

CEA ELISA Kit

Enzyme-linked immunosorbent assay for the detection CEA in serum or plasma

INTENDED USE

The CEA Enzyme Immunoassay test kit is intended for the quantitative determination of CEA in human serum.

SUMMARY AND PRINCIPLE OF THE TEST

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.

The CEA ELISA 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 10 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.

REAGENTS

Materials provided with the kits:

- 12X8 well microtiter strip: 1 plate, coated with anti-CEA.
- Wash Buffer Concentrate (20 x): 40 ml for 96 tests, containing PBS, Tween. The buffer should be diluted 20 times with distilled water before use.
- Enzyme Conjugate: 6ml, monoclonal anti-CEA-HRP conjugate.
- Standard: 5ng/ml, 20ng/ml. Add 1ml distilled water before use.
- Substrate Solution A: urea peroxide.
- Substrate Solution B: TMB.
- Stop Solution: 1N Sulfuric Acid

Materials required but not provided:

- Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Humidified Box capable of maintaining 37°C
- Absorbent paper or paper towel.
- Microtiter plate or strip-well washer
- Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

- 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.
- Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with the test procedures and should be avoided.
- 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.

PRECAUTIONS

- Caution: Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human

blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.

- Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.
- Dispose off all specimens and materials used to perform the test as if they contained infectious agents.
- Do not mix reagents from kits with different lot numbers.
- Cross contamination between reagents will invalidate the test results.
- All reagents and components except the conjugate must be equilibrated at room temperature prior to use.

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

WORKING WASH BUFFER PREPARATION

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water.

STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C. Exposure of the plates to humidity drastically reduces the shelf life.

ASSAY PROCEDURE:

Allow all components to reach room temperature before use.

- Dispense 50 ul of 5ng/ml and 20ng/ml into respective wells. Set one blank well as background control, and 50ul of serum or plasma samples into respective wells
- Add one drop (50 ul) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
- Place the microtiter plate into a humidified box and incubate at 37°C for 60 min.
- Wash each well 5 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
- Add one drop (50 ul) of Substrate Solution A (HRP substrate) to each well, then add one drop (50 ul) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 15min.
- Add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Weak Positive: sample OD \geq 5ng/ml OD but < 20ng/ml OD

Strong Positive: sample OD \geq 20ng/ml OD

Negative: sample OD < 5ng/ml OD

LIMITATIONS OF THE ASSAY

- 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 practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

- Heterophilic antibodies such as human anti-mouse antibodies (HAMA) are frequently found in the serum of human subjects. Those antibodies can cause severe interference in many immunodiagnostic procedures. This assay has been designed to minimize that kinds of interference. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.











RELATED READING MATERIALS

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- Zamcheck N. and Martin E.W. Sequential Carcinoembryonic Antigen Levels in Pancreatic Cancer: Some Clinical Correlations. **Cancer** 1981;47:1620-1627.

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	<i>In vitro</i> diagnostic device		Lot code
	Consult instructions for use		Catalogue number
	Keep dry		Contains sufficient for <n> tests
	Temperature limitation		Manufacturer
	Use by		Do not use if package damaged