1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (PSA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

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. PSA derives its name from the observation that it is a tumor 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; detection of 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 due to increased sensitivity.3

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 different and distinct 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 unbound antigen by decantation or aspiration. The activity enzyme in the antibody-bound fraction is directly proportional to the amount of antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:
A. PSA Calibrators – 1 ml/vial – icons A-F
B. Enzyme Reagent – 13 ml/vial – Icon
C. (see Reagent Preparation Section).
D. Wash Solution Concentrate – 20 ml/vial – Icon
E. Stop Solution – 8 ml/vial – Icon
F. PSA Calibrators – 1 ml/vial – Icons A-F
G. Product Instructions.

Pour the contents of the amber vial labeled Solution ' A' into a clear vial for easy identification. Mix and label accordingly.

Note 2: Do not use reagents that are contaminated or have been refrigerated or frozen. Samples may be refrigerated at 2-8°C for a maximum period of one year (365 days) from the expiration date. When assayed in duplicate, 0.050 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in the same manner as unknown and low controls. The average absorbance of the duplicates for each unknown should be used for determining the specimen.

8.0 REAGENT PREPARATION

A. Wash Buffer

Dilute contents of wash concentrate to 100 ml with distilled or deionized water in a suitable container. Store diluted buffer at 2-30°C for up to 60 days.

B. Working Substrate Solution – Stable for one year

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

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the bottle.

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

3. Add 0.100ml (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. Incubate the microplate for 20-30 seconds to mix and cover.

5. Incubate 30 minutes at room temperature.

6. Incubate the contents for an additional 20 minutes by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (and blot) and 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 0.100ml (100µl) of substrate solution to each well (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. Read the absorbance within thirty (30) minutes of adding the stop solution.

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

4.1 Required But Not Provided: 

1. Pipette(s) capable of delivering 0.025, 0.050 & 0.100 ml (25, 50, & 100 µl) with a precision of better than 1.5%.

2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.

3. Microplate washers or a squeeze bottle (optional).

4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.

5. Absorbant paper for blotting the microplate wells.

6. Manual plate washer can be used. Follow the manufacturer’s instructions 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.

1. 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.

6. Quality control materials

**For In Vitro Diagnostic Use**

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can completely assess if the virus agents are present, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory practice and handwashing should be followed in all laboratory procedures.


Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6. SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual sample volume for the test. Serum samples from the in vitro diagnostic procedures sample should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red top venipuncture tube without additives or anti-coagulants. Allow the clot to clot. Centrifugate the specimen to separate the serum from the cells.

In patients receiving therapy with high blood doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Note 2: Do not use reagents that are contaminated or have been refrigerated or frozen. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. The specimen cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 ml (50 µl) of the specimen is required.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PSA for an unknown. Compare the dose response curve of the unknown with those of the known. The activity enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

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 mg/ml or ng/ml on a linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best fit line 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 horizontal axis, and read the corresponding PSA concentration mg/ml or ng/ml. Significant deviation from established performance can be determined depending on the graph (the duplicates of the unknowns may be averaged as indicated). In the following example, the average absorbance (1.142) intersects the dose response curve at 2.050 mg/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 LD.</th>
<th>Well Number</th>
<th>Abs (A)</th>
<th>Mean Abs (B)</th>
<th>Value (mg/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>B1</td>
<td>0.279</td>
<td>0.276</td>
<td>5</td>
</tr>
<tr>
<td>Cal C</td>
<td>C1</td>
<td>0.567</td>
<td>0.563</td>
<td>10</td>
</tr>
<tr>
<td>Cal D</td>
<td>D1</td>
<td>1.248</td>
<td>1.213</td>
<td>25</td>
</tr>
<tr>
<td>Cal E</td>
<td>E1</td>
<td>2.051</td>
<td>1.999</td>
<td>50</td>
</tr>
<tr>
<td>Cal F</td>
<td>F1</td>
<td>2.892</td>
<td>2.833</td>
<td>100</td>
</tr>
<tr>
<td>Patient</td>
<td>G1</td>
<td>1.186</td>
<td>1.186</td>
<td>23.6</td>
</tr>
</tbody>
</table>
In the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the assay on request from Monobind Inc.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. 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.
7. 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 conditions.
8. Due to the variation in the calibration used in PSA/PSA test kits and differences in epitopic recognition of different antibodies, it is always suggested that the patient sample should be tested with PSA/PSA tests made by the same manufacturer.

14.0 PERFORMANCE CHARACTERISTICS

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

TABLE 1

Expected Values for PSA AccuBind® ELISA Test System

<table>
<thead>
<tr>
<th>PSA Values in ng/ml</th>
<th>Normal</th>
<th>≤ 1 ng/ml</th>
<th>1.01-4.01 ng/ml</th>
<th>4.01-10 ng/ml</th>
<th>&gt; 10 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Males</td>
<td>1.06</td>
<td>0.05</td>
<td>2.12</td>
<td>3.18</td>
<td>3.28</td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of normal persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

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

TABLE 2

Within Assay Precision (Values in ng/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>2.06</td>
<td>10.2%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>3.56</td>
<td>18.1%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>23.07</td>
<td>88.4%</td>
</tr>
</tbody>
</table>

TABLE 3

Between Assay Precision (Values in ng/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>Sigma</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>0.98</td>
<td>0.08</td>
<td>8.5%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>3.35</td>
<td>0.19</td>
<td>5.7%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>23.17</td>
<td>0.95</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

Only slight margins of bias between the PSA AccuBind® ELISA test system and the reference method are indicated by the closeness of the means. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity:

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

15.0 REFERENCES


Revision: Date: 2022-MAY-01, DCO: 1557, MP2125, Product Code: 2125-300