

CALDON BIOTECH INC. Free Prostate Specific Antigen (fPSA) ELISA

Catalog No. PS092F
(96 tests)

INTENDED USE

The CALDON BIOTECH INC (CBI), fPSA ELISA kit is used for the quantitative measurement of fPSA 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. In men with a total PSA test result in the "gray zone," between 4 and 10 ng/mL, there is still a one in four chance of having prostate cancer. fPSA, can help to distinguish between cancer and benign prostatic hyperplasia. Men with BPH will have a higher percentage of fPSA, while those with cancer will have a lower percentage. Some experts recommend use of fPSA to weed out men with BPH, thus avoiding the more costly and invasive transrectal ultrasound-guided biopsy. Applying this strategy to men with PSA levels between 2.5 and 10.0 ng/mL may lead to detection of early disease in a larger number of men and may result in a lower biopsy rate compared with older strategies."

PRINCIPLE OF THE TEST

The fPSA is a two-site sandwich ELISA method. Samples and diluent are added to the wells coated with Anti-fPSA MAb. fPSA in the patient's serum binds to anti- MAb on the well. Unbound

proteins are washed off by wash buffer. Anti-fPSA 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 fPSA in the samples. A standard curve is prepared relating color intensity to the concentration of the fPSA.

MATERIALS PROVIDED

1. Microwells coated with fPSA MAb (12x8x1 wells). Total of 96 wells.
2. Standard: 6 vials (2 mL each). lyophilized
3. Enzyme Conjugate: 1 bottle (22 mL). Ready to use.
4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 1 bottle (12 mL). Ready to use.

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

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Reconstitute each lyophilized standard with 2.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards should be stored sealed and are stable for 30 days at 2-8°C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 200µl of standards, specimens, and controls into appropriate wells.
3. Gently mix for 10 seconds.
4. Incubate at room temperature (18-25°C) for 60 minutes.
5. Remove the incubation mixture by emptying plate contents into a suitable waste container.
6. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 200µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
9. Incubate at room temperature for 60 minutes.
10. Remove the incubation mixture by emptying plate contents into a suitable waste container.
11. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
12. Strike the wells sharply onto absorbent paper to remove residual water droplets.

13. Dispense 100µl of TMB Reagent into each well. Gently mix for 5 seconds.
14. Incubate at room temperature for 20 minutes.
15. Stop the reaction by adding 100µl of Stop Solution to each well.
16. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
17. Using a microtiter plate reader, read the optical density at 450 nm within 30 minutes.

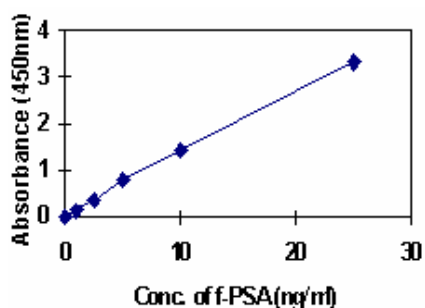
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) 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. Using the mean absorbance value for each sample, determine the corresponding concentration of f-PSA in ng/ml from the standard curve.

EXAMPLE OF THE STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against fPSA 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.

f-PSA (ng/ml)	Absorbance (450nm)	Corrected Absorbance (450nm)
0	0.072	0.000
1.0	0.199	0.127
2.5	0.434	0.362
5.0	0.866	0.794
10.0	1.502	1.430
25.0	3.392	3.320



EXPECTED VALUES

As discussed in the introduction, the important diagnostic parameter is not the level of fPSA, but rather the ratio of fPSA to total PSA. Percent fPSA offered the greatest advantage to the total PSA test when the total PSA values were between 3.0 and 10.0 ng/ml.

SENSITIVITY

The sensitivity of this kit is estimated to be 0.1 ng/ml.

LIMITATIONS OF THE TEST

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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