PROSTATE SPECIFIC ANTIGEN (PSA) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00022

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Enzyme Immunoassay for the Quantitative Determination of Prostate Specific Antigen (PSA) in Human Serum

NOTE: When determining PSA values using assays from different manufacturers, the concentration of PSA in a given specimen can vary due to differences in assay methods and reagent specificity. Values obtained with different assay methods cannot be used interchangeably, and the results reported to the physician must include the identity of the PSA assay used. If in the course of monitoring a sample, the method used for serial determination of PSA is changed, additional sequential testing should be carried out, and the baseline value of the sample MUST be confirmed.

FOR RESEARCH PURPOSES ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE ASSAY

PSA ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[18-20] 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 reacted 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 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 1N HCl changing 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 AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (1 96-well plate, 12 x 8 strips)*
   Microtiter wells coated with goat anti-PSA.
2. *Zero Buffer (7 mL)*
   Contains bovine serum proteins in Tris buffer (pH = 7.60) with preservatives.
3. *Enzyme Conjugate Reagent (12 mL)*
   Contains monoclonal anti-PSA conjugated to horseradish peroxidase in Tris buffer (pH = 7.60) with preservatives.
4. *Reference Standard Set (1 mL/vial)*
   Contains 0, 2, 4, 15, 60, and 120 ng/mL human prostate specific antigen in bovine serum with preservatives. 1 mL each, lyophilized.
5. *TMB Reagent (1 bottle, 11 mL)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
6. *Stop Solution (1N HCl) (1 bottle, 11 mL)*
   Contains diluted hydrochloric acid.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 0.02, 0.05, 0.1, 0.2 and 1 mL
3. Disposable pipette tips
4. Microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper
8. Quality control material (e.g., BioRad Lyphochek Control sera)

STORAGE OF TEST KIT & 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 it is 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 in absorbance measurement.

REAGENT PREPARATION

All reagents should be allowed to reach 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 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.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by flicking well contents into a suitable waste container.
7. Rinse the wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply on absorbent paper to remove residual water droplets.

9. Dispense 100 μL Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 60 minutes.
11. Remove the incubation mixture by flicking well contents into a suitable waste container. Follow procedure in steps 7-8.
12. Dispense 100 μL TMB solution into each well. Gently mix for 5 seconds.
13. Incubate at room temperature for 20 minutes.
14. Stop the reaction by adding 100μL of Stop Solution (1N HCl) into each well.
15. Gently mix for 30 seconds. ***It is important to ensure that all the blue color changes to yellow completely.***
16. Read OD at 450nm with a microtiter well reader **within 15 minutes**.

PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, all pipetting should be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. Avoid microbial contamination of reagents by using a clean, disposable pipette tip for each reagent, Standard, Control or specimen.
5. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

CALCULATION OF RESULTS

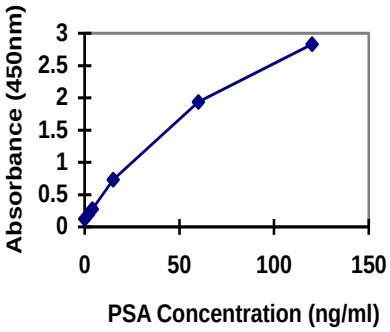
1. Calculate the mean absorbance value (OD_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on graph paper, with absorbance on the vertical (y) axis and 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. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

EXAMPLE OF STANDARD CURVE

1. Results of a typical standard run of the assay are shown below:

PSA (ng/mL)	Absorbance(450nm)
0	0.079
2	0.163
4	0.250
15	0.708
60	2.034
120	3.043

2. This standard curve is for illustration only, and should not be used to calculate unknowns.



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