# Total PSA

Enzyme immunoassay for the quantitative
determination of total PSA in human serum

Only for in-vitro diagnostic use

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Product Number: GWB-F8000C (96 Determinations)

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## 1. INTRODUCTION

Prostate Specific antigen (PSA) is a serine protease with chymotrypsin-like activity. The protein is a single chain glycoprotein with a molecular weight of 28.4 kDA. PSA derivesits 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 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. Due to increased sensitivity, serum PSA levels are more useful than prostatic acid phosphatise (PAP) in diagnosis and management of patients.

## 2. INTENDED USE

Total PSA kit is a direct solid phase enzyme immunoassay for quantitative determination of Total Prostatic Specific Antigen (tPSA) in human serum or plasma.

## 3. PRINCIPLE OF THE ASSAY

In this method, standards, patient specimens and/or controls (containing the native tPSA antigen) are first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labelled antibodies are then added and the reactants are mixed: these antibodies have high affinity and specificity and are directed against distinct and different epitopes of tPSA.
Reaction between the various tPSA antibodies and native tPSA occurs in the microwells without competition or steric hindrance, forming a soluble sandwich complex. The interaction is illustrated by the following equation:

$$\text{EnzAb} + \text{Ag tPSA} + \text{BtnAb(m)} \underset{\text{k-a}}{\overset{\text{ka}}{\leftrightarrow}} \text{EnzAb - AgPSA-BtnAb(m)}$$

BtnAb(m) = Biotinylated Monoclonal Antibody (Excess Quantity)
AgtPSA = Native Antigen (Variable Quantity)
EnzAb = Enzyme labelled Antibody (Excess Quantity)
EnzAb-AgtPSA-BtnAb(m) = Antigen-Antibodies Sandwich Complex
ka = 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:

$$\text{EnzAb -AgtPSA-BtnAb(m)} + \text{StreptavidinC.W.} \Rightarrow \text{Immobilized complex}$$

StreptavidinC.W. = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

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. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce colour. By utilizing several different calibrators of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4. MATERIALS

### 4.1. Reagents supplied

- **Coated Mircoplate:** 12 breakapart 8-well snap-off strips coated with streptavidin; in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Conjugate:** 1 bottle containing 13 ml of horseradish peroxidase labelled anti-PSA antibodies and biotinylated mouse-anti-tPSA antibodies.
- **TMB Substrate Solution**: 1 bottle containing 15 ml 3, 3´, 5, 5´-tetramethylbenzidine ($H_2O_2$-TMB 0.25 g/l) (avoid any skin contact).
- **Wash solution 50x conc.:** 1 bottle containing 20 ml phosphate buffer (50 mM, pH 7.4) and Tween (20 g/l)
- **tPSA Standards:** 6 bottles with 1 ml standard each.
- Standard 0: 0 ng/ml
- Standard 1: 5 ng/ml
- Standard 2: 10 ng/ml
- Standard 3: 25 ng/ml
- Standard 4: 50 ng/ml
- Standard 5: 100 ng/ml

## 4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

## 4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm, 620 - 630 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver a volume of 25 µl with a precision of better than 1.5%
- Dispensers for repetitive deliveries of 100 µl and 300 µl with a precision of better than 1.5%
- Quality control materials
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

# 5. STABILITY AND STORAGE

The closed reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark.
Opened reagents are stable for 60 days when stored at 2…8°C.

# 6. REAGENT PREPARATION

*It is very important to bring all reagents, samples and standards to room temperature (22…28°C) for at least 30 minutes before starting the test run! At the end of the assay, store immediately the reagents at 2-8°C; avoid long exposure to room temperature.*

## 6.1. Coated microplate

The ready to use break apart snap-off strips are coated with streptavidin. Store at 2…8 °C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °. Do not remove the adhesive sheets on the unused strips.*

## 6.2. Conjugate

The conjugate is ready to use.

## 6.3. tPSA Standards

The standards are ready to use. The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 1st IS 96/670.

| | |
|---|---|
| Standard 0: | 0 ng/ml |
| Standard 1: | 5 ng/ml |
| Standard 2: | 10 ng/ml |
| Standard 3: | 25 ng/ml |
| Standard 4: | 50 ng/ml |
| Standard 5: | 100 ng/ml |

## 6.4. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.*

## 6.5. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

## 6.6 Wash Solution

Dilute the concentrated wash solution to a volume of 1000 ml with distilled or deionised water in a suitable container.

## 7. 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 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 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, 50µl of the specimen is required.

