standard curve from which the amount of PSA in patient samples and controls can be directly read.

**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 α1-antichymotrypsin, and to a lesser extent with other serum proteins. Only a small fraction of PSA is present as the free form (fPSA).

The expected normal level of PSA in male serum is lower than 4.0 ng/mL. A rise in the concentration of PSA correlates pathologically, including benign prostatic hyperplasia (BPH) and prostate cancer.

Free PSA (fPSA) has been studied in attempts to help distinguish BPH from untreated prostate cancer. These studies have shown that the ratio of free PSA to total prostate-specific antigen (PSA) is lower in untreated prostate cancer than in patients with BPH.

**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 control 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. Control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.

5. 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 PRECAUTIONS**

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If touched with any of the reagents, wash with plenty of water. TMB is a suspected carcinogen.

**SPECIMEN COLLECTION AND STORAGE**

Approximately 0.2 mL of serum is required for determination. Collect 4-5 mL of blood into an appropriately labeled 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 possibly 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**

- 1. Mouse Anti-PSA Antibody-Coated Break-Apart Well Microplate — Ready To Use
- 3. Substrate Concentrate — Ready To Use
- 4. Controls
- 5. Stop Solution
- 6. A calibrator curve must be established for every run. The controls should be included in every run and fall within the established confidence limits. If contacted with any of the reagents, wash with plenty of water. TMB is a suspected carcinogen.

**CHEMICAL HAZARDS**

- Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If touched with any of the reagents, wash with plenty of water. TMB is a suspected carcinogen.

**SPECIMEN COLLECTION AND STORAGE**

- Approximately 0.2 mL of serum is required for determination. Collect 4-5 mL of blood into an appropriately labeled 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 possibly biohazardous materials and take appropriate precautions when handling.

**SPECIMEN PRETREATMENT**

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

**REAGENTS PROVIDED**

- Mouse Anti-PSA Antibody-Coated Break-Apart Well Microplate — Ready To Use
- Mouse Anti-PSA Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X10
- Substrate Concentrate — Ready To Use
- Controls
- Stop Solution
- Calibration Standards:
  - Calibrator A 0 ng/mL 2.0 mL
  - Calibrator B 0.1 ng/mL 0.5 mL
  - Calibrator C 0.5 ng/mL 0.5 mL
  - Calibrator D 2.0 ng/mL 0.5 mL
  - Calibrator E 5 ng/mL 0.5 mL
  - Calibrator F 15 ng/mL 0.5 mL

**STORAGE**

- Refrigerate at 2–8°C.
- Store in the original unopened vials or as indicated on label.

**CONTROLS**

- Contents: Two vials containing PSA in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of PSA. Refer to vial label for the acceptable range.
- Volume: 0.5 mL/vial
- Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 24 hours of thawing and stored frozen. Avoid multiple freezing and thawing cycles.

**WASH BUFFER CONCENTRATE**

- Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
- Volume: 16 mL/bottle
- Stability: 12 months or as indicated on label.
- Storage: Refrigerate at 2–8°C.

**ASSAY BUFFER**

- Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
- Volume: 48 mL/bottle
- Stability: 12 months or as indicated on label.
- Storage: Refrigerate at 2–8°C.

**TMB SUBSTRATE**

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

**STOPPING SOLUTION**

- Contents: One bottle containing 1M sulfuric acid.
- Volume: 6 mL/bottle
- Stability: 12 months or as indicated on label.
- Storage: Refrigerate at 2–8°C.
1. Prepare working solutions of the anti-PSA-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reassemble the bag and return any unused strips to the refrigerator.
3. Pipette 50 µL of each calibrator, control and specimen sample into a corresponding labelled well in duplicate.
4. Pipette 100 µL of the conjugate working solution 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 plates 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbant paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
9. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 8.
10. Read the plate on a microplate reader at 450 nm filter.

**CALCULATIONS**

1. Calculate the mean optical density of each calibrator sample.
2. Calculate the mean optical density of each unknown sample.
3. Subtract the mean absorbance value of the "0" calibrator curve. The results (in ng/mL) are tabulated below:
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 or 5-parameter curve is recommended.
5. The DBC Direct fPSA ELISA kit (y) was compared with a competing fPSA ELISA kit (x). The concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. The sensitivity of the DBC Direct fPSA ELISA kit is 0.05 ng/mL.

**INTRA-ASSAY PRECISION**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.24</td>
<td>0.02</td>
<td>9.6</td>
</tr>
<tr>
<td>2</td>
<td>0.19</td>
<td>0.08</td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td>0.19</td>
<td>0.10</td>
<td>5.6</td>
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</table>

**INTER-ASSAY PRECISION**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.24</td>
<td>0.03</td>
<td>12.2</td>
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<tr>
<td>2</td>
<td>1.15</td>
<td>0.13</td>
<td>11.1</td>
</tr>
<tr>
<td>3</td>
<td>3.13</td>
<td>0.21</td>
<td>6.6</td>
</tr>
</tbody>
</table>

**COMPARATIVE STUDIES**

The DBC Direct fPSA ELISA kit (y) was compared with a competitor’s fPSA ELISA kit (x). The comparison of 16 serum samples yielded the following linear regression results:

\[ y = 0.8406x + 0.7096 \]

**HIGH DOSE HOOK EFFECT**

The DBC Direct fPSA ELISA kit did not experience a high dose hook effect when it was tested up to a fPSA concentration of 200 ng/mL.

**REFERENCES**


**SYMBOLS**

**REF** Catalogue Number

**LOT** Lot number

**WD** In vitro diagnostic device for use

**LM** Contains information for legal use

**X R** Dilute 1:5 before use