پادتن علم
PADTAN ELM

THE PADTAN ELM EIA KIT FOR 96 TESTS

FreePSA EIA Kit

Intended use:
Quantitative determination of PSA levels
in human serum and plasma.

For *in vitro* diagnostic use.

I.Introduction

Prostate Specific Antigen (PSA) is a monomeric glycoprotein of 237 amino acids containing 7-8% carbohydrate as a single N-linked oligosaccharide side chain. PSA has a molecular weight of approximately 30 kDa [1].
This glycoprotein, produced by the epithelial cells of the prostate gland, is a serine protease of the kallikrein family, having chymotrypsin- and trypsin- like enzyme activity [2,3].
PSA plays a functional role in the proteolytic cleavage of seminal vesicle proteins, liquefaction of the seminal gel and augmentation of the sperm mobility.
In blood, PSA exists in two forms: (a) as a major component complexed with a proteinase inhibitor: a1-antichymotrypsin or a2-macrogloboulin, and (b) as a minor free component. Both PSA/ a1-antichymotrypsin and free PSA can be detected by conventional immunoassays. By contrast, due to the engulfment and subsequent masking of the PSA epitopes by the a2-macrogloboulin, the PSA/a2-macrogloboulin complexes are not determined by conventional immunoassays, such as EIA[4].
PSA is present in normal, benign hyperplastic and malignant prostatic tissue, in metastatic prostatic carcinoma and also in prostatic fluid and seminal plasma [5]. Serum levels of PSA are undetectable in women and are low in apparently healthy men, as well as in men with non-prostatic carcinomas such as lung, colon, rectum, stomach and thyroid cancers. Moderate to high levels of PSA are found in the serum of diagnosed prostate cancer patients or in other abnormal prostate conditions such as prostatitis or benign prostatic hyperplasia [6,7,8].
Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer deaths in men in developed countries. A number of studies have found that the percentage of free PSA (% free PSA / total PSA) is lower in prostate cancer than in benign prostatic hyperplasia[9,10]. This percentage can be used as an aid in distinguishing prostate cancer from benign prostatic hyperplasia when the total PSA concentration is between 4 and 10 ng/ml and the digital rectal examination is not suspicious for prostate cancer (immulite)[11-14].

II.Principle of the test

The Free PSA quantitative test kit is based on solid phase enzyme immunoassay (EIA). This assay system uses two mouse monoclonal antibodies directed against distinct antigenic determinants on the Free PSA molecule. The polystyrene wells are coated with captured mouse monoclonal antibodies against Free PSA. Standards, controls and patient samples are added to the wells (solide phase) and incubate. The Free PSA present in the wells is bound to the anti-Free PSA antibodies. The unbound material is removed by aspiration and washing. After washing, the HRP labeled anti-Free PSA Mab is added to the wells. After second incubation and washing, a solution of TMB (3,3',5,5' tetra-methylbenzidine) is added to each well, resulting in the development of a blue color. The intensity of the color is proportional to the amount of Free PSA present in the sample. The color development is stopped by addition of Stop solution, causing the blue color to change to yellow. The color intensity is determined in a microtiter plate spectrophotometer at 450 nm. Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard. The Free PSA concentrations of patient samples are then read from the standard curve.

III.Kit contents

The reagents provided with the Free PSA Kits (Cat.No.P-FPI) are sufficient for 96 wells. The expiry date of each reagent is shown on the vial label.

Store the Kit at 2-8 C.

1.Coated microtiter wells: 96 wells, coated with mouse monoclonal anti- Free PSA antibodies.

2.Zero standard: 1 vial (4 ml) serum matrix with thimerosal. The zero standard should also be used as sample diluents.

3.Standards: 6 vials (1.5 ml) of Human Free PSA in serum matrix with thimerosal as a preservative. The exact Free PSA concentration for each standard is specified on the label of each vial. The standards supplied with the kit were calibrated against the international standard 1ST. IRP 96/668.

4.Serum controls: 2 vials (1.5 ml) of human Free PSA in processed human serum containing thimerosal. The nominal concentrations of Free PSA in the controls are specified on the label of each vial.

5.Assay buffer: 1 vial (6ml) of PBS with proteins and thimerosal.

6.Enzyme tracer: 1 vial (12 ml) of monoclonal anti- Free PSA antibody conjugated to horseradish peroxidase (HRP) in phosphate buffered saline (PBS) with proteins and thimerosal.

