

Product Information

Carcinoembryonic Antigen (CEA) ELISA

Catalog Number **SE120025**

Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Carcinoembryonic antigen (CEA), a 180 kDa intercellular adhesion molecule expressed in high concentrations in the fetus but normally not found in adult serum because the synthesis of this protein ceases after birth. However, it reappears in a high concentration in the sera of patients with colorectal (57%), gastric (41%), hepatocellular (45%), pancreatic (59%), and biliary (59%) carcinoma. The serum concentration of CEA can also be elevated in benign diseases of the colorectum (inflammatory bowel disease 17%), stomach (chronic gastritis and peptic ulcer 14%), liver (cirrhosis and hepatitis 17%), and pancreas (21%). Elevated levels of CEA have also been observed in patients with inflammatory nonmalignant diseases like pulmonary emphysema, alcoholic cirrhosis, pancreatitis, and in heavy smokers. In contrast to cancer these elevations are transitory. The serum levels drop back into the normal range within a few weeks. The primary use of CEA is to monitor patients after surgery for recurrent colorectal carcinoma. Serum CEA has sensitivity between 60–95% in detecting recurrences prior to clinical detection and a lead-time between 2–10 months (positive predictive value 65%; negative predictive value 70%). False-positive results are usually below 10.0 ng/mL.

The Carcinoembryonic Antigen (CEA) ELISA Kit is intended for the quantitative measurement of CEA in human serum. The CEA is a solid phase direct sandwich ELISA method. The samples and diluted anti-CEA-HRP conjugate are added to the wells coated with Mab to the CEA beta subunit. CEA in the patient's serum binds to anti-CEA Mab on the well and the anti-CEA-HRP second antibody then binds to CEA. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of CEA in the samples. A standard curve is prepared relating color intensity to the concentration of the CEA.

Components

Materials Provided	96 Tests
Microwells coated with CEA MAb	12 x 8 x 1
CEA Standards: 6 vials (ready to use)	0.5 mL
CEA Enzyme Conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 °C) for 5 days. If storage time exceeds 5 days, store frozen at (–20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Reagent Preparation

Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

It is recommended that standards, control, and serum samples be run in duplicate.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µL of CEA standards, control, and sera into the appropriate wells.
3. Add 100 µL of Enzyme Conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18–26 °C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1x wash buffer. Blot on absorbent paper towels.
6. Add 100 µL of TMB Substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µL of Stop Solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

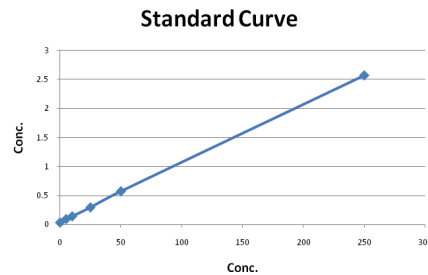
Results**Calculations**

The standard curve is constructed as follows:

1. Check CEA standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the CEA standards (vertical axis) versus the CEA standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a standard curve

	OD 450 nm	Concentration ng/mL
Std 1	0.032	0
Std 2	0.092	5
Std 3	0.139	10
Std 4	0.293	25
Std 5	0.570	50
Std 6	2.564	250

**Expected values**

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

CEA Normal Value: Less than 5 ng/mL. Most people have a CEA value of below 2.5 ng/mL. In a small percentage of the population the level extends up to 5 ng/mL. Smokers have in general a higher CEA level than non-smokers.

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.

References

1. Bates, S.E., Clinical applications of serum tumor markers. *Ann. Intern. Med.*, 1991;115:623-38.
2. Kuusela, P. et al., Comparison of a new tumour marker CA 242 with CA 19- 9, CA 50 and carcinoembryonic antigen (CEA) in digestive tract diseases. *Br. J. Cancer*, 1991;63:636-40.
3. Nilsson, O. et al., Sensitivity and specificity of CA242 in gastro- intestinal cancer. A comparison with CEA, CA50 and CA 19-9. *Br, J, Cancer*, 1992;65:215-21.
4. Barillari, P. et al., Role of CEA, TPA, and CA 19-9 in the early detection of localized and diffuse recurrent rectal cancer. *Dis. Colon Rectum*, 1992;435:471-6.
5. Camuñas. J. et al., Value of follow-up in the management of recurrent colorectal cancer. *Eur. J. Surg. Oncol.*, 1991;17:530-5.
6. Moertel, C.G. et al, An evaluation of the carcinoembryonic antigen (CEA) test for monitoring patients with resected colon cancer. *JAMA*, 1993;270:943-7.
7. Chevinsky, A.H., CEA in tumors of other than colorectal origin. *Semin Surg Oncol* 1991;7:162-6.

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