

4. Duplication of Standards & 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 RLU values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the RLU 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 results.

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SYMBOL KEYS

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

Manufactured by:
Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa- 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

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0422/VER-01

electra™
●●●●●●●●●● tPSA

Chemiluminescence Assay 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

ELECTRA™ tPSA CLIA 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

ELECTRA™ tPSA Quantitative CLIA assay is for use on **ELECTRA™** analyzer. **ELECTRA™ tPSA CLIA** works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at $\lambda=425\text{nm}$.

The **ELECTRA™ PSA** Quantitative Test kit is based on a solid phase two-site immunoassay. The assay system utilizes one anti-PSA antibody for solid phase (microtitre wells) immobilization and another antibody labeled with horseradish peroxidase which is used as the tracer. The test sample is allowed to react simultaneously with the antibodies, resulting in the formation of the sandwiched molecule, antibody-antigen-antibody-enzyme complex. After incubation, the wells are washed with wash buffer to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and luminescence is measured in RLU. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of PSA in the sample. By reference to a series of PSA standards assayed in the same way, the concentration of PSA in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Anti- PSA antibody.
- PSA Enzyme Conjugate. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- tPSA 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).

Materials required but not provided:

- Precision pipettes: 10-100 μl , 20-200 μl , 100-1000 μl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

STORAGE AND STABILITY

1. **ELECTRA™ PSA** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. In case the desiccant pouch changes color from blue to pink, the strips should not be used.
3. Diluted wash buffer is stable up to one week at 2-8°C.
4. Working Substrate (A+B) must be used immediately.

electra™ Chemiluminescence assay

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electra™ Chemiluminescence assay

electra™ Chemiluminescence assay

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 and standards 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.

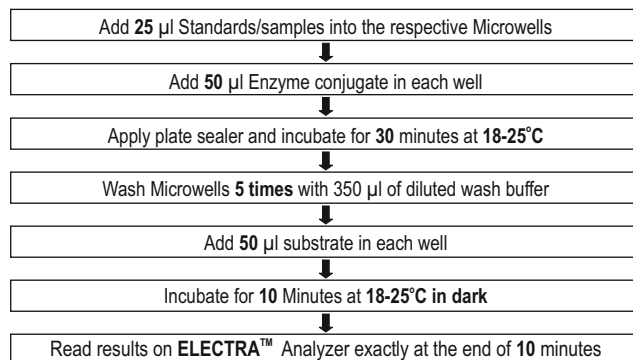
REAGENT PREPARATION

- 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.
- Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate- A μ l	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate- B μ l	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 25 μ l of Standards & serums into the appropriate wells.
2. Dispense 50 μ l of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for 30 minutes.
3. After incubation, empty the microtitre wells and wash the plate 5 times with 350 μ l of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
4. Add 50 μ l of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
5. Cover the ELECTRA™ microplate and incubate for 10 minutes at room temperature (18-25°C) in dark.
6. Read the ELECTRA™ micro-plate exactly at 10 minutes in ELECTRA™ Analyzer.



CALCULATIONS

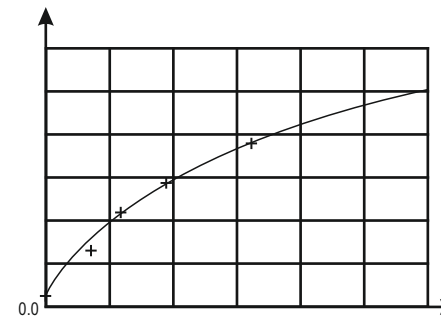
Construct a standard curve by plotting the RLU obtained from each reference standards against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration 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 RLU's 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)	RLU's
A	40
B	367472
C	815187
D	2377584
E	4404289
F	6919672



This Standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own Standard curve and data.

Expected Ranges of values

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

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **Electra™ tPSA** was evaluated against commercially available licensed kit with 90 random clinical samples, & **Electra™ tPSA** has demonstrated 100% clinical correlation with the commercially available licensed kit.
2. Precision: **Electra™ tPSA** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with Electra™ tPSA	Coefficient of Variation (CV)
Level 1	10	0.361	5.72
Level 2	10	5.895	3.35
Level 3	10	21.20	3.39

B) External Evaluation:

Electra™ tPSA CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ tPSA** has demonstrated 98% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd).

Important Note:

1. The **Electra™ tPSA** assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU readings.
3. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipette is available.