

**LIMITATIONS OF THE TEST**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

**REFERENCES:**

1. Christensson A, Bjork T, Nilsson O, et al. Serum Prostate Specific Antigen Complexed to  $\alpha$ 1-Antichymotrypsin As An Indicator of Prostate Cancer. J. of Urol. 150:100-105; 1993.
2. Lilja H, Christensson A, Dahlen U, et al. Prostate-specific antigen in serum occurs predominantly in complex with  $\alpha$ -1-antichymotrypsin. Clin Chem 37:1618-1625, 1991.
3. Stenman U-H, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and  $\alpha$ -1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostate cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res. 51:222-226, 1991.
4. Catalona WJ, Smith DS, Ratliff TL, Basler JW. Detection of organ-confined prostate cancer is increased through prostate-specific antigen-based screening. JAMA. 270:948-954, 1993.
5. Stamey TA, Yang N Hay AR, McNeal JE, Freiha, FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med. 317-: 909-916, 1987.
6. Junker R, Brandt B, Zechel C, and Assmann, G. Comparison of Prostate-specific antigen (PSA) measured by four combinations of free-PSA and total PSA assays. Clinical Chemistry 43:1588-1594, 1997.
7. Luderer AA, Chen Y-T, Soriano TF, et al. Measurements of the proportion of free to total prostate-Specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total prostate-specific antigen. Urology 46:187-194, 1995.
8. Carter HB, Pearson JD, Metter J, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. JAMA. 267:2215-2220, 1992.
9. Smith DS, Catalona WJ. Rate of change in serum prostate-specific antigen levels as a method for prostate cancer detection. J Urol. 152:1163-1167, 1994
10. Benson MC, Whang IS, Pantuck A, et al. Prostate specific antigen density: a means of distinguishing benign prostatic hypertrophy and prostate cancer. J Urol. 147:815-816, 1992.
11. Catalona WJ, Hudson MA, and Scardino PT, et al. Selection of optimal prostate specific antigen cut-offs for early detection of prostate cancer: receiver operating characteristic curves. J Urol. 152:2037-2042, 1994.
12. Smith DS, Catalona WJ. The nature of prostate cancer detected through prostate specific antigen based screening. J Urol. 152:1732-1736, 1994.
13. Catalona, et al. Evaluation of Percentage of Free Serum Prostate-Specific Antigen to Improve Specificity of Prostate Cancer Screening. JAMA 274:1214, 1995.
14. Vashi AR, Wojno KJ, Henricks W, et al: Determination of the "reflex range" and appropriate cutpoints for percent free-PSA in 413 men referred for prostatic evaluation using the AxSYM system. Urology 49:19-27, 1997.
15. Bangma, CH, Kranse R, Blijenberg B, et al: The value of screening tests in the detection of prostate cancer. Part I: Results of a retrospective evaluation of 1726 men. Urol. 46:773-778, 1995.
16. Yemoto CM, Nolley R, Prestigiacomo AF, et al: Free (f) and total (t) PSA density in patients with prostate cancer (CaP) and benign prostatic hyperplasia (BPH). J Urol 155:347A, 1996.
17. Bangma, CH, Kranse R, Blijenberg B, et al: The value of screening tests in the detection of prostate cancer. Part II: Retrospective analysis of free-total prostate-specific analysis ration, age-specific reference ranges, and PSA density. Urol 46:779-785, 1995.

2008-03-17

**Warning**

All of Bio-Quant ELISA Kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of Bio-Quant customers to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

**BIO-QUANT DOES NOT MAKE ANY OTHER WARRANTY OR REPRESENTATION WHATSOEVER, WHETHER EXPRESS OR IMPLIED, WITH RESPECT TO THESE PRODUCTS. IN PARTICULAR Bio-Quant, INC. DOES NOT MAKE ANY WARRANTY OF SUITABILITY, NON-INFRINGEMENT, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OF ANY PRODUCT.**



## Free Prostate Specific Antigen (f-PSA) ELISA

Catalog No. BQ142C (96 tests)

**INTENDED USE**

The f-PSA ELISA kit is used for the quantitative measurement of f-PSA in human serum or plasma.

**SUMMARY AND EXPLANATION**

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).<sup>1-3</sup> 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.<sup>4</sup> 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.<sup>5</sup> A potential solution to this problem involves the determination of free PSA levels.<sup>6-17</sup> Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than those with benign prostatic hyperplasia.<sup>2</sup> 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<sup>6</sup>

**PRINCIPLE OF THE TEST**

The f-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 in the samples. A standard curve is prepared relating color intensity to the concentration of the f-PSA.

MATERIALS PROVIDED		96 TESTS
1.	Microwell coated with f-PSA MAb	12x8x1
2.	f-PSA Standard: 6 vials ( ready to use)	0.5 ml
3.	Anti-f-PSA Enzyme Conjugate: 1 bottle (ready to use)	12 ml
4.	TMB Substrate: 1 bottle (ready to use)	12 ml
5.	Stop Solution: 1 bottle (ready to use)	12 ml
6.	20X Wash concentrate: 1 bottle	25 ml

**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 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.
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

1. Prepare 1X wash buffer by adding the content of the bottle (25 ml, 20X) 475 of distilled or de-ionized water. Store at room temperature (18 °C – 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 f-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

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of f-PSA in ng/ml from the standard 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.

f-PSA (ng/ml)	Absorbance (450 nm)
0	0.02
1.0	0.14
2.0	0.26
5.0	0.57
10.0	1.13
20.0	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.<sup>14</sup> For a given patient 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.<sup>14</sup> 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.<sup>14</sup> With respect to free PSA levels and prostate volume; the available information is again limited. Catalona et al were the first to demonstrate the importance of prostate size in selecting the cutoff value for percent free-PSA.<sup>13</sup> In their 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.<sup>16</sup> 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.<sup>2, 14, 17</sup>

#### CORRELATION WITH A REFERENCE ELISA KIT:

A total of 60 sera were tested by CRP ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.99	0.79	0.011

#### SENSITIVITY

The sensitivity of this kit is estimated to be 0.1 ng/ml.