LIMITATIONS OF THE TEST

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

REFERENCES

1. Espana F; Sanchez-Cuenca J; Estelles A; Gilabert J; Griffin JH; Heeb MJ. Quantitative immunoassay for complexes of prostate-specific antigen with alpha2-macroglobulin. Clin Chem 1996; 42(4):545-50.
2. Corey E; Wegner SK; Stray JE; Corey MJ; Arfman EW; Lange PH; Vessella RL. Characterization of 10 new monoclonal antibodies against prostate-specific antigen by analysis of affinity, specificity and function in sandwich assays. Int J Cancer 1997; 71(6):1019-28.
3. Barak M; Cohen M; Mecz Y; Stein A; Rashkovitzki R; Laver B; Lurie A. The additional value of free prostate specific antigen to the battery of age-dependent prostate-specific antigen, prostate-specific antigen density and velocity. Eur J Clin Chem Clin Biochem 1997; 35(6): 475-81.
4. Vogl M; Muller MM; Holtl W. Clinical usefulness of percentage of free serum prostate specific antigen. Clin Chim Acta 1997; 258(1):79-90.
5. Stenman UH; Leinonen J; Zhang WM. Problems in the determination of prostate specific antigen. Eur J Clin Chem Clin Biochem 1996; 34(9):735-40. For Research Use Only. Not for use in Diagnostic Procedures.

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Cat#: IB19126 (96 Tests)
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Prostate Specific Antigen (PSA) ELISA

Catalog No.: IB19126 (96 Tests)

INTENDED USE

The PSA ELISA Kit is intended for the determination of PSA in human serum. For research use only, not for use in diagnostic procedures.

PRINCIPLE OF THE TEST

The PSA ELISA test 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 that are coated with streptavidin. PSA in the serum forms a sandwich between two highly specific anti-PSA antibodies, labeled with biotin and HRP. Simultaneously, the biotinylated antibody is immobilized onto the wells through a high affinity streptavidin-biotin interaction. Unbound protein and excess biotin/enzyme conjugated reagent are washed off by washing buffer. Upon addition of the substrate, the intensity of the 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)	0.5ml
3. Anti-PSA Conjugate Reagent: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. 20X Wash concentrate: 1 bottle	25ml

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 the 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. 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
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 standards, control and 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

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).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature (18-26°C).
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 25 µl of PSA standards, controls (if using) and unknowns to selected wells.
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 (18-26° C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper or paper towel.
7. Add 100 µl of TMB substrate into 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

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.
2. To construct the calibration 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 to determine the corresponding concentration of PSA from the calibration 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.

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