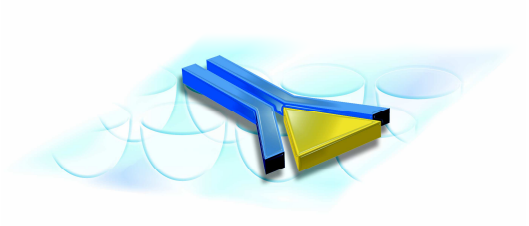
NovaTec
Immundiagnostica GmbH



CEA

Enzyme immunoassay for the quantitative
determination of CEA in human serum

Only for in-vitro diagnostic use

CE

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Product Number: DNOV060 (96 Determinations)

CONTENTS

1. INTRODUCTION

Carcinoembryonic antigen (CEA) is a glycoprotein, with a molecular weight of 180 kDa, involved in cell adhesion. It is normally produced during fetal development, but the production of CEA stops before birth. Therefore, it is not usually present in the blood of healthy adults, although levels are raised in heavy smokers. CEA was identified in human colon cancer tissue extracts . It was later found that serum from individuals with colorectal and other carcinomas had higher levels of CEA than healthy individuals and can be used to monitor the response to colon cancer treatment.
CEA and related genes make up the CEA family belonging to the immunoglobulin superfamily.
The most frequent cancer which causes an increased CEA is cancer of the colon and rectum. Others include cancers of the pancreas, stomach, breast, lung, and certain types of thyroid and ovarian cancer. Benign conditions which can elevate CEA include smoking, infections, inflammatory bowel disease, pancreatitis, cirrhosis of the liver, and some benign tumors in the same organs in which an elevated CEA indicates cancer. Chemotherapy and radiation therapy can cause a temporary rise in CEA due to the death of tumor cells and release of CEA into the blood stream. Benign tumours do not usually cause an increase above 10 ng/ml.

2. INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of CEA in human serum.

3. PRINCIPLE OF THE ASSAY

In this method, CEA standards, patient specimens and/or controls containing the native antigen are first added to streptavidin coated wells. Biotinylated monoclonal and horseradish peroxidase (HRP) labeled antibodies are added and the reactants are mixed. The different types of antibodies used have high affinity and specificity and are directed against distinct and different epitopes of CEA. Reaction between the various CEA antibodies and native CEA occurs in the microwells without competition or sterics hindrance forming a soluble sandwich complex.

The interaction is illustrated by the following equation:

$$\text{E-Ab + AgCEA + BtnAb (m)} \underset{\text{K-a}}{\overset{\text{Ka}}{\leftrightarrow}} \text{E-Ab-AgCEA-BtnAb(m)}$$

BtnAb(m)	Byotinilated Monoclonal Antibody (Excess Quantity)
AgCEA	Native Antigen (Variable Quantity)
E-Ab	enzyme labeled Antibody (Excess Quantity)
HRP-Ab(p)-AgCEA-BtnAb(m)	Antigen-Antibodies Sandwich Complex
Ka	Rate Constant of Association
K-a	Rate Constant of Dissociation

Simultaneously, the complex is fixed to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$$\text{E-Ab- AgCEA -BtnAb (m)+Streptavidin CW} \rightarrow \text{Immobilized Complex}$$

Streptavidin CW	Streptavidin immobolized on well.
Immobilized Complex	Antibodies-Antigen sandwich bound.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by aspiration. The native antigen concentration is directly proportional to the HRP activity in the antibody-bound fraction. The activity of the conjugated HRP is quantitated by reaction with TMB substrate to produce blue colour. The reaction is terminated by adding stop solution which turns the blue colour into yellow. The absorbance is measured on a plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Coated Mircoplate:** 12 breakapart 8-well snap-off strips coated with streptavidin; in aluminium foil.
- **Conjugate:** 1 bottle containing 13 ml of horseradish peroxidase labelled anti- CEA antibodies and biotinylated monoclonal mouse anti-CEA antibodies.
- **TMB Substrate Solution**: 1 bottle containing 12 ml 3, 3´, 5, 5´-tetramethylbenzidine (H_2O_2-TMB 0.25 g/l) (avoid any skin contact).
- **Wash solution 50x conc.:** 1 bottle containing 20 ml (NaCl 9 g/l, Tween 20 1 g/l)
- **Stop Solution**: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).

- **Standards**: 6 bottles containing 1 ml standard solution with the approx. the following concentration:

Standard 0	0 ng/ml
Standard 1	5 ng /ml
Standard 2	10 ng /ml
Standard 3	25 ng /ml
Standard 4	50 ng /ml
Standard 5	250 ng /ml

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- 37 °C incubator
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver a volume of 100 µl
- Distilled water
- Timer

5. STABILITY AND STORAGE

The closed reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark.
Opened reagents are stable for 60 days when stored at 2÷8°C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (20…25°C) before starting the test run!

