DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F,
Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

	See external label	2°C-8°C	Σ=96 tests	Cat # 9075-16

CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
BREAST CANCER ANTIGEN (CA15-3)

CA-153

Cat # 9075-16

Enzyme Immunoassay for the Quantitative Measurement of Breast Cancer Antigen (CA15-3) in Human Serum.

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).
ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.
Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.
The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panals, such as T3, T4, TSH, Hormone panals, such as hCG, LH, FSH, and other panals. They can be used to replace conventional colorimetric

ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the ***MPL1*** *and* ***MPL2*** microplate luminometers from Berthold Detection System, ***Lus2*** microplate luminometer from Anthos, ***Centro LB960*** microplate luminometer from Berthold Technologies, and ***Platelumino*** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

INTRODUCTION OF CA15-3 IMMUNOASSAY

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 CA-125, 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.

TEST PRINCIPLE

The CA15-3 EIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The CA15-3 molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by adding the substrate reagents and undergoing the chemiluminescent reactions. The intensity of the emitting light from the associated well is proportional to the amount of enzyme present and is directly related to the amount of CA15-3 antigen in the sample. By reference to a series of CA15-3 standards assayed in the same way, the concentration of CA15-3 in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

1. Anti-CA15-3 antibody coated 96 well microtiter plate.
2. Sample Diluent, 100 ml
3. Enzyme conjugate reagent, 22 ml.
4. CA15-3 reference standards, containing 0, 15, 30, 60, 120 and 240 U/ml CA15-3, prediluted liquid, ready for use.
5. 50x Wash Buffer Concentrate, 15 ml
6. Chemiluminescence Reagent A, 6.0 ml
7. Chemiluminescence Reagent B, 6.0 ml

Materials required but not provided:

1. Distilled water.
2. Precision pipettes: 0.05ml, 0.1ml, 0.2ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well luminometer.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

REAGENT PREPARATION

1. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

Important Note:

The CA15-3 standards have already been prediluted and are ready for use. Please DO NOT dilute again!

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.
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. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
6. Strike the wells sharply onto absorbent paper to remove residual water 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 step 4, 5 and 6 above.
10. Dispense **100**μl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
11. Read wells with a chemiluminescence microwell reader 5 minuters later. (between 5 and 20 min. after dispensing the substrates).

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bubbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

CALCULATION OF RESULTS

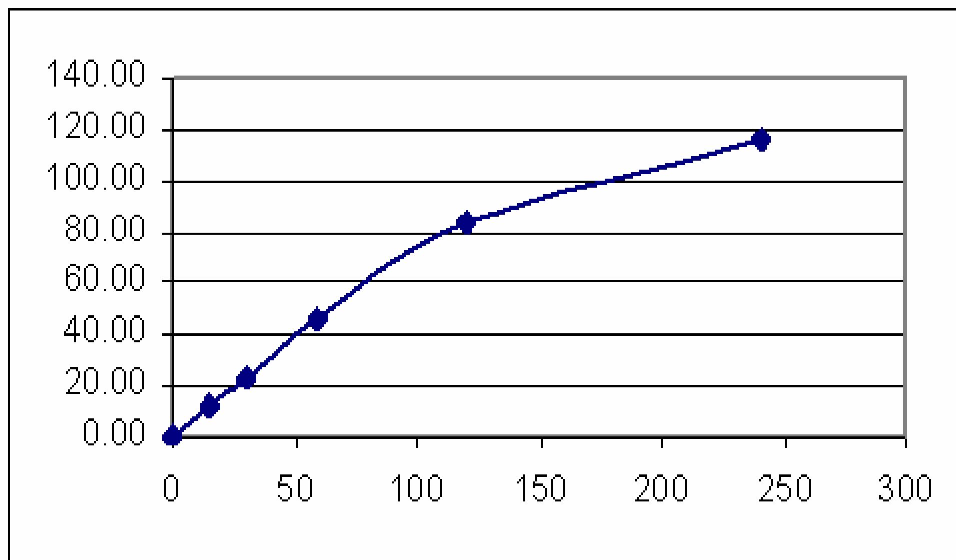
1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend using proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regrassion or cubic spline regaression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against CA15-3 concentration in Units/ml on linear graph paper, with RLU 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 Units/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is runing at the same time.

CA15-3 (Unit/ml)	Relative Light Units (RLU) (10^5)
0	0.64
15	12.21
30	22.90
60	45.90
120	83.96
240	115.39



EXPECTED VALUES AND SENSITIVITY

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

REFERENCES

1. Aziz DC, Rittenhouse HJ, Ranken R. Use and interpretation of tests in oncology. Santa Monica: **Specialty Laboratories**, 1991.
2. Aziz DC. Quantitation of estrogen and progesterone receptors by immunocytochemical and image analyses. **A J Clin Pathol** 1992;98:105-11

3. Aziz DC, Peter JB. DNA ploidy and cell-cycle analysis. Tools for assessment of cancer prognosis. **J Clin Pathol** 1991;5:422-38.
4. 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.
5. Elledge RM, McGuire WL. Prognostic factors and therapeutic decisions in axillary node-negative breast cancer. **Annu Rev Med** 1993;44:201-10.
6. 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.
7. Isola J, Visakorp 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.
8. 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.
9. McGuire WL, Tandon AK, Allred D, Chamnes GC, Clark GM. How to use prognostic factors in axillary node
negative breast cancer patients. **J Natl Cancer Inst** 1990;82:1006-7.
10. 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.
11. 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.
12. Ueronese S, Gambacorta M. Detection of Ki-67 rate in breast cancer. **Am J Clin Pathol** 1991;95:30-4.
13. Lotnicker M, Pavesi F, Scarabelli M. Tumor associated antigens CA15-3 and CA-125 in ovarian cancer. **Int. J. Biolog Markers** 1991; 6:115

Date Adopted :	Reference No.
2008-05-28	DA-CA-153-2009

DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003

Revision Date: 02/16/09