

Human Carcinoembryonic Antigen (CEA) ELISA Kit

For the Quantitative Determination of Human Carcinoembryonic
Antigen (CEA) Concentrations in Human Serum and Plasma Samples

Catalogue Number: EL10052

96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



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INTENDED USE

This Human Carcinoembryonic Antigen ELISA Kit is designed for *in vitro* quantitative determination of Carcinoembryonic Antigen (CEA) concentrations in human serum and plasma samples. The kit is intended for RESEARCH USE ONLY and should not be used in any diagnostic or therapeutic procedures.

INTRODUCTION

Carcinoembryonic Antigen (CEA) is a 180-200 KD glycoprotein expressed at high level in the epithelial cells of the embryonic colon. The expression is stopped after birth. CEA serum content is low in normal population and only slightly elevated in heavy smokers. Increased serum CEA level was found associated with colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, lung carcinoma, and breast carcinoma (3, 4, 5, 6,). CEA has been used as a biomarker for carcinoma. However elevated CEA level not necessarily indicates neoplastic conditions, since high CEA expression has been observed in non-neoplastic conditions such as ulcerative colitis, pancreatitis and cirrhosis. Clinically, CEA level is mainly used to monitor the tumor recurrence after surgical resection of carcinoma (1, 2, 7,).

PRINCIPLE OF THE ASSAY

This CEA enzyme linked immunosorbent assay (ELISA) kit uses a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for CEA. During the assay, standards or samples are added to the microtiter plate wells and CEA, if present, will bind to the CEA antibody pre-coated on the wells. Then, a preparation of horseradish peroxidase (HRP)-conjugated CEA antibody is added to each well. The conjugated antibody will bind to the CEA immobilized on the plate after incubation. All unbound components will be removed by subsequent washing procedure. Afterwards, 3, 3', 5, 5' tetramethyl-benzidine(TMB), a substrate for HRP is added to each well. The colorimetric reaction is proportional to the CEA content in each well. The reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at the wavelength of 450nm \pm 2nm.

In order to quantify the amount of CEA present in the sample, the calibration standards should be assayed at the same time as the samples to allow the operator to produce a standard curve of Optical Density (O.D.) versus CEA concentration (ng/mL). The concentration of CEA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

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1. **CEA MICROTITER PLATE** (Part EL52-1) _____ **96 wells**
Microtiter plate pre-coated with anti-human CEA monoclonal antibody.
2. **CEA CONJUGATE** (Part EL52-2) _____ **12 mL**
Horseradish peroxidase conjugated anti-human CEA antibody, *Ready-to-use*
3. **CEA STANDARD – 160 ng** (Part EL52-3) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 160ng/ml with 0.6 ml of dH2O for use.
4. **CEA STANDARD – 80 ng** (Part EL52-4) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 80ng/ml with 0.6 ml of dH2O for use.
5. **CEA STANDARD – 40 ng** (Part EL52-5) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 40ng/ml with 0.6 ml of dH2O for use.
6. **CEA STANDARD – 20 ng** (Part EL52-6) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 20ng/ml with 0.6 ml of dH2O for use.
7. **CEA STANDARD – 10 ng** (Part EL52-7) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 10ng/ml with 0.6 ml of dH2O for use.
8. **CEA STANDARD – 5 ng** (Part EL52-8) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 5ng/ml with 0.6 ml of dH2O for use.
9. **CEA STANDARD – 2.5ng** (Part EL52-9) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 2.5ng/ml with 0.6 ml of dH2O for use.
10. **CEA STANDARD – 0 ng** (Part EL52-10) _____ **1 vial**
Lyophilized human CEA in protein matrix. Reconstitute to 0ng/ml with 0.6 ml of dH2O for use.
11. **SUBSTRATE A** (Part EL52-11) _____ **10 mL**
Buffered solution with H₂O₂
12. **SUBSTRATE B** (Part 30007) _____ **10 mL**
Buffered solution with TMB
13. **STOP SOLUTION** (Part 30008) _____ **15 mL**
2N Sulphuric Acid (H₂SO₄). Caution: Caustic Material!
14. **WASH BUFFER (20X)** (Part 30005) _____ **60 mL**
20-fold concentrated solution of buffered surfactant

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μL and 50-200 μL for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Incubator ($37\pm 2^\circ\text{C}$).
8. Microtiter plate reader ($450\text{ nm}\pm 2\text{ nm}$).
9. Automatic microtiter plate washer or squirt bottle.
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water.
12. Plastic plate cover.
13. Disposable gloves.
14. Absorbent paper.
15. 37°C incubator.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature ($22\text{-}25^\circ\text{C}$) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at $2\text{-}8^\circ\text{C}$ in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
Solid Wastes: Autoclave for 60 minutes at 121°C .
Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat or flame.

