CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
BREAST CANCER ANTIGEN (CA15-3)
Catalog Number: 8004
(For Research use only)
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, hapten 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 CLIA is based on the 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 Immunoassays using a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancer.

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 homogenization.
2. To prepare wash buffer, mix 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
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 \( \mu l \) serum with 1.0 ml Sample Diluent.
2. Secure the desired number of coated wells in the holder. Dispense 200 \( \mu l \) 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. Strike the wells sharply onto absorbent paper to remove residual water droplets.
6. Dispense 200 \( \mu l \) of enzyme conjugate reagent into each well. Gently mix for 10 seconds.
7. Incubate at 37°C for 1 hour.
8. Remove the contents and wash the plates as described in step 4.
9. Strike the wells sharply onto absorbent paper to remove residual water droplets.
10. Dispense 200 \( \mu l \) of each 5x Wash Buffer into each well. Gently mix for 10 seconds.

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.
10. Dispense 100µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.

11. Read wells with a chemiluminescence microtiter 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 bubbles existing in the wells, the false readings will be created. Please use distilled water to remove the bubbles 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 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.
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 running at the same time.

<table>
<thead>
<tr>
<th>CA15-3 (Unit/ml)</th>
<th>Relative Light Units (RLU) $10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.64</td>
</tr>
<tr>
<td>15</td>
<td>12.21</td>
</tr>
<tr>
<td>30</td>
<td>22.90</td>
</tr>
<tr>
<td>60</td>
<td>45.90</td>
</tr>
<tr>
<td>120</td>
<td>83.96</td>
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</tbody>
</table>

**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**

13. Lotniker M, Pavesi F, Scarabelli M. Tumor associated