

Prostate Specific Antigen (PSA) Enzyme Immunoassay Test Kit

Intended Use

For the quantitative determination of the Prostate Specific Antigen (PSA) in serum. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

Principle

The PSA 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 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 Reagent is added and incubated at room temperature for 20 minutes, resulting in the developing of a blue color. The color development is stopped with the addition of Stop Solution 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 450nm.

Materials Provided

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

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 it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm 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

- Secure the desired number of coated wells in the holder.
- Dispense 50µl of standards, specimens, and controls into appropriate wells.
- Dispense 50µl of Zero Buffer into each well.
- Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this step.
- Incubate at room temperature (18-25°C) for 60 minutes.
- Remove the incubation mixture by emptying plate contents into a suitable waste container.
- Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Do not use tap water.)
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

- Dispense 100µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
- Incubate at room temperature (18-25°C) for 60 minutes.
- Remove the incubation mixture by emptying plate contents into a suitable waste container.
- Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Do not use tap water.)
- Strike the wells sharply onto absorbent paper to remove residual water droplets.
- Dispense 100µl of TMB Reagent into each well. Gently mix for 10 seconds.
- Incubate at room temperature for 20 minutes.
- Stop the reaction by adding 100µl of Stop Solution to each well.
- Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
- Using a microtiter plate reader, read the optical density at 450nm within 15 minutes.

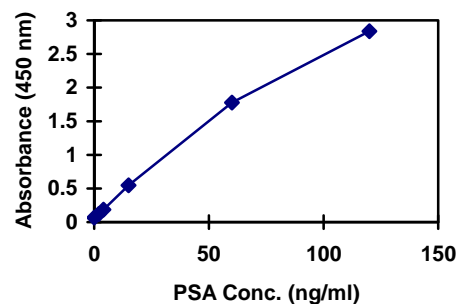
Calculations

- Calculate the average absorbance values (A_{450}) for each set of reference standards, control, and samples.
- Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- 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 450nm shown in the y axis against PSA concentrations shown in 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 his or her own data and standard curve.

PSA (ng/ml)	Absorbance (450nm)
0	0.066
2	0.119
4	0.184
15	0.545
60	1.777
120	2.840



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