The employment of several serum references of known prostate specific antigen (PSA) levels permits the construction of a dose response curve of activity vs. 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 high affinity and specificity antigen, an excess, and native antigen. In this procedure, the immobilization takes place during the reaction on 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:

\[ \text{EnzAb(p)} - \text{AgfPSA} - \text{BtnAb(m)} = \text{Antigen-Antibodies Complex} \]

The rate constant of association and dissociation are given as:

\[ k_a = \text{Rate Constant of Association} \]
\[ k_a = \text{Rate Constant of Dissociation} \]

The antibodies that are biotinylated are immobilized through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[ \text{AgfPSA} = \text{Native Antigen (Variable Quantity)} \]
\[ \text{BtnAb(m)} = \text{Biotinylated Antibody (Excess Quantity)} \]
\[ \text{EnzAb(p)} = \text{Enzyme Labeled Antibody (Excess Quantity)} \]
\[ \text{EnzAb(p)} - \text{AgfPSA} - \text{BtnAb(m)} = \text{Antigen-Antibodies Complex} \]

The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing monoclonal antibodies of different antigen epitopes, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. IPSA Calibrators – 1ml/vial - Icos A-F

Six (6) vials of serum references free PSA antigen at levels of

0(A), 0.5(B), 1(C), 2.5(D), 5.0(E) and 10.0(F) ng/ml. A preservative has been added. Store at 2-8°C.

B. IPSA Tracer Reagent – 13 ml/vial - Icon

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

C. Light Reaction Wells 96 wells – Icon

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

D. Wash Solution Concentrate – 20ml/vial - Icon

One (1) vial containing a surfactant in buffer saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7ml/vial - Icon G

One (1) vial containing luminescent buffer. Store at 2-8°C. (See Reagent Preparation Section)

F. Signal Reagent B – 7ml/vial - Icon G

One (1) vial containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. (See Reagent Preparation Section)

G. Product Instructions

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

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the pack.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette(s) capable of delivering 0.050 & 0.100ml (50 & 100µl) volumes 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% (optional).

3. Microplate washer and a squeeze bottle for incubation steps.

4. Microplate Luminometer

5. Absorbent Paper for blotting the microplate wells.

6. Plastic wrap or micropipette cover for incubation steps.

7. Vacuum aspirator (optional) for wash steps.

8. Timer

9. Quality control materials

5.0 PRECAUTIONS

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive with Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can confirm conclusively that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, “Biobasics in Microbiological and Biomedical Laboratories,” 2nd Edition, National Institutes of Health, 89-8395.

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

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual specimen should be collected in a plain red-top venipuncture tube without additives or anti-coagulants. All plasma should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. To determine the reason for the variations. Significant deviation from established performance can be determined using a reference preparation, which was assayed accordingly.

7.0 QUALITY CONTROL

Each laboratory should assure 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 all other test procedures performed. Quality control charts can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute concentrated Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C.

2. Reagent Signal Reagent - Store at 2 - 8°C.

Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B.

Note: For In Vitro Diagnostic Use

For Internal or External Use in Humans or Animals

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

9.0 CALCULATION OF RESULTS

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

1. Record the RLUs obtained from the printout of microplate reader.

2. Plot the RLUs for each duplicate serum reference versus the corresponding dose in ng/ml for standard curve (ng/ml). A straight line should be observed. For accurate comparison to established normal values, a fasting morning sample should be obtained. The blood should be processed and manipulations on white blood cell tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid exposure to contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100 µl) of the specimen is required.

9.0 TEST PROCEDURE

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

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

1. Format the microplate’s wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into plastic wrap bag, see procedure at 2-8°C.

2. Pipette 0.050ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.

3. Add 0.100ml (100µl) of the IPSA Tracer Reagent to each well.

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

5. Incubate 45 minutes at room temperature.

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

7. Add 0.100ml (100µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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. Discard the wash and repeat four (4) additional times.

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

9. Incubate for five (5) minutes at room temperature in the dark.

10. Incubate for five (5) minutes at room temperature in the dark.

11. Add 0.350ml (350µl) of luminol in buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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. Discard the wash and repeat four (4) additional times.

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
7. Use components from the same lot. No intermixing of reagents.
6. Failure to remove adhering solution adequately in the device usage.
5. The addition of signal reagent initiates a kinetic reaction, and if more than one (1) plate is used, it is recommended to repeat measurement of light output.
4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the photon counting system are within established ranges.
3. The reagents for AccuLite® CLIA procedure have been formulated to eliminate maximal 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. 1983; 29:272-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and other clinical findings.
2. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
1. The Dose Response Curve (80%; 50% & 20% intercepts) should be evaluated by the interpolation of the mean plus two standard deviations of each control sera.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>0.46</td>
<td>0.05</td>
<td>10.3%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>1.77</td>
<td>0.08</td>
<td>4.5%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

11.0 QC. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve (80%; 50% & 20% intercepts) should be evaluated by the interpolation of the mean plus two standard deviations of each control sera.
2. 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.
3. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the photon counting system are within established ranges.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
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 imperative that the predicted values for the photon counting system are within established ranges.
7. If the total PSA (PSA) reads 4-10 ng/ml the ratio is useful in the differential diagnosis of BPH and PC (Prostate Cancer). Depending on the ratio the probability can be determined as follows:

<table>
<thead>
<tr>
<th>PSA/PSA Ratio</th>
<th>Probability of Prostate Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10%</td>
<td>55%</td>
</tr>
<tr>
<td>10-15%</td>
<td>25%</td>
</tr>
<tr>
<td>15% -20%</td>
<td>15%</td>
</tr>
<tr>
<td>&gt; 20%</td>
<td>10%</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>0.46</td>
<td>0.05</td>
<td>10.3%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>1.77</td>
<td>0.08</td>
<td>4.5%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form 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 dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-during reaction.
6. Failure to remove adhering solution adequately in the device usage may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.

13.0 EXPECTED RANGES OF VALUES

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Acetylsalicic Acid</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Caffeine</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Dexametshasone</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Methotroxate</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Prostagl</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>TSH</td>
<td>100 µM/Liter</td>
</tr>
</tbody>
</table>

15.0 REFERENCES