



Signosis
BioSignal Capture

CEA ELISA

Catalog Number EA-0104

(For Research Use Only)

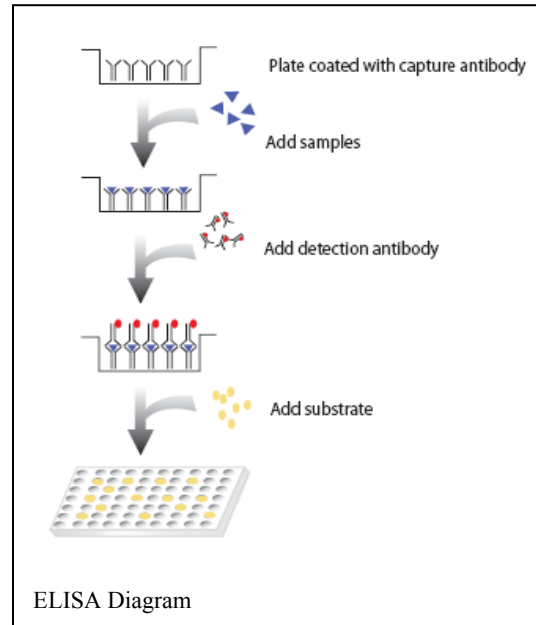
Introduction

Carcinoembryonic antigen (CEA) is a cell-surface 200-kd glycoprotein (1-2). CEA is normally produced during the development of a fetus. The production of CEA stops before birth, and it usually is not present in the blood of healthy adults. CEA is found in certain kinds of cancers, especially large intestine (colon and rectal) cancer. It may also be present in people with cancer of the pancreas, breast, ovary, or lung. When the CEA level is abnormally high before surgery or other treatment, it is expected to fall to normal following successful surgery to remove all of the cancer. A rising CEA level indicates progression or recurrence of the cancer (3-6).

The Signosis CEA Immunoassay provides a rapid, sensitive, and reliable assay for the quantitative measurement of CEA level. The antibodies developed for the test will determine a minimal concentration of 1.0 ng/ml.

Principle of the assay

The CEA ELISA 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 monoclonal anti-CEA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with these antibodies, resulting in CEA being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then 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.



Materials provided with the kits:

- Monoclonal anti-CEA coated microtiter plate with 96 wells
- Enzyme Conjugate Reagent, 13 ml
- Lyophilized CEA reference standards containing; 0, 3, 12, 30, 60 and 120 ng/ml CEA
- TMB Reagent, 11 ml
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Specimen Collection and Handling

Serum should be separated from the red blood cells as soon as possible. Specimens should be stored for up to 48 hours or -20°C for up to 6 months prior to assay.

Preparation for Assay

- All reagents should be taken to room temperature (18-25 °C) before use.
- Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8 °C.
- Hook Effect: In order to avoid effect, samples with expected CEA concentrations over 9,000 ng/ml may be quantitated by 100 fold dilution with CEA- free human serum

Assay Procedure

1. Add 50µl of standard, specimens, and controls into appropriate wells.
2. Add 100µl of Enzyme Conjugate Reagent to each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at room temperature (18-25°C) for 60 minutes.
5. Remove the incubation mixture by emptying plate content into a waster container.
6. Rinse and flick the microtiter wells 5 times with distilled or deionized water. Put the wells sharply onto paper towel to remove all residual water droplets.
7. Add 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
8. Incubated at room temperature for 30 minutes.
9. Remove the incubation mixture by flicking plate contents into a waster container.
10. Rinse and flick the microtiter wells 5 times with distilled water
11. Strike the plate sharply onto paper towel to remove residual water droplets.
12. Dispense 100 µl TMB Reagent into each well, and mix gently for 10 seconds.
13. Incubate at room temperature in the dark for 20 minutes.
14. Add 100 µl Stop Solution to each well to stop reaction.
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 well reader within 15 minutes.

References

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Example of standard curve

CEA (ng/ml)	Absorbance (450 nm)
0	0.096
3	0.177
12	0.450
30	0.971
60	1.677
120	2.881

