



Abnova

CEA (Human) ELISA Kit

Catalog Number KA0204

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

For the quantitative determination of the Cancer Antigen CEA concentration in human serum.

Background

Carcinoembryonic 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.

Principle of the Assay

The CEA ELISA test 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 1 hour incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of 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.

General Information

Materials Supplied

List of component

Component	Amount
Antibody-coated microtiter plate with 96 wells.	1
CEA standards containing; 0, 3, 12, 30, 60, and 120 ng/ml CEA. read to use.	1 ml each
Enzyme Conjugate Reagent	13 ml
TMB Reagent (One-Step)	11 ml
Stop Solution (1N HCl)	11 ml

Storage Instruction

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.

Materials Required but Not Supplied

- ✓ Precision pipettes: 50 µl, 100 µl, and 1 ml.
- ✓ Disposable pipette tips.
- ✓ Distilled water.
- ✓ Vortex mixer or equivalent.
- ✓ Absorbent paper or paper towel.
- ✓ Graph paper.
- ✓ Microtiter plate reader. (A 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 is acceptable for use in absorbance measurement.)

Precautions for Use

- Limitation of procedure
- ✓ 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.

Assay Protocol

Reagent Preparation

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

Sample Preparation

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.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent to each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by emptying plate content 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 the optical density at 450 nm with a microtiter plate reader within 15 minutes.

Data Analysis

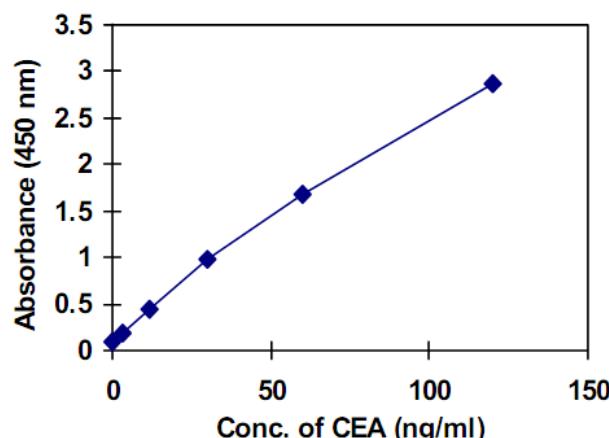
Calculation of Results

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 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.

Example of standard curve

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against CEA 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.

CEA (ng/ml)	Absorbance (450 nm)
0	0.057
3	0.235
12	0.637
30	1.388
60	2.144
120	3.050



Resources

References

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Plate Layout

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