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Product Information

CA15-3 ELISA kit

Catalog Number: EA100885
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The CA15-3 ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA15-3 concentration in human serum.

Background

Breast cancer is the most common life-threatening malignant lesion in women of many developed countries today, with approximately 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.

Principle of the Test

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 is used for solid phase immobilization (on the microtiter wells). 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
1. Microwells coated with muriine monoclonal Anti-CA15-3	12x8x1
2. Sample Diluent	100 ml
3. Enzyme Conjugate Concentrate (22X)	1 ml
4. Enzyme Conjugate Diluent	21 ml
5. CA 15-3 Standards 6 vials	2 ml
6. TMB Solution	11 ml
7. Stop Solution	11 ml
8. Wash Concentrate 20x: 1 Bottle	25 ml

Materials 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 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

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 (1:21 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).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- 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 standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Patient serum 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. PLEASE 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 microtiter 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 450nm with a microtiter plate reader within 15 minutes.

Calculation of Results

1. Calculate the average absorbance values (A450) 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 a Standard Curve

Results of a typical standard run with optical density readings at 450nm shown in the Y-axis against CA15-3 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.

	CA15-3 Values (U/ml)	Absorbance (450 nm)
Std 1	0	0.021
Std 2	15	0.425
Std 3	30	0.693
Std 4	60	1.214
Std 5	120	1.956
Std 6	240	2.845

Expected Values and Sensitivity

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.

References

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