IVD

# Carcinoembryonic Antigen (CEA) ELISA

**Catalog No. E0205-2**

**96 tests**

## *INTENDED USE*

The Autobio CEA ELISA kit is intended for the quantitative determination of carcinoembryonic antigen (CEA) concentration in human serum.

## *INTRODUCTION*

Carcinoembroyonic antigen (CEA) is a cell-surface 200 kD glycoprotein. It was first described by Gold and Freedman in 1965 as a complex immunoreactive glycoprotein found in epithelial adenocarcinomas of the colon and fetal colon[1, 2]. 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. Its main use is in the monitoring of cancer patients after surgery, chemotherapy or radiotherapy [3]. Successful removal of the tumor is usually followed by a decrease in the concentration of circulating CEA[4], whereas recurrence of the primary tumor or metastasis is accompanied by increasing CEA concentrations[5]. Elevated serum levels of CEA may be found in a variety of benign and malignant conditions other than carcinoma of the colon. Conditions that may cause elevated levels of CEA include hepatic cirrhosis, hepatitis, heavy smoking, bronchitis, pancreatitis, gastritis, inflammatory bowel disease and renal disease[6, 7].

## *PRINCIPLE OF THE TEST*

The CEA quantitative assay is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-CEA antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-CEA antibody (anti-CEA MAb) in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the anti-CEA coated microtiter wells. Then the anti-CEA labeled horseradish peroxidase (HRP) is added. If human CEA is present in the specimen, it will combine with the antibody on the well and the conjugate will bind immunologically to the CEA on the well, resulting in the CEA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with wash fluid to remove unbound conjugate. Substrate solution and chromogen solution are then added and incubated, resulting in the development of a blue color. The color development is terminated with stop solution, and the color turns to yellow and is measured spectrophotometrically at the wavelength of 450 nm. The concentration of CEA is directly proportional to the color intensity of the test sample.

## *MATERIALS PROVIDED*

1. Antibody Coated Microtiter Plate: microplate with murine anti-CEA MAb coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: HRP labeled murine anti-CEA MAb in stabilizing buffer (1 vial, 7.2 mL)
3. Reference Standards: 5, 10, 20, 40, 80 ng/mL CEA in human serum with preservatives. (5vials, 1 mL/ea)
4. Wash Fluid Concentrate: PBS-Tween (1 vial, 8.0 mL, 62.5×)
5. Substrate Solution: hydrogen peroxide (1 vial, 7.4 mL)
6. Chromogen Solution: tetramethylbenzidine (TMB) (1 vial, 7.4 mL)
7. Stop Solution: 1.0 M H2SO4 (1 vial, 7.4 mL)

## *MATERIALS REQUIRED BUT NOT PROVIDED*

1. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper

6. Incubator
7. Disposable reagent troughs
8. Instrumentation
    1. Automated microplate strip washer
    2. Microplate reader

    or

    3. Fully automated microplate processor

## *STORAGE OF TEST KIT AND INSTRUMENTATION*

1. Unopened test kits should be stored at 2 – 8℃ upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Reference Standards should be stored sealed at 2 – 8℃. The remaining Reference Standards opened should be used within 30 days and be frozen at -20℃ for long term storage. Avoid multiple freeze-thaw cycles of Reference Standards.
3. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, or until the expiration date, whichever is earlier, provided it is stored as prescribed above.

## *SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE*

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2 – 8℃. Specimens going to be stored or transported for more than 48 hours must be stored frozen (- 20℃ or lower). Avoid multiple freeze-thaw cycles. After thawing, ensure specimens are thoroughly mixed and brought to room temperature before being assayed.

## *PRECAUTIONS AND WARNINGS*

1. for *in vitro* diagnostic use only
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. Handling of reagents, serum specimens should be in accordance with local safety procedures.
4. Reference Standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported. Nevertheless, the Reference Standards and components containing animal substances should still be treated as potentially infectious.
5. Avoid any skin contact with all reagents. Stop Solution contains $H_2SO_4$, in case of contact, wash thoroughly with water.
6. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

## *REAGENT PREPARATION*

1. All reagents should be brought to room temperature (18 - 25°C) before use. All reagents should be mixed through gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute the Wash Fluid Concentrate with 500 mL of distilled water prior to use.

## *IMPORTANT NOTES*

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all Reference Standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.

4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

## *ASSAY PROCEDURE*

1. Secure the desired number of coated wells in the holder. Dispense 50μL of each Reference Standard and specimens into appropriate wells.
2. Dispense 50 μL of Enzyme Conjugate Reagent to each well.
3. Incubate at 37℃ for 60 minutes.
4. Remove the incubation mixture by emptying plate content into a waste container. Rinse and empty the microtiter wells 5 times with the diluted Wash Fluid. The volume of each well is about 350 μL. Dry the plate by striking it sharply onto absorbent paper or paper 0towels after the last wash cycle. Alternatively, wash it in an automated microplate strip washer 5 times.
5. Dispense 50 μL of Substrate Solution into each well, then 50 μL of Chromogen Solution into each well. Gently mix and incubate for 10 minutes at room temperature in the dark.
6. Terminate the reaction by adding 50 μL of Stop Solution to each well.
7. Gently mix for 10 seconds to ensure that the blue color completely turns to yellow.
8. Read absorbance at a wavelength of 450 nm in a microplate reader within 10 minutes.

