CANCER ANTIGEN 15-3 (CA15-3) CHEMILUMINESCENCE

IMMUNOASSAY KIT
Catalog No. CL0210-2

INTENDED USE
The Autobio cancer antigen 15-3 (CA15-3) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of CA15-3 concentration in human serum.

INTRODUCTION
Breast cancer is the most common life-threatening malignant lesion in women of many developed countries today, with approximately 180,000 new cases diagnosed every year. Roughly half of these newly diagnosed patients are node-negative, however 30% of these cases progress to metastatic disease. Research studies have indicated that CA 15-3 assay values are frequently elevated in patients with breast cancer. These studies have suggested that the CA 15-3 assay may be of clinical value for monitoring the response of patients undergoing therapy because increasing and decreasing values correlated with disease progression and regression, respectively. Additional published studies have suggested that increasing CA 15-3 assay values in patients at risk for breast cancer recurrence after primary therapy may be indicative of recurrent disease before it can be detected clinically. Non-mammary malignancies in which elevated CA 15-3 assay values have been reported include lung, colon, pancreatic, primary liver, ovarian, cervical, and endometrial. The CA 15-3 assay values are not elevated in most normal individuals.

PRINCIPLE OF THE TEST
The CA15-3 CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The CA15-3 molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The related light unit (RLU) of the reaction is proportional to the concentration of CA15-3 present in the sample.

MATERIALS PROVIDED
1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to cancer antigen (anti-CA15-3 MAB) (1 plate, 48 wells/96 wells)
2. Sample diluent: (1 vial, 50 ml/ea)
3. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-CA15-3 MAB in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
4. Reference Standards: 0, 15, 30, 60, 120, and 240 U/ml CA15-3 in Stabilizing Buffer (6 vials, 0.5ml/ea)
5. Substrate A (1 vial, 3.5ml/6.0ml)
6. 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°C 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°C 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°C. For longer storage, freeze the specimens at -20°C. Thawed samples must be mixed prior to testing.
6. Patient serum and control serum should be diluted 51-fold before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20 μl serum with 1.0 ml Sample Diluent.

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 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 BSE has not been reported. Nevertheless, the standards 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°C) 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°C.

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
The CA15-3 standards have already been prediluted and are ready for use. Please DO NOT dilute again!
1. Secure the desired number of coated wells in the holder. Dispense 50 μl of CA15-3 standards, diluted specimens, and diluted controls into appropriate wells.
2. Incubate at 37°C for 60 min.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the well is about 300 μl.
4. Dispense 100 μl of enzyme conjugate reagent to each well. Mix gently for 30 seconds.
5. Incubate at 37°C for 60 min.
6. At the end of the 60 minutes incubation, remove the incubation reagent by emptying the plate content into a waste container. Repeat step 4.

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 CA15-3 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.

<table>
<thead>
<tr>
<th>CA15-3 (U/ml)</th>
<th>RLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34.272</td>
</tr>
<tr>
<td>15</td>
<td>1684.31</td>
</tr>
<tr>
<td>30</td>
<td>3471.285</td>
</tr>
<tr>
<td>60</td>
<td>6730.955</td>
</tr>
<tr>
<td>120</td>
<td>12964.02</td>
</tr>
<tr>
<td>240</td>
<td>22661.25</td>
</tr>
</tbody>
</table>

**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 CA15-3 levels less than 35U/ml.

**PERFORMANCE**

A. Sensitivity
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean RLU of Standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio CA15-3 CLIA kit is not higher than 1.0U/ml.

B. Specificity
No interference was detected with the performance of Autobio CA15-3 CLIA upon addition of massive amounts of the following substances to a human serum pool.

<table>
<thead>
<tr>
<th>Interferents</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Albumin</td>
<td>100mg/ml</td>
</tr>
<tr>
<td>CEA</td>
<td>500ng/ml</td>
</tr>
<tr>
<td>CA125</td>
<td>400U/ml</td>
</tr>
<tr>
<td>CA19-9</td>
<td>500U/ml</td>
</tr>
</tbody>
</table>

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 31.26 2.16 6.91  
High titer 20 129.71 7.16 5.52

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

<table>
<thead>
<tr>
<th>Serum</th>
<th>Number</th>
<th>Mean</th>
<th>SD</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low titer</td>
<td>10</td>
<td>30.43</td>
<td>2.21</td>
<td>7.26</td>
</tr>
<tr>
<td>High titer</td>
<td>10</td>
<td>124.82</td>
<td>8.96</td>
<td>7.18</td>
</tr>
</tbody>
</table>

D. Accuracy  
For 122 specimens in the range of 0U/ml to 240U/ml, the correlation between the Autobio CA15-3 CLIA kit and Roche Elecsys assay was as follows:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of Specimens</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche® (ECLIJA)</td>
<td>122</td>
<td>$y = 1.0306x -1.0241$</td>
<td>0.963</td>
</tr>
</tbody>
</table>

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

REFERENCES:
1. Hayes DF, Zurawski V, Kufe D. Comparison of Circulating CA15-3 and Carcinoembryogenic Antigen


For order and inquiries, 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