Prostate Specific Antigen (Free, human) ELISA Kit

(Catalog # K7432-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

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. BioVision's free-PSA kit is a solid phase direct sandwich ELISA method. The samples and diluted anti-f-PSA-HRP conjugate are added to the wells coated with MAb to f-PSA. The f-PSA molecules present in the standard solution or sera are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the 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 f-PSA. Sensitivity: 0.1 ng/ml

II. Application:

Quantitative measurement of free PSA

III. Specificity:

Human PSA

IV. Sample Type:

- Serum

V. Kit Contents:

Components	K7432-100	Part No.
Microwells coated with f-PSA MAb	12x8x1	K7432-100-1
f-PSA Standard	6x0.5 ml	K7432-100-2
Anti-f-PSA Enzyme Conjugate	12 ml	K7432-100-3
Wash Buffer (20X)	25 ml	K7432-100-4
TMB Substrate	12 ml	K7432-100-5
Stop Solution	12 ml	K7432-100-6

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{o}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Buffer:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).

VIII. Warning & Precautions:

- Potential biohazardous materials: The Standard and control contains 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, 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.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that serum samples be run in duplicate.
- 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.

IX. Sample Preparation and Storage:

Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 5 days. Store frozen at (-20°C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Don't use grossly lipemic specimens.

X. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 50 µl of f-PSA Standards, control, or samples into designated wells.
3. Add 100 µl of enzyme conjugate into all wells. Shake gently for 10-30 sec. to mix.
4. Cover the plate and incubate for 60 min. at room temperature (18-26°C).
5. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.

6. Add 100 µl of TMB substrate to all wells, cover the plate & incubate for 15 min. at room temperature.
7. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
8. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stop solution.

XI. Calculation: Construct the standard curve; plot the absorbance for the f-PSA standards (vertical axis) versus f-PSA standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Example of a Standard Curve:

Standard	OD (450 nm)
Standard 1 (0 ng/ml)	0.02
Standard 2 (1 ng/ml)	0.14
Standard 3 (2 ng/ml)	0.26
Standard 4 (5 ng/ml)	0.57
Standard 5 (10 ng/ml)	1.13
Standard 6 (20 ng/ml)	2.22

Expected Values:

As discussed in the introduction, the important diagnostic parameter is not the level of free PSA, but rather the ratio of free PSA to total PSA. Percent free-PSA offered the greatest advantage to the total PSA test when the total PSA values were between 3.0 and 10.0 ng/ml.14 For a given sample, different commercial test kits of total PSA and free-PSA may give different values of total PSA and free-PSA. Users should keep this in mind while calculating the percentage. The following information is cited from References 6, 7, 10, 11, 13, 14-17. For total PSA levels between 3.0 and 4.0 ng/ml, using a 19% cutoff point for percent free-PSA would result in detection of 90% of all cancers. 14 For total PSA levels between 4.1 and 10.0 ng/ml, the most appropriate cutoff point for free-PSA is 24%. At this cutoff point, 95% of the cancers would be detected.14 With respect to free PSA levels and prostate volume; the available information is again limited. In Catalona et al study, men with prostate cancer and a prostate volume of 400 cc or less had a median free-to-total PSA proportion of 0.092 (9.2%), a value statistically lower than the 0.159 (15.9%) found for patients with prostate cancer and a gland >40.0 cc. Yemoto et al, in a recent study of 200 men, showed no correlation between percent free-PSA and prostate volume.16 Data from several studies have demonstrated an inverse relationship between percent free-PSA and total PSA. This observation suggests that higher PSA levels are more commonly associated with lower percent free-PSA values and these men most frequently have more aggressive or advanced prostate cancers.

Correlation with a reference ELISA Kit:

A total of 60 sera were tested by BioVision f-PSA ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.99	0.79	0.011

XII. RELATED PRODUCTS:

Prostate Specific Antigen (Total, human) ELISA Kit (K7431)
Progeststerone (human) ELISA Kit (K7414)
Progesterone (mouse/rat) ELISA Kit (4715)
Testosterone (mouse/rat) ELISA Kit (K7418)

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