Atlas Medical

PROSTATE SPECIFIC ANTIGEN (PSA) ENZYME IMMUNOASSAY TEST KIT

IVD *For in vitro diagnostic use only*

2°C 8°C **Store at 2 to 8°C.**

Σ 96 Tests

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 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 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 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

- Goat anti-PSA coated microtiter plate with **96 wells.**
- Zero Buffer, **12 ml dark green cap vial.**
- Reference standard containing **0 (white cap), 2 (golden cap), 4 (green cap), 15 (blue cap), 50 (purple cap) and 100 (red cap)** ng/ml PSA, **1ml** each vial, ready to use.
- Enzyme Conjugate Reagent, **12 ml red cap vial.**
- TMB Reagent solution, **12 ml brown cap vial.**
- Stop Solution, **12 ml transparent cap vial.**
- Wash buffer concentrate (50X), **15ml faint green cap vial.**

Materials required but not provided:

- 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

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 KIT

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 setup.
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 withwashing 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 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 wash buffer (1X).
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 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. It is important to make sure that all the blue color changes to yellow color completely.
18. Using a microliter plate reader, read the optical density at 450nm within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) 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. Use the mean absorbance value for each sample, determine the corresponding concentration of PSA in ng/ml from the standard curve.

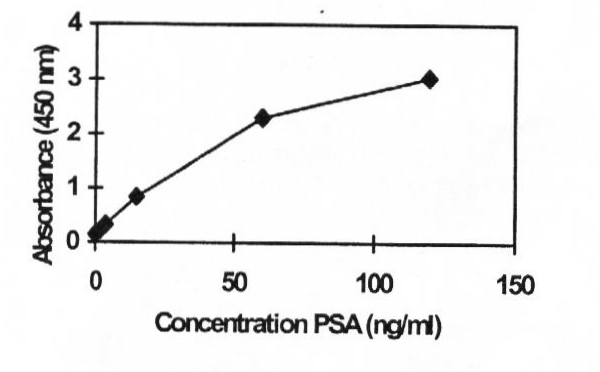
WASH PROCEDURE

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.

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	0.003
2	0.106
4	0.206
15	0.750
50	2.102
100	3.038



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 0.5ng/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 (Commercial) = 6.78

2. Precision:

- Intra-Assay:

Concentration	Replicates	Mean	S.D.	% CV
Level 1	20	2.64	0.117	4.42
Level 2	20	5.70	0.191	0.829
Level 3	20	36.13	0.829	2.29

- Inter-Assay:

Concentration	Replicates	Mean	S.D.	% CV
Level 1	50	2.54	0.155	6.10
Level 2	50	6.30	0.320	5.08
Level 3	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	% Recovery
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	% Recovery
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	1000 ng/ml	0.00	0.00
AFP	10000 ng/ml	0.00	0.00
CEA	1000 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.

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REF	Catalogue Number		Temperature limit
IVD	In Vitro diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry