



PROSTATE SPECIFIC ANTIGEN (PSA) ENZYME IMMUNOASSAY TEST KIT

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

FOR INVESTIGATIONAL USE ONLY

Store at 2 to 8°C.

INTENDED USE

For the quantitative determination of the Cancer Antigen PSA concentration in human serum.

INTRODUCTION

Human prostate-specific antigen (PSA) is a serine protease, a single chain glycoprotein with a molecular weight of approximately 34,000 daltons containing 7% carbohydrate by weight. PSA is immunologically specific for prostatic tissue, it is present in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid and seminal plasma. PSA is not present in any other normal tissue obtained from men, nor is it produced by cancers of the breast, lung, colon, rectum, stomach, pancreas or thyroid. Besides, it is functionally and immunologically different from prostatic acid phosphatase (PAP). Elevated serum PSA concentrations have been reported in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues, 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 serve 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).

PRINCIPLE OF THE TEST

The PSA ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a rabbit 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 rabbit antibody at room temperature for 60 minutes. The wells are washed to remove any unbound antigen. The monoclonal anti-PSA-HRP conjugate then reacts 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 by 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 450 nm.

REAGENTS

Materials provided with the kits:

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

Materials required but not provided:

- Precision pipettes: 50 µl, 100 µl and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- 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.

SPECIMEN COLLECTION AND

PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

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 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

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, specimens, 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 in this setup.
5. Incubate at room temperature (18-25°C) 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.** (Please 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.** (Please 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 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 make sure that all the blue color changes to yellow color completely.**
18. Using a microtiter plate reader, read the optical density at 450nm **within 15 minutes.**

CALCULATION OF RESULTS

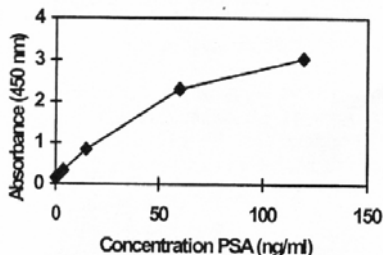
1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
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 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 (450 nm)
0	0.141
2	0.219
4	0.320
15	0.832
60	2.300
120	3.031



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 1 ng/ml.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

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