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**Warning**

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## Prostate Specific Antigen (PSA) ELISA

Catalog No. **BQ 067T** (96 Tests)**INTENDED USE**

The PSA ELISA Kit is intended for the quantitative measurement of PSA in human serum.

**SUMMARY AND EXPLANATION**

Prostate Specific Antigen (PSA) is a single chain glycoprotein produced by epithelial cells of the prostate gland. PSA is useful in the management of patients with prostate cancer. 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. Current indications suggest that men over 50 years should be screened with digital rectal examination and PSA. Men with a high risk of prostate cancer, such as a family history or of African heritage, should begin annual testing at age 40 years. If both are normal, the patient can be followed with annual evaluations and monitoring to determine the rate of change. Slight elevations in PSA (4.1 ng/ml to 10.0 ng/ml) warrant a transrectal ultrasound (TRUS) to evaluate prostate volume and echogenicity of the gland. Hypo-echogenic lesions should be biopsied. Elevated PSA density (>0.15 ng/ml/cc), very high PSA (>10 ng/ml) or a free-to-total PSA ratio of <16% warrants systemic biopsy.

**PRINCIPLE OF THE TEST**

The PSA ELISA test is a solid phase two-site immunoassay. An anti-PSA monoclonal antibody is coated on the surface of the microtiter wells and a mouse anti-PSA antibody labeled with horseradish peroxidase is used as the tracer. The 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 proteins and antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the color formed is proportional to the concentration of PSA present in the sample.

<b>MATERIALS PROVIDED</b>		<b>96 Tests</b>
1.	Microwell coated with PSA MAbs	12x8x1
2.	PSA Standard: 6 vials ( ready to use)	0.7ml
3.	PSA Enzyme Conjugate: 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. 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.

**WARNINGS AND PRECAUTIONS**

1. 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
2. This test kit is designed for Research Use Only. Not for use in diagnostic procedures.
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 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.  
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 50 µl of PSA standards, control and patient's sera to selected wells.
3. Add 100 µl of enzyme conjugate 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 (18-26° 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**

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
Std 1	0.01	0
Std 2	0.11	2
Std 3	0.32	5
Std 4	0.81	10
Std 5	2.07	25
Std 6	3.07	50

**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 may be used as initial guideline ranges only:

PSA Normal Range = Less Than 4 ng/ml.

**LIMITATIONS OF THE TEST**

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.