Carcinoembryonic Antigen (CEA) ELISA Quantitation Kit

Manual

Catalog number: 40-052-115008

For the quantitative determination of Human Carcinoembryonic Antigen (CEA) in serum.

This kit is for research use only, and is not for use in diagnostic procedures.

INTENDED USE

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

INTRODUCTION

Carcinoembroyonic 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 Test

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 monoclonal anti-CEA 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 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.

REAGENTS

Materials provided with the kit:

- *Antibody-Coated Wells (1 plate, 96 wells)*
  Microtiter Wells coated with CEA MoAb
- *Reference Standard Set (1.0 ml/vial)*
  Contains 0, 3, 12, 30, 60, and 120 ng/ml of CEA in bovine serum with preservatives; lyophilized. See instructions for reconstitution under Reagent Preparation.
- *Enzyme Conjugate Reagent (13 ml)*
  Contains CEA MoAb conjugated to horseradish peroxidase with preservatives
- *TMB Reagent (11 ml)*
  Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- *Stop Solution -1N HCl (11 ml)*
  Diluted hydrochloric acid

Materials required but not provided:

- 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

STORAGE CONDITIONS

1. 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.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[25]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For research use only.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

SPECIMEN COLLECTION AND PREPARATION

1. The use of SERUM samples is required for this test.
2. Specimens should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Specimens which cannot be assayed within 24 hours of collection should be frozen at –20°C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or samples (after centrifugation).
5. Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel). Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. 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.

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***.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

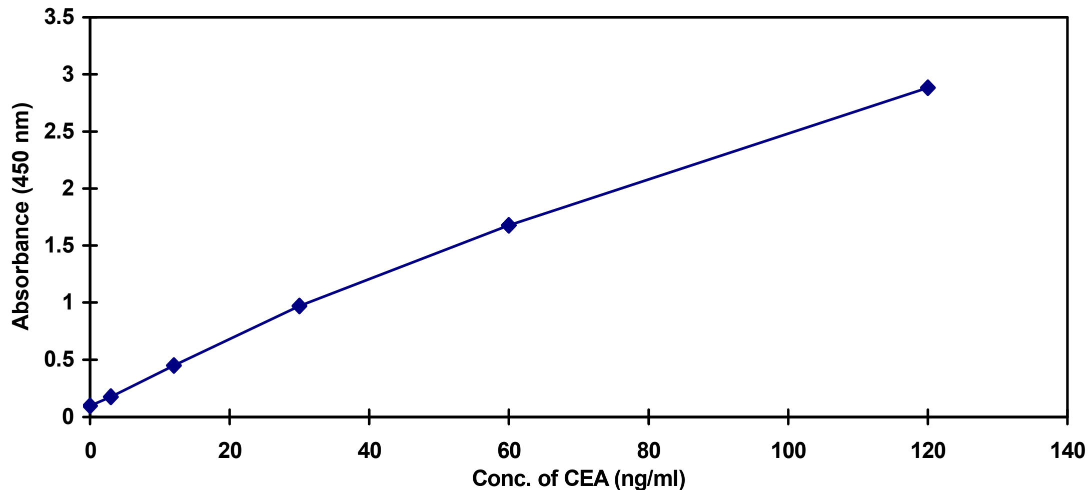
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) 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	0.096
3	0.177
12	0.450
30	0.971
60	1.677
120	2.881



EXPECTED VALUES

The most complete study of CEA is a compilation of collaborative studies in which CEA values in 35,000 samples from more than 10,000 patients and controls were analyzed. Of 1425 normal persons who did not smoke, 98.7% had values less than 5.0 ng/ml. It is recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

1. Accuracy

A statistical study using patient samples demonstrated good correlation of results with the commercially available kits as shown below:
Comparisons between GenWay Biotech CEA ELISA and Abbott AxSym CEA ELISA kits provide the following data:

N = 68
Correlation coefficient = 0.971
Slope = 0.883
Intercept = 0.034
GenWay Biotech Mean = 10 ng/mL
Abbott AxSym Mean = 9 ng/mL

2. Sensitivity

The minimum detectable concentration of CEA by this assay is estimated to be 1.0 ng/ml.

3. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different control sera in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4
Number of Replicates	24	24	24	24
Mean CEA (ng/mL)	6	23	58	120
Standard Deviation	0.2	0.6	1.3	1.7
Coefficient of Variation (%)	3.3	2.7	2.2	1.4

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of four different control sera in several different assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4
Number of Replicates	32	32	32	32
Mean CEA (ng/mL)	7	24	59	129
Standard Deviation	0.6	0.6	1.6	4.7
Coefficient of Variation (%)	9.7	2.5	2.8	3.7

4. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known CEA levels were mixed and assayed in duplicate. The average recovery was 95.9%.

	Expected Concentration (ng/ml)	Observed Concentration (ng/ml)	% Recovery
1.	56.5	54.1	95.9
2.	38.9	39.1	100.7
3.	24.2	24.4	100.9
4.	17.4	15.7	90.2
5.	11.4	10.9	95.7
6.	12.4	11.4	92.0
	Average Recovery = 95.9%		

b. Linearity

Three patient samples were serially diluted with the zero standard in a linearity study. The average recovery was 97.2%.

