PROSTATE SPECIFIC ANTIGEN (PSA)

ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 10109

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

Intended use

The PSA Enzyme Immunoassay test kit is intended for the quantitative determination of PSA in human serum.

Introduction

Human prostate-specific antigen (PSA) is a serine protease, a sigle 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 malllignant 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 serves 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 enhancce early prostate cancer detection when combined with digital rectal examination (DRE).

Principle of the test

The PSA EIA test is a solid phase two-site immunoassay. Rabbit anti-PSA is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the for mation of the coated antibody-antigen-antibody-enzyme Complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the color formed is proportional to the concentration of PSA present in the sample.

Materials and components

Materials provided with the test kits:

- Antibody-coated microtiter plate with 96 wells.
- Zero buffer, 12 mL.
- Reference standard containing 0, 2, 4, 15, 50, and 100 ng/mL PSA, Liquid standards, ready for use. 1 set.
- Enzyme Conjugate Reagent, 12 mL.
- TMB Substrate Solution, 12mL
- Stop Solution, 12 mL.
- Wash Buffer Concentrate(50X), 15 mL.

Materials required but not provided:

- Precision pipettes: 0.04~0.2 mL, 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 10nm or less And an optical density range of 0-2 OD or greater at 450nm

Specimen collection and preparation

1. Blood should be drawn using standard venipuncture Techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, Lipemic or turbid samples.

2. Plasma samples collected in tubes containing EDTA, Heparin, or oxalate may interfere with the test procedures and should be avoided.

3. Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer Time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

Storage of test kits

Unopened test kits should be stored at 2-8°C upon receipt. 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, provided it is stored as described above.

Reagent preparation

1. All reagents should be brought to room temperature(18-22°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.

2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

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 **100**µL of zero buffer into each well.

4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this step.

5. Incubate at room temperature (18-22°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 washing buffer (1X).

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 5 seconds.

10. Incubate at room temperature 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 washing buffer (1X).

13.Strike the wells sharply onto absorbent paper to remove residual water droplets.

14.Dispense **100**µL TMB solution into each well. Gently mix for 5 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 to make sure that the blue color completely changes to yellow.

18.Using a microtiter plate reader, read the optical density at 450nm within 15 minutes.

Important Note

1.The wash procedure is critical. Insufficient washing will Result in a poor precision and falsely elevated absorbance readings.

2.It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.

3.Duplication of all standards and specimens, although not required, is recommended.

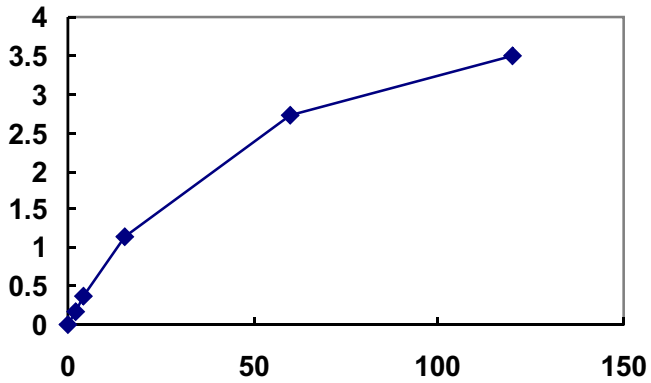
Calculation of results

Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls, and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/mL on graph paper. The absorbance values are placed on the vertical or Y-axis and concentrations on the horizontal or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of PSA in ng/ mL from the standard curve.

Example of standard curve

Results of typical standard run with optical density reading at 450nm shown in the Y-axis against PSA concentrations shown in the X-axis.

PSA (ng/mL)	Absorbance (450nm)
0	0.003
2	0.106
4	0.206
15	0.750
50	2.102
100	3.038



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 standard curve and data.

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 0.5 ng/mL.

Performance characteristics

1. Accuracy: Comparison between Our Assay and commercial available Kits
   provide the following data
   N = 70
   Correlation Coefficient = 0.995
   Slope = 0.94
   Intercept = 0.29
   Mean (Our) = 6.93
   Mean (Abbott) = 6.78
2. Precision.
1]. Intra-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level	20	2.64	0.117	4.42
LevelI	20	5.70	0.191	0.829
LevelII	20	36.13	0.829	2.29

2]. Inter-Assay

Concentrations	Replicates	Mean	S.D.	% CV
Level	50	2.54	0.155	6.10
LevelI	50	6.30	0.320	5.08
LevelII	50	35.32	1.460	4.14

3.Linearity
Two patient sera were serially diluted with 0 ng/mL standard in a linearity study. The average recovery was 97.10 %.

Sample A			
Dilution	Expected	Observed	% Recov.
Undiluted	34.96	34.96	
2x	17.48	16.09	92.07
4x	8.74	8.13	92.96
8x	4.37	4.30	98.33
16x	2.19	2.31	105.86
Average Recovery: 97.30 %			

Sample B			
Dilution	Expected	Observed	% Recov.
Undiluted	62.40	62.40	
2x	31.20	29.79	95.46
4x	15.60	15.04	96.40
8x	7.80	7.31	93.64
16x	3.90	3.98	102.15
Average Recovery: 96.9 %			

4.Sensitivity
The sensitivity is defined as the concentration of PSA that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the zero calibrator. The minimum detectable concentration of this assay is estimated to be 0.5 ng/mL.