SAMPLE PREPARATION

1. Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) followed by serum extraction.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

2. If sample concentration exceeds the standard curve range, PBS containing 10%BSA can be used to dilute the samples.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (22-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

CEA Standards: Reconstitute each CEA Standard vial with **0.6 ml** of distilled or de-ionized water. Allow to sit for at least 15 minutes with gentle agitation. The reconstituted CEA standards can be aliquoted to store at -20°C for at least 1 month. Avoid freeze-thaw cycles.

Wash Buffer (1X): Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

Substrate Solution: Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80wells)	6.0	6.0	12.0
12 strips (96wells)	7.0	7.0	14.0

ASSAY PROCEDURE

1. Prepare all CEA Standards and Wash Buffer (1x) before starting assay procedure (see Preparation of Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*
2. Secure a desired number of strips from the coated microtiter plate to the holder. Add 50 μ l Standards or Samples to the appropriate wells and 100 μ l of Conjugate to each well. **IMPORTANT: COMPLETE MIXING SHOULD BE ACHIEVED BEFORE PROCEEDING.** Cover and incubate for **one hour at Room Temperature (23°C \pm 2°C).**
3. Wash the Microtiter Plate using one of the specified methods described below:

Manual Washing: Remove the incubation mixture by aspirating the contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with de-ionized or distilled water and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate and blot dry by hitting the plate onto absorbent paper or paper towel until no moisture is visible. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain in the frame.

Automated Washing: Aspirate all wells and wash plates **FIVE times** using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μ L/well/wash (range: 350-400 μ L). After final wash, invert plate and blot dry by hitting the plate onto absorbent paper or paper towel until no moisture is visible. *It is recommended that the washer be set for soaking time of 10 seconds or shaking time of 5 seconds between washes.*
4. Add 100 μ L freshly mixed Substrate Solution to each well. Cover and incubate for **15 minutes at Room Temperature.**
5. Add 100 μ L of Stop Solution to each well. Mix well.
6. Read the Optical Density (O.D.) at 450nm using a microtiter plate reader within 10 minutes.

CALCULATION OF RESULTS

A standard curve should be generated for each run. The standard curve is used to determine the amount of CEA in an unknown sample. The standard curve is generated by plotting the average O.D. (450nm) obtained for each standard concentrations on the vertical (Y) axis versus the corresponding CEA concentration (ng/mL) on the horizontal (X) axis.

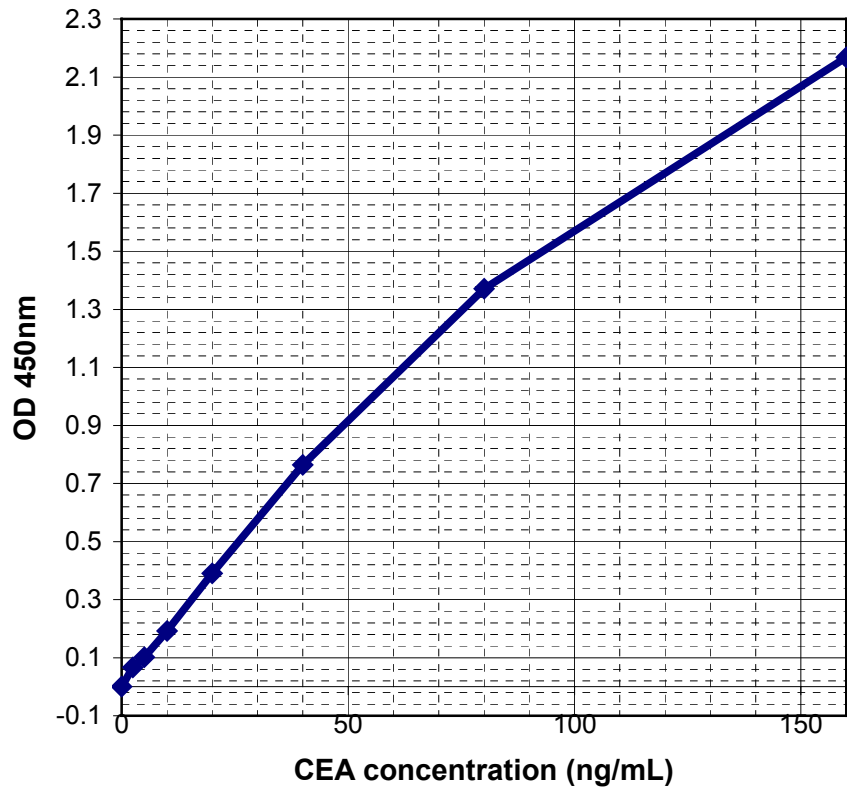
1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0ng/ml) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of CEA in each sample, locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding CEA concentration. If samples generate values greater than the highest standard, dilute the samples with the Sample Diluent and repeat the assay. The concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