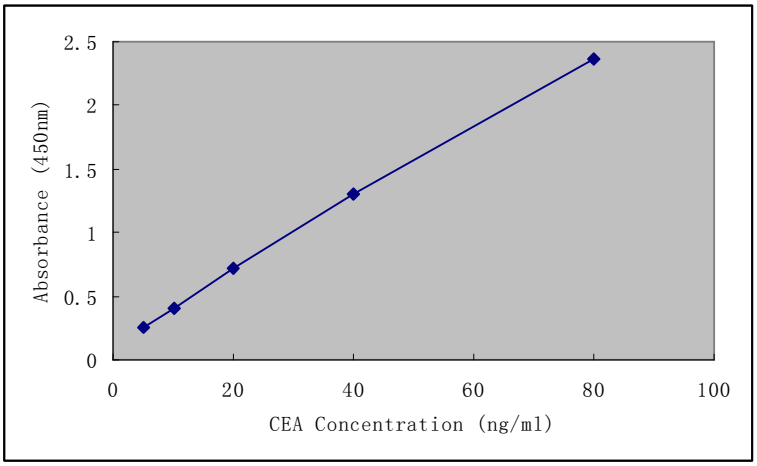
## *CALCULATION OF RESULTS*

1. Calculate the mean values from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance for each sample, determine the corresponding CEA concentration in ng/mL from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

## *EXAMPLE OF STANDARD CURVE*

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

| CEA(ng /mL) | Absorbance (450 nm) |
|---|---|
| 5 | 0.248 |
| 10 | 0.406 |
| 20 | 0.713 |
| 40 | 1.302 |
| 80 | 2.368 |




## *REFERENCE NORMAL RANGE*

Each laboratory should establish its own reference normal range. These values are given only for guidance.

| | | | |
|---|---|---|---|
| **Sample Numbers（smokers）** | 326 | **Sample Numbers（smokers）** | 248 |
| **Average Value（ng/mL）** | 1.36 | **Average Value（ng/mL）** | 3.46 |
| **Standard Deviation ( )** | 1.82 | **Standard Deviation ( )** | 3.27 |
| **Normal Range (+2 , ng/mL)** | 5.0 | **Normal Range (+2 , ng/mL)** | 10.0 |

## *PERFORMANCE CHARACTERISTICS*

### *1. Analytical Sensitivity*

The sensitivity of the assay, defined as the concentration of CEA equivalent to the mean absorbance of 20 replicates of the zero Reference Standard plus two standard deviations, is typically 2.0 ng/mL.

### *2. Specificity*

No interference was detected with the performance of Autobio CEA ELISA upon addition of massive amounts of the following substances to a human serum pool.

| **Substance** | **Concentration** |
|---|---|
| AFP | 500ng/ml |
| CA125 | 400U/ml |

### *3. Precision*

#### *3.1. Intra-assay precision*

Intra-assay precision was determined by assaying 20 replicates of control, respectively.

| **Serum** | **Number** | **Mean** | **SD** | **RSD（%）** |
|---|---|---|---|---|
| control | 20 | 24.36 | 1.43 | 5.87 |

#### *3.2. Inter-assay precision*

Inter-assay precision was determined by assaying 1 serum pools in duplicate across 20 separate runs, using a standard curve constructed for each run.

| **Serum** | **Number** | **Mean** | **SD** | **RSD（%）** |
|---|---|---|---|---|
| control | 20 | 23.87 | 2.35 | 9.84 |

### *4. High Dose Hook Effect*

No interfere with CEA values up to 6000 ng/mL.

### *5. Accuracy*

For 128 samples in the range of 5 ng/mL to 80 ng/mL, the relationship between the Autobio CEA ELISA and the Elecsys CLIA assay is described by the equation below:

| **Reference** | **Number of Specimens** | **Least Square Regression Analysis** | **Correlation Coefficient** |
|---|---|---|---|
| Roche（ECLIA） | 128 | y = 1.0057x - 1.9464 | 0.945 |

## *LIMITATIONS*

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis,or turbidity should not be used with this assay.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

## *QUALITY CONTROL*

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

## *SYMBOLS*

| | |
|---|---|
| LOT | **BATCH CODE** |

| | |
|---|---|
|  | **USE BY** |
|  | **MANUFACTURER** |
|  | **CONTAINS SUFFICIENT FOR <n> TESTS** |
|  | ***IN VITRO* DIAGNOSTIC MEDICAL DEVICE** |
|  | **TEMPERATURE LIMITATION** |
|  | **CATALOGUE NUMBER** |
|  | **CONSULT INSTRUCTIONS FOR USE** |

***REFERENCES***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