



PSA ELISA

Catalog Number EA-0105

(For Research Use Only)

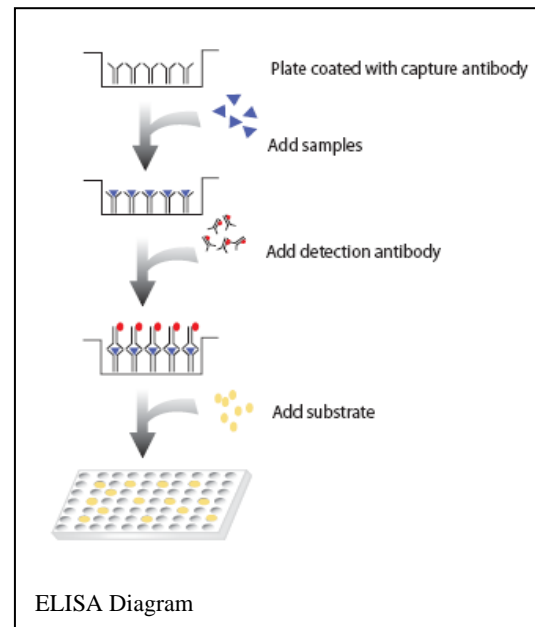
Introduction

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. 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.

The Signosis PSA Immunoassay provides a rapid, sensitive, and reliable assay for the quantitative measurement of PSA level. The antibodies developed for the test will determine a minimal concentration of 1.0 ng/ml.

Principle of the assay

The PSA ELISA 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 simultaneously with these antibodies in PSA being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then 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 450 nm.



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:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Specimen Collection and Handling

Serum should be separated from the red blood cells as soon as possible. Specimens should be stored for up to 48 hours or -20°C for up to 6 months prior to assay.

Preparation for Assay

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

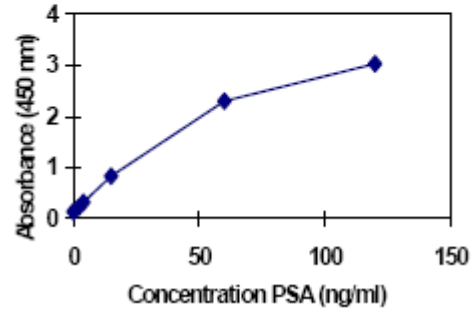
Samples of patient serum, plasma need to be diluted before use for best bet results

1. Add 50 µl of standard, specimens, and controls into appropriate wells.
2. Add 50 µl of Zero Buffer into each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at room temperature (18-25°C) for 30 minutes.
5. Remove the incubation mixture by flicking plate content into a waste container.
6. Rinse and flick the microtiter wells 5 times with distilled or deionized water. Put the wells sharply onto paper towel to remove all residual water droplets.
7. Add 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
8. Incubated at room temperature for 30 minutes.
9. Remove the incubation mixture by flicking plate contents into a waster container.
10. Rinse and flick the microtiter wells 5 times with distilled water
11. Tap the plate sharply onto paper towel to remove residual water droplets.
12. Dispense 100 µl TMB Reagent into each well, and mix gently for 10 seconds.
13. Incubate at room temperature in the dark for 20 minutes.
14. Add 100 µl Stop Solution to each well to stop the reaction.
15. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
16. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

References

1. Hara, M. and Kimura, H. Two prostate-specific antigens, gamma-seminoprotein and beta-

PSA (ng/ml)	Absorbance (450 nm)
0	0.141
2	0.219
4	0.320
15	0.832
60	2.300
120	3.031



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