Controls	No. of testings	Mean Control values with Electra™ fPSA	Coefficient of Variation (CV)
Level 1	10	0.353	8.70
Level 2	10	2.863	8.78
Level 3	10	15.640	8.32

B) External Evaluation:

Electra™ fPSA CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ fPSA** has demonstrated 100 % correlation with the reference method.

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

Important Note:

1. The **Electra™ fPSA** 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.
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	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF 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.

An ISO 13485 Certified Company

0422/VER-01

electra™ fPSA

Chemiluminescence Assay for the Quantitative Determination of Free Prostate Specific Antigen (fPSA) in Human Serum.
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ fPSA CLIA test is intended for the quantitative determination of Free Prostate Specific Antigen (fPSA) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Human Prostate Specific Antigen (PSA) is a 33KD serine proteinase which, in human serum, is predominantly bound to alpha 1- antichymotrypsin (PSA-ACT) and alpha 2-macroglobulin (PSA-AMG). Trace amounts of alpha 1-antitrypsin and inter-alpha trypsin inhibitor bound to PSA can also be found. Any remaining PSA is in the free form (f-PSA). Current methods of screening menfor 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. Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than those with benign prostatic hyperplasia. Thus, the measurement of free serum PSA in conjunction with total PSA, can improve specificity of prostate cancer screening in selected men with elevated total serum PSA levels, which would subsequently reduce unnecessary prostate biopsies with minimal effects on cancer detection rates.

PRINCIPLE

ELECTRA™ fPSA Quantitative CLIA assay is for use on **ELECTRA™** analyzer. **ELECTRA™ fPSA 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 λ=425nm.

In **ELECTRA™ fPSA** a certain amount of anti-fPSA antibody is coated on microtiter wells. A measured volume of patient serum, and a constant volume of fPSA conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, fPSA antibody in the samples and conjugated fPSA compete for the limited binding sites on the anti- fPSA antibody of the wells. After incubation the wells are washed and bound enzyme is detected by adding the chemiluminescent substrate. The bound enzyme converts substrate to a reaction product that emits a photon of light. Chemiluminescence is measured in Relative Light Units (RLU). The amount of light emitted is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fPSA in the sample. By reference to a series of fPSA standards assayed in the same way, the concentration of fPSA in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Anti- fPSA antibody.
- Sample Diluent. Ready to use.
- Enzyme Conjugate. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- fPSA 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: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

STORAGE AND STABILITY

1. **ELECTRA™ fPSA** 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.

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 µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate- B µl	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 **100** µl of Standards & serums into the appropriate wells.
2. Dispense **100 µl** of Sample diluent into each well. Incubate at 37° C for **15 minutes.**
3. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
4. Dispense **150**µl of Enzyme Conjugate into each well. Incubate at 37°C for **15 minutes**.
5. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
6. Add **50** µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
7. Cover the **ELECTRA™** microplate and incubate for **10 minutes** at room temperature (**18-25°C**) in dark.
8. Read the **ELECTRA ™** micro-plate exactly at **10 minutes** in **ELECTRA ™ Analyzer.**

Add **100**µl Standards/samples into the respective Microwells

↓

Add **100** µl Sample Diluent in each well

↓

Apply plate sealer and incubate for **15** minutes at **37°C**

↓

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

↓

Add **150** µl Enzyme conjugate in each well

↓

Apply plate sealer and incubate for **15** minutes at **37°C**

↓

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

↓

Add **50** µl substrate in each well

↓

Incubate for **10** Minutes at **18-25°C in dark**

↓

Read results on **ELECTRA™** Analyzer exactly at the end of **10** minutes

CALCULATIONS

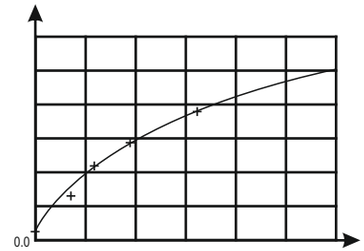
Construct a standard curve by plotting the RLU obtained from each reference standards against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of fPSA 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 fPSA concentrations shown in the X axis.

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

fPSA (ng/ml)	RLU's
A	55
B	34133
C	353788
D	1548041
E	3709640
F	6287156



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 values:

The relationship between f-PSA/t-PSA ratio and risk of prostrate cancer is also age related.

When Total PSA is in the range of 4.0-10.0 ng/ml the following risk interpretation is indicated:

- f-PSA/t-PSA ratio ≤ 0.10 indicates 49% - 65% risk of prostate cancer
- f-PSA/t-PSA ratio > 0.25 indicates 9% - 16% risk of prostate cancer

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

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

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