IVD

CARBOHYDRATE ANTIGEN 50 (CA50) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL0207

INTENDED USE

The Autobio carbohydrate antigen 50 (CA50) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of CA50 concentration in human serum.

INTRODUCTION

Tumor cells express substances in the cell membrane which are not usually produced in healthy cell membranes. The determination of these tumor-associated structures is a valuable tool in the diagnosis of malignant disorders.

Carbohydrate antigen 50 (CA50), a cancer associated carbohydrate marker, is not organ-specific and its elevated levels in serum can be observed in a variety of malignancies, especially gastrointestinal cancers (*e.g.* pancreatic, stomach, hepatic and colorectal cancers) [1–3]. Benign liver and biliary disease also are associated with increased CA50 serum levels [4-7,8-11]. The mechanism whereby CA50 increases in these patients is unclear.

CA50 is a tumor marker defined by the monoclonal antibody C50 that recognizes two different structures: sialylated Lewis ganglioside antigen and sialylated lacto-N-tetraose[12,13]. The CA50 antigens occur in the cell membrane in a lipid-bound form (as ganglioside) and in a form bound to a high molecular weight protein (as glycoprotein). The CA50 antigens are released by the tumors into the blood stream where they can be specifically detected by means of immunological techniques based on C50 MAB.

PRINCIPLE OF THE TEST

The CA50 CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes one anti-CA50 monoclonal antibody for solid phase (microtiter wells) immobilization and as antibody-enzyme (horseradish peroxidase) conjugate reagent. CA50 in the standards or in the patient's specimens binds to anti-CA50 MAB on the well and the anti-CA50 antibody enzyme conjugate then binds to CA50. 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 chemiluminescence reaction. The related light unit (RLU) of the reaction is proportional to the concentration of CA50 presented in the specimen.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to carbohydrate antigen 50 (anti-CA50 MAB) (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-CA50 MAB in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 0, 5, 15, 30, 90, and 180U/ml CA50 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, 100-1000µl (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. Reconstituted standards should be used within 14 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.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2～8℃.

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 25μl of CA50 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 wells sharply onto absorbent paper to remove residual water droplets. The volume of the well is about 300μl.
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.

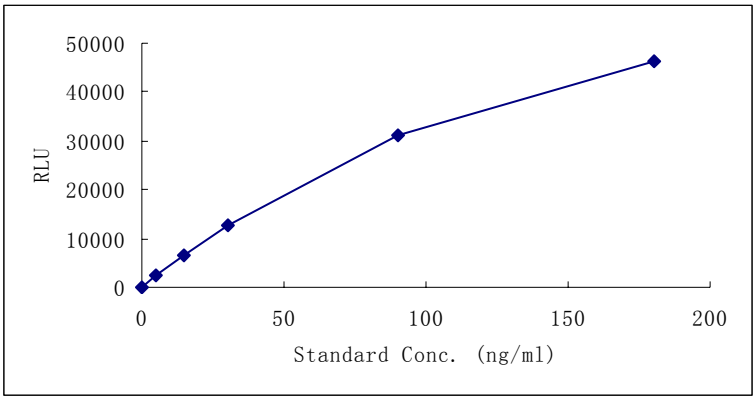
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the RLU (ordinate) obtained from each reference standard against the corresponding concentration of CA50 in U/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a point to point function curve fitting is recommended.
5. 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.

CA50 (U/ml)	RLU
0	108.19
5	2344.82
15	6624.24
30	12867.70
90	31082.80
180	46315.15



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance. Approximately 95% of the normal healthy population has CA50 levels less than 25U/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 CA50 CLIA kit is 1.0U/ml.

B. Specificity

No interference was detected with the performance of Autobio CA50 CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	**Concentration**
human albumin	100mg/ml
CEA	500ng/ml
CA125	400U/ml
CA15-3	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	34.26	2.56	7.47
High titer	20	114.03	5.67	4.97

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	35.72	2.23	6.24
High titer	10	117.06	5.46	4.66

D. High Dose Hook Effect

No hook effect occurred with CA50 concentration up to 1200U/ml.

E. Accuracy

For 84 specimens in the range of 0U/ml to 180U/ml, the correclation between the Autobio CA50 CLIA kit and CISBIO RIA-gnost® CA-50 assay was as follows:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
CISBIO RIA-gnost®	84	Y = 1.026x -0.8764	0.955

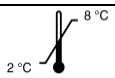
LIMITATIONS

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**

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