Important Note:

1. The **Electra™ CEA** 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.

BIBLIOGRAPHY

1. Abelev G I. Alpha-fetoprotein as a marker of embryo-specific differentiation in normal and human tissues. Transplant Rev 1974;20:3-37. irai H. Alpha fetoprotein. In: Chu T M, ed. Biochemical markers for cancer. New York: Marcel Dekker, 1982:23-59.
2. Chan D W, Miao Y C. Affinity chromatographic separatoin of alpha- fetoprotein variants: Development of a mini-column procedure and application to cancer patients. Clin Chem 1986;32:2143-2146.
3. Sell L S. Cancer markers of the 1990s. Clin Lab Med 1990;10:1-37.
4. Hirai H, Nishi S, Watabe H et al. Some chemical, experimental and clinical investigations on alpha fetoprotein. In: Hirai H, Miyaji T, eds. Alpha-fetoprotein and hepatoma. Gann Monogr 1973:14:19-34.

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™ CEA

Chemiluminescence Assay for the Quantitative Determination of Carcinoembryonic Antigen (CEA) in Human Serum.

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

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

INTRODUCTION

Carcinoembroyonic antigen (CEA) is a cell-surface 200-kd glycoprotein. In 1969, it was reported that plasma CEA was elevated in 35 of 36 patients with adenocarcinoma of the colon and that CEA titers decreased after successful surgery. Normal levels were observed in all patients with other forms of cancer or benign diseases. Subsequent studies have not confirmed these initial findings, and it is now understood that elevated levels of CEA are found in many cancers. Increased levels of CEA are observed in more than 30% of patients with cancer of the lung, liver, pancreas, breast, colon, head or neck, bladder, cervix, and prostate. Elevated plasma levels are related to the stage and extent of the disease, the degree of differentiation of the tumor, and the site of metastasis. CEA is also found in normal tissue.

PRINCIPLE

ELECTRA™ CEA Quantitative CLIA assay is for use on **ELECTRA™** analyzer. **ELECTRA™ CEA 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™ CEA** a certain amount of anti-CEA antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of CEA conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, CEA antibody in the samples and conjugated CEA compete for the limited binding sites on the anti- CEA 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 CEA in the sample. By reference to a series of CEA standards assayed in the same way, the concentration of CEA in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Anti- CEA antibody.
- Enzyme Conjugate: Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- CEA Standard set of 6 standards labeled as A to F in liquid form. 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™ CEA** 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 **25**µl of Standards & serums into the appropriate wells.
2. Dispense **50**µl 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 350µl 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.**

Add **25** µl Standards/samples into the respective Microwells

⬇

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

⬇

Apply plate sealer and incubate for **30** minutes at **18-25°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

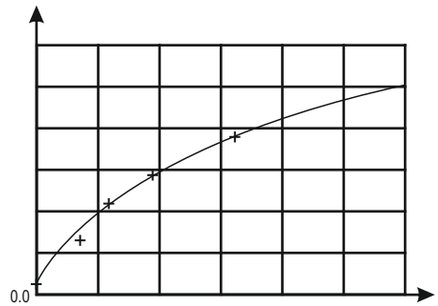
Construct a standard curve by plotting the RLU obtained from each reference standards against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration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

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

CEA (ng/ml)	RLU's
A	46
B	106252
C	646411
D	1697143
E	3129094
F	4781394



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

The most complete study of CEA is a compilation of collaborative studies in which CEA values in 35,000 samples from more than 10,000 patients and controls were analyzed. Of normal persons who did not smoke, 98.7% had values less than 5.0ng/ml. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of CEA by this assay is estimated to be 1.0 ng/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **Electra™ CEA** was evaluated against commercially available licensed kit with 89 random clinical samples, & **Electra™ CEA** has demonstrated 100% clinical correlation with the commercially available licensed kit.
2. Precision: **Electra™ CEA** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with Electra™ CEA	Coefficient of Variation (CV)
Level 1	10	2.05	3.28
Level 2	10	25.83	1.28
Level 3	10	92.95	1.43

B) External Evaluation:

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

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