



Prostate Specific Antigen (Total, human) ELISA Kit

03/19

(Catalog # K7431-100, 100 assays; Store at 2-8°C)

I. Introduction:

Prostate Specific Antigen (PSA) is useful in the management of patients with prostate cancer. It is a single chain glycoprotein produced by epithelial cells of the prostate gland. 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. BioVision's PSA ELISA kit is a solid phase assay based on a streptavidin-biotin principle. The standards, samples and a reagent mixture of Anti-PSA Enzyme and Biotin conjugates (conjugate reagent) are added into the wells, coated with Streptavidin. PSA in the serum sample forms a sandwich between two highly specific Anti-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 PSA in the samples. A standard curve is prepared relating color intensity to the concentration of the PSA.

II. Application:

Quantitative measurement of PSA

III. Specificity:

Human PSA

IV. Sample Type:

- Serum

V. Kit Contents:

Components	K7431-100	Part No.
Microwells coated with Streptavidin	12x8x1	K7431-100-1
PSA Standard*	6x0.5 ml	K7431-100-2
Anti-PSA Conjugate Reagent	12 ml	K7431-100-3
Wash Buffer (20X)	25 ml	K7431-100-4
TMB Substrate	12 ml	K7431-100-5
Stop Solution	12 ml	K7431-100-6

* Check PSA standard value on each standard vial. This value might vary from lot to lot.

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°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. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

- **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 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.

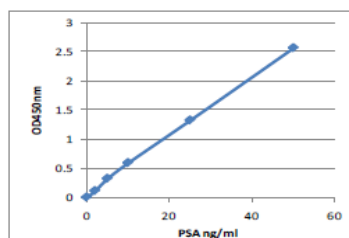


2. Pipet 25 µl of PSA Standards, control, or samples into designated wells.
3. Add 100 µl of Anti-PSA conjugate reagent into all wells. Shake gently for 10-30 sec. to mix.
4. Cover the plate and incubate for 60 min. at room temperature (18-26°C).
5. Remove liquid from all wells & wash wells three times 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 PSA standards (vertical axis) versus 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)	0.049
Standard 2 (3 ng/ml)	0.266
Standard 3 (6 ng/ml)	0.444
Standard 4 (10 ng/ml)	0.658
Standard 5 (25 ng/ml)	1.505
Standard 6 (50 ng/ml)	2.79



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 were obtained from literature and may be used as initial guideline ranges only:
PSA Normal Range = Less than 4 ng/ml

Sensitivity

The minimum detectable concentration for the PSA ELISA as measured by 2 X SD from the mean of 20 zero standards is estimated to be 0.0144 ng/ml.

XII. RELATED PRODUCTS:

Prostate Specific Antigen (Free, human) ELISA Kit (K7432)
Progesterone (human) ELISA Kit (K7414)

Progesterone (mouse/rat) ELISA Kit (4715)
Testosterone (mouse/rat) ELISA Kit (K7418)

FOR RESEARCH USE ONLY! Not to be used on humans.