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See external label  2°C-8°C



Σ=96 tests



Cat # 5201Z

CARCINOEMBRYONIC ANTIGEN (CEA) ENZYME IMMUNOASSAY TEST KIT

MICROWELL ELISA

CEA

Cat # 5201Z

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

Intended use

CEA enzyme immunoassay test kit is intended for the quantitative determination of CEA concentration in human serum.

Introduction

Carcinoembryonic antigen (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 it 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.

Principle of the test

The CEA Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti-CEA antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-CEA antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the CEA antibody coated microtiter wells. Then CEA antibody labeled with horseradish peroxidase (conjugate) is added. If human CEA is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate 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 is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl. The color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of CEA is directly proportional to the color intensity of the test sample.

Materials and components

Materials provided with the test kits:

- Antibody-coated microtiter plate with 96 wells.
- CEA standards containing: 0, 3, 12, 30, 60, and 120 ng/ml, 1 set.
- Enzyme Conjugate Reagent, 12 ml.
- Substrate TMB, 12 ml.
- Stop Solution, 12ml.
- Wash Buffer Concentrate (50X), 15 ml

Materials required but not provided:

- Precision pipettes: 0.04ml~0.2ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

Specimen collection and preparation

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with the test procedures and should be avoided.
3. Specimens 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 of test kits and instrumentation

Unopened test kits should be stored at 2-8°C upon receipt. The microtiter plate should be stored at 2-8°C, in a sealed bag with desiccants, to minimize exposure to damp air. Opened test kits will remain stable until the expiration date, provided they are stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater, at 450nm wavelength, is acceptable for use in the absorbance measurement.

Reagent preparation

1. All reagents should be brought to room temperature (18-22°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use

Assay procedures

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of enzyme conjugate reagent to each well.
4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-22°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 washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds to ensure that the blue color completely changes to yellow.
13. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

Important Note:

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

Calculation of results

5. Mughal A.W., Hortobagyi G. N., Fritsche H.A., Buzdar A.U. Yap H-Y., and Blumenschein G.R. Serial Plasma Carcinoembryonic Antigen Measurements During Treatment of Metastatic Breast Cancer. **JAMA** 1983; 259:1881-1886.

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