7.Wash solution (concentrate 20X): 1 vial (25 ml) of PBS-Tween 20 and thimerosal.

8.TMB HRP-Substrate: 1 vial (12 ml) of buffered H_20_2 and 3,3',5,5' tetra-methyl benzidine.

9.Stop solution: 1 vial (12ml) of 2N H_2S0_4.

IV. Materials required (but not supplied with the kit)

1.Microtiter plate spectrophotometer reader with a wavelength of 450 nm(with reference wavelength at 630 nm) and an absorbance range of 0 to 3.0.

2.Precision micropipettes (50 and 100 µL).

3.Distilled or deionized water for preparation of diluted Wash Solution.

V. Specimen collection and preparation

The assay can be performed on serum or heparinized plasma samples. Keep samples at 2-8°C for 4 days; for longer periods it is recommended to store the sample in aliquot form at -20° C. Avoid repeated freezing and thawing of samples. Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand. Do not vortex patient samples. Serum sample with Free PSA greater than the last standard should be diluted with "zero" standard and reassayed to give a quantitative result. The value obtained must be multiplied by the dilution factor to give the correct Free PSA concentration.

VI. Assay procedures

All reagents should be brought to room temperature prior to use

Concentrated wash solution must be diluted with distilled or deionizer water (Dilute one part concentrated wash solution with 19 parts of water). Diluted wash solution is stable for 7 days at 2 to 8°C. In presence of undissolved crystals, resuspend the solution by placing the vial at 37° C for a few minutes.

1.Dispense 100 µl Free PSA standards, control serums and patient samples into appropriate wells. Pipette 50 µl of Assay Buffer to each well. Thoroughly mix the plate for 15 seconds.

2.Incubate the strips for 30 minutes at room temperature.

3.Aspirate and wash each well 4 times with 300 µl of diluted wash solution.

4.Add 100 µl of HRP anti-Free PSA conjugate to each well. Then incubate for 30 minutes at room temperature.

5.Aspirate and wash each well 4 times with 300 µl of diluted wash solution.

6.Dispense 100 µl of TMB HRP-Substrate into each well.

7.Incubate at room temperature in the dark for 15 minutes.

8.Add 100 µl of Stop Solution and mix for 10 seconds.

9.Read absorbance at 450 nm (with reference wavelength at 630 nm) in a microtiter plate readerwithin 15 minutes after addition of Stop solution.

SUMMARY OF ASSAY PROCEDURE

Pipette 100 µl standard, control or sample
Pipette 50 µl Assay buffer
Incubate 30 min. at RT
Wash 4 x (300 µl)
Pipette 100 µl Anti-Free-PSA HRP
Incubate 30min at RT
Wash 4 x (300 µl)
Pipette 100 µl TMB
Incubate 15min at RT
Pipette 100 µl Stop Solution
Read at 450/630 nm

پادتن علم
PADTAN ELM

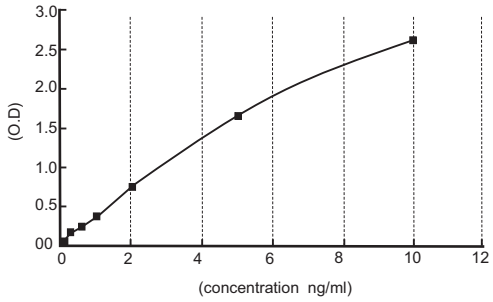
VII. Calculation of results

The results can be calculated by either microtiter plate spectrophotometer reader or manual evaluation. If a microtiter plate spectrophotometer reader with data calculation program is used, refer to the plate reader and create a program using the concentration of each of the Free PSA standards (in ng/ml).
For manual evaluation, a standard curve is constructed by plotting the absorbance (A) values obtained for each Free PSA standard against the corresponding Free PSA concentrations in ng/ml. The unknown Free PSA concentration, in ng/ml, can then be read from the standard curve using the absorbance value of each patient specimen.

