

Carcinoembryonic Antigen (CEA) ELISA Kit

48 Tests Kit

Enzyme Immunoassay for the Quantitative Determination of CEA Concentration in Human Serum/Plasma (For In Vitro Diagnostic Use Only) Catalogue No. PT-CEA-48

PISHTAZ TEB DIAGNOSTICS

Introduction

Carcinoembryonic antigen (CEA) is a 150-300 kD glycoprotein molecule which has been found by Gold and Freedman at 1965 and it is also known as major component of CEA glycoprotein family. This family consists of 36 different glycoproteins with the common feature of close similarity of their structural domain to IgG heavy chain structure. Carbohydrate constitutes 45-55% of the CEA whole molecule and its polypeptide chain formed by 641 aminoacids. During the fetal period and prior to birth, endothelial cells and after birth epithelial cells are mainly contribute to CEA production and secretion. It is also shown that adenocarcinoma cells of the colon and fetal intestinal cells are produce CEA during their life period. Several factor affect CEA serum level including CEA gene expression in source tissue, its synthesis and secretion level, CEA half life in circulation, tumor necrosis and vascularization level and finally its catabolism in liver. Normal level in healthy nonsmoker individuals is about 2.5-5 ng/ml (95-98% of healthy people are under 5 ng/ml) but in smoker individuals, elevated levels (5-10 ng/ml) have been reported. It is also shown that males CEA level is more than females and olds individuals have higher level than youths. Elevated levels of CEA are found in many cancers. More than 80% of patients with advance adenocarcinoma of the colon and more than 30% of patients with cancer of the lung, liver, pancreas, breast, head or neck,

bladder, cervix, and prostate have been showed to have increased levels of CEA. However, CEA assay per se should not used as final cancer diagnostic test since its increase is also reported in various benign conditions. Chronic liver disease (90%), acute liver disease (50%), liver cirrohsis (45%), pulmonary emphysema (30%), intestinal polyps (5%) and ulcerative colitis (15%) are benign conditions which were reported with high CEA level . However in all benign conditions CEA level is not more than 10 ng/ml. Elevated plasma levels are related to the stage and extent of the disease, the degree of differentiation of the tumor, and the site of metastasis. Normally the CEA assay can be used for the diagnosis of colon adenocarcinoma cancer stage, treatment followup and prognosis of the disease but not as screening test.

The CEA Quantitative Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for CEA level.

Test Principle

The CEA Quantitative Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes an anti-CEA antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-CEA antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwiched formation of CEA between solid phase and conjugated antibodies. After second wash step a solution of TMB is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of CEA is directly proportional to the color intensity of the test sample.



Materials provided with the kit

1. Antibody coated wells (1 plate, 48 wells) microtiter wells coated with monoclonal anti CEA
2. Enzyme conjugate: (1 vial, 6 ml)
Monoclonal anti CEA labeled with HRP in buffer contain protein as stabilizer and Thiomerosal as preservative, ready to use
3. Standards set (0.5 ml / vial): contain 0, 2, 5, 20, 50 and 100 ng/ml of CEA calibrated against WHO 1st IPR 73/601.
diluted in buffer contain protein as stabilizer and 0.05% Thiomerosal as preservative
4. Low control serum (0.5 ml / vial)
Certain amount of CEA diluted in buffer contain protein as stabilizer and 0.05% Thiomerosal as preservative the exact concentration is printed on the labels
5. High control serum (0.5 ml / vial)
Certain amount of CEA diluted in buffer contain protein as stabilizer and 0.05% Thiomerosal as preservative the exact concentration is printed on the labels
6. Assay buffer (1 vials, 6 ml) Contain Phosphate buffer solution with protein as stabilizer and Kathon CG as preservative, ready to use
7. Chromogen Substrate Reagent (1 vials, 6 ml) Contain Tetramethyl benzidine and hydrogen peroxide, ready to use solution.
8. Wash solution: (1 vial, 6 ml) contain Phosphate Buffer salt Solution with 0.05 % Tween 20 as detergent, PH = 6
Concentrated (20X).
Stop solution: (1 vial, 6 ml) 1 molar hydrochloric acid solution, PH = < 1
9. Cardboard sealer

Materials required but not provided

1. Precision pipettes: 50 µl and 100 µl
2. Disposable pipette tips.
3. Distilled water.
4. Absorbent paper or paper towel.
5. Graph paper.
6. Microtiter well reader.

General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C (maximum 30 days). Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Test Procedure

1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 50 µl of standard, serum

control and specimen in appropriate wells in duplicate.

