ab108653

Cancer Antigen CA125
Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Cancer Antigen CA125 concentrations in serum

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

ab108653 is for the quantitative determination of the Cancer Antigen CA125 concentration in human serum.

Cancer Antigen 125 (CA125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA125 is associated with a high molecular weight glycoprotein. Published studies have indicated that elevated serum CA125 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma.

The serum CA125 concentration is greater than 35 units per ml in 60% of women with ovarian cancer and >80% of patients with disseminated ovarian cancer. The serum CA125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, and 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salpingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serum CA125 concentration may be useful in monitoring treatment and distinguishing between good response to treatment and progressive malignant disease with poor therapeutic response. To date, CA125 is the most sensitive marker for residual epithelial ovarian cancer.
2. Assay Summary

*ab108653 is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA125 molecule.*

A mouse monoclonal anti-CA125 antibody is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA125 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution.

The test sample is allowed to react simultaneously with the two antibodies, resulting in the CA125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at 37°C for 90 minutes, the wells are washed with Washing Solution 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 Stop Solution changing the color to yellow. The concentration of CA125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.
3. Kit Contents

- Murine Monoclonal anti-CA125 coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml
- CA125 Reference Standard 0 (1 ml, 0 U/ml)
- CA125 Reference Standard 1 (1 ml, 15 U/ml)
- CA125 Reference Standard 2 (1 ml, 50 U/ml)
- CA125 Reference Standard 3 (1 ml, 100 U/ml)
- CA125 Reference Standard 4 (1 ml, 200 U/ml)
- CA125 Reference Standard 5 (1 ml, 400 U/ml)
- 20X Washing Solution (1 x 50 ml)
- TMB Substrate Solution, 11 ml
- Stop Solution, 11 ml

4. Storage and Handling

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.
5. Additional Materials Required

- Precision pipettes and tips: 100 μl
- Disposable pipette tips
- Distilled water
- Vortex mixer
- Absorbent paper or paper towel
- Microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength
- Graph paper

6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

2. To prepare Washing Solution (1X): Add 50 ml of 20X Washing Solution to 950 ml of DI water. The diluted Washing Solution 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 Specimens

1. 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.

8. Assay Method

1. Secure the desired number of coated wells in the holder. Dispense 100 μl of CA125 standards, specimens, and controls into appropriate wells.

2. Dispense 100 μl of Enzyme Conjugate Reagent into each well.

3. Mix gently for 30 seconds. It is very important to mix completely.

4. Incubate at 37°C for 90 minutes.

5. Remove the incubation mixture by flicking plate contents into a waste container.

6. Rinse and empty the microtiter plate 5 times with Washing Solution (1X).

7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.

8. Dispense 100 μl of TMB Reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.

9. Stop the reaction by adding 100 μl of Stop Solution to each well.
10. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*

11. Read the optical density at 450nm with a microtiter plate reader *within 15 minutes.*

**9. Data Analysis**

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. 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.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CA125 in U/ml from the standard curve.
A. Typical Data

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against CA125 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

<table>
<thead>
<tr>
<th>CA125 (U/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.071</td>
</tr>
<tr>
<td>15</td>
<td>0.205</td>
</tr>
<tr>
<td>50</td>
<td>0.551</td>
</tr>
<tr>
<td>100</td>
<td>0.936</td>
</tr>
<tr>
<td>200</td>
<td>1.746</td>
</tr>
<tr>
<td>400</td>
<td>2.824</td>
</tr>
</tbody>
</table>
B. Sensitivity

The minimum detectable concentration of CA125 in this assay is estimated to be 5 U/ml.

C. Expected Values

Healthy women are expected to have CA125 assay values below 35 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.
## 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 addition when</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>concentration of target is too high</td>
<td></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>
<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 STOP solution</td>
<td>Read plate immediately after adding STOP solution</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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