DRG® Carcinoembryonic Antigen (CEA) (EIA-1871)

USA: RUO

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This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Principle of the Test

The CEA ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.
The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CEA molecule is used for solid phase immobilization (on the microtiter wells). A monoclonal anti-CEA conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CEA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of CEA is directly proportional to the color intensity of the test sample.
Absorbance is measured spectrophotometrically at 450 nm.

Reagents

Materials provided with the kit:

1. **Antibody-Coated Wells** (1 plate, 96 wells) Microtiter Wells.
2. **CEA Standards** Containing 0, 3, 12, 30, 60, and 120 ng/ml of CEA. 1 ml each, ready to use.
3. **Enzyme Conjugate** Reagent (13 ml)
4. **TMB Reagent** (One-Step) (11 ml)
5. **Stop Solution** 1N HCl (11 ml)

Materials required but not provided

- Distilled water.
- Precision pipettes: 5 µl, 100 µl and 1 ml.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph Paper.
- Microtiter plate reader.

Storage of Test Kit and Instrumentation

Unopened test kits should b stroed at 2-8° upon receipt and the microtieter patle should be kept in a sealed bag with descants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter patle reader with a bandwidth of 10 nm or less and an optical density range of 02- OD or greater at 450 nm wavelength is acceptable for us in absorbance measurement.

Warnings and Precautions

1. CAUTION: This kit contains human material. The course material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For Research Use Only.

Reagent Prepration

All reagents should be brought to room temperature (18-25°C) before use.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent to each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by emptying plate content into a waste container.
7. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
13. Read the optical density at 450 nm with a microtiter plate reader ***within 15 minutes***.

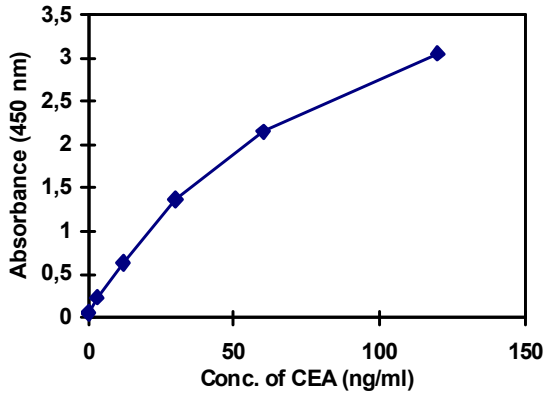
Calculation of Results

1. Calculate the average absorbance values (A_{450}) for each set of reference standards, control, and samples.
2. Construct a 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. Using the mean absorbance value for each sample, determine the corresponding concentration of CEA in ng/ml from the standard curve.

I. Example of Standard Curve

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against CEA concentrations shown in the X axis.
This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

CEA (ng/ml)	Absorbance (450 nm)
0	0.057
3	0.235
12	0.637
30	1.388
60	2.144
120	3.050



Limitations of the Procedure

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory price.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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