<table>
<tr><th>#</th><th>Dilution</th><th>Expected Conc. (ng/mL)</th><th>Observed Conc. (ng/mL)</th><th>% Recovery</th></tr>
<tr><td>1.</td><td>Undiluted
1:2
1:4
1:8
1:16
1:32</td><td>----
42.6
20.3
10.2
5.1
2.5</td><td>81.8
44.2
21.9
10.6
4.6
1.9</td><td>….
108.7
107.7
104.0
90.5
76.1</td></tr>
<tr><td colspan="5">Mean = 97.4 %</td></tr>
<tr><td>2.</td><td>Undiluted
1:2
1:4
1:8
1:16
1:32</td><td>….
61.2
30.6
15.3
7.6
3.8
2.394</td><td>122.4
61.9
30.6
15.1
6.1
3.1
2.300</td><td>….
110.1
99.9
98.7
80.2
80.4
96.1</td></tr>
<tr><td colspan="5">Mean = 92.1 %</td></tr>
<tr><td>3.</td><td>Undiluted
1:2
1:4
1:8
1:16
1:32</td><td>….
61.1
30.6
15.3
7.6
3.8</td><td>135.3
61.1
29.7
15.0
7.4
3.6</td><td>----
100.0
97.2
98.2
96.9
94.8</td></tr>
<tr><td colspan="5">Mean = 97.4%</td></tr>
</table>

<table>
<tr><td>4.</td><td>Undiluted
1:2
1:4
1:8
1:16
1:32</td><td>….
22.6
11.4
5.7
2.8
1.4</td><td>45.5
24.1
11.3
5.8
3.1
1.3</td><td>----
106.1
99.5
102.3
108.3
93.0</td></tr>
<tr><td colspan="5">Mean = 101.8%</td></tr>
</table>

5. Specificity

The following substances were tested for cross-reactivity:

Analyte Tested	Concentration	Produced Intensity Equibalent to CEA (ng/mL)
AFP	5,000 ng/mL	0

	10,000 ng/mL 25,000 ng/mL 50,000 ng/mL	0 0 0
hCG	5,000 mIU/mL 10,000 mIU/mL 50,000 mIU/mL 100,000 mIU/mL 250,000 mIU/mL 500,000 mIU/mL	0 0 0 0 0 0
PAP	1,000 ng/mL 2,500 ng/mL 5,000 ng/mL 10,000 ng/mL	0 0 0 0
PSA	1,000 ng/mL 2,500 ng/mL 5,000 ng/mL	0 0 0
CA 125	1,000 U/mL 2,500 U/mL 5,000 U/mL 10,000 U/mL	0 1.3 2.6 8.6

6. Hook Effect

No hook effect is observed in this assay at CEA concentrations up to 20,000 ng/mL.

LIMITATIONS OF THE PROCEDURE

1. 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.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. 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

REFERENCES

[1] Engvall, E., Enzyme immunoassay ELISA and EMIT. In: Van Vunakis, H. and Langone, J.J. eds., *Methods in Enzymol.,* Academic Press, New York, 1980; 70: 419-439.

[2] Uotila, M., Ruoslahti, E. and Engvall, E., Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. *J. Immunol. Methods.* 1981; 42: 11-15.

[3] Gold, P., Freedman, S.O., Demonstration of tumor-specific antigens in human colonic carcinoma by immunologic tolerance and absorption techniques. *J. Exp. Med*., 1965; 121: 439-462.

[4] Thompson, D.M.P., Krupey J., Freedman, S.O., Gold, P., The radioimmunoassay of circulating carcinoembryonic antigen of the human digestive system. *Proc. Natl. Acad. Sci*., 1969; 64: 161-167.

[5] Schwartz, M.K., Tumor markers in diagnosis and screening. In: Ting, S.W., Chen, J.S., Schwartz, M.K., eds. *Human tumor markers*, Amsterdam: Elsevier Science; 1988: 3-16.

[6] Zamcheck, N., and Martin, E.W., Factors controlling the circulating CEA levels in pancreatic cancer: Some clinical correlations. *Cancer*, 1981; 47: 1620-1627.
[7] Mughal, A.W., Hortobagyi, G.N., Fritsche, H.A., et al., Serial plasma carcinoembryonic antigen measurements during treatment of metastatic breast cancer. *JAMA*, 1983; 249:1881-1886.

Troubleshooting

The following are some common problems encountered with the use of ELISA kits, and some of the causes of these problems.

1. **Problem: Low absorbance**
   - ☐ Incorrect dilutions or pipetting errors.
   - ☐ Improper incubation times
   - ☐ Improper mixing of the TMB substrate. Each component is mixed in equal parts.
   - ☐ Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
   - ☐ Kit materials or reagents are contaminated or expired.
   - ☐ Incorrect reagents used.

2. **Problem: High Absorbance**
   - ☐ Cross contamination from other samples or positive control.
   - ☐ Incorrect dilutions or pipetting errors.
   - ☐ Improper washing.
   - ☐ Wrong filter on microtiter reader.
   - ☐ Contaminated buffers or enzyme substrate.
   - ☐ Improper incubation times.
   - ☐ Kit materials or reagents are contaminated or expired.

3. **Problem: Poor Duplicates**
   - ☐ Poor mixing of specimens.
   - ☐ Incorrect dilutions or pipetting errors.
   - ☐ Technical error.
   - ☐ Inconsistency in following ELISA protocol.
   - ☐ Inefficient washing.

4. **Problem: All wells are positive**
   - ☐ Contaminated buffers or enzyme substrate.
   - ☐ Incorrect dilutions or pipetting errors.
   - ☐ Kit materials or reagents are contaminated or expired.
   - ☐ Inefficient washing.

5. **Problem: All wells are negative**
   - ☐ Procedure not followed correctly.
   - ☐ Contaminated buffers or enzyme substrate.
   - ☐ Contaminated conjugate.
   - ☐ Kit materials or reagents are contaminated or expired.