IVD

CARCINOEMBRYONIC ANTIGEN (CEA) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL0205-2

INTENDED USE

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

INTRODUCTION

Carcinoembroyonic antigen (CEA) is a cell-surface 200kD 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 tumour or metastasis is accompanied by increasing 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 CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes one anti-CEA monoclonal antibody for solid phase (microtiter wells) immobilization and another anti-CEA monoclonal antibody as antibody-enzyme (horseradish peroxidase) conjugate reagent. CEA in the standards or in the patient's specimens binds to anti-CEA MAB on the well and the anti-CEA second antibody then binds to CEA. Unbound protein and HRP conjugate are removed by washing. Upon the addition of the substrate, the horseradish peroxidase activity bound on the wells is then assayed by a chemiluminescence reaction. The Related Light Unit (RLU) of the reaction is proportional to the concentration of CEA presented in the specimen.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to Carcinoembroyonic antigen (anti-CEA MAB) (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled anti-CEA MAB in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 5, 10, 20, 40, 80, and 160ng/ml CEA in Stabilizing Buffer (6 vials, 0.5ml/ea)
4. Substrate A: (1 vial, 3.5ml/6.0ml)
5. Substrate B: (1 vial, 3.5ml/6.0ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Distilled water
2. Precision pipettes for delivery of 20-200µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent
5. Washer for microplate
6. Quality control specimens
7. Incubator
8. Absorbent paper

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 (6 months from the date of manufacture). Refer to the package label for the expiration date.
2. Liquid standards should be stored sealed at 2～8℃. The remaining standards opened should be used within 30 days and be frozen at -20℃ for long term storage. Avoid repeated freezing and thawing of the standards. 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, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

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℃. For longer storage, freeze the specimens at -20℃. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The satandards 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 BSE has not been reported. Nevertheless, the satandards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. 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℃) 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 pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette 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 invalid results.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 20μl of CEA standards, specimens, and controls into appropriate wells.
3. Dispense 100μl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
5. Incubate at 37℃ for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container，Rinse and flick the microtiter wells 5 times with distilled water，Strike the plate sharply onto absorbent paper to remove residual water droplets.
7. Dispense 50μl of Substrate A, then 50μl of Substrate B into each well. Gently mix for 10 seconds.
8. Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

CALCULATION OF RESULTS

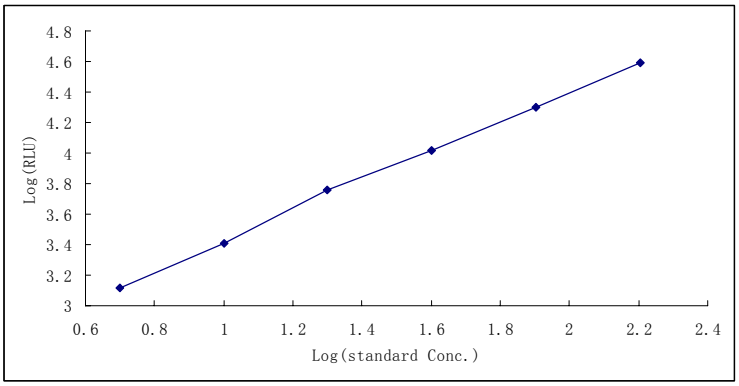
1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.

2. On logarithmic graph paper plot the log_{10}RLU (ordinate) obtained from each reference standard against the common logarithm of corresponding concentration of CEA in ng/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with a curved line.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Any diluted specimens must be corrected by the appropriate dilution factor.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression logistic function curve fitting is recommended.

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)	RLU
5	1316.4
10	2579.1
20	5743.5
40	10477.9
80	20036
160	39426.2



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information are given only for guidance. Approximately 95% of the normal healthy population has CEA levels less than 5ng/ml.

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio CEA CLIA kit is not higher than 2.5ng/ml.

B. Specificity

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

Substance	**Concentration**
AFP	500ng/ml
CA125	400U/ml
CA15-3	500U/ml
CA19-9	500U/ml

C. Precision

a. Intra-Assay Precision

Intra-Assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV(%)
Low titer	20	33.78	2.06	6.10
High titer	20	99.16	5.23	5.27

b. Inter-Assay Precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV(%)
Low titer	10	34.86	2.66	7.63
High titer	10	102.34	5.34	5.22

D. High Dose Hook Effect

No hook effect occurred with CEA concentration up to 3200ng/ml.

E. Accuracy

For 126 specimens in the range of 5ng/ml to 160ng/ml, the correclation between the Autobio CEA CLIA kit and Roche Elecsys assay was as follows:

Reference	**Number of Specimens**	**Least Square Regression Analysis**	**Correlation Coefficient**
Roche® (ECLIA)	126	y = 1.0032x -0.9175	0.948

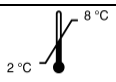
LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert 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 test.
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

	**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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3. SMITH, A.M., MACDONALD, D.J., Enzyme immunoassay for carcinoembryonic antigen. Clin. Chem. 29: 2019, 1983.
4. LOKICH, J.J., ZAMCHECK, N., LOWENSTEIN, M., Sequential carcinoembryonic antigen levels in the therapy of metastatic breast cancer. Ann. Inter. Med. 89: 902, 1978.
5. FALKSON, H.C., FALKSON, G., PORTUGAL, M.A., VAN DER WATT, J.J., SCHOEMAN, H.S., Carcinoembryonic antigen as a marker in patients with breast cancer receiving postsurgical adjuvant chemotherapy. Cancer 49: 1869, 1982.
6. TOROSIAN, M.H., The clinical usefulness and limitations of tumor markers. Surg. Gynecol. Obstet. 166: 567, 1988.
7. SUGARBAKER, P.H., Role of carcinoembryonic antigen assay in the management of cancer. Adv. Immuno. Cancer Ther. 5. 167, 1985.

For order and inquires, please contact

AUTOBIO DIAGNOSTICS CO., LTD.
ADD: No.87 Jingbei Yi Road, National Eco & Tech Development Area,
Zhengzhou , China 450016
Tel: +86-371-67985313 Fax: +86-371-67985804
Web: www.autobio.com.cn