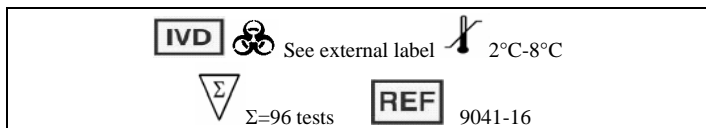
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CEA

CAT No: 9041-16

Chemiluminescence Immunoassay

Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of CARCINOEMBRYONIC ANTIGEN (CEA) 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 *MPL2* microplate luminometer 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 CEA Immunoassay

Carcinoembryonic antigen (CEA) is a cell-surface 200-kd glycoprotein. In 1969, it was reported that plasma CEA was elevated in 35 of 36 patients with adenocarcinoma of the colon and that CEA titers decreased after successful surgery. Normal levels were observed in all patients with other forms of cancer or benign diseases. Subsequent studies have not confirmed these initial findings, and it is now understood that elevated levels of CEA are found in many cancers. Increased levels of CEA are observed in more than 30% of patients with cancer of the lung, liver, pancreas, breast, colon, head or neck, bladder, cervix, and prostate. Elevated plasma levels are related to the stage and extent of the disease, the degree of differentiation of the tumor, and the site of metastasis. CEA is also found in normal tissue.

Principle of the test

The CEA Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti-CEA antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-CEA antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the CEA antibody coated microtiter wells. Then CEA antibody labeled with horseradish peroxidase (conjugate) is added. If human CEA is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the CEA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies.

A solution of chemiluminescent substrate is added and then read relative light units (RLU) in the appropriate Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of CEA in the sample. By reference to a series of CEA standards assayed in the same way, the concentration of CEA in the unknown sample is quantified.

Materials and components

Materials provided with the test kits:

1. Antibody-coated microtiter plate with 96 wells.
2. Lyophilized CEA standards containing; 0, 3, 12, 30, 60, and 120ng/ml CEA. 1 set.
3. Enzyme Conjugate Reagent, 12 ml.
4. 20x Wash Buffer, 30 ml
5. Chemiluminescence Reagent A, 6.0 ml.
6. Chemiluminescence Reagent B, 6.0 ml.

Materials required but not provided:

- Precision pipettes: 50µl~200µl
- Disposable pipette tips.

- Distilled water.
- Glass tubes or flasks to mix Chemiluminescence Reagent A and Chemiluminescence Reagent B.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate Luminometer

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

1. 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. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Add 1 ml of distilled water to reconstitute the lyophilized standards. Allow the reconstituted materials to stand for at least 20 minutes. Mix gently. The reconstituted standards should be stored sealed at 2-8 °C.
3. 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.
4. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

Assay procedures

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
4. Incubate at room temperature for 60 minutes.
5. Remove the incubation mixture by flicking plate contents into a waste container.
6. Rinse and flick the microtiter wells 5 times with wash buffer.
7. Strike the wells sharply onto absorbent paper to remove residual water droplets.
8. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
9. Read wells with a chemiluminescence microwell reader 5 minutes 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 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

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend use proper software to calculate the results. The best curve fitting used in the assays are quadratic regression or 4-parameter regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against CEA concentration in ng/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 CEA in ng/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.

CEA (ng/ml)	RLU (10 ⁵)
0	0.1
3	1.3
12	4.6
30	9.5
60	21.5
120	29.3

Expected values and sensitivity

The most complete study of CEA is a compilation of collaborative studies in which CEA values in 35,000 samples from more than 10,000 patients and controls were analyzed. Of 1425 normal persons who did not smoke, 98.7% had values less than 5.0 ng/ml. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of CEA by this assay is estimated to be 1.0 ng/ml.

Literatures

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