The activity of the enzyme present on the surface of the well is its name from the observation that it is a normal antigen of the Serum by a Microplate Enzyme Immunoassay, Colorimetric well.

The employment of several serum references of known total PSA forms a reaction between the various tPSA antibodies and native tPSA forms a complex. After the completion of the required incubation period, the enzyme-labeled antibodies (directed against distinct and different epitopes) are added and the reaction is mixed. Reaction between the various PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin-coated well.

After the completion of the required incubation period, the enzyme-PSA antibody complex is separated from the unbound enzyme-PSA antibodies by decantation or aspiration. 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 total prostate specific antigen (tPSA) 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 tPSA concentration.

### 3.0 PRINCIPLE

**Immunoenzymometric assay (TYPE 3):**

The essential variables for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immunoconjugates), with different and distinct epitope recognition, in absence, and native antigen. In this procedure, the immunometric assay 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 enzyme-antibody complex. The interaction is illustrated by the following equation:

\[
\frac{d[A]}{dt} = k_{on} [E][A] - k_{off} [E][A]
\]

5.0 PRECAUTIONS

2. Pipette 0.025mL (25µL) of the appropriate serum reference calibrator, control or specimen into the assigned well.

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

5. Incubate the microplate gently for 20-30 minutes to mix and equalize.

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

7. Add 0.350mL (350µL) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat twice (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.

8. Wash the plate and repeat (2) additional times.

10. Add 0.100mL (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.

10.0 CALCULATION OF RESULTS

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

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

2. Plot a standard curve for each serum reference versus the corresponding iPSA concentration in ng/mL on linear graph paper (do not average the duplicates of the serum references before plotting the points).

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

4. To determine the concentration of iPSA for an unknown, locate the average blank absorbance value and 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 for the unknown is determined to be 2.833 ng/mL.

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

### EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Well Number</th>
<th>Abs (A)</th>
<th>Mean Abs (B)</th>
<th>Value (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>0.019</td>
<td>0.019</td>
<td>0</td>
</tr>
<tr>
<td>Cal B</td>
<td>C1</td>
<td>0.276</td>
<td>0.276</td>
<td>5</td>
</tr>
<tr>
<td>Cal C</td>
<td>E1</td>
<td>0.567</td>
<td>0.563</td>
<td>10</td>
</tr>
<tr>
<td>Cal D</td>
<td>G1</td>
<td>1.248</td>
<td>1.213</td>
<td>25</td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>2.051</td>
<td>1.999</td>
<td>50</td>
</tr>
<tr>
<td>Cal F</td>
<td>D2</td>
<td>2.775</td>
<td>2.833</td>
<td>100</td>
</tr>
<tr>
<td>Patient</td>
<td>E2</td>
<td>1.186</td>
<td>1.143</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>1.099</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The reagents for AccuBind® ELISA procedure have been formulated to eliminate random interference; however, potential interactions between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato, LM, Stuart, MC. "Heterophilic antibodies: a problem for all immunoassays?" Clin. Chem. 1988: 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges.

5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

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

7. PSA is elevated in benign prostate hyperplasia (BPH). Clinically, an elevated 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.

8. Due to the variation in the calibration used in tPSA/ fPSA test kits and differences in epitope recognition of different antibodies, it is always suggested that the patient sample should be tested with tPSA/ fPSA tests made by the same manufacturer. (Monobind Inc. offers a tPSA ELISA test that should be used for consistency reasons, when needed.)

9. PSA is elevated in benign prostate hypertrophy (BPH). Clinically, an elevated 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.

10. Accurate and precise pipetting, as well as following the exact laboratory is located.

11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

12. Interpretation: Measurements and interpretation of results must be performed by a skilled individual or trained professional. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.


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.

15.0 REFERENCES


Revision: D  Date: 2019-Jul-16 DOC: 1353 MP: 2125 Product Code: 2125-100