

CALDON BIOTECH INC. Prostate Specific Antigen (PSA) ELISA

Catalog No. PS067T
(96 tests)

INTENDED USE

The CALDON BIOTECH INC (CBI), PSA ELISA kit is used for the quantitative measurement of PSA in human serum or plasma.

SUMMARY AND EXPLANATION

Prostate Specific Antigen (PSA) is a single chain glycoprotein produced by epithelial cells of the prostate gland. PSA is useful in the management of patients with prostate cancer. The measurement of serum PSA has become the most accepted test to indicate men who are at risk of having prostate cancer and who should be examined by other tests. Using a cut-off of 4 ng/mL, 92% of men over 50 years of age with malignant prostatic tissues, 8% of healthy men and 28% of men with benign prostate hyperplasia (BPH) test positive for PSA. Three major forms of PSA exist in the serum: free PSA, bound PSA and complex PSA. Bound PSA is found in higher concentrations in patients with prostate cancer; whereas, free PSA is detected in higher concentrations in patients with BPH. If the free PSA to total PSA ratio is >25%, it is unlikely that the patient has prostate cancer; whereas, if free PSA is <16% then prostate cancer is likely to be the cause. Serial measurement of PSA concentration in the serum is an important tool in monitoring patients with prostatic cancer and determining the potential and actual effectiveness of surgery or other therapies, or may allow for earlier discovery of residual or recurrent carcinoma after radical prostatectomy or radiotherapy. Current indications suggest that men over 50 years should be screened with digital rectal examination and PSA. Men with a high risk of prostate cancer, such as a family history or of African heritage, should begin annual testing at age 40 years. If both are normal, the patient can be followed with annual evaluations and monitoring to determine the rate of change. Slight elevations in PSA (4.1 ng/mL to 10.0 ng/mL) warrant a transrectal ultrasound (TRUS) to evaluate prostate volume and echogenicity of the gland. Hypo-echogenic lesions should be biopsied. Elevated PSA density (>0.15 ng/mL/cc), very high PSA (>10 ng/mL) or

a free -to-total PSA ratio of <16% warrants systemic biopsy.

PRINCIPLE OF THE TEST

The PSA is a two-site sandwich ELISA method. Samples and diluent are added to the wells coated with Anti-PSA MAb. PSA in the patient's serum binds to anti- MAb on the well. Unbound proteins are washed off by wash buffer. Anti-PSA HRP labeled second antibody is then added. Unbound protein and HRP conjugate are washed off by wash buffer. 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 relating color intensity to the concentration of the PSA.

MATERIALS PROVIDED

1. Microwell strips coated with PSA MAb (12x8x1 wells). Total of 96 wells.
2. Standard: 6 vials (0.7 mL each). Ready to use.
3. Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
4. Assay Diluent: 1 bottle (12 mL). Ready to use.
5. TMB Substrate: 1 bottle (12 mL). Ready to use.
6. Stop Solution: 1 bottle (8 mL). Ready to use.
7. 10X Wash Concentrate: 1 bottle (50 mL).

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes

- of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for in vitro diagnostic use only.
 3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
 4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
 5. It is recommended that serum samples be run in duplicate.
 6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

ASSAY 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 50 µL of PSA standards, controls and patient's sera.
3. Pipet 50 µL of assay diluent into each well.
4. Cover the plate and incubate for 60 minutes at room temperature (18 - 26° C).
5. Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 µL Enzyme Conjugate into each well.

7. Incubate for 30 minutes at room temperature.
8. Wash wells as step # 5.
9. Add 100 µL of TMB substrate into each well.
10. Incubate for 15 minutes at room temperature.
11. Add 50 µL of stop solution into each well. Shake the plate gently for 30 seconds to mix the solution. Make sure that the blue color completely changes to yellow.
12. Read absorbance on ELISA Reader at 450 nm within 30 minutes from adding 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. Make sure you check the value on every kit. See example of the standard attached.
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.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for PSA may be used as initial guideline ranges only:

PSA Normal Range = Less Than 4 ng/mL.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

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

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

2. Precision Intra-Assay

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

Inter-assay

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

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 follows:

Serum	Original Value (ng/mL)	Percentage of Recovery		
		1:2	1:4	1:8
1	25	94	91	102
2	4	105	96	99
3	2	112	115	106

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Caldon Biotech Inc.

www.caldonbiotech.com

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2270-K Camino Vida Roble, Carlsbad 92009

Tel #: 800-257-2812