

**REFERENCES**

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## Prostate Specific Antigen(PSA) ELISA

Catalog No. PS235T (96 Tests)

**INTENDED USE**

The Calbiotech, Inc PSA ELISA Kit is intended for the quantitative measurement of PSA in human serum. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

**Summary AND EXPLANATION**

Prostate Specific Antigen (PSA) is a kallikrein-family serine protease secreted primarily by epithelial cells of the prostate gland. In research, PSA is widely used as a molecular marker in prostate biology studies, androgen regulation research, and investigations of gene expression in prostate-derived cell lines. It is also employed in molecular biomarker panels exploring prostate pathophysiology, drug response in preclinical models, and the biology of kallikrein-related peptidases.

**PRINCIPLE OF THE TEST**

The 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 patient's serum 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.

MATERIALS PROVIDED		96 TESTS
1.	Microwells coated with Streptavidin	12x8x1
2.	PSA Standard: 6 vials (ready to use)	0.5 mL
3.	PSA Control: 2 vials (ready to use)	0.5 mL
4.	Anti-PSA Conjugate Reagent: 1 bottle (ready to use)	12 mL
5.	TMB Substrate: 1 bottle (ready to use)	12 mL
6.	Stop Solution: 1 bottle (ready to use)	12 mL
7.	20X Wash Concentrate: 1 bottle	25 mL

**MATERIALS NOT PROVIDED**

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

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**WARNINGS AND PRECAUTIONS**

Potential biohazardous materials:

1. The standards 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. 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**

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

**REAGENTS PREPARATION**

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

**Example of a Standard Curve**

	OD 450 nm	Conc. ng/mL
<b>Std 1</b>	0.01	0
<b>Std 2</b>	0.12	2
<b>Std 3</b>	0.33	5
<b>Std 4</b>	0.60	10
<b>Std 5</b>	1.33	25
<b>Std 6</b>	2.57	50

**LIMITATIONS OF THE TEST**

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