Prostate Specific Antigen ELISA Kit

Cat. No. 99-0067
Size: 96 Tests
For Research Use Only
Lot No.

The Prostate Specific Antigen ELISA Kit is an enzyme-linked immunosorbent sandwich assay for quantitative detection of human Prostate Specific Antigen (PSA) in human serum or plasma.

INTRODUCTION

Prostate Specific Antigen (PSA) is a single chain glycoprotein produced by epithelial cells of the prostate gland. Three major forms of PSA exist in the serum: free PSA, bound PSA, and complex PSA. PSA immunoassays are widely used to detect early-stage prostate cancer, to evaluate disease progression, and to assess therapeutic response.[1] In addition to the total serum PSA level, the ratio of free to total PSA has become an important variable for distinguishing between males with benign and malignant prostate. The percentage of free serum PSA is lower in males with prostate carcinoma than in those with benign prostate hyperplasia or with no apparent prostate disease.[2]

REAGENTS AND MATERIALS PROVIDED

Microwell strips coated with PSA monoclonal antibody (12 x 8 wells)
Standard: 6 vials (0.7 mL each), Ready to use
Enzyme Conjugate: 1 vial (12 mL), Ready to use
Assay Diluent: 1 vial (12 mL), Ready to use
TMB Substrate: 1 vial (12 mL), Ready to use
Stop Solution: 1 vial (12 mL), Ready to use
20X Wash Buffer Concentrate: 1 vial (25 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

5 mL and 10 mL graduated pipettes, beakers, flasks, and cylinders
10 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
50 µL to 300 µL adjustable multichannel micropipette, disposable tips, and reservoir
Microwell strip reader capable of reading at 450 nm

STORAGE

2° - 8°C

PRINCIPLE OF TEST

This ELISA Kit is based a two-site sandwich ELISA method. Samples and diluent are added to the wells coated with monoclonal antibody against PSA. PSA in the serum binds to the antibody coated on the well. Unbound proteins are washed off. HRP labeled anti-PSA antibody is then added to the mixture. Unbound protein and HRP conjugate are washed off. Upon the addition of the substrate, the intensity of color is proportional to the concentration of PSA in the samples. A standard curve is prepared by plotting color intensity and the concentration of the PSA.

PREPARATION OF REAGENTS

20X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 475 mL of distilled water. Store at room temperature.

PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder.
2. Pipet 50µL of PSA standards, controls and samples into appropriate wells.
3. Pipet 50µL of assay diluent into each well.
4. Cover the plate and incubate for 60 minutes at room temperature.
5. Remove incubation mixture from all wells. Wash wells with wash buffer for three times. Blot on absorbent paper towels.
6. Add 100µL Enzyme Conjugate into each well.
7. Incubate for 30 minutes at room temperature.
8. Remove incubation mixture from all wells. Wash wells with wash buffer for three times.
9. Add 100µL of TMB substrate into each well.
10. Incubate for 15 minutes at room temperature.
11. Add 50µL of stop solution into each well. Shake the plate gently for 30 seconds to mix the solution. It is important to make sure that the blue color completely changes to yellow.
12. Read absorbance on ELISA Reader at 450 nm within **15 minutes** after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check PSA standard value on each standard vial. This value might vary from lot to lot.
2. To construct the standard curve, plot the absorbance for the PSA standards (vertical axis) against its concentration in ng/mL (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Use the absorbance for controls and each unknown sample to determine the corresponding concentration of PSA from the standard curve.

PERFORMANCE CHARACTERISTICS

1. Correlation with a Reference ELISA kit:

A total of 108 sera were tested by this PSA ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.93	0.77	-0.103

2. Precision

Intra-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
High	16	23	1.51	6.56
Normal	16	3.8	0.30	7.89
Low	16	1.2	0.09	7.50

Inter-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
High	10	27	2.10	7.77
Normal	10	4.2	0.35	8.33
Low	10	1.3	0.15	11.53

3. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.185	0.20	0.585 ng/mL

4. Recovery

Known quantities of PSA were added to a serum that contained a low concentration of PSA.

Expected Value (ng/mL)	Recovered (ng/mL)	Percent of Recovery
25.0	23.6	94
4.0	3.65	91
1.5	1.20	80

5. Linearity

Three different patient samples were diluted with the “0” calibrator to 1:2, 1:4 and 1:8. PSA values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follow:

Serum	Original Value	Percentage of Recovery		
		1:2	1:4	1:8
1	25	27	2.10	7.77
2	4	4.2	0.35	8.33
3	2	1.3	0.15	11.53

REFERENCES:

1. Armbruster DA. *Clin Chem* 39:181-195, 1993.
2. Stenman U-H, et al. *Cancer Res 51:*222-226, 1991.