BREAST CANCER ANTIGEN CA15-3 ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00004

Aviva Systems Biology
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Enzyme Immunoassay for the Quantitative Determination of CA15-3 in Human Serum

FOR Research Purposes Only

NOT FOR USE IN DIAGNOSTIC PROCEDURES

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 (HRP) 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 Wash Buffer 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.

REAGENTS

Materials provided with the kit:

- *Antibody-Coated Wells (1 plate, 96 wells)*
  Microtiter wells coated with CA15-3 MoAb
- *Reference Standard Set (2.0 ml/vial)*
  Contains 0, 15, 30, 60, 120, and 240 Unit/ml of CA15-3 in bovine serum with preservatives; liquid, ready to use
  These standards have been pre-diluted 51-fold. Please do not dilute them again.
- *CA15-3 Enzyme Conjugate Concentrate (22x), 1.0 ml*
  Contains CA15-3 MoAb conjugated to horseradish peroxidase with preservatives
- *CA15-3 Conjugate Diluent, 21 ml*
  Contains bovine serum, tris buffer and preservatives
- *CA 15-3 Sample Diluent, 100 ml*
  Tris buffer with preservatives
- *Wash Buffer Concentrate (20x), 50 ml*
  Potassium phosphate buffer with tween 20
- *TMB Reagent (11 ml)*
  Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- *Stop Solution -1N HCl (11 ml)*
  Diluted hydrochloric acid

Materials required but not provided:

- Precision pipettes and tips: 20 µl, 100 µl, 200 µl, and 1 ml
- Distilled water
- Disposable pipette tips
- Vortex mixer
- Absorbent paper or paper towel
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater
- Graph paper

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.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. 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.
4. To prepare **Wash Buffer (1X)**: Add 50 ml of Wash Buffer (20X) to 950 ml of DI water. The diluted Wash Buffer is stable at 2-8°C for 30 days. Mix well before use. Note: Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.

ASSAY PROCEDURE

1. ***Sample 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.

3. Dispense 200 µl of CA15-3 standards, **diluted** specimens, and **diluted** controls into the appropriate wells. Gently mix for 10 seconds.
4. Incubate at 37°C for 1 hour.
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X).
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds
9. Incubate at 37°C for 1 hour.
10. Remove the contents and wash the plate as described in steps 6-7 above.
11. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
12. Incubate at room temperature in the dark for 20 minutes.
13. Stop the reaction by adding 100 µl of Stop Solution to each well.
14. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
15. 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.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

EXAMPLE OF 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)
0	0.067
15	0.338
30	0.587
60	1.081
120	1.880
240	2.640