6.1. Coated microplate

The ready to use break apart snap-off strips are coated with streptavidin. Store at 2…8 °C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °. Do not remove the adhesive sheets on the unused strips.*

6.2. Conjugate

The conjugate is ready to use.

6.3. CEA Standards

The standards are ready to use.

6.4. TMB Substrate Solution

The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.*

6.5. Stop Solution

The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

6.6 Wash Solution

Dilute the concentrated wash solution to 1000 ml distilled or deionised water. Store at room temperature (22 - 28°C) for up to 60 days.

7. SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.
Samples may be refrigerated at 2÷8°C for a maximum period of 5 days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of –20°C for up to 30 days. Avoid repetitive freezing and thawing.
Patient specimens with CEA concentrations above 250 ng/ml may be diluted (for example 1/10 or higher) with Standard 0 and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
When assayed in duplicate, 0.050ml of the specimen is required.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well	e.g. A1)	for blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(e.g. F1+G1)	for standard 2
2 wells	(e.g. H1+A2)	for standard 3
2 wells	(e.g. B2+C2)	for standard 4
2 wells	(e.g. D2+E2)	for standard 5

It is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 25 µl standards and samples (and controls) into their respective wells.
2. Dispense 100 µl conjugate in each well. Cover with a foil.
3. **Incubate for 60 min at room temperature (22 – 28°C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100 µl TMB Substrate Solution into all wells.
6. **Incubate for 15 min at room temperature (+22…+28°C) in the dark.**
7. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue colour developed during the incubation turns into yellow.*
8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of stop solution against blank.

9. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CEA for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

10. RESULTS

10.1. Note

The optical densities (O.D.) of some standards and samples may be higher than 2.0, in such a case, they could be out of the measurement range of the microplate reader. It is therefore necessary, for O.D.s higher than 2.0, to perform a reading at 405 nm (= wavelength of peak shoulder) in addition to 450 nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).
For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:
- Read the microplate at 450 nm and at 620 nm.
- Read again the plate at 405 nm and 620 nm.
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/OD 405 = 3.0), that is: OD 450 nm = OD 405 nm x 3.0.

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his own reader.
The OD of standard 5 should be ≥ 1.3.

10.2. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic or smoothed cubic spline function).
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

10.3. Reference values

Non-smoker < 5 ng/ml
Smoker < 10 ng/ml

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Sensitivity

The lowest detectable concentration of CEA that can be distinguished from the standard 0 is 0.1 ng/ml at the 95 % confidence limit.

11.2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham is:

Analyte	**Concentration**	**% Cross Reaction**
CEA		100 %
Acetylsalicylic Acid	100 µg/ml	ND
Ascorbic Acid	100 µg/ml	ND
Caffeine	10 µg/ml	ND
HCG	250 ng/ml	ND
AFP	10 µg/ml	ND
CA-125	10,000 U/ml	ND
PSA	1000 ng/ml	ND
Prolactin	100 µg/ml	ND
hLH	10 IU/ml	ND
hTSH	100 mIU/ml	ND

11.3. Precision

Intra Assay Variation
Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 4.6%.

Inter Assay Variation
Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 7.5%.

11.4. Correlation with RIA

The NovaTec CEA ELISA was compared to another commercially available CEA assay**.** Serum samples of 32 females and 4 males were analysed according in both test systems.
The linear regression curve was calculated
y = 1.04 x 0.41
r = 0.99 (r^2 = 0.98)

11.4. Accuracy

The recovery of 12,5 – 25 – 50 – 100 ng/ml of CEA added to samples gave an average value (±SD) of 99.5% ± 3.7% with reference to the original concentrations.

11.5. Hook Effect

The CEA ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 60,000 ng/ml.

12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
CEA has a low clinical sensitivity and specificity as a tumour marker. Clinically an elevated CEA value alone is not of diagnostic value as a test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV 1+2 antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Do not use heavily haemolysed or highly lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- This method allows the determination of CEA from 5.0 to 250 ng/ml.
- Avoid contact with reagents containing hydrogen peroxide, sulphuric and preservatives, which may be toxic if ingested. Do not pipette by mouth.

WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE

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15. ORDERING INFORMATION

Prod. No.:	DNOV060	CEA Determination (96 Determinations)

SCHEME OF THE ASSAY

CEA

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.

Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Blank	**Standard 0 - 5**	**Sample**
Standard 0 - 5	-	25 µl	-
Sample	-	-	25 µl
Conjugate	-	100 µl	100 µl
Cover wells with foil supplied in the kit **Incubate for 60 min at room temperature (22 - 28 °C)** Wash each well **three times** with **300 µl** diluted Wash Solution			
TMB	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature in the dark			
Stop solution	100 µl	100 µl	100 µl
Photometric measurement at 450 nm			

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