

**REF**

GD7145 00

CEA**Enzyme-immunoassay for the quantitative
determination of Cercinoembrionic antigen
in human serum****IVD****INDICATION**

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. Levels are raised in heavy smokers.

CEA is considered a tumor marker, 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. Benign tumours does not usually cause an increase above 10 ng/ml. 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.

PRINCIPLE OF THE ASSAY

In the present "sandwich" method standards, samples and/or controls are first added to streptavidin coated wells. Two high affinity and specificity antibodies, one biotinylated (Ab1-Biotin) and one labelled with the enzyme horseradish peroxidase (Ab2-HRP), directed against distinct and different epitopes of CEA, are then added to the reaction mixture. The immunological reaction between the analyte and the two antibodies occurs on the solid phase, forming a stable soluble sandwich complex through the high affinity reaction of biotin and streptavidin:

Streptavidin—Biotin—Ab1—CEA—Ab2—HRP

After a washing step, that separates the antibody bound fraction from the unbound antigen, the measurement of enzyme activity is performed by adding a chromogen/substrate solution. The developed colour intensity, measured at 450 nm, is proportional to the quantity of antigen present in the sample.

KIT CONTENT

- 1. Reagent A – Microplate**
12x8 strips.
8 wells breakable strips, coated with Streptavidin. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.
- 2. Reagent B – Enzymatic Tracer**
1 vial of 13 ml.
Ready to use liquid reagent containing anti-CEA, Horseradish peroxidase (HRP) conjugate and anti-CEA conjugate biotinylated.
- 3. Reagent C – Washing Solution 50x**
1 vial of 20 ml.
Concentrated solution to be diluted 1:50 with distilled water. It contains NaCl 9 g/l, Tween 20 1 g/l.
- 4. Reagent D/E – Chromogen/Substrate**
1 vial of 12 ml.
Ready to use solution containing Tetramethylbenzidine (TMB) 0.25 g/l.
Avoid any skin contact and light exposure.
- 5. Reagent F – Stop Solution**
1 vial of 12 ml.
Ready to use solution containing Sulphuric acid 0.15 M.
Avoid any skin contact.
- 6. CEA Standards**
6 vials of 1 ml each.
Ready to use liquids containing CEA at the following concentrations:
S₀: 0 ng/ml, S₁: 5 ng/ml, S₂: 10 ng/ml, S₃: 25 ng/ml, S₄: 50 ng/ml, S₅: 250 ng/ml
(Calibrated against 1st IRP 73/601 standard)
Actual concentrations to be used for calculation are stated on the labels of the vials.
- 7. Cardboard sealers**
2 cardboard sealers to be used to cover the plate during the incubations.
- 8. Package insert:** instruction for use GD7145 00 it/ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes of 10, 200 and 1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm (620- 630 nm).
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

SAMPLES

The blood should be collected in 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 specimens cannot be assayed within this time, the samples may be stored at -20 °C for up to 30 days. Avoid repetitive freezing and thawing.

Turbid, highly lipemic, haemolysed, microbiologically contaminated sera should not be used.

REAGENTS PREPARATION

- **WASHING SOLUTION:** dilute 1:50 with distilled or ELISA grade water (ex.: 20 ml of Reagent C + 980 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for one week at room temperature or 3 weeks at +2-8 °C. It is recommended to store diluted washing solution at room temperature for immediate use.

WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 2-3 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 3 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blanking" of the instrument is to be carried out in the blank reagent well (well A1).

ASSAY SCHEME

- Put the desired number of microstrips into the frame.
- If suggested analyte concentration in the sample exceeds 250 ng/ml, dilute this sample accordingly, using Standard 0.
- Follow the scheme:

	Microplate wells coated with Streptavidin			
	REAGENTS	Blank	Standard	Sample
Immunological reaction	Standard	-	25 µl	-
	Sample	-	-	25 µl
	Reagent B (Enzymatic Tracer)	-	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate 60 minutes at room temperature (22-28 °C)			
Washing	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 3 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Reagent D/E (Chromogen-Substrate)	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate 15 minutes at room temperature (22-28 °C), avoiding light exposure			
	Reagent F (Stop Solution)	100 µl	100 µl	100 µl
	Read the absorbance of each well against Blank at 450 (620-630 nm)			

QUALITY CONTROL

It is recommended, in each analytical run, to use control sera with known CEA values, to check the correspondence of the obtained results with those expected and consequently validate the data.

CALCULATION OF RESULTS

- Calculate the mean of the absorbance (E_m) for each point of the standard curve and of each sample.
- Use the 4 parameters logistic, preferred, or the smoothed cubic spline function as calculation algorithm.
- Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.
- If computer controlled data reduction is used to calculate the results of the tests, it is imperative that the predicted values for the calibrators fall within 10% of assigned concentrations

The optical density of Standard S_5 should be ≥ 1.3 .

Conversion of the optical densities

The optical densities (OD) of some calibrators 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 in addition to 450 nm 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:
 $OD_{450\text{ nm}} = OD_{405\text{ nm}} \times 3.0^*$

* 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.

EXPECTED VALUES

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

It is recommended that each laboratory establishes its own reference range.

Note

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.

ANALYTICAL PERFORMANCES

Sensitivity

The lowest detectable concentration of CEA is 1 ng/ml at the 95 % confidence limit.

Precision

a. Intra-assay

Within-run variation was determined by 16 replicate determinations of two different control sera in one assay. The %CV calculated is 6.5

b. Inter-assay

Between-run variations was determined by replicate measurements of three different control sera in 2 different lots. The %CV calculated is 6.5.

Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

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

Accuracy

The recovery of amounts of CEA > 60000 ng/ml in linear dilutions added to samples gave an average value (\pm SD) of 101.7% \pm 9.7% with reference to the original concentrations.

Correlation with RIA

The present kit 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.

$$y = 1.0324 x - 0.1164 \text{ ng/ml} \quad r = 0.97$$

Hook Effect

The CEA ELISA shows no Hook Effect up to 60000 ng/ml.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentrations of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

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