ab108633
Cancer Antigen CA15-3
Human ELISA Kit

Instructions for Use

For the quantitative determination of the Cancer Antigen CA15-3 concentration in Human serum

This product is for research use only and is not intended for diagnostic use.
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1. Introduction

ab108633 Cancer Antigen CA15-3 Human ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA15-3 concentration in human serum.

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.

There are a number of tumor markers that can help clinicians to identify and diagnose which breast cancer patients will have aggressive disease and which will have an indolent course. These markers include estrogen and progesterone receptors, DNA ploidy and percent-S phase profile, epidermal growth factor receptor, HER-2/neu oncogene, p53 tumor suppressor gene, cathepsin D, proliferation markers and CA15-3. CA15-3 is most useful for monitoring patients post-operatively for recurrence, particularly metastatic diseases. 96% of patients with local and systemic recurrence have elevated CA15-3, which can be used to predict recurrence earlier than radiological and clinical criteria. A 25% increase in the serum CA15-3 is associated with progression of carcinoma. A 50% decrease in serum CA15-3 is associated with response to treatment. CA15-3 is more sensitive than CEA in early detection of breast cancer recurrence.
2. Assay Summary

*ab108633 is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes two unique antibodies directed against distinct antigenic determinants on the intact CA15-3 molecule.*

A monoclonal antibody directed against a distinct antigenic determinant on the intact CA15-3 molecule is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA15-3 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution.

The test sample is allowed to react sequentially with the two antibodies, resulting in the CA15-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate 1-hour incubation steps at 37 °C, the wells are washed with Wash Buffer to remove unbound labeled antibodies.

A solution of tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of a blue color.

The color development is stopped with the addition of 1N hydrochloric acid (HCl) changing the color to yellow. The concentration of CA15-3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.
3. Kit Contents

- Murine Monoclonal Anti-CA15-3 coated microtiter plate with 96 wells.
- Sample Diluent, 100 ml.
- Enzyme Conjugate Concentrate (22x), 1.0 ml.
- Enzyme Conjugate Diluent, 21 ml.
- CA15-3 reference standards, containing 0, 15, 30, 60, 120, and 240 Unit/ml. Liquid. 1 set. **These standards have been prediluted 51-fold. Please do not dilute them again.**
- Wash Buffer Concentrate (20X), 50 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

4. Storage and Handling

Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.
5. Additional Materials Required

- Distilled or deionized water
- Precision pipettes: 20 µl, 100 µl, 200 µl and 1.0 ml
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450 nm with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
- Vortex mixer, or equivalent
- Absorbent paper
- Graph paper

6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare working CA 15-3 Conjugate Reagent: Add the entire 1.0 ml of Conjugate Concentrate (22x) to 21 ml of the Enzyme Conjugate Diluent (1:21 dilution) and mix well. The diluted Enzyme Conjugate Reagent is stable at 4°C for at least 4 months.
3. To prepare Wash Buffer (1X): Add 50 ml of Wash Buffer (20X) to 950 ml of DI water. The diluted Wash Buffer is stable at 2-8°C for 30 days. Mix well before use. Note: Any crystals that
may be present due to high salt concentration must be redissolved at room temperature before making the dilution.

7. Preparation and Collection of Specimen

1. The use of serum samples is required for this test. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

2. Samples 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.

8. Assay Method

Assay Procedure:

1. Secure the desired number of coated wells in the holder.

2. Dispense 200 µl of CA15-3 standards, diluted specimens, and diluted controls into appropriate wells.


4. Incubate at 37°C for 1 hour.

5. Remove the incubation mixture by flicking plate contents into a waste container.
6. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X).
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 200 µl Enzyme Conjugate Reagent into each wells.
10. Incubate at 37°C for 1 hour.
11. Remove the contents and wash the plate as described in steps 6-7 above.
12. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
13. Incubate at room temperature in the dark for 20 minutes.
14. Stop the reaction by adding 100 µl of Stop Solution to each well.
15. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
16. Read absorbance at 450nm with a microtiter plate reader *within 15 minutes.*
9. Data Analysis

17. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.

18. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

19. Using the mean absorbance value for each sample, determine the corresponding concentration of CA15-3 in U/ml from the standard curve.

A. Typical Data

Results of a typical standard run with optical density readings at 450nm shown on the Y axis against CA15-3 concentrations shown on the X axis.

NOTE: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.
<table>
<thead>
<tr>
<th>CA15-3 Values (U/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.067</td>
</tr>
<tr>
<td>15</td>
<td>0.338</td>
</tr>
<tr>
<td>30</td>
<td>0.587</td>
</tr>
<tr>
<td>60</td>
<td>1.081</td>
</tr>
<tr>
<td>120</td>
<td>1.880</td>
</tr>
<tr>
<td>240</td>
<td>2.640</td>
</tr>
</tbody>
</table>

![Graph showing the relationship between CA15-3 concentration and absorbance at 450 nm.](image-url)
B. Sensitivity

Healthy women are expected to have CA15-3 assay values below 35 U/ml. The minimum detectable concentration of CA15-3 in this assay is estimated to be 5 U/ml.

10. Limitations

- 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.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- Serum samples demonstrating gross lipemia, gross hemolysis or turbidity should not be used with this test.
- The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
## 11. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>addition when concentration of target is</td>
<td></td>
</tr>
<tr>
<td></td>
<td>too high</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs.</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>cell extract)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td></td>
<td>STOP solution</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
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