PSA ELISA Kit

96 Tests Kit
Enzyme Immunoassay for the
Quantitative Determination of
PSA
Concentration in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. MA_PSA_96_01

PISHTAZ TEB DIAGNOSTICS

Introduction

Prostate Specific Antigen (PSA) is a single chain glycoprotein and a member of Kallekerin family molecules which has similar enzymatic activity as chemotrypsin molecule. PSA glycoprotein monomer (molecular weight of 30 kDa) produced by prostate epithelium and normally secreted into the seminal fluid. PSA is mainly responsible for gel dissolution in freshly ejaculated semen by proteolysis of the major gel forming proteins. The majority (70-90%) of PSA in serum is complexed to alpha1-antichymotrypsin (ACT) which has molecular weight of 90-100 kDa. Total PSA (free PSA + PSA-ACT-complex) is increased in both benign prostate hyperplasia and malignant prostate cancer. The ratio of free to complex PSA is also variable in different people. PSA is present in small detecting level in blood circulation of normal individuals but its increase is a good indicator of benign or malignant prostate tumor.
PSA determination is a reliable diagnostic tool for the diagnosis of prostate cancer and for the monitoring of patients after cancer treatment or surgery. Prostate cancer is the second most prevalent cancer in developed countries with high mortality rate. Since PSA level are also increased in benign prostate tumor like Benign Prostate Hyperplasia (BPH), PSA determination can not be used as confirmatory test for the prostate cancer and combination of PSA test with other diagnostic tests is highly valuable in those cases. After prostatectomy, serum PSA level become nil and levels higher than 0.1 ng/ml should be considered as possible incomplete prostate removal or cancer metastases. The current PSA ELISA kit detects both free and complex PSA level.

Test Principle

The PSA Quantitative test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes an anti-PSA antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-PSA antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwiched formation of PSA between solid phase and conjugated antibodies. After second wash step a solution of TMB is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of PSA is directly proportional to the color intensity of the test sample.

Materials provided with the kit

1. Anti-PSA coated microplate. 96 wells per bag.
2. Anti-PSA-HRP conjugate: 12 ml, ready to use.
3. Standards set: 0, 1, 4, 10, 25, and 50 ng/ml of PSA.
4. High and low PSA level control serum: 1ml each, the expected concentration is specified on their labels.
5. Assay buffer: 12 ml, ready to use.
6. TMB reagent: 12 ml, ready to use.
7. Wash solution: 50 ml, concentrated (20X).
8. Stop solution: 12 ml.
9. Cardboard sealer

Materials required but not provided

- Precision pipettes: 20µl, 100µl
- Disposable pipette tips.
- Distilled water.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C (maximum 30 days). Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Test Procedure

1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 20 µl of each standard, serum control and specimen in appropriate wells.
3. Add 100 µl of assay buffer into the wells and shake them gently for 15 seconds. Cover the microtiter wells with cardboard sealer. Leave wells for 30 minutes at room temperature (22-28°C).
4. Remove the sealer and take out wells contents by flicking the microplate into a waste container. Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Add 100 µl of Anti-PSA-HRP conjugate into the wells.
7. Seal the plate with cardboard sealer again. Incubate wells for 30 minutes at room temperature (22-28°C).
8. Repeat step 4 and 5.
9. Dispense 100 µl of chromogen/ substrate (TMB) into the microplate wells.
10. Leave the microplate wells at room temperature and dark for 15 minutes, to develop color.
11. Stop the reaction by adding 100 µl of stop solution to the microplate wells.

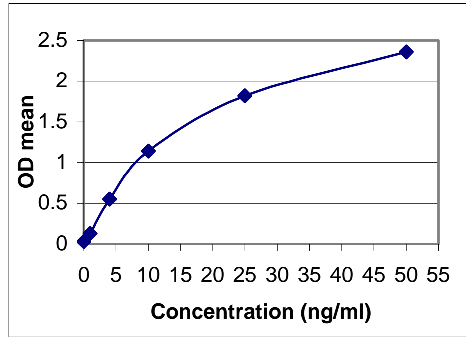
12. Measure absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available).

Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm (Use 630 nm filter as reference filter if it's available)
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
3. vertical (y) axis and concentration on the horizontal (x) axis.
4. Use the mean absorbance value for each sample, determine the corresponding concentration of PSA in ng/ml from the standard curve.

