

BREAST CANCER ANTIGEN CA15-3 ENZYME IMMUNOASSAY TEST KIT

Enzyme Immunoassay for the Quantitative Determination of Breast Cancer Antigen CA15-3 in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2 to 8°C.

INTENDED USE

For the quantitative determination of the Cancer Antigen CA15-3 concentration in human serum.

INTRODUCTION

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 are 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 (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 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.

REAGENTS

Materials provided with the kit:

- Murine Monoclonal Anti-CA15-3 coated microtiter plate with 96 wells.
- Sample Diluent, 100 ml.
- Enzyme Conjugate Concentrate (22x), 1.0 ml.
- Enzyme Conjugate Diluent, 21 ml.
- CA15-3 reference standards, containing 0, 15, 30, 60, 120, and 240 IU/ml. Liquid. 1 set. **These standards have been pre-diluted 51-fold. Please do not dilute them again.**
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

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.

SPECIMEN COLLECTION AND 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.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter 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.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. 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

ASSAY PROCEDURE

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.
3. Dispense 200µl 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 distilled or deionized water. (Please do not use tap water.)
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.

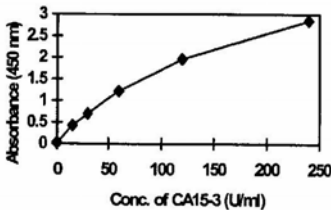
CALCULATION OF RESULTS

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 inIU/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 inIU/ml from the standard curve.

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 (IU/ml)	Absorbance (450 nm)
0	0.021
15	0.425
30	0.693
60	1.214
120	1.956
240	2.845



EXPECTED VALUES AND SENSITIVITY

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

LIMITATIONS OF THE PROCEDURE

1. 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

2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. 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.

REFERENCES

1. Aziz DC. Quantitation of estrogen and progesterone receptors by immunocytochemical and image analyses. *J Clin Pathol* 1992;98:105-11
2. Aziz DC, Peter JB. DNA ploidy and cell-cycle analysis. Tools for assessment of cancer prognosis. *J Clin Pathol* 1991;5:422-38.
3. Clark GM, Dressler LG, Owens MA, Dounds G, Oldaker T, McGuire WL. Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. *N Engl J Med* 1989;320:627-33.
4. Elledge RM, McGuire WL. Prognostic factors and therapeutic decisions in axillary node-negative breast cancer. *Ann Rev Med* 1993;44:201-10.
5. Foekens JA, Rio C, Seguin P, et al. Prediction of relapse and survival in breast cancer patients by pS2 protein. *Cancer Res* 1990; 50-3832-7.
6. Isola J, Visakorpi T, Holli K, Kallioniemi D. Association of p53 expression with other prognostic factors and long term survival in node-negative breast cancer. *J Cell Biochem* 1992;(Suppl 16D):101.
7. Kute TE, Shao ZM, Snugg NK, Long RT, Russell GB, Case LD. Cathepsin D as a prognostic indicator for node-negative breast cancer patients using both immunoassays and enzymatic assays. *Cancer Res* 1992;52-198-203.
8. McGuire WL, Tendon AK, Alfred D, Chames GC, Clark GM. How to use prognostic factors in axillary node negative breast cancer patients. *J Natl Cancer Inst* 1990;82:1006-7.
9. Nicholson S, Richard J, Sainsbury C, et al. Epidermal growth factor receptor (EGFr): results of a 6 year follow up study in operable breast cancer with emphasis on the node-negative subgroup. *Br J Cancer* 1991;63:146-50.
10. Somerville JE, Clarke LA, Biggart JD. C-erb B-2 overexpression and histological type of in-situ and invasive breast carcinoma. *J Clin Pathol* 1992;45-16-20.
11. Ueronese S, Gambacorta M. Detection of Ki-67 rate in breast cancer. *Am J Clin Pathol* 1991;95:30-4.
12. Lotnick M, Pavesi F, Scarabelli M. Tumor associated antigens CA15-3 and CA125 in ovarian cancer. *Int. J. Biolog Markers* 1991; 6:115

Atlas Medical
 William James House, Cowley Rd
 Cambridge, CB4 0WX
 Tel: ++44 (0) 1223 858 910
 Fax: ++44 (0) 1223 858 524

PP1322A01
 Revision A (13.05.2006)

