

**PERFORMANCE CHARACTERISTICS****A) Internal Evaluation:**

- Accuracy: In an internal study **Electra™ CA-125** was evaluated against commercially available licensed kit with 90 random clinical samples, & **Electra™ CA-125** has demonstrated 100% clinical correlation with the commercially available licensed kit.
- Precision: **Electra™ CA-125** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with <b>Electra™ CA-125</b>	Coefficient of Variation (CV)
Level 1	10	16.72	3.78
Level 2	10	68.21	3.48
Level 3	10	209.70	2.23

**B) External Evaluation:**

**Electra™ CA-125** CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ CA-125** has demonstrated 100% correlation with the reference method.

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

**Important Note:**

- The **Electra™ CA-125** assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU readings.
- 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.
- 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.

**BIBLIOGRAPHY**

(1) Kenemans P, Yedema CA, Bon GG, von Mensdorff-Pouilly S. Ca125 in gynecological pathology a review. Eur J Obstet Gynecol 1993;49:115-124. (2) Saksela F. Prognostic markers in epithelial ovarian cancer. Intl J Gynecol Pathol 1993;12:156-161. (3) Farghaly SA. Tumor markers in gynecologic cancer. Gynecol & Obstet Invest 1992;34:65-72. (4) Welander CE. What do CA 125 and other antigens tell us about ovarian cancer biology. Acta Obstet Gynecol Scand Sup 1992;155:85-93. (5) McGowan L. Pathology of the ovary. Curr Opin on Obstet Gynecol 1991;3:580-586. (6) Niloff JM. Ovarian malignancy. Curr Opin on Obstet Gynecol 1991;3:66-72. Olt G, Berchuck A, Bast RC. The role of tumor markers in gynecologic oncology. Obstet Gynecol Survey 1990;45:570-577. (7) Diez M, Cerdan FJ, Ortega MD, Torres A, Picardo A, Balibrea JL. Evaluation of serum CA-125 as a tumor marker in non-small cell lung cancer. Cancer 1991;67:150-154.8. (8) Niloff JM, Klug TL, Schaetzl E. Elevation of serum CA-125 in carcinomas of the fallopian tube, endometrium, and endocervix. AM J. Obstet Gynecol 1984;148:1057.

**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.

An ISO 13485  
Certified Company

0422/VER-01

**electra™**  
●●●●●●●●●● **CA-125**

**Chemiluminescence Assay for the Quantitative Determination of Ovarian Cancer Antigen (CA-125) in Human Serum.**

**FOR IN VITRO DIAGNOSTIC USE ONLY**

**Store at 2°C to 8°C**

**INTENDED USE**

**ELECTRA™ CA-125** CLIA test is intended for the quantitative determination of Ovarian Cancer Antigen (CA-125) in human serum. For In Vitro Diagnostic Use only.

**INTRODUCTION**

Ovarian cancer is the most malignant type of gynecological cancers, with an overall 5-year survival rate of only 30%. This is because diagnosis is often not made until the advanced stage. Cancer Antigen 125 (CA-125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA-125 is associated with a high molecular weight glycoprotein. Serum concentrations of this tumor marker can be detected and measured by a murine monoclonal antibody. Published studies have indicated that elevated serum CA-125 levels can be found in individuals with serious endometrioid, clear-cell and undifferentiated ovarian carcinoma. Serum CA-125 levels higher than normal can also be found in individuals with adenocarcinoma of the fallopian tube endometrium, certain non-gynecologic malignancies and some non-malignant conditions. Serial determinations of serum CA-125 further enhance the positive predictive value of the test for ovarian cancer. Serum CA-125 concentration may be useful in monitoring patients with diagnosed ovarian cancer. A persistently high serum CA-125 may be associated with progressive malignant disease and poor therapeutic response. On the other hand, a declining CA-125 value appears to be indicative of a favorable prognosis and a good response to treatment. Residual disease is confirmed in 95% of patients with serum CA-125 concentrations greater than 35 units per ml. however, negative results do not necessarily exclude the disease. To date, CA-125 is the most sensitive marker for residual epithelial ovarian cancer. CA-125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

**PRINCIPLE**

**ELECTRA™ CA-125** Quantitative CLIA assay is for use on **ELECTRA™** analyzer. **ELECTRA™ CA-125 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  $\lambda=425\text{nm}$ .

In **ELECTRA™ CA-125**, the assay system utilizes one monoclonal anti-CA 125 antibody for solid phase (microtiter wells) immobilization and another monoclonal anti-CA 125 antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the CA 125 antibody coated microtiter wells. Then CA 125 antibody labeled with horseradish peroxidase (conjugate) is added. If human CA 125 is present in the specimen, it will combine with the antibody on the wells and the enzyme conjugate resulting in the CA 125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at 37°C, the wells are washed with wash buffer 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 directly related to the amount of CA 125 antigen in the sample. By reference to a series of CA 125 standards assayed in the same way, the concentration of CA 125 in the unknown sample is quantified.

**MATERIALS & COMPONENTS****Materials provided with the test kits:**

- Coated Microwells: Microwells coated with Anti- CA-125 antibody.
- Enzyme Conjugate. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- CA-125 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 $\mu\text{l}$ , 50-200 $\mu\text{l}$ , 100-1000 $\mu\text{l}$
- Disposable pipette tips
- Distilled water

- Disposable Gloves
- **ELECTRA™ Analyzer**

#### STORAGE AND STABILITY

1. **ELECTRA™ CA-125** 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.

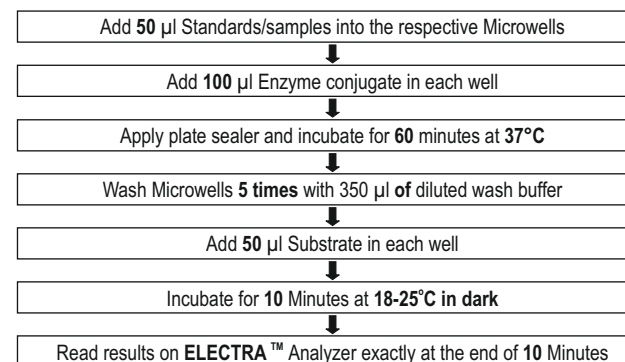
#### REAGENT PREPARATION

- 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 **50 µl** of Standards & serums into the appropriate wells.
2. Dispense **100 µl** of Enzyme Conjugate into each well. Incubate at 37°C for **60 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. Add **50 µl** 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

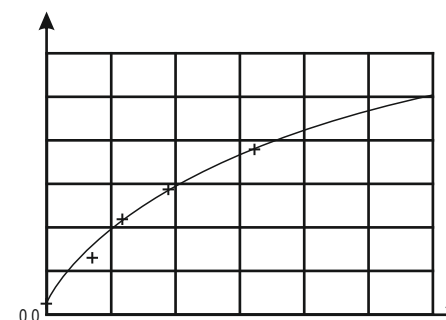
Construct a standard curve by plotting the RLU obtained from each reference standards against its concentration in U/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of CA-125 in U/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 CA-125 concentrations shown in the X axis.

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

CA-125 (U/ml)	RLU's
A	34
B	105539
C	549435
D	1066430
E	2487227
F	3697773



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 women are expected to have CA-125 assay values below 35 U/ml. The minimum detectable concentration of CA-125 in this assay is estimated to be 5 U/ml.