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CEA ELISA Kit

Name	CEA ELISA Kit
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
Microwells coated with Streptavidin	12x8x1
CEA Standard: 7 vials (ready to use)	0.5ml
CEA Enzyme Conjugate: 1 bottle (ready to use)	12ml
TMB Substrate: 1 bottle	12ml
Stop Solution: 1 bottle (ready to use)	12ml
20X Wash concentrate: 1 bottle	25ml

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

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8C.

Keep microwells sealed in a dry bag with desiccants.

The reagents are stable until the expiration of the kit.

Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

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 Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. 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.
SPECIMEN COLLECTION HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8C) for 5 days. If storage time exceeds 5 days, store frozen at (-20C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. 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 (20-25C).
ASSAY PROCEDURE	Prior to assay, allow reagents to stand at room temperature (20-25C). Gently mix all reagents before use. Place the desired number of coated strips into the holder Pipet 25 l of CEA standards, control and patient specimens into designated wells. Add 100 l of ready to use enzyme conjugate to all wells. Shake for (10-30) sec. Cover the plate and incubate for 60 minutes at room temperature (20-25C).

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Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbent paper towels.

Add 100 l of TMB substrate to all wells.

Incubate for 15 minutes at room temperature.

Add 50 l of stop solution to all wells. Shake the plate gently to mix the solution.

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:

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.

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.

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

LIMITATIONS OF THE TEST

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

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

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