Total Prostate-Specific Antigen ELISA (PSA ELISA)

For the quantitative determination of PSA in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 25-PSAHU-E01
Size: 96 wells
**PRINCIPLE OF THE TEST**

The PSA (Total) ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a goat anti-PSA antibody directed against intact PSA for solid phase immobilization (on the microtiter wells). A monoclonal anti-PSA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react first with the immobilized goat antibody at room temperature for 60 minutes. The wells are washed to remove any unbound antigen. The monoclonal anti-PSA-HRP conjugate is then added and allowed to react with the immobilized antigen for 60 minutes at room temperature resulting in the PSA molecules being sandwiched between the solid phase and enzyme-linked antibodies.

The wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution which changes the color to yellow. The concentration of PSA is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

**REAGENTS**

*Materials provided with the kits:*

- Goat anti-PSA coated microtiter plate with 96 wells.
- Zero Buffer, 7 ml.
- Standards containing 0, 2, 4, 15, 60, and 120 ng/ml PSA, 1 ml each, ready to use.
- Enzyme Conjugate Reagent, 12 ml.
- TMB Reagent (one step), 11 ml.
- Stop Solution (1 N HCl), 11 ml.

**STORAGE OF TEST KIT AND INSTRUMENTATION**

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided they are stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use.

**REAGENT PREPARATION**

All reagents should be brought to room temperature (18-25°C) before use.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, samples, and controls (optional, not included with kit) into appropriate wells.
3. Dispense 50 µl of Zero Buffer into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this step.
5. Incubate at room temperature for 60 minutes.
6. Remove the incubation mixture by emptying plate contents into a waste container.
7. Rinse and empty the microtiter wells 5 times with *distilled or deionized water. (Do not use tap water)*.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature (18-25°C) for 60 minutes.
11. Remove the incubation mixture by emptying plate contents into a waste container.
12. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Do not use tap water.)
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature (18-25°C) for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. It is important to confirm that all the blue color changed completely to yellow.
18. Using a microtiter plate reader, read the optical density at 450 nm within 15 minutes.

CALCULATION OF RESULTS
1. Calculate the average absorbance value \( A_{450} \) for each standard, control (optional, not included with kit), and sample.
2. Construct a standard curve by plotting the mean absorbance obtained for each standard against its concentration in ng/ml on linear graph paper (or appropriate computer program), with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of PSA in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE
Results of a typical standard run with optical density readings at 450 nm shown on the Y-axis against PSA concentrations shown on the X-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own data and standard curve for each assay run.

<table>
<thead>
<tr>
<th>PSA (ng/ml)</th>
<th>Absorbance (450 nm)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0.066</td>
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<tr>
<td>2</td>
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<tr>
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<td>0.545</td>
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