Atlas Medical

**FREE PROSTATE SPECIFIC ANTIGEN (f-PSA)**
**ENZYME IMMUNOASSAY TEST KIT**

IVD *For in vitro diagnostic and professional use only*

2°C 8°C **Store at 2 to 8°C.**

Σ 96 Tests

## INTENDED USE

For the quantitative determination of f-PSA concentration in human serum.

## INTRODUCTION

Prostate Specific Antigen PSA, is a 33KD serine protease, a single chain glycoprotein is an enzyme found in the blood produced exclusively by prostate cells. Normal levels of PSA in the blood are small amounts between 0-2.5 ng/ml. Higher than normal levels, greater than 2.5 ng/ml, can be caused by cancer or benign, non-cancerous conditions such as enlarged prostate, prostate inflammation, infection, or trauma. All elevated readings of PSA should be checked.

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.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. A potential solution to this problem involves the determination of free PSA levels.

## PRINCIPLE OF THE TEST

The f-PSA ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. Anti-f-PSA monolonal is coated on the microtiter wells and another anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The f-PSA molecules present in the standard solution or serum 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.

## MATERIALS PROVIDED

- Anti-f-PSA coated microtiter plate with **96 wells.**
- Sample diluent (zero buffer), **12 ml transparent cap bottle with yellow color reagent.**
- Reference standard set containing :
  **0 (silver cap vial) , 0.1 (golden cap vial), 0.5 (green cap vial ), 2.0 (blue cap vial) ,5.0 (purple cap vial) , and 10.0 (red cap vial )** ng/ml ,(liquid ready to use) or lyophilized form, **1ml** each.
- Enzyme Conjugate Reagent, **22 ml red cap bottle.**
- TMB Reagent solution, **12 ml brown cap bottle.**
- Stop Solution, **12 ml white cap bottle.**
- Wash buffer concentrate (50X), **15ml green cap bottle.**
- Control set **(optional).**

**Materials required but not provided:**

- Vortex mixer .
- Precision pipettes:0.10,0.20 and 10.0ml.
- Disposable pipette tips.
- Graph paper.
- Distilled water.
- Absorbent paper or paper towel.
- 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.

## SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture Techniques and the serum should be separated from the red blood cells as soon as possible. Avoid grossly hemolytic, Lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, Heparin, or oxalate may interfere with the test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

## STORAGE OF TEST KIT

1. Unopened test kits should be stored at 2-8°C upon receipt. The microtiter plate should be kept in a sealed bag with desiccants, to minimize exposure to damp air.
2. Opened test kits will remain stable until the expiration date, provided it is stored as described above.
3. A microtiter plate reader with a band width of 10 nm or less and an optical density (O.D) range of 0-2.5 or greater at 450 nm Wavelength is accebtable for use in absorbance measurement.

## REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) and mixed by gently inverting or swirling prior to use. Do not induce Foaming.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of Distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

## ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of sample diluent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this step.
5. Incubate at room 37°C for 60 minutes.
6. Remove the incubation mixture by emptying plate contents into a waste container.
7. Rinse and empty the microtiter wells 5 times withwashing buffer (1X).
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 5 seconds.
10. Incubate at 37°C 60 minutes.
11. Remove the incubation mixture by emptying plate contents into a waste container.
12. Rinse and empty the microtiter wells 5 times with wash buffer (1X).
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 5 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 microliter plate reader, read the optical density at 450nm within 15 minutes.

## WASH PROCEDURE

1. The wash procedure is critical. Insufficient washing will Result in a poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, 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 ($A_{450}$) for each set of

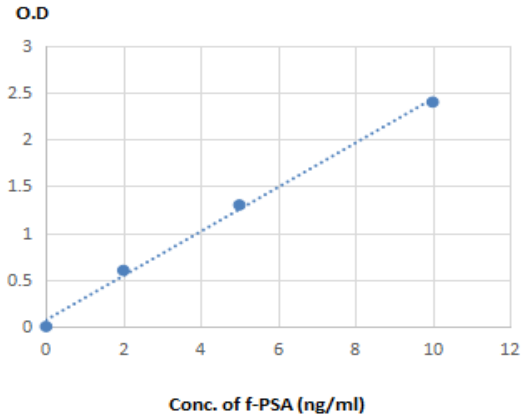
reference standards, control, and samples.

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. Use the mean absorbance value for each sample, determine the corresponding concentration of 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 | 0.006 |
| 0.1 | 0.032 |
| 0.5 | 0.155 |
| 2.0 | 0.597 |
| 5.0 | 1.302 |
| 10.0 | 2.361 |




## EXPECTED VALUES AND SENSITIVITY

Clinical studies have shown that higher total PSA levels and lower percentages of free PSA are associated with higher risks of prostate cancer. of free PSA are associated with higher risks of prostate cancer. In one study, 52 individuals with benign prostate hyperplasia (BPH) and 77 individuals with prostate cancer were analyzed. The f-PSA/t-PSA ratio were summarized in the table below.

| | f-PSA/t-PSA Ratio | | | |
|---|---|---|---|---|
| | Median | Min | Max | Mean (95% confidence interval) |
| BPH | 0.18 | 0.04 | 0.42 | 0.19 (0.17-0.21) |
| Prostate Cancer | 0.09 | 0.02 | 0.53 | 0.12 (0.10-0.14) |