3. Add 50 µl of assay buffer into each well and shake wells gently for 15 seconds to mix well.
4. Cover the microtiter wells with cardboard sealer firmly. Leave wells for 30 minutes at room temperature (22-28°C).
5. Remove the sealer and take out wells contents by flicking the microplate into a waste container.
Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 100 µl of Anti-CEA-HRP conjugate into the wells.
8. Seal the plate with cardboard sealer again. Leave wells for 30 minutes at room temperature (22-28°C).
9. Repeat step 5
10. Dispense 100 µl of chromogen/substrate (TMB) into the microplate wells.
11. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
12. Stop the reaction by adding 100 µl of stop solution to the microplate wells.
13. Measure absorbance at 450 nm by ELISA reader. (Use 630 nm filter as reference filter if it's available).

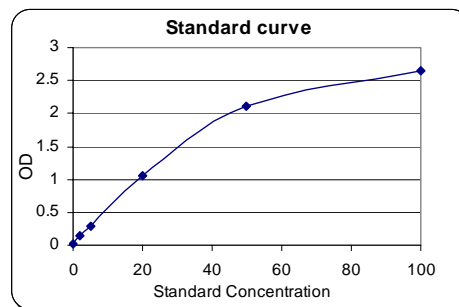
Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample; determine the corresponding concentration of CEA in ng/ml from the standard curve.

Example of Standard curve

Standards ng/ml	OD
0	0.02
2	0.142
5	0.286
20	1.05
50	2.103
100	2.65

Note: All absorbances shown in above curve and table are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve.



Expected Values

It is important for each laboratory to establish the normal range limits. The following normal range should be considered as a guideline only:

Normal range ng / ml		
Expected Value	Healthy Nonsmokers	Up to 5.0
	Smoker	Up to 10.0

Performance Characteristics

1. Minimum Detection Limit

The minimum detectable concentration of CEA by this assay is estimated to be 0.2 ng/ml.

2. Test Precision

Four serum samples with different concentrations of CEA were repeatedly tested. Results are shown in table 1 and 2:

Table no. 1 (Intra-assay)

No.	No. of tests performed	Means ng/ml	SD ng/ml	CV %
1	24	4.5	0.15	3.3
2	24	23.2	0.96	4.1
3	24	67.3	2.5	3.7

Table No. 2 (Inter-assay)

No.	No. of tests*	Means ng/ml	SD ng/ml	CV %
1	10	5.5	0.28	5.2
2	10	22.2	1.19	5.3
3	10	74	5.1	7.0

*Each test has been run in duplicate

3. Test Recovery

The certain amount of CEA was added into 4 different serums with known concentration of CEA and then their recovery were determined. The results shown below:

Table no. 3: Test recovery

N o.	CEA level ng/ml	CEA added ng/ml	Exp. ng/ml	Obs. ng/ml	Rec. (%)
1	1.2	2.5	1.85	1.75	94
1	1.2	23	12.1	12.9	106
1	1.2	59	30.1	32.0	106
2	8.3	2.5	5.4	5.1	94
2	8.3	23	15.6	16.8	107
2	8.3	59	33.6	34.1	101
3	22.8	2.5	12.6	12.5	100
3	22.8	23	22.9	22.1	96
3	22.8	59	40.5	39.3	97
4	67.2	2.5	34.8	36.6	105
4	67.2	23	45.1	44.5	98
4	67.2	59	63.1	69.0	109

Exp.: Expected , Obs.: Observed,
Rec.: Recovery

4. Test Linearity

To verify test linearity, three different serum samples with known CEA concentration were diluted sequentially by zero standard. Then the serums were tested by CEA ELISA test. The results and serum recovery were determined considering dilution factor.:

Table no. 4: Test linearity

No.	CEA undiluted specimen	Recovery (%)				
		1:2	1:4	1:8	1:16	1:32
1	84	104	98	89	106	93
2	57	99	100	92	95	90
3	38	102	95	98	110	108

5. Hook effect

To rule out possible hook effect occurrence, the CEA assay was done on serums with high concentration of CEA (up to 5000 ng/ml) and no "hook effect" was seen.

References

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- 5 - Oikawa S, Nakazato H, Kosaki G. Primary structure of human carcinoembryonic antigen (CEA) deduced from cDNA sequence. *Biochemical & Biophysical Research Communications* 142(2): 511-518; 1987.
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


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CEA Test Procedure

Step 1




Reagent	Standard	Serum Control	Sample
			
Standard	50 µl	None	None
Serum Control	None	50 µl	None
Sample	None	None	50 µl
Assay Buffer	50 µl	50µl	50 µl

Gently mixed for 15 seconds and cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature.

Step 2

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.




Step 3

Anti CEA- HRP conjugate	100 µl 	100 µl 	100 µl 
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Cover the microplate wells again with cardboard sealer and incubate them for 30 minutes at room temperature.




Step 4

Remove the microplate wells cover and remove contents by flicking of the wells into a waste container. Rinse and flick the microtiter wells 5 times according to test manual.

TMB	100 µl 	100 µl 	100 µl 
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Incubate wells for 15 minutes at room temperature in dark.

Step 5

Stop Solution	100 µl 	100 µl 	100 µl 
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Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).