Example of calculation:

The values shown below are examples and must not he used in place of experimental data

Specimen	Absorbance	Free PSA concentration(ng/ml)
Standard A	0.03	0
Standard B	0.1	0.2
Standard C	0.2	0.5
Standard D	0.37	1.0
Standard E	0.78	2.0
Standard F	1.71	5.0
Standard G	2.75	10.0
Sample	0.58	1.4



VIII. Normal values

A normal range study for Padtan Elm free PSA was performed using serum samples from healthy adult volunteer men ranging from 20 to 70 years of age. Medians and 95th percentiles are tabulated below:

Age	n	Mean (ng/ml)	95%
20-40	111	0.11	0.09
41-60	298	0.18	0.15
>61	153	0.45	0.39

The probability of prostate cancer was significantly decreased in patients with free/total PSA ratio higher than 23%. The free/total PSA values between 16.3 and 23 % was assessed as equivocal; results below 16.3 indicated significantly increased probability of prostate cancer presence (immunotech):

Interpretation of results

The clinical evaluation of results is based on the concentration ratio of free PSA and PSA (in percents):

$$\frac{\text{free PSA concentration (ng/ml)}}{\text{total PSA concentration (ng/ml)}} \times 100$$

The level of free/total PSA ratio cannot be used as absolute evidence for the presence or absence of malignant disease and should not be used as a screening test. The results should be interpreted only in conjunction with other investigations.

free/total PSA ratio(%)	probability of prostate cancer
>23.0	negative
16.3 - 23.0	equivocal
<16.3	positive

It is suggested that each laboratory establish its own normal range for distinguishing benign and malignant prostatic disease, on the basis of a sufficient number of clinically proved samples.

Important note:
Complex formation between PSA and ACT results in exposure of a limited number of the antigenic epitopes of PSA, whereas complex formation with 2-macroglobulin encapsulates the antigenic epitopes of PSA. Differences in recognition of these multiple forms of PSA by coated antibodies have contributed to discrepancies between various commercial PSA assays . Thus these determinations must be made using assays developed by the same manufacturer since combining tests from different manufacturers to determine tPSA and fPSA can produce erroneous values[15-17].

IX. Specific assay characteristics

1.Sensitivity: The sensitivity of this kit was calculated based upon the standard curve and expressed as the minimal concentration of FreePSA showing a significant difference from the "zero" standard. This minimal detectable concentration of FreePSA is 0.02 ng/ml.
2.Hook effect: The samples with very high antigen concentration are tested undiluted. With this kit, no hook effect has been noticed with samples containing Free PSA concentration up to 10µg/ml.

3.Precision: Precision was evaluated for intra- and inter-assay variability.
Intra-assay reproducibility was determined by measurement of 10 replicates of 3 samples in a single run.
Inter-assay reproducibility was determined by replicate measurement of 3 samples in 10 separate runs.

Reproducibility

	Inter-assay			Intra-assay		
Sample	Mean(ng/ml)	SD	%CV	Mean(ng/ml)	SD	%CV
1	0.72	0.04	5.86	0.71	0.03	4.25
2	1.12	0.08	7.04	1.06	0.07	6.34
3	3.26	0.16	4.84	3.22	0.17	5.17

4. Recovery and dilution: Recovery is defined as the increase in concentration seen when a known concentration of an analyte is added to a sample. Spiked samples were prepared by mixing the appropriate aliquot of a concentrated free PSA solution with a serum sample containing a low free PSA concentration. The percentage recovery is calculated as:

[(Measured conc. (ng/ml)/ Expected conc. (ng/ml)] X100

The percentage recovery ranged from 92.6 to 112.1.

Recovery Test

Added Conc. (ng/ml)	Expected Conc. (ng/ml)	Measured Conc. (ng/ml)	% Recovery
-	-	1.0	-
2.3	3.3	3.7	112.1
4.3	5.4	5.1	94.4
6.3	7.3	7.0	95.9
11.2	12.1	11.2	92.6

For dilution test, several serum samples containing elevated Free PSA concentrations were tested after serially diluting with the zero standard. The results of one of these samples are shown in the following table. The percentage recovery ranged from 84.2 to 104.1.

Dilution test

Dilution	Expected Cone. (ng/ml)	Measured Cone. (ng/ml)	% Recovery
-	-	7.6	-
1:2	3.8	3.9	102.6
1:4	1.9	1.7	89.5
1:8	0.95	0.8	84.2
1:16	0.48	0.5	104.1

5.Comparison with Free-PSA electrochemiluminescence methods: Correlation studies on more than 1000 random serum samples, were performed using the quantitative results from the ROCHE electrochemiluminescence (Elecsys). The correlation coefficient of test results was 0.92.

