

CARCINOEMBRYONIC ANTIGEN (CEA) ENZYME IMMUNOASSAY TEST KIT

Catalog Number: OKBA00007



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Enzyme Immunoassay for the Quantitative Determination of Carcinoembryonic Antigen (CEA) in Human Serum

FOR IN Research Purposes 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:

- Antibody-Coated Wells (1 plate, 96 wells)
Microtiter Wells coated with CEA
- Reference Standard Set (1.0 ml/vial)
Contains 0, 3, 12, 30, 60, and 120 ng/ml of CEA; ready to use
- Enzyme Conjugate Reagent (13 ml)
Contains CEA MoAb conjugated to horseradish peroxidase with preservatives
- TMB Reagent (11 ml)
Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- Stop Solution -1N HCl (11 ml)
Diluted hydrochloric acid

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Materials required but not provided:

- Precision pipettes: 50 µl, 100 µl, and 1 ml
- Disposable pipette tips
- Distilled water
- Vortex mixer or equivalent
- Absorbent paper or paper towel
- Graph paper
- Microtiter plate reader

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

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.**

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

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.

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 |

