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2°C-8°C



Σ=96 tests



9075-16

CA – 15-3

Chemiluminescence Immunoassay

CAT No: 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 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 panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. 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. 20x Wash Buffer, 30 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 reader.

- Vortex mixer or equivalent.
- Absorbent paper.
- Graph paper.

REAGENT PREPARATION

- To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
- Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

ASSAY PROCEDURE

Important Note :

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

- 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.
- Secure the desired number of coated wells in the holder. Dispense 200µl of CA15-3 standards, diluted specimens, and diluted controls into the appropriate wells. Gently mix for 10 seconds.
- Incubate at 37°C for 1 hour.
- Remove the incubation mixture by emptying the plate content into a waste container.
- Rinse and flick the microtiter wells 5 times with washing buffer.
- Strike the wells sharply onto absorbent paper to remove residual water droplets.
- Dispense 200 µl of enzyme conjugate reagent into each well. Gently mix for 10 seconds
- Incubate at 37°C for 1 hour.
- Remove the contents and wash the plate as described in step 4, 5 and 6 above.
- Dispense 100µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
- Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

Important Note:

- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- If there are bobbles 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

- Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
- We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. 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.
- Using the mean absorbance value for each sample, determine the corresponding concentration of CA15-3 in Units/ml from the standard curve.

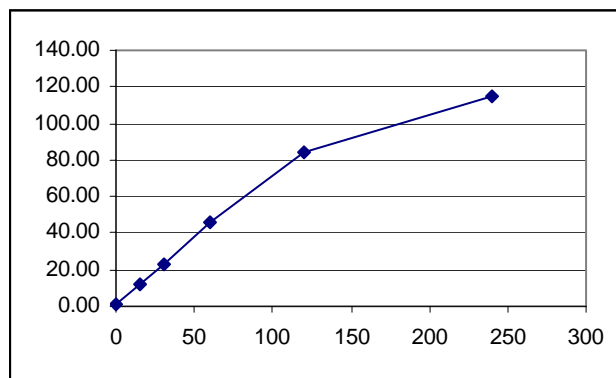
EXAMPLE OF STANDARD CURVE

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 running at the same time.

CA15-3 (Units/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.



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Revision Date:10/16/06