X. References

1.Belanger A, Van Halbeek H, Graves HCB, et al. Molecular Mass and Carbohydrate structure of prostate specific antigen: Studies for Establishment of an International PSA Standard. Prostate. 27:187-97,1995.
2.Mc Cormack RT, Rittenhouse HG, Finlay JA, et al. Molecular Forms of prostate specific antigen and the Human Kallikrein Gene Family: A New Era. Urology 45:729-44, 1995.
3.Watt KWK, Lee P-J, K, Timkulu T, et al. Human of prostate specific antigen: Structural and functional similarities with serine proteases. Proc Natl Acad Sci UDS. 83:3166-70, 1986.
4.Liedtke, R. J., Batijer J. D., Measurement of prostate specific antigen by RIA. Clin. Chem., 30:649, 1984.
5.Papsidero L. D., Wang M. C., Valenzuela, L. A. Murphy G.P. Chu T. M. A prostate antigen measurement in sera of prostatic cancer antigen. Cancer Res. 40:2428, 1980.
6.Pontes J. E., Chu T. M., Slack N., Karr J., G. P. Murphy. Serum prostatic antigen measurement in localized prostatic cancer: Correlation with clinical course. J. Urol. 138:1216, 1982.
7.Samey T. A. Et al. PSA as a serum marker for adenocarcinoma of the prostate. N. E. J. Med., 317:909, 1987.
8.Parker ST, Tong T, Bolden S, et al. Cancer Statistics, CA Cancer J. Clin 47:5-27, 1997.
9. Luderer AA, Chen Y-T Soriano TF, et al. Measurements of the proportion of free to total prostate-specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total prostate-specific antigen. Urology 46:187-194, 1995
10. Yemoto CM, Nolley R, Prestigiacomo AF, et al. Free (f) and total (t) PSA density in patients with prostate cancer and benign prostatic hyperplasia. J. Urol., 155:347A, 1996.
11. Correale M, Pagliarulo A, Donatuti G, Sturda F, Capobianco AM, Stigliani V, et al. Preliminary clinical evaluation of free/total PSA ratio by the IMMULITE system. Int J Biol Markers 1996;11:24-8
12. Wymecga LF, Duisterwinkel FJ, Groenier K, Visser-van Brummen P, Marrink J, Mensink HJ. Clinical implications of free-to-total immunoreactive prostate-specific antigen ratios. Scand J Urol Nephrol 2000;34:181-7
13. McArdle PA, Pollock MA, Wallace Ma, McMillan DC, Crooks JE, Underwood MA. Comparison of total, complexed and free prostate-specific antigens and their ratios in the detection of prostate cancer in a non-screened population. Ann Clin Biochem 2004;41:201-6
14. Martinez-Pineiro L, Garcia Mediero JM, Gonzalez Gancedo P, Tabercero A, Lozano D, et al. Probability of prostate cancer as a function of the percentage of free prostate-specific antigen in patients with a non-suspicious rectal examination and total prostate-specific antigen of 4-10 ng/ml. World J Urol 2004;22:124-31
15. Zhou AM, et al. Multiple forms of prostate-specific antigen in serum: differences in immunorecognition by monoclonal and polyclonal assays. Clin Chem 1993;39:2483-91
16. Wolff JM, Stocker G, Borchers H, Haubeck H, Greiling H, Jakse G. Critical aspects related to the interpretation of the free-to-total PSA-ratio. Anticancer Res 1999;19:2633-6
17. Patel D, White PA, Milford Ward A. A comparison of six commercial assays for total and free prostate specific antigen (PSA): the predictive value of the ratio of free to total PSA. BJU Int 2000;85:686-9

WARNING!

This kit contains toxic materials, animal and human sourced components. Since no method can completely rule out the presence of blood-borne disease(e.g. HIV,HCV and HBV) therefore,all human sourced material must be considered potentially infectious.
In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary. Never pipette by month. Do not eat, smoke or apply cosmetics in the laboratory.

Rev: Jun.2016

PADTAN ELM Co.
No.26, Shahid Sarparast Ave.
West Taleghani Ave.
Tehran 14168-IRAN

Tel & Fax: (+98-21) 63481
Email: office@padtan.com