Total Prostate Specific Antigen (tPSA)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for the Quantitative Determination of Total Prostate Specific Antigen (tPSA) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

tPSA Sandwich ELISA test is intended for the quantitative determination of Total Prostate Specific Antigen (tPSA) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Human prostate-specific antigen (PSA) is a serine protease. PSA is immunologically specific for prostatic tissue; it is present in normal, benign hyperplastic and malignant prostatic tissue, in metastatic prostatic carcinoma and also in prostatic fluid and seminal plasma. PSA is not present in any other normal tissue obtained from men, nor is it produced by cancers of the breast, lung, colon, rectum, stomach, pancreas or thyroid. Besides, it is functionally and immunologically different from prostatic acid phosphatase (PAP).

Elevated serum PSA concentrations have been reported in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues, but not in apparently healthy men, men with non-prostatic carcinoma, apparently healthy women, or women with cancer. Serum PSA is one of the most useful tumor markers in oncology. It may serve as an accurate marker for assessing response to treatment in patients with prostatic cancer. Therefore, measurement of serum PSA concentrations can be an important tool in monitoring patients with prostatic cancer and in determining the potential and actual effectiveness of surgery or other therapies. PSA measurements can enhance early prostate cancer detection when combined with digital rectal examination (DRE).

PRINCIPLE OF THE ASSAY

tPSA Quantitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. The test employs anti-PSA antibody for solid phase (microtiter wells) immobilization and another anti-PSA monoclonal antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the PSA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation the wells are washed and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The concentration of PSA is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with anti- PSA antibody.
- PSA Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use.
- Stop Solution. Ready to use.
- PSA Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).
- Control Set
- Pack Insert
- Plate sealers
- Protocol Sheet
- Microwell Holder

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **tPSA** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to pink at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water), Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **25 µl** of standards, controls and sera into the appropriate wells.
2. Dispense **50 µl** of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C), for **45 minutes**.
3. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
4. Dispense **100 µl** of TMB Substrate into each well. Incubate at room temperature(18-25°C) in the dark, for **20 minutes**.
5. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.

Add **25** µl standards/controls/samples into the respective microwells

↓

Add **50** µl Enzyme Conjugate in each well

↓

Apply plate sealer and incubate for **45** minutes at **18-25°C**

↓

Wash Microwells **5 times** with **350** µl of diluted wash buffer

↓

Add **100** µl substrate in each well

↓

Incubate for **20** Minutes at **18-25°C in dark**

↓

Add **100** µl stop solution in each well

↓

Read results @450nm (Ref.600-700nm) within **15** Minutes

CALCULATION OF RESULTS

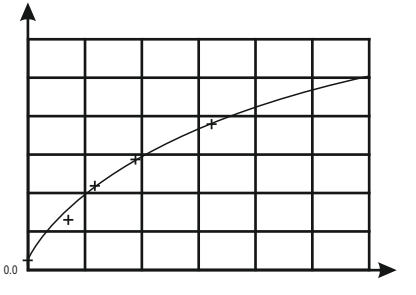
Construct a standard curve by plotting the absorbance obtained from each reference standards against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of tPSA in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against tPSA concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

tPSA (ng/ml)	Absorbance (450nm)
A	0.009
B	0.156
C	0.288
D	0.626
E	1.101
F	1.555



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 standard curve and data.

Expected values and sensitivity

Healthy males are expected to have tPSA values below 4ng/ml.
The minimum detectable concentration of tPSA in this assay is estimated to be 0.5ng/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **tPSA** was evaluated against commercially available licensed kit with 90 random clinical samples, & **tPSA** has demonstrated100% clinical correlation with the commercially available licensed kit.
2. Precision: **tPSA** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with tPSA	Coefficient of Variation (CV)
Level 1	10	0.264	6.43
Level 2	10	5.583	4.60
Level 3	10	18.969	2.56

B) External Evaluation:

tPSA ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **tPSA** ELISA has demonstrated 100% correlation with the reference method.
*Data file: Orchid Biomedical Systems Private Limited.

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards, controls and samples is not mandatory but may provide information on reproducibility & application errors.

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. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

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SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up		Contains sufficient for <n> tests
	Do not reuse		

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