ab108635

Carcino embryonic Antigen Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Carcino embryonic Antigen (CEA) concentrations in serum.

This product is for research use only and is not intended for *in vitro* diagnostic use.

www.abcam.com
# Table of Contents

1. Introduction 2  
2. Assay Summary 3  
3. Kit Contents 4  
4. Storage and Handling 4  
5. Additional Materials Required 5  
6. Preparation of Reagents 5  
7. Preparation and Collection of Specimen 5  
8. Assay Method 6  
9. Data Analysis 7  
10. Troubleshooting 9
1. Introduction

ab108635 Carcino embryonic Antigen Human ELISA Kit is intended for the quantitative determination of Carcino embryonic Antigen concentration in human serum.

Carcino embryonic antigen (CEA) is a cell-surface 200-kd glycoprotein. In 1969, it was reported that plasma CEA was elevated in 35 of 36 patients with adenocarcinoma of the colon and that CEA titers decreased after successful surgery. Normal levels were observed in all patients with other forms of cancer or benign diseases. Subsequent studies have not confirmed these initial findings, and it is now understood that elevated levels of CEA are found in many cancers. 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. CEA is also found in normal tissue.
2. Assay Summary

*ab108635 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 CEA molecule is used for solid phase immobilization (on the microtiter wells). A goat anti-CEA 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 CEA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed with water 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 CEA is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.
3. Kit Contents

- Antibody-Coated microtiter plate with 96 wells.
- CEA Reference Standard Set (1 set, 1.0 ml/vial); contains 0, 3, 12, 30, 60 and 120 ng/ml CEA; ready to use.
- Enzyme Conjugate Reagent (13 ml)
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 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

- Distilled or deionized water
- Precision pipettes: 50 µl, 100 µl and 1.0 ml
- Disposable pipette tips
- Microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm.
- Vortex mixer, or equivalent
- Absorbent paper
- Graph paper

6. Preparation of Reagents

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

7. Preparation and Collection of Specimen

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

Assay Procedure:

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450nm with a microtiter well reader within 15 minutes.
9. Data Analysis

1. Calculate the average absorbance values (A450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/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 CEA in ng/ml from the standard curve.

4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculation.

A. Typical Data

Results of a typical standard run with optical density readings at 450nm shown on the Y axis against CEA 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>CEA (ng/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.057</td>
</tr>
<tr>
<td>3</td>
<td>0.235</td>
</tr>
<tr>
<td>12</td>
<td>0.637</td>
</tr>
<tr>
<td>30</td>
<td>1.388</td>
</tr>
<tr>
<td>60</td>
<td>2.144</td>
</tr>
<tr>
<td>120</td>
<td>3.050</td>
</tr>
</tbody>
</table>

![Graph showing the relationship between CEA concentration and absorbance](graph.png)
## 10. 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>Curve doesn't fit scale</td>
<td></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 concentration of target is too high</td>
<td>Increase dilution factor of sample</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>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>Reason</td>
<td>Solution</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
<td></td>
</tr>
<tr>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
<td></td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
<td></td>
</tr>
<tr>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
<td></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).
ab108635 Carcino embryonic Antigen Human ELISA Kit

**Abcam in the USA**
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge, MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

**Abcam in Japan**
Abcam KK
1-16-8 Nihonbashi
Kakigaracho,
Chuo-ku, Tokyo
103-0014
Japan

Tel: +81-(0)3-6231-094
Fax: +81-(0)3-6231-0941

**Abcam in Europe**
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

**Abcam in Hong Kong**
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: Tel: 400 921 0189 / +86 21 2070 0500
Fax: (852) 3016-1888

Copyright © 2011 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.
All information / detail is correct at time of going to print.