## 8. ASSAY PROCEDURE

### 8.1. Test Preparation

Bring all reagents to room temperature (22-28°C). Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

| | | |
|---|---|---|
| 1 well | (e.g. A1) | for substrate blank |
| 2 wells | (e.g. B1+C1) | for standard 0 |
| 2 wells | (e.g. D1+E1) | for standard 1 |
| 2 wells | (e.g. F1+G1) | for standard 2 |
| 2 wells | (e.g. H1+A2) | for standard 3 |
| 2 wells | (e.g. B2+C2) | for standard 4 |
| 2 wells | (e.g. D2+E2) | for standard 5 |

*It is recommended to determine standards and patient samples in duplicate.*

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 25 µl standards and samples into their respective wells.
2. Add 100 µl Conjugate to each well. Leave well A1 for substrate blank.
3. Cover wells with the foil supplied in the kit.
4. **Incubate for 30 min at room temperature (22…28°C).**
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl diluted wash solution. Avoid overflows from the reaction wells
6. **Important note**: During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

   **Automatic washer**: In case you use automatic equipment, wash the wells at least 5 times.Dispense 100 µl TMB Substrate Solution into all wells.
7. **Incubate for exactly 15 min at room temperature (2…28°C) in the dark.**
8. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue colour developed during the incubation turns into yellow.*
9. Measure the absorbance of the specimen at 450 nm. against a reference wavelength of 620-630 nm or against blank within 5 minutes.

## 9. RESULTS

### 9.1. Validity of the Assay

The OD of standard 5 should be ≥ 1.3.

### 9.2. Quality Control

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve 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.

### 9.3. OD Conversion

The optical densities (O.D.s) of some calibrators and samples may be higher than 2.0. In such a case, they could be out of the measurement range of the microplate reader. It is therefore necessary, for O.D.s higher than 2.0, to perform a reading at 405 nm (= wavelength of peak shoulder) in addition to 450 nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).
For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm.
- Read again the plate at 405 nm and 620 nm.
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/OD 405 = 3.0), that is: OD 450 nm = OD 405 nm x 3.0.

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for its own reader.

## 9.4. Data Reduction-Automated Method

Use the 4 parameters logistic- preferred – or the smoothed cubic spline function as calculation algorithm.

NOTE: If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

## 9.5. Data Reduction-Manual Method

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

1. Record the OD obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the OD for each duplicate standards versus the corresponding tPSA concentration in ng/ml on linear graph paper (do not average the duplicates of the standards before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of tPSA of 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 OD (1.142) intersects the dose response curve at (23.6 ng/ml) tPSA concentration (See Figure 1).

| **EXAMPLE 1** | | | | |
|---|---|---|---|---|
| **Sample ID** | **Well number** | **OD** | **Mean OD** | **Value ng/ml** |
| **Std 0** | A1 | 0.019 | 0.019 | 0 |
| | B1 | 0.019 | | |
| **Std 1** | C1 | 0.279 | 0.276 | 5 |
| | D1 | 0.273 | | |
| **Std 2** | E1 | 0.563 | 0.561 | 10 |
| | F1 | 0.559 | | |
| **Std 3** | G1 | 1.248 | 1.213 | 25 |
| | H1 | 1.179 | | |
| **Std 4** | A2 | 2.051 | 1.999 | 50 |
| | B2 | 1.947 | | |
| **Std 5** | C2 | 2.892 | 2.833 | 100 |
| | D2 | 2.775 | | |
| **patient** | E2 | 1.186 | 1.142 | **23.6** |
| | F2 | 1.089 | | |

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

## 9.6. Measurement Range

Patient specimens with tPSA concentrations above 100 ng/ml may be diluted (for example 1/10 or higher) with zero standard (tPSA 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).

## 9.7. Interpretation

PSA is elevated in benign prostrate hypertrophy (BPH). Clinically an elevated PSA value alone is not of diagnostic value as a specific test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures (prostate biopsy). Free PSA determinations may be helpful in regard to the discrimination of BPH and prostrate cancer conditions.

## 9.8. Expected Ranges of Values

Healthy males are expected to have values < 4 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.

## 10. SPECIFIC PERFORMANCE CHARACTERISTICS

### 10.1. Precision

The intra- and interassay precision of the tPSA kit were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (SD) and coefficient of variation for each of these control sera are presented below:

| Intra assay Precision (Values in ng/ml) | | | | |
|---|---|---|---|---|
| Sample | n | mean | SD | C.V. |
| Level 1 | 20 | 0.7 | 0.05 | 7.1% |
| Level 2 | 20 | 4.5 | 0.20 | 4.4% |
| Level 3 | 20 | 28.3 | 1.07 | 3.7% |
| | | | | |

| Inter assay Precision* (Values in ng/ml) | | | | |
|---|---|---|---|---|
| Sample | n | mean | SD | C.V. |
| Level 1 | 10 | 0.8 | 0.09 | 11.3% |
| Level 2 | 10 | 4.3 | 0.25 | 5.8% |
| Level 3 | 10 | 27.5 | 1.42 | 5.2% |
| *As measured in ten experiments in duplicate | | | | |

### 10.2. Specificity

No interference was detected with the performance of tPSA Elisa upon addition of massive amounts of the following substances to a human serum pool.

