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Calbiotech Inc..

CALBIOTECH
A life science company

Free Prostate Specific Antigen (f-PSA) ELISA

Catalog No. PS233T (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) f-PSA ELISA kit is used for the quantitative measurement of f-PSA in human serum.

Summary AND EXPLANATION

Human Prostate Specific Antigen (PSA) is a 33 kD serine proteinase which, in human serum, is predominantly bound to alpha 1-antichymotrypsin (PSA-ACT) and alpha 2-macroglobulin (PSAAMG). Trace amounts of alpha 1-antitrypsin and inter-alpha trypsin inhibitor bound to PSA can also be found. Any remaining PSA is in the free form (f-PSA).1-3 Current methods of screening men for prostate cancer utilize the detection of the major PSA-ACT form. Levels of 4.0 ng/ml or higher are strong indicators of the possibility of prostatic cancer.4 However, elevated serum PSA levels have also been attributed to benign prostatic hyperplasia and prostatitis, leading to a large percentage of false positive screening results.5 A potential solution to this problem involves the determination of free PSA levels.6-17 Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than those with benign prostatic hyperplasia.2 Thus, the measurement of free serum PSA in conjunction with total PSA, can improve specificity of prostate cancer screening in selected men with elevated total serum PSA levels, which would subsequently reduce unnecessary prostate biopsies with minimal effects on cancer detection rates.

PRINCIPLE OF THE TEST

The f-PSA ELISA kit is a solid phase assay based on a streptavidin-biotin principle. The standards, samples and a reagent mixture of Anti-f-PSA Enzyme and Biotin conjugates (conjugate reagent) are added into the wells, coated with Streptavidin. f-PSA in the patient's serum forms a sandwich between two highly specific Anti-f-PSA antibodies, labeled with Biotin and HRP. Simultaneously, the biotinylated antibody is immobilized onto the well through a high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin/enzyme conjugated reagent are washed off, by washing buffer. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of f-PSA in the samples. A standard curve is prepared relating color intensity to the concentration of the f-PSA.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated with Streptavidin	12x8x1
2.	f-PSA Standard: 6 vials (ready to use)	0.5 ml
3.	f-PSA Conjugate Reagent: 1 bottle (ready to use)	12 ml
4.	TMB Substrate: 1 bottle (ready to use)	12 ml
5.	Stop Solution: 1 bottle (ready to use)	12 ml
6.	20X Wash concentrate: 1 bottle	25 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

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.
4. Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. 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
4. This test kit is designed for Research Use Only.
5. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
6. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
7. It is recommended that serum samples be run in duplicate.
8. 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. Typically, 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. Prepare 1X wash buffer by adding the content of the bottle (25 ml, 20X) 475 of distilled or de-ionized water. Store at room temperature (20-25°C)

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. Pipette 50 µl of f-PSA standards, control and patient's sera to selected wells.
3. Add 100 µl of conjugate reagent to all wells.
4. Mix the content of the plate, gently, for 30 seconds.
5. Cover the plate and incubate for 60 minutes at room temperature (20-25°C).
6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

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 STANDARD CURVE

This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory should obtain its own data and standard curve.

f-PSA (ng/ml)	Absorbance (450 nm)
0	0.01
0.5	0.19
1.0	0.39
2.5	0.75
5.0	1.55
10.0	2.32

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.
3. Sodium azide inhibits the activity of enzyme conjugate; hence do not use samples with Na-Azide preservative.