Example of Standard curve

Standards (ng/ml)	OD
0	0.03
1	0.13
4	0.55
10	1.14
25	1.82
50	2.36



Note: All absorbances shown in above curve are for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own data and standard curves.

Expected Values

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on randomly selected clinical laboratory samples:

Normal Range
Up to 4.0 ng/ml

Performance Characteristics

1. Minimum Detection Limit

Based on mean absorbance of standard 0+3SD, the minimum detectable concentration of PSA by this assay is estimated to be 0.1 ng/ml.

2. Test Precision

To assess precision of this assay kit, four serum samples with different concentrations of PSA were repeatedly tested.
Results are shown in table 1 and 2:

Table no. 1 (Intra-assay)

No.	No. of tests performed	Means ng/ml	SD ng/ml	CV %
1	24	0.6	0.03	5.0
2	24	2.2	0.08	3.6
3	24	9.9	0.35	3.5
4	24	30	1.3	4.3

Table No. 2 (Inter-assay)

No.	**No. of tests performed**	**Means** ng/ml	**SD** ng/ml	**CV %**
1	10	0.6	0.05	8.2
2	10	2.9	0.2	7.0
3	10	19.2	1.2	6.3
4	10	37	2.4	6.5

Each test has been run in duplicate

3. Test Recovery

The certain amount of PSA was added into 4 different serum samples with known PSA concentration.

Table no. 3: Test recovery

No.	Serum PSA level ng/ml	PSA added ng/ml	Exp. ng/ml	Obs. ng/ml	Rec. (%)
1	1.5	1	1.25	1.15	92
1	1.5	10	5.7	5.9	103
1	1.5	25	13.2	13	98
2	4.3	1	2.6	2.4	92
2	4.3	10	7.1	6.8	96
2	4.3	25	14.6	14.1	96
3	12.8	1	6.9	6.5	95
3	12.8	10	11.4	12.1	106
3	12.8	25	18.9	19.3	102
4	37.2	1	19.1	20	105
4	37.2	10	23.6	24.5	104
4	37.2	25	31.1	29	93

Exp.: Expected , Obs.: Observed,
Rec.: Recovery

4. Test Linearity

To verify test linearity, 3 different serum samples with known PSA concentrations were sequentially diluted with 0.0 standard reagent. Then the serums were tested with PSA ELISA test and the results are shown below (considering dilution factor):

Table no. 4: Test linearity

		Recovery (%)				
No.	PSA level in undiluted specimen	1:2	1:4	1:8	1:16	1:32
1	80	104	98	89	106	93
2	47	99	100	92	95	90
3	25	102	95	98	110	108

6. Hook effect

To rule out possible hook effect occurrence, the PSA assay was done on serums with high concentration of PSA (up to 50 µg/ml) and no “hook effect” was seen.

References

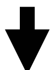
1. Belanger A., Van Harbeek H., et al. Molecular mass and carbohydrate structure of prostate specific antigen. Studies for establishment of an international PSA. Prostate 1995; 27: 187-197.
2. Zhov A. M., Tewari P.C., Card Weu G. W. Multiple forms of PSA in serum; Differences in immunorecognition by monoclonal and polyclonal assay. Clin. Chem. 1993; 39:2483.

PSA Test Procedure

Step 1

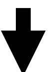
Reagent	Standard	Serum Control	Sample
Standard	20 µl	None	None
Serum Control	None	20 µl	None
Sample	None	None	20 µl
Assay buffer	100 µl	100 µl	100 µl

Shake wells gently for 15 seconds. Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature.



Step 2

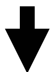
Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.



Step 3

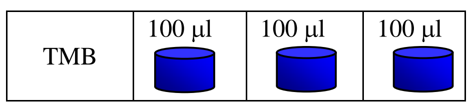
Anti PSA - HRP conjugate	100 µl	100 µl	100 µl

Cover the microplate wells again with cardboard sealer and incubate them for 30 minutes at room temperature.



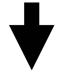
Step 4

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

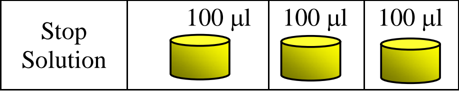


TMB	100 µl	100 µl	100 µl

Incubate wells for 15 minutes at room temperature and dark.



Step 5



Stop Solution	100 µl	100 µl	100 µl

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).