Acetylsalicylic Acid 100 µg/ml
Ascorbic Acid 100 µg/ml
Caffeine 100 µg/ml
CEA 10 µg/ml
AFP 10 µg/ml
CA-125 10,000 U/ml
hCG 1000 IU/ml
hLH 10 IU/ml
hTSH 100 mIU/ml
hPRL 100 µg/ml

### 10.3. Analytic Sensitivity

The analytical sensitivity is 0.012 ng tPSA. This is equivalent to a sample containing 0.5 ng/ml tPSA.

### 10.4. Accuracy-Method comparison

The tPSA kit was compared with a reference Elisa method. 241 biological specimens with normal and elevated concentrations were assayed. The least square regression equation and the correlation coefficient were computed for the tPSA kit in comparison with the reference method. The data obtained is displayed in Table 4.

| Method | Mean | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| GenWay tPSA kit | 5.62 | y = -0.0598+0.98(X) | 0.987 |
| Reference method | 5.57 | | |

## 11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

## 12. PRECAUTIONS AND WARNINGS

- Only for in-vitro diagnostic use.
- The kit is not for internal or external use in humans or animals.
- All components of human origin used for the production of these reagents have been tested for anti-HIV 1+2 antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Highly lipemic or hemolysed specimen(s) should not be used.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- It is important that the time of reaction in each well is held constant for reproducible results.
- Pipetting of samples should not extend beyond 10 minutes to avoid assay drift.
- If more than 1 plate is used, it is recommended to repeat the dose response curve.
- 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.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- Follow good laboratory procedures for handling blood products.
- Avoid contact with reagents containing hydrogen peroxide, sulphuric acid and preservatives which may be toxic if ingested. Do not pipette by mouth.
- Avoid exposure of TMB/$H_2O_2$ reagent to direct sun-light, metal or oxidants.
- Failure to remove adhering solution adequately in the aspiration or decantation wash steps may result in poor replication and spurious results.

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

### 12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

## 13. LITERATURE


Christensson A, Laurell CB, Lilja H., Eur J Biochem, 194, 755- 63 (1990).

Watt KW, et. al., Proc Nat Acad Sci USA, 83, 3166-70 (1986).

Chen Z, Prestiglacomo A, Stamey T., Clin Chem,, 41, 1273-82 (1995).

Wild D, The Immunoassay Handbook., Stockton Press p452 (1994).

Junker R, Brandt B, Zechel C, Assmann G, Clin Chem, 43, 1588- 94 (1997).

Prestigiacomo AF, Stamey TA, ' Physiological variations of serum prostate antigen in the (4-10 ng/ml) range in male volunteers.' J. Urol (1996); 155:1977-80.

Stamey TA, McNeal JE, Yemoto CM, Sigal BM, Johnstone IM,'Biological determinants of cancer progression in men with prostate cancer.' JAMA (19999;281:1395-1400.

Chen Z, Prestigiacomo A, Stamey T, 'Purification and characterization of Prostate Specific Antigen (PSA) Complexed to a1-Anticymotrypsin:Potential reference Material for International Standardization of PSA Immunoassays.' Clin Chem. (1995);41/9:1273-1282.

Horton GL, Bahnson RR, Datt M, Cfhan KM, Catalona WJ and Landenson JH.'Differences in values obtained with two assays of Prostate Specific Antigen.' J. Urol. (1988);139:762-72.

Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen Kand Alfthan O.'A complex between prostate specific antigen anda1-anticymotrypsin is the major form of prostate specific antigenin serum of patients with prostate cancer:assay of complex improves clinical sensitivity for cancer.' Cancer Res.(1991);51:222-26.


## 14. ORDERING INFORMATION

Prod. No.: GWB-F8000C total PSA Determination (96 Determinations)

# SCHEME OF THE ASSAY

Total PSA

## Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.

Select the required number of microtiter strips or wells and insert them into the holder.

## Assay Procedure

| | Substrate blank | Stand. 0 | Stand. 1 | Stand. 2 | Stand. 3 | Stand. 4 | Stand. 5 | Sample |
|---|---|---|---|---|---|---|---|---|
| Stand. 0 | - | 25 µl | - | - | - | - | - | - |
| Stand. 1 | - | - | 25 µl | - | - | - | - | - |
| Stand. 2 | - | - | - | 25 µl | - | - | - | - |
| Stand. 3 | - | - | - | - | 25 µl | - | - | - |
| Stand. 4 | - | - | - | - | - | 25 µl | - | - |
| Stand. 5 | - | - | - | - | - | - | 25 µl | - |
| Sample | - | - | - | - | - | - | - | 25 µl |
| Conjugate | - | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| Cover wells with foil supplied in the kit<br>**Incubate for 30 min at room temperature**<br>Wash each well **three** times with **300 µl** diluted Wash Solution | | | | | | | | |
| TMB Substrate | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| **Incubate for exactly 15 min at room temperature in the dark** | | | | | | | | |
| Stop Solution | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| Photometric measurement at 450 nm | | | | | | | | |

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