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AccuBind
ELISA Microwells

# Free Prostate Specific Antigen (fPSA)

## *Product Code: 2325-300*

**Intended Use: The Quantitative Determination of Free Prostrate Specific Antigen (fPSA) Concentration in Human Serum by a Microplate Immunoenzymometric assay**

## SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific antigen (PSA) is a serine protease with chymotrypsin-like activity (1,2). The protein is a single chain glycoprotein with a molecular weight of 28.4 kDA (3). PSA derives its name from the observation that it is a normal antigen of the prostrate but is not found in any other normal or malignant tissue. PSA is released from the normal prostate and appears at low serum concentrations in healthy men. Studies with reverse transcription-PCR have shown that PSA also is expressed at a low concentration in peripheral blood cells and other tissues (4). High serum concentrations can be detected in patients with advanced prostate cancer (PCA) (5). Therefore PSA is applied as a tumor marker for the clinical management of PCA (6). However, increased PSA concentrations in serum also occur in patients with benign prostate hyperplasia (BPH) (7). Hence the goal is to discriminate clearly between BPH and PCA in the clinical laboratory to spare the patient invasive diagnostic procedures, such as a prostate biopsy.

In human serum PSA occurs in two forms: free PSA (f-PSA) and complexed PSA. The major form is a complex of PSA and $\alpha_1$-antichymotrypsin (ACT). The fraction of f-PSA was shown to be substantially smaller in patients with untreated PCA than in patients with BPH. Therefore combined measurements of f-PSA and total PSA (t-PSA) may lead to a better discrimination between BPH and PCA Some recent studies have already shown that the f-PSA/t-PSA ratio is helpful in the differential diagnosis of BPH and PCA.

PSA is found in benign, malignant and metastatic prostrate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity (4).

In this method, fPSA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different free epitopes of fPSA) are added and the reactants mixed. Reaction between the various PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-fPSA antibody bound conjugate is separated from the unbound enzyme-fPSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known prostate specific antigen (fPSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with fPSA concentration.

## PRINCIPLE

**Immunoenzymometric assay (TYPE 3):**

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$Enz_{Ab_{(P)}} + Ag_{FPSA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Enz_{Ab_{(p)}}\text{-}Ag_{FPSA}\text{-}{}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ =Biotinylated Antibody (Excess Quantity)

$Ag_{FPSA}$ = Native Antigen (Variable Quantity)

$Enz_{Ab_{(p)}}$ = Enzyme labeled Antibody (Excess Quantity)

$Enz_{Ab_{(p)}}\text{-}Ag_{FPSA}\text{-}{}^{Btn}Ab_{(m)}$ =Antigen-Antibodies Complex

$k_a$ = Rate Constant of Association

$k_{-a}$ = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$${}^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow \text{Immobilized complex}$$

$Streptavidin_{C.W.}$ = Streptavidin immobilized on well

Immobilized complex = complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## REAGENTS

**Materials Provided:**

**A. f-PSA Calibrators- 1ml/vial - Icons A-F**
Six (6) vials of references free PSA antigen at levels of 0(**A**), 0.5(**B**), 1.0(**C**), 2.5(**D**), 5.0(**E**) and 10.0(**F**) ng/ml. Store at 2-8°C. A preservative has been added.

**Note**: The calibrators, protein based buffered matrix, were calibrated using a reference preparation, which was assayed against the WHO 1st International Standard 96/668.

**B. fPSA Enzyme Reagent—13 ml/vial - Icon** Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated specific free PSA monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

**C. Streptavidin Coated Plate -- 96 wells - Icon** ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**D. Wash Solution – 20 ml - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

**E. Substrate A --7ml/vial - Icon $S^A$**
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

**F. Substrate B -- 7ml/vial - Icon $S^B$**
One (1) bottle containing hydrogen peroxide ($H_2O_2$) in buffer. Store at 2-8°C.

**G. Stop Solution -- 8ml/vial - Icon** STOP
One (1) bottle containing a strong acid (1N HCl). Store at 2-30°C.

**I. Product Instructions.**

**Note 1:** Do not use reagents beyond the kit expiration date.
**Note 2:** Opened reagents are stable for sixty (60) days when stored at 2-8°C.
**Note 3:** See end of this product insert for various configurations of reagents by kit size.

**Materials Required But Not Provided:**

1. Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

## SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

## REAGENT PREPARATION:

1. **Wash Buffer**
   Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days.
2. **Working Substrate Solution**
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

***Note: Do not use the working substrate if it looks blue.***

## PRECAUTIONS

**For In Vitro Diagnostic Use**
***Not for Internal or External Use in Humans or Animals***

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

## TEST PROCEDURE

*Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).*

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the fPSA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature (20-27°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

   **DO NOT SHAKE THE PLATE AFTER SUBTRATE ADDITION**
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

## QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of fPSA in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding fPSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of fPSA for an unknown, locate the average absorbance of the duplicates for each

unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.6483) intersects the dose response curve at (2.28ng/ml) fPSA concentration (See Figure 1).

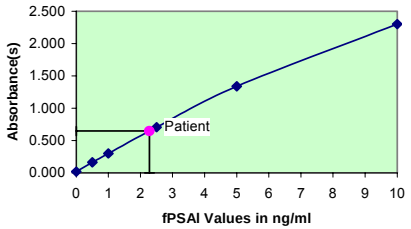
**Note:** Computer data reduction software designed for ELISA assays may also be used for the data reduction.

