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Product Information

CA15-3 ELISA

Catalog Number **SE120153**
Storage Temperature

TECHNICAL BULLETIN

Product Description

Breast cancer is the most common life-threatening malignant lesion in women of many developed countries today, with ~180,000 new cases diagnosed every year. Roughly half of these newly diagnosed patients are node-negative; however, 30% of these cases progress to metastatic disease.

There are a number of tumor markers that can help clinicians to identify and diagnose which breast cancer patients will have aggressive disease and which will have an indolent course. These markers include estrogen and progesterone receptors, DNA ploidy, and percent-S phase profile, epidermal growth factor receptor, HER-2/neu oncogene, p53 tumor suppressor gene, cathepsin D, proliferation markers, and CA15-3. CA15-3 is most useful for monitoring patients post-operatively for recurrence, particularly metastatic diseases. 96% of patients with local and systemic recurrence have elevated CA15-3, which can be used to predict recurrence earlier than radiological and clinical criteria. A 25% increase in the serum CA15-3 is associated with progression of carcinoma. A 50% decrease in serum CA15-3 is associated with response to treatment. CA15-3 is more sensitive than CEA in early detection of breast cancer recurrence. In combination with CA125, CA15-3 has been shown to be useful in early detection of relapse of ovarian cancer. CA15-3 levels are also increased in colon, lung and hepatic tumors.

The CA15-3 ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA15-3 concentration in human serum. The CA15-3 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 CA15-3 molecule. It is used for solid phase immobilization on the multiwell plate). A rabbit anti-CA15-3 antibody conjugated to horseradish peroxidase (HRPO) is in the antibody-enzyme conjugate solution.

The test sample is allowed to react sequentially with the two antibodies, resulting in the CA15-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate 1-hour incubation steps at 37 °C, 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 CA15-3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Components

Materials Provided	96 Tests
Microwells coated with murine monoclonal Anti-CA15-3	12 x 8 x 1
Sample Diluent	100 mL
Enzyme Conjugate Concentrate (22x)	1 mL
Enzyme Conjugate Diluent	21 mL
CA 15-3 Standards 6 vials	2 mL
TMB Solution	11 mL
Stop Solution	11 mL
Wash Concentrate 20x: 1 Bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbant paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Reagent preparation

1. To prepare working CA 15-3 Conjugate Reagent, add the entire 1.0 mL of Conjugate Concentrate (22x) to 21 mL of the Enzyme Conjugate Diluent (22-fold dilution) and mix well. The diluted Enzyme Conjugate Reagent is stable at 4 °C for at least 4 months.
2. Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that serum samples be run in duplicate.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Serum sample and control serum should be diluted, 51-fold before use. Prepare a series of small tubes (such as 1.5 mL microcentrifuge tubes) and mix 20 µL serum with 1.0 mL Sample Diluent.
   Note: **Do not dilute the standards**.
2. Secure the desired number of coated wells in the holder. Dispense 200 µL of CA15-3 standards, diluted specimens, and diluted controls into the appropriate wells. Gently mix for 10 seconds.
3. Incubate at 37 °C for 1 hour.
4. Remove the incubation mixture by emptying the plate content into a waste container.
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer. Blot on absorbance paper or paper towel.
6. Strike the multiwell plate sharply onto absorbent paper or paper towels to remove all residual liquid droplets.
7. Dispense 200 µL of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds
8. Incubate at 37 °C for 1 hour.
9. Remove the contents and wash the plate as described in steps 6-7 above.
10. Dispense 100 µL of TMB Reagent into each well. Gently mix for 10 seconds.
11. Incubate at room temperature in the dark for 20 minutes.
12. Stop the reaction by adding 100 µL of Stop Solution to each well.
13. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
14. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

Results

Calculations

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 U/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 CA15-3 in U/mL from the standard curve.

Example of standard curve

Typical results of a standard run with optical density readings at 450 nm. These results are 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.

CA15-3 Values (U/mL)	Absorbance (450 nm)
0	0.021
15	0.425
30	0.693
60	1.214
120	1.956
240	2.845

Note: Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the 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. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

Product Profile

Healthy women are expected to have CA15-3 assay values below 35 U/mL. The minimum detectable concentration of CA15-3 in this assay is estimated to be 5 U/mL.

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