Results of a typical standard run of CEA ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variations in the results. The following examples are for the purpose of illustration only. To calculate the results of samples, a standard curve must run using the same lot of reagents

EXAMPLE

Standard (ng/ml)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.062, 0.057	0.059	0.000
2.5	0.117, 0.113	0.115	0.066
5	0.164, 0.158	0.161	0.102
10	0.255, 0.247	0.251	0.192
20	0.451, 0.448	0.449	0.390
40	0.831, 0.815	0.823	0.764
80	1.406, 1.455	1.430	1.371
160	2.218, 2.237	2.227	2.168



PERFORMANCE CHARACTERISTICS

1. PRECISION

Within-run (Intra-assay) coefficients of variation (CV) were determined by 20 replicate tests of three serum samples in one assay.

Samples	Mean Concentration (ng/mL)	Coefficients of Variation (CV)
1	8.5	5.5%
2	24	4.43%
6	68.4	2.38%

Between-run (Inter-assay) coefficients of variation (CV) were determined by testing 4 serum samples in 5 different assays.

Samples	Mean Concentration (ng/mL)	Coefficients of Variation (CV)
1	22.3	6.7%
2	42.4	3.7%
3	23.8	6.7%
4	46.7	5.4%

2. SENSITIVITY

The minimal detectable dose of this CEA assay was calculated by adding two standard deviations to the mean optical density value of 20 zero standard replicates and determining the corresponding concentration from the standard curve. The minimal detectable dose for this human CEA assay generated by this method is 0.724ng/ml.

3. SPECIFICITY

Cross-reaction with other cancer markers was tested.

	Concentration	Mean OD	Detected Value
CA19-9	10,000unit/mL	0.084	0.392ng/mL
PSA	50 μ g/ML	0.043	Under detection limit
CA125	100,000unit/mL	0.134	1.673ng/mL
Beta-HCG	14unit/ML	0.067	Under detection limit
AFP	218 μ g/ML	0.076	Under detection limit

4. RECOVERY

The recovery rate of CEA adding to human sera ranged from 89.5% to 108.4% with three different samples containing high, median and low CEA concentrations.

5. HOOK EFFECT

No hook effect was observed with sample containing up to 5,000ng/ml CEA.

6. NORMAL VALUES

Over 50 serum and plasma samples from normal blood donors were tested using this assay. The CEA contents were below 8.14ng/ml. It is recommended that each laboratory should establish its own normal value for the population tested.

LIMITATIONS OF THE PROCEDURE

The assay is for research use only.

The CEA value should be used in conjunction with information available from clinical evaluation and other procedures.

REFERENCES

1. Chen CC, Yang SH, Lin JK, Lin TC, Chen WS, Jiang JK and Wang LW. Is it reasonable to add preoperative level of CEA and CA19-9 to staging for colorectal cancer? *J Surg Res* 2005; 124: 169–174

2. Gobbi PG, Valentino F, and Berardi E *et al.* New insights into the role of age and carcinoembryonic antigen in the prognosis of colorectal cancer. *Br. J. Cancer* 2008;98: 328-334

3. Khoo SK and MacKay FR. Carcinoembryonic antigen in serum in diseases of the liver and pancreas. *J. Clin. Path.* 1973; 26: 470-475.
4. Laurence DJR, Stevens U, and Bellelheim R *et al.* Evaluation of the role of carcinoembryonic antigen in the diagnosis of gastro-intestinal, mammary and bronchial carcinoma. *Br Med J* 1972; 3: 605-609.
5. Moore TL, Kupchik HZ, Marcon N, and Zamchek N. Carcinoembryonic antigen assay in cancer of the colon and pancreas and other digestive tract disorders. *Am. J. Dig Dis* 1971; 16: 1-7.
6. Vincent RG, Chu TM, Fergen TB, and Ostrander M. Carcinoembryonic antigen in 228 patients with carcinoma of the lung. *Cancer* 1975; 36: 2069-2076.
7. Wanebo HJ, Rao B, Pinsky CM, Hoffman RG, Stearns M, Schwartz MK, and Oettgen HF. Preoperative carcinoembryonic antigen level as a prognostic indicator in colorectal cancer. *N Engl J Med* 1978; 299: 448-451