**EXAMPLE 1**

| Sample I.D. | Well Number | Abs (A) | Mean Abs (B) | Value (ng/ml) |
|---|---|---|---|---|
| **Cal A** | A1 | 0.019 | 0.021 | 0 |
| | B1 | 0.022 | | |
| **Cal B** | C1 | 0.167 | 0.164 | 0.5 |
| | D1 | 0.161 | | |
| **Cal C** | E1 | 0.300 | 0.302 | 1.0 |
| | F1 | 0.304 | | |
| **Cal D** | G1 | 0.701 | 0.707 | 2.5 |
| | H1 | 0.714 | | |
| **Cal E** | A2 | 1.353 | 1.337 | 5.0 |
| | B2 | 1.321 | | |
| **Cal F** | C2 | 2.286 | 2.300 | 10.0 |
| | D2 | 2.314 | | |
| **Patient** | E2 | 0.647 | 0.648 | **2.28** |
| | F2 | 0.648 | | |

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

**Figure 1**




## Q.C. PARAMETERS

**In order for the assay results to be considered valid the following criteria should be met:**

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

## LIMITATIONS OF PROCEDURE

**A. Assay Performance**

1. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
2. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
3. Plate readers measure vertically. Do not touch the bottom of the wells.
4. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
5. Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or hemolysed specimen(s) should similarly not be used.
6. Patient specimens with fPSA concentrations above 10 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).

**B. Interpretation**

1. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2. fPSA is elevated in benign prostatic hyperplasia (BPH). Clinically an elevated fPSA value alone is not of diagnostic value as a specific test for differential diagnosis of BPH. The ratio of fPSA/tPSA is a better marker and should be used in conjunction with other clinical observations (DRE) and diagnostic procedures (prostate biopsy).
3. When the total PSA (tPSA) reads 4-10 ng/ml the fPSA/tPSA ratio is useful in the differential diagnosis of BPH and PC (Prostate Cancer). Depending on the ratio the probability can be determined as follows:

| fPSA/tPSA Ratio | Probability of Prostate Cancer |
|---|---|
| 0-10% | 55% |
| 10-15% | 28% |
| 15- 20% | 25% |
| > 20% | 10% |

## EXPECTED RANGES OF VALUES

**TABLE I**
**Expected Values for the PSA Elisa Test System**

| | |
|---|---|
| Healthy Males | ≤ 1.3 ng/ml |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## PERFORMANCE CHARACTERISTICS

**A. Precision**

The within and between assay precisions of the fPSA AccuBind™ ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**TABLE 2**
**Within Assay Precision (Values in ng/ml)**

| Sample | N | X | S.D. | C.V. |
|---|---|---|---|---|
| Level 1 | 20 | 0.43 | 0.04 | 9.3% |
| Level 2 | 20 | 2.57 | 0.20 | 7.8% |
| Level 3 | 20 | 8.20 | 0.73 | 8.9% |

**TABLE 3**
**Between Assay Precision* (Values in ng/ml)**

| Sample | N | X | S.D. | C.V. |
|---|---|---|---|---|
| Level 1 | 10 | 0.52 | 0.04 | 7.7% |
| Level 2 | 10 | 2.34 | 0.22 | 9.4% |
| Level 3 | 10 | 7.70 | 0.68 | 8.8% |

*As measured in ten experiments in duplicate.

**B. Sensitivity**

The theoretical sensitivity, or minimum detection limit, calculated by the interpolation of the mean plus two standard deviations of 16 replicates of the 0 ng/ml fPSA calibrator, is 0.052 ng/ml..

**C. Specificity:**

The following substances did not interfere with the performance of fPSA determination using the fPSA AccuBind™ ELISA test system. These substances were added to the pooled sera in concentrations 10-100 times more than normal.

| *Compound* | *Concentration Added* |
|---|---|
| AFP | 10 µg/ml |
| Atropine | 100 µg/ml |
| Acetylsalicylic Acid | 100 µg/ml |
| Ascorbic Acid | 100 µg/ml |
| Caffeine | 100 µg/ml |
| Dexamethasone | 10 µg/ml |
| Flutamide | 100 µg/ml |
| hCG | 100 IU/ml |
| hLH | 100 IU/ml |
| Methotrexate | 100 µg/ml |
| Prolactin | 100 µg/ml |
| TSH | 100 mIU/ml |

**C. Accuracy**

The fPSA AccuBind™ ELISA method was compared with a reference automated chemiluminescence method. Clinical and non-clinical biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 167. The least square regression equation and the correlation coefficient were computed for the fPSA AccuBind™ ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

**TABLE 4**

| Method | Mean | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| This Method (x) | 1.62 | x = 0.0189 + 0.9649(y) | 0.957 |
| Reference (y) | 1.66 | | |

Only slight amounts of bias between the fPSA AccuBind™ ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

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**Revision: B** **Date: 102506**

**Cat #: *2325-300***

| Size | | 96(A) | 192(B) |
|---|---|---|---|
| Reagent (fill) | A) | 1ml set | 1ml set |
| | B) | 1 (13ml) | 2 (13ml) |
| | C) | 1 plate | 2 plates |
| | D) | 1 (20ml) | 1 (20ml) |
| | E) | 1 (7ml) | 2 (7ml) |
| | F) | 1 (7ml) | 2 (7ml) |
| | G) | 1 (8ml) | 2 (8ml) |

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