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 for CEA may be used as initial guideline ranges only:

Classification	Normal Range (ng/ml)
None Smokers	0.0 - 5.0
Smokers	1.0 - 7.0

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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Cat#: CE236T (96 Tests)
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New Streptavidin Format

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. For research use only.

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: 6 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

Note: The opened reagents are stable for 60 days at 2-8°C

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. Potential biohazardous materials: The standards set 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 kit is designed for research 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
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 (18-26°C).

2. Pour the contents of Solution A into the vial labeled Solution B. Mix and label accordingly. Store at 2-8°C.

ASSAY PROCEDURE
Prior to assay, allow reagents to stand at room temperature (18-26°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 (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 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	0.023	0
Std 2	0.092	5
Std 3	0.139	10
Std 4	0.340	25
Std 5	0.660	50
Std 6	2.564	250

Standard Curve