LIMITATIONS OF THE TEST

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

REFERENCES

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4. Barillari P, Bolognese A, Chirletti 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.
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7. Chevinsky AH. CEA in tumors of other than colorectal origin. Semin Surg Oncol 1991;7:162-6.

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Calbiotech Inc.

CALBIOTECH
A life science company

Carcinoembryonic Antigen (CEA) ELISA

Catalog No.: CE236T (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) CEA ELISA Kit is intended for the quantitative measurement of CEA in human serum.

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 CBI CEA is a solid phase sandwich ELISA method. The samples, and anti-CEA-HRP/Biotin conjugate are added to the wells coated with Streptavidin. CEA in the patient's sample forms a sandwich between two specific antibodies 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	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. CEA Standard: 7 vials (ready to use)	0.5ml
3. CEA Enzyme Conjugate: 1 bottle (ready to use)	12ml
4. TMB Substrate: 1 bottle	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. 20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

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

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 the expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

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

1. 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 (20-25°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature (20-25°C).
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 25 µl of CEA standards, control and patient specimens into designated wells.
3. Add 100 µl of ready to use enzyme conjugate to all wells. Shake for (10-30) sec.
4. Cover the plate and incubate for 60 minutes at room temperature.
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 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.

Example of Standard Curve

	OD 450 nm	Conc. ng/mL
Std 1	0.017	0
Std 2	0.101	5
Std 3	0.183	10
Std 4	0.451	25
Std 5	0.872	50
Std 6	1.437	100
Std 7	2.710	250