

CALDON BIOTECH INC.
Carcinoembryonic
Antigen(CEA)
ELISA

Catalog No. CE062T
(96 tests)

INTENDED USE

The CALDON BIOTECH INC (CBI), CEA ELISA kit is used for the quantitative measurement of CEA in human serum or plasma.

SUMMARY AND EXPLANATION

Carcinoembryonic antigen (CEA), a 180 kD 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 reappear 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% and 95% in detecting recurrences prior to clinical detection and a lead-time between 2 and 10 months (positive predictive value 65%; negative predictive value 70%). False- positive results are usually below 10.0 ng/mL.

PRINCIPLE OF THE TEST

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 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.

MATERIALS PROVIDED

1. Microwell coated CEA MAb (12x8x1 wells). Total 96 wells.
2. CEA Standard: Six vials (0.7 mL each). Ready to use
3. Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 1 bottle (8 mL). Ready to use.
6. Wash Concentrate: One Bottle (50 mL, 10X).

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. 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.

SPECIMEN COLLECTION HANDLING

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.

REAGENTS PREPARATION

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 mL of distilled water. Store at room temperature.

ASSAY PROCEDURE

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 patient's sera.
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. Fill wells with wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 µL of TMB substrate to all wells.
7. Incubate for 10 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 20 minutes after adding the stopping solution.

CALCULATION OF RESULTS

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 you check the value on every kit. See example of the standard attached.
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.

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.

LIMITATIONS OF THE TEST

1. 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.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS**1. Correlation with a Reference ELISA kit:**

A total of 96 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.94	0.84	-2.198

2. Precision**Intra-Assay**

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation%
Normal	16	36.0	1.80	5.0
Low	16	10.9	0.57	5.2
High	16	2.5	0.13	5.2

Inter-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation%
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Normal	10	33	2.30	6.9
Low	10	12	1.00	8.3
High	10	2.6	0.25	9.6

- Chevinsky AH. CEA in tumors of other than colorectal origin. *Semin Surg Oncol* 1991;7:162-6. Jun-99

3. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.32	0.16	0.64 ng/mL

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