**INTRODUCTION OF PSA IMMUNOASSAY**

Chemiluminescence Immunoassay (CLIA) detection plays an important part in the Diagnostic and Research areas that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advancements, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do. But not in apparently healthy men, men with non-prostatic carcinoma, apparently healthy women, or women with cancer. Reports have suggested that serum PSA is one of the most useful tumor markers in oncology. It may serves as an accurate marker for assessing response to treatment in patients with prostatic cancer. Therefore, measurement of serum PSA concentrations can be an important tool in monitoring patients with prostatic cancer and in determining the potential and actual effectiveness of surgery or other therapies. Recent studies also indicate that PSA measurements can enhance early prostate cancer detection when combined with digital rectal examination (DRE).

**TEST PRINCIPLE**

Immunospec PSA CLIA test is a solid phase two-site immunoassay. One antibody is coated on the surface of the microtiter wells and another antibody labeled with horseradish peroxidase is used as the tracer. The PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by adding the substrate reagents and undergoing the chemiluminescent reactions. The intensity of the emitting light from the associated well is proportional to the amount of enzyme present and is directly related to the amount of PSA antigen in the sample. By reference to a series of PSA standards assayed in the same way, the concentration of PSA in the unknown sample is quantified.

**MATERIALS AND COMPONENTS**

**Materials provided with the test kits:**
1. Anti-PSA antibody coated 96 well microtiter plate.
2. Assay buffer; 12 ml.
3. Enzyme conjugate reagent; 12 ml.
4. PSA reference standards set, contains 0, 2, 4, 15, 50, and 100 ng/ml PSA, liquid; Ready for use.
5. 20x Wash Buffer; 30 ml.
6. Chemiluminescence Reagent A; 6.0 ml
7. Chemiluminescence Reagent B; 6.0 ml

**Materials required but not provided:**
1. Distilled water.
2. Precision pipettes: 0.05ml, 0.1ml, 0.2ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well reader.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

**REAGENT PREPARATION**

1. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder. Dispense 50μL of PSA standards, specimens, and controls into appropriate wells.
2. Dispense 100μL of Assay Buffer to each well. Mix gently for 30 seconds.
3. Incubate at room temperature for 45 minutes.
4. Remove the incubation mixture by emptying the plate content into a waste container.
5. Rinse and flick the microtiter wells 5 times with washing buffer.
6. Strike the wells sharply onto absorbent paper to remove residual water droplets.
7. Dispense 100μL of enzyme conjugate reagent into each well. Mix well.
8. Incubate at room temperature for 60 minutes.
10. Remove the contents and wash the plate as described in step 4,5, and 6 above.
11. Dispense 100µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
12. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

Important Note:
1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bubbles existing in the wells, the false readings will be created. Please use distilled water to remove the bubbles before adding the substrate.

CALCULATION OF RESULTS
1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regrression or cubic spline regaression. If the software is not available, construct a standard curve by parameter regrassion or cubic spline regaression. If the results. The best curve fitting used in the assays are 4-
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EXAMPLE OF STANDARD CURVE
Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

<table>
<thead>
<tr>
<th>PSA (ng/ml)</th>
<th>Relative Light Units (RLU) (10^4)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>2</td>
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<td>50</td>
<td>93.50</td>
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<tr>
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<td>150.03</td>
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</tbody>
</table>

EXPECTED VALUES AND SENSITIVITY
Healthy males are expected to have PSA values below 4 ng/ml. The minimum detectable concentration of PSA in this assay is estimated to be 0.5 ng/ml.

REFERENCES
10. McCarthy, R.C.; Jakubowski, H.V. and Markowitz, H. Human prostate acid phosphatase concentration on the horizontal (x) axis.