CEA (Carcinoembryonic Antigen) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIACEA1	CEA Elisa	96 Tests

Intended Use:

CEA Elisa is intended to be used for the quantitative determination of CEA in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

CEA is a cell-surface 200 Kd glycoprotein. In 1969, it was reported that plasma CEA was elevated in 35 of 36 patients with adenocarcinoma of the colon and that CEA titers decreased after successful surgery. Normal levels were observed in all patients with other forms of cancer or benign diseases. Subsequent studies have not confirmed these initial findings, and is now understood that elevated levels of CEA are found in many cancers. Increased levels of CEA are observed in more than 30% of patients with cancer of the lung, liver, pancreas, breast, colon, head or neck, bladder, cervix and prostate. 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. CEA is also found in normal tissue.

The CEA Elisa is based on a solid phase EIA. The assay system utilizes one anti-CEA antibody for solid phase immobilization and another mouse monoclonal anti-CEA antibody in the antibody enzyme conjugate solution. The test sample is allowed to react simultaneously with the antibodies resulting in the CEA molecules being sandwiched between the solid phase and enzyme linked antibodies. After 60 minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes resulting in the development of a blue colour. The colour development is stopped with the addition of 2N HCl, and the colour is changed to yellow and measured at 450nm. The concentration of CEA is directly proportional to the intensity of colour developed.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal anti-CEA antibody. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable until expiry date at 2-8°C.
CEA Calibrators	6x2x0.5 ml	6 vials containing CEA at concentrations of 0.0, 3, 12, 30, 60 and 120 ng/ml made up in a human serum matrix. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable until expiry date at 2-8°C.
Enzyme Conjugate	1x12ml	1 vial containing 12ml of HRP labelled monoclonal Anti-CEA antibody in Buffered saline. Once open, stable until expiry date at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable until expiry date at 2-8°C.
Stop Solution	1x12ml	2N HCl Ready to use. Once open, stable until expiry date at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. Avoid grossly haemolytic, lipaemic or turbid samples. Plasma samples collected in tubes containing EDTA, heparin or oxalate may interfere with the test procedures and should be avoided. Specimen should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable until the labelled expiry date. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained until the labelled expiry date.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature (18-22°C) prior to use.
2. Lyophilised standards are reconstituted using 0.5ml of distilled water. Ensure that the measurement of the distilled water is exact. Set aside for 20 minutes, gently swirling every 5 minutes. Use after ensuring that all the lyophilised material has gone into solution and the solution is homogeneous.
3. Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 50 µl of Calibrators and Samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100 µl of the Enzyme Conjugate to each well. Shake the plate for 5 seconds to ensure that the added components are well mixed. Complete mixing at this step is very important.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at room temperature (18-22°C).

STEP 5

Washing: At the end of the incubation period, remove the plate cover and discard the contents of the wells. Wash each well 5 times with diluted washing buffer of 350 µl. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100 µl of Substrate Solution to each well. Mix gently for 5 seconds.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 100 µl of Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Note down the absorbances.

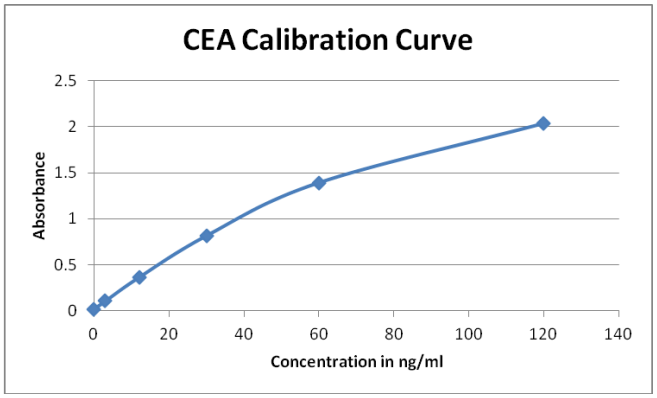
Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. It is recommended that no more than 32 wells are used for each assay run if manual pipetting is used since pipetting of all calibrators, specimens, controls should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available. Duplication of calibrators and specimens although not required is recommended.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in ng/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.019	0.0 ng/ml
CAL B	0.105	3.0 ng/ml
CAL C	0.362	12.0 ng/ml
CAL D	0.814	30.0 ng/ml
CAL E	1.390	60.0 ng/ml
CAL F	2.032	120.0 ng/ml



This calibration curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own curve and data.