5.Cross-reactivity
The following cancer marker antigens at high concentrations, as seen in cancer patients, were assayed to determine the possible reactivity.

Antigens	Concentration	Equivalent PSA	% Cross-reactivity
HCG	400 IU/mL	0.00	0.00
PAP	1,000 ng/mL	0.00	0.00
AFP	10,000 ng/mL	0.00	0.00
CEA	1,000 ng/mL	0.00	0.00

6. Hook Effect
This PSA ELISA test showed no hook effect at concentrations as high as 579,670 ng/mL.

Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

1)As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

2)Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse Monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.

3)The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

References

1.Hara, M. And Kimura, H. Two prostate-specific antigens, Gamma - seminoprotein and beta - microseminoprotein. **J. Lab. Clin. Med.** 113:541-548;1989.

2.Yuan, J.J.; Coplen, D.E.; Petros, J.A.; Figenshau, R.S.; Ratliff, T.L.; Smith, D.S. and Catalona, W.J. Effects of rectal examination, prostatic massage, ultrasonography and needle Biopsy on serum prostate specific antigen levels. J. Urol. 147:810-814; 1992.

3.Wang, M.C.; Papsidero, L.D.; Kuriyama, M.; Valenzuela, L.A.; Murphy, G.P. and Chu, T.M. Prostatic antigen: a new Potential marker for prostatic cancer. Prostate 2:89-93; 1981.

4.Stowell, L.I.; Sharman, I.E. and Hamel, K. An Enzyme-Linked Immunosorbent Assay (ELISA) for Prostate-specific Antigen. Forensic Science Intern. 50:125-138; 1991.

5.Frankel,A.E.;Rouse,R.V.;Wang,M.C.;Chu, T.M. And Herzenberg, L.A.Monoclonal antibodies to a human Prostate antigen.**Canc. Res.** 42:3714; 1982.

6.Benson, M.C.; Whang, I.S.; Pantuck, A.; Ring, K.; Kaplan, S.A.; Olsson, C.A. and Cooner, W.H. Prostate specific Antigen density: a means of distinguishing benign prostatic Hypertrophy and prostate cancer. J. Urol. 147:815-816; 1992.

7.Gorman,C.The private pain of prostate cancer.Time 10(5):77-80; 1992.

8.Walsh, P.C. Why make an early diagnosis of prostate Cancer. **J. Urol.** 147:853-854; 1992.

9.Labrie,F.;Dupont, A.;Suburu,R.; Cusan, L.; Tremblay,M.; Gomez, J-L and Emond,J. Serum prostate specific antigen as pre-screening test for prostate cancer.**J. Urol.**147:846-852; 1992.

10.McCarthy, R.C.; Jakubowski, H.V. And Markowitz,H. Human Prostate acid phosphatase : purification, characterization, and Optimization of conditions for Radioimmunoassay. **Clin. Chim. Acta.** 132:287-293; 1983.

11.Heller, J.E. Prostatic acid phosphase: its current clinical status. **J. Urol.** 137:1091-1099; 1987.

12.Filella, X.; Molina, R.; Umbert, J.J.B.; Bedini, J.L. And Ballesta, A.M. Clinical usefulness of prostate - specific Antigen. **Tumor Biol.** 11:289-294; 1990.

13.Shin, W.J.; Gross, K.; Mitchell, B.; Collins, J.; Wierzbinski, B.; Magoun, S. and Ryo, U.Y. Prostate adenocarcinoma Using Gleason scores correlates with prostate - specific Antigen and prostate acid phosphatase measurements. **J. Nat. Med. Assoc.** 84:1049-1050; 1992.

14.Wirth, M.P. and Frohmuller, H.G. Prostate-specific antigen and prostate acid phosphatase in the detection of early prostate cancer and in the prediction of regional lymph node Metastases. **Eur. Urol.** 21:263-268; 1992.

15.Campbell, M.L. More cancer found with sensitive PSA assay. **Urol. Times.** 20:10; 1992.

16.Vessella, R.L.; Noteboom, J. and Lange, P.H. Evaluation of the Abbott Imx Automated immunoassay of Prostate-Specific Antigen. **Clin. Chem.** 38:2044-2054; 1992.

17.Brawer, M.K.; Chetner, M.P.;Beatie, J.;Buchner,D.M.; Vessella, R.L. and Lange,P. H. Screening for prostatic carcinoma with Prostate specific antigen. **J. Urol.** 147:841-845; 1992.

18.Benson, M.C. Whang, I.S.; Olsson, C.A.; McMahon, D.J. And Cooner, W.H. The use of prostate specific antigen density to Enhance the predictive value of intermediate levels of Serum prostate specific antigen. **J. Urol.** 147:817-821; 1992.

19.Oesterling, J.E. and Hanno, P.M. PSA still finding niches in cancer diagnosis. **Urol. Times** 20:13-18; 1992.

20. Babaian, R.J.; Fritsche, H.A . And Evans, R.B. Prostate-specific antigen and the prostate gland volume: correlation and Clinical application. **J. Clin. Lab. Anal.** 4:135-137; 1990.

7/2014

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