Free Prostate Specific Antigen (f-PSA) ELISA Kit Protocol

(Cat. No.:EK-310-21)

PHOENIX PHARMACEUTICALS, INC.
330 Beach Road, Burlingame CA
Tel: 650-558-8898 Fax: 650-558-1686
E-Mail: info@phoenixpeptide.com
www.PhoenixPeptide.com

INTENDED USE

For the quantitative determination of the Cancer Antigen f-PSA concentration in human serum. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

LIMITATIONS OF THE PROCEDURE

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. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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INTRODUCTION AND PROTOCOL OVERVIEW

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

PRINCIPLE OF THE TEST

The f-PSA ELISA test is a solid phase two-site immunoassay. An anti-f-PSA monoclonal antibody is coated on the surface of the microtiter wells and a goat anti-PSA antibody labeled with horseradish peroxidase is used as the tracer. 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 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 f-PSA present in the sample.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

STORAGE

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement. DO NOT FREEZE

LIST OF COMPONENTS

Materials Provided with the Kit:

- Murine Monoclonal Anti-Free PSA coated microtiter plate with 96 wells.
- Reference standards containing 0, 1.0, 2.5, 5.0, 10.0, and 25.0 ng/ml Free PSA, 1 ml each, ready to use.
- Free PSA Zero Buffer, 13 ml.
- Enzyme Conjugate Reagent, 22 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes: 50 µl, 100 µl, 200 µl, and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towels.
- Microtiter plate shaker
- Graph paper.
- A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

REAGENT PREPARATION

All reagents should be brought to room temperature (18-25°C) before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of f-PSA Zero Buffer into each well.
4. Gently mix for 30 seconds.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by emptying plate contents into a suitable waste container.
7. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 60 minutes.
11. Remove the incubation mixture by emptying plate contents into a suitable waste container.
12. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
18. Using a microtiter plate reader, read the optical density at 450 nm **within 15 minutes.**

Important Notes:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that if manual pipetting is used, no more than 32 wells be used for each assay run, since pipetting of all standards, specimens and controls should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

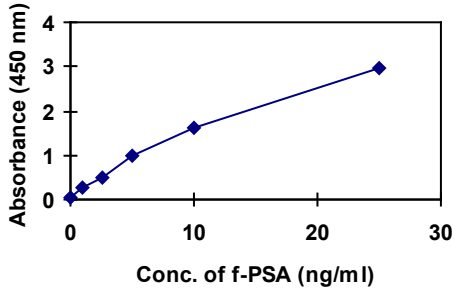
CALCULATION OF RESULTS

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

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against f-PSA concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

f-PSA (ng/ml)	Absorbance (450 nm)
0	0.045
1.0	0.252
2.5	0.496
5.0	0.967
10.0	1.618
25.0	2.948



EXPECTED VALUES AND SENSITIVITY

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. (14)

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. (14) 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. (14)

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. (13) 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.(16)

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. (2,14,17)

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

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ASSAY DIAGRAM