Expected Values:

The most complete study of CEA is a compilation of collaborative studies which CEA values in 35000 samples from more than 10,000 patients and controls were analyzed. Of 1425 normal persons who did not smoke, 98.7% had values less than 5 ng/ml. It is recommended that each laboratory establish its own normal range.

Performance Characteristics:

1. **Intra assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	24	5.07 ng/ml	0.173	3.4%
2	24	20.3 ng/ml	0.744	3.7%
3	24	35.44 ng/ml	1.09	3.1%

2. **Inter assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	20	4.94 ng/ml	0.243	4.9%
2	20	19.82 ng/ml	1.24	6.3%
3	20	35.36 ng/ml	1.33	3.8%

3. **Sensitivity:**

The minimum detectable concentration of CEA by this assay was found to be 1 ng/ml.

Linearity:

A patient serum was serially diluted with 0 ng/ml standard in a linearity study. The average recovery was 99.7%

Sample A			
Dilution	Expected	Observed	% Recovery
Sample undil	103.5 ng/ml	103.5 ng/ml	
2x	51.75 ng/ml	50.89 ng/ml	98.3 %
4x	25.88 ng/ml	26.26 ng/ml	101.5 %
8x	12.94 ng/ml	13.22 ng/lm	102.2 %
16x	6.47 ng/ml	6.25 ng/ml	96.6 %
32x	3.23 ng/ml	3.51 ng/ml	108.5 %
Average Recovery: 99.7%			

Method Comparison:

Method comparison between this assay and a commercially available assay yielded the following data:

N=46, Correlation Coeff: 0.946, Slope: 0.788, Intercept: 0.74

Mean values: This assay: 7.59 ng/ml and comparator: 6.03 ng/ml

Recovery:

Various patient serum samples of known CEA levels were mixed and assayed in duplicate. The average recovery was 100.7%

Expected conc ng/ml	**Observed Conc ng/ml**	**% Recovery**
54.28	53.21	98.0
28.42	29.13	102.5
23.85	22.98	96.4
16.53	15.99	96.7
20.90	21.36	102.2
18.62	19.65	105.5
28.77	29.78	103.5
Average Recovery: 100.7%		

Cross Reactivity:

The following materials were tested for cross reactivity and the results are as follows:

Antigens	Concentration	Equivalent CEA	% Cross reactivity
HCG	400 IU/ml	0.0 ng/ml	0.0 %
PAP	1000 ng/ml	0.0 ng/ml	0.0 %
PSA	1000 ng/ml	0.0 ng/ml	0.0 %
AFP	1000 ng/ml	0.0 ng/ml	0.0 %

No High dose hook effect was observed up to 40,000 ng/ml CEA in this assay.

References:

1. Gold P, Freedman SO. Demonstration of tumor specific antigen in human colonic carcinomata by immunologic tolerace and absorption techniques. J Exp Med 1965; 127: 439-462.
2. Thompson D PM, Krupey J, Freedman SO et al. The radioimmunoassay of circulating Carcinoembryonic antigen of the human digestive system. Proc Natl Acad Sci USA 1969; 64: 161-167.
3. Schawrtz MK. Tumor markers in diagnosis and screening IN: Ting SW, Chen JS Schwartz MK eds, Human tumor mnarkers Amsterdam: Elsevier science, 1987; 3-16.
4. Zamcheck N and Martin E.W Sequential Carcinoembryonic antigen levels in Pancreatic cancer: some clinical correlation. Cancer. 1981;47: 1620-1627.
5. Mughal A.W, Hortobagyi GN Fritsche HA, Buzdar A.U Yap H-Y and Blumschein G.R. Serial Plasma Carcinoembryonic antigen measurements during treatment of metastatic breast cancer. JAMA 1983; 259: 1881-1886.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

Prestige Diagnostics U.K. Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

V3: rev Feb 2017

CE