The relationship between f-PSA/t-PSA ratio and risk of prostate cancer is also age related. When total PSA is in the range of 4.0-10.0 ng/mL, a f-PSA/t-PSA ratio ≤ 0.10 indicates 49% to 65% risk of prostate cancer depending on age ; a f-PSA/t-PSA ratio > 0.25 indicates a 9% to 16% risk of prostate cancer, depending on age.

| | Percent probability of prostate cancer | | |
|---|---|---|---|
| f-PSA/t-PSA ratio | 50-59 years | 60-69 years | =70 years |
| =0.10 | 49.2 | 57.5 | 64.5 |
| 0.11-0.18 | 26.9 | 33.9 | 40.8 |
| 0.19-0.25 | 18.3 | 23.9 | 29.7 |
| >0.25 | 9.1 | 12.2 | 15.8 |

Multiple factors such as population, age, specificity of test method may affect interpretation of f-PSA and t-PSA values. The seranges should be used as guidelines only. Each laboratory should establish its own reference values.

### Sensitivity

The minimum detectable concentration of f-PSA in this assay is estimated to be 0.05 ng/ml.

### Cross Reactivity

| Antigens | Concentration | %Cross-react |
|---|---|---|
| PSA-ACT | 500 ng/mL | 0.2 |
| AFP | 10,000 ng/ mL | 0 |
| CEA | 5,000 ng/ mL | 0 |
| CA 125 | 1,000 U/ mL | 0 |
| CA 15-3 | 1,000 U/ mL | 0 |
| CA 19-9 | 1,000 U/ mL | 0 |
| -HCG | 1,000 ng/ mL | 0 |
| β-HCG | 1,000 ng/ mL | 0 |
| HCG | 50,000 mIU/ mL | 0 |

### Precision

| Intra- Assay | | | |
|---|---|---|---|
| | Replicates | S.D. | %CV |
| level | 20 | 0.005 | 13.1 |
| level | 20 | 0.011 | 4.4 |
| level | 20 | 0.128 | 3.2 |

### Linearity

Two patient sera were serially diluted with 0 ng/ml standard. The average recovery was 108.7%.

**Sample A**

| Dilution | Expected | Observed | % Recov |
|---|---|---|---|
| undiluted | 8.691 | 8.691 | 100 |
| 2X | 4.346 | 4.455 | 102.5 |
| 4X | 2.173 | 2.290 | 105.4 |
| 8X | 1.086 | 1.258 | 115.8 |
| 16X | 0.543 | 0.617 | 113.6 |
| 32X | 0.272 | 0.294 | 108.1 |
| 64X | 0.136 | 0.147 | 108.1 |

Average Recovery: 107.6%

**Sample B**

| Dilution | Expected | Observed | % Recovery |
|---|---|---|---|
| undiluted | 7.015 | 7.015 | 100 |
| 2X | 3.508 | 3.516 | 100.2 |
| 4X | 1.754 | 1.834 | 104.6 |
| 8X | 0.877 | 0.970 | 110.6 |
| 16X | 0.438 | 0.509 | 116.2 |
| 32X | 0.219 | 0.249 | 113.7 |
| 64X | 0.110 | 0.136 | 123.6 |

Average Recovery: 109.8%

### Recovery

Equal parts of diluted patient sera were mixed to test for interference by unknown materials, such as drugs or hormones, in the assay. Concentrations of Free PSA were determined before (original and added) and after (observed). The average recovery was 99.2%.

**Sample 1**

| Sample | Orig.Conc | Added | Expected | Obsearved | Recovery |
|---|---|---|---|---|---|
| A | 9.802 | 0.141 | 4.972 | 4.540 | 91.3 |
| B | 9.802 | 0.281 | 5.042 | 4.638 | 92.0 |
| C | 4.699 | 2.168 | 3.434 | 3.342 | 97.3 |
| D | 2.168 | 1.170 | 1.669 | 1.661 | 99.5 |
| E | 0.563 | 0.281 | 0.422 | 0.423 | 100.2 |
| F | 4.699 | 1.125 | 2.912 | 2.727 | 93.6 |
| G | 0.563 | 0.141 | 0.352 | 0.399 | 113.4 |

Average Recovery: 98.2%

**Sample 2**

| Sample | Orig.Conc | Added | Expected | Obsearved | Recovery |
|---|---|---|---|---|---|
| A | 6.825 | 0.098 | 3.462 | 3.301 | 95.3 |
| B | 6.825 | 0.195 | 3.510 | 3.156 | 89.9 |
| C | 3.342 | 1.563 | 2.453 | 2.503 | 102.0 |
| D | 1.706 | 0.781 | 1.244 | 1.205 | 96.9 |
| E | 0.391 | 0.195 | 0.293 | 0.327 | 111.6 |
| F | 3.342 | 0.781 | 2.062 | 1.964 | 95.2 |

| | | | | | |
|---|---|---|---|---|---|
| **G** | 0.391 | 0.098 | 0.245 | 0.271 | 110.6 |

Average Recovery: 100.2%

**Limitations of the Procedure**

There are some limitations of the assay:

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who ha ve received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

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**PPI1660A01**

**Rev A (02.09.2019)**


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|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | In Vitro diagnostic medical device | | Caution |
| Σ | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |