Total Prostate Specific Antigen (tPSA) Test System

Product Code: 2125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (tPSA) Concentration in Human Serum by a Microparticle Immunoenzymometric assay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific antigen (PSA) is a serine protease with chymotrypsin-like activity (1,2). The protein is a single chain glycoprotein with a molecular weight of 28.4 kDa (3). PSA derives its name from the observation that it is a normal antigen of the prostate but is not found in any other normal or malignant tissue. PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients with prostate cancer (4).

In this method, PSA-calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PSA) are added and the reagents mixed. Reaction between the various PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-PSA antibody bound conjugate is separated from the unbound enzyme-PSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known prostate specific antigen (PSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with PSA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{EnzAb(p)} + \text{AgPSA} + \text{BtnAb(m)} \rightarrow \text{EnzAb(p)}\text{-AgPSA-BtnAb(m)}
\]

\[\text{EnzAb(p)}\text{-AgPSA-BtnAb(m)} + \text{Streptavidin} \rightarrow \text{Immobilized complex}
\]

\[\text{Immobilized complex} \rightarrow \text{complex bound to the solid surface}
\]

Note 1: Do not use reagents beyond the kit expiration date.

4.0 REAGENTS

Materials Provided:

A. Prostate Specific antigen (PSA) 1ml/vial – Icons A-F

Six (6) vials of references PSA antigen at levels of 0(A), 5(B), 10(C), 25(D), 50(E) and 100(F) ng/ml. Store at 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 1st IS 96/670.

B. PSA Enzyme Reagent – 13ml/vial - Icon

One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells - Icon

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

E. Substrate A – 7ml/vial - Icon

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon

One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon

One (1) bottle containing a strong acid (1N HCl). Store at 2-30°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.

5.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).**

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of the PSA Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 30 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well-to-well variations) in a suitable microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PSA in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding PSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of PSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (11.42) intersects the dose response curve at (23.6 ng/ml) PSA concentration (See Figure 1).
Highly lipemic, hemolysed or grossly contaminated specimen(s) should not be used.

5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

6. PSA is elevated in benign prostrate hypertrophy (BPH). The PSA value alone is not of diagnostic value as a specific test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures (prostate biopsy). Free PSA determinations may be helpful in regard to the discrimination of BPH and prostate cancer conditions (5).

13.0 EXPECTED RANGES OF VALUES

Healthy males are expected to have values below 4 ng/ml (4).

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within- and between-assay precisions of the tPSA AccuBind™ ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

### Table 2: Within Assay Precision (Values in ng/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>0.8</td>
<td>11.3%</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>4.3</td>
<td>5.8%</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>27.5</td>
<td>14.2%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.*

14.2 Sensitivity

The tPSA AccuBind™ ELISA test system has a sensitivity of 0.012 ng. This is equivalent to a sample containing 0.5 ng/l tPSA concentration.

14.3 Accuracy

The tPSA AccuBind™ ELISA method was compared with a reference Elisa method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the tPSA AccuBind™ ELISA test method in comparison with the reference method. The data obtained is shown in Table 4.

### Table 4: Least Square Regression Analysis

<table>
<thead>
<tr>
<th>Method Mean</th>
<th>y = -0.0598 + 0.987x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>5.57</td>
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Only slight amounts of bias between the tPSA AccuBind™ ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

No interference was detected with the performance of tPSA AccuBind™ ELISA test system upon addition of massive amounts of the following substances to a human serum pool.

- Acetylsalicylic Acid: 100 μg/ml
- Ascorbic Acid: 100 μg/ml
- Caffeine: 100 μg/ml
- CEA: 10 μg/ml
- AFP: 10 μg/ml
- CA-125: 10,000 U/ml
- NGC: 1000 U/ml
- NLD: 10 U/ml
- NTSH: 100 mIU/ml
- HPRL: 100 μg/ml

15.0 REFERENCES
