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 # 9057-16

CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
OVARIAN CANCER ANTIGEN (CA-125)

CA-125

Cat # 9057-16

Enzyme Immunoassay for the Quantitative Determination of OVARIAN CANCER ANTIGEN (CA-125) 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 CA-125 IMMUNOASSAY

One in every 70 American women will develop ovarian cancer in her life. There are approximately 20,000 new cases of ovarian cancer diagnosed every year and more than 12,000 women die each year because of it. Ovarian cancer is the most malignant type of gynecological cancers, with an overall 5-year survival rate of only 30%. This is because diagnosis is often not made until the advanced stage. Cancer Antigen 125 (CA-125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA-125 is associated with a high molecular weight glycoprotein. Serum concentrations of this tumor marker can be detected and measured by a murine monoclonal antibody. Published studies have indicated that elevated serum CA-125 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma. Serum CA-125 levels higher than normal can also be found in individuals with adenocarcinoma of the fallopian tube endometrium, certain non-gynecologic malignancies and some non-malignant conditions.
The serum CA-125 concentration is greater than 35 units per ml in about 60% of women with ovarian cancer. More than 80% of patients with disseminated ovarian cancer, have serum CA-125 concentrations greater than 35 units per ml. The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serum levels of CA-125 greater than 35 units per ml. combined with pelvic examination increases the test specificity. Serial determinations of serum CA-125 further enhances the positive predictive value of the test for ovarian cancer. Serum CA-125 concentration may be useful in monitoring patients with diagnosed ovarian cancer. A persistently high serum CA-125 may be associated with progressive malignant disease and poor therapeutic response. On the other hand, a declining CA-125 value appears to be indicative of a favorable prognosis and a good response to treatment. Residual disease is confirmed in 95% of patients with serum CA-125 concentrations greater than 35 units per ml., however, negative results do not necessarily exclude the disease. To date, CA-125 is the most sensitive marker for residual epithelial ovarian cancer. CA-125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

PRINCIPLE OF THE TEST

The assay system utilizes one monoclonal anti-CA-125 antibody for solid phase (microtiter wells) immobilization and another monoclonal anti-CA 125 antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the CA-125 antibody coated microtiter wells. Then CA-125 antibody labeled with horseradish peroxidase (conjugate) is added. If human CA-125 is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the CA-125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 3 hour incubation at 37°C, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in the Berthold Detection Systems MPL2 Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of CA-125 antigen in the sample. By reference to a series of CA-125 standards assayed in the same way, the concentration of CA-125 in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials Provided with Test Kit

1. Anti-CA-125 antibody coated microtiter plate , 96 wells.
2. Enzyme conjugate reagent, 12 ml.
3. CA-125 reference standards set,containing; 0,15,50, 100, 200 and 400 Unit/ml . Liquid, ready for use..
4. 50x Wash Buffer Concentrate, 15 ml
5. Chemiluminescence Reagent A, 6.0 ml
6. 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

1. Secure the desired number of coated wells in the holder. Dispense **100μl** of CA-125 standards, specimens, and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
2. Dispense **100μl** of enzyme conjugate reagent into each well. Mix gently for 30 seconds. It is very important to have a complete mixing in this setup. Incubate at 37°C for 2 hours.
3. Remove the incubation mixture by emptying the plate content into a waste container.
4. Rinse and flick the microtiter wells 5 times with washing buffer.
5. Strike the wells sharply onto absorbent paper to remove residual water droplets.
6. Dispense 100 μl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
7. 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 bubbles before adding the substrate.

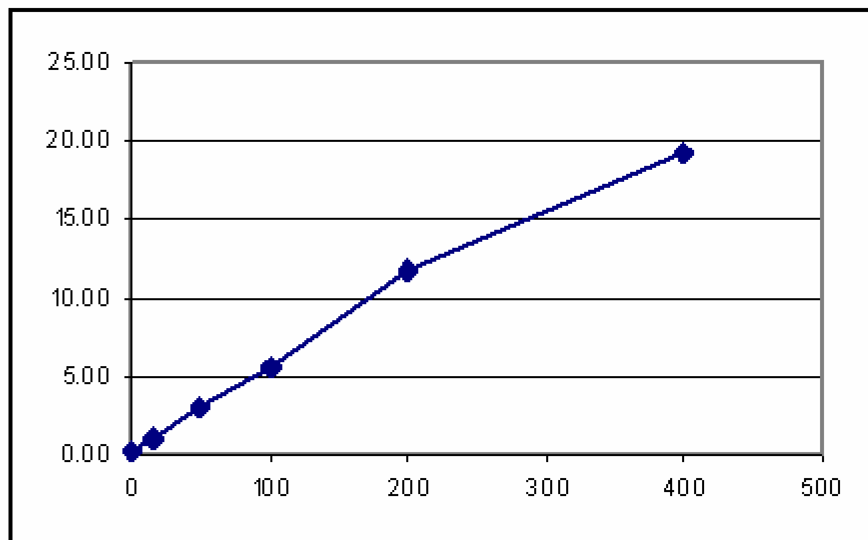
CALCULATION OF RESULTS

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 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 CA-125 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 CA-125 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.

CA-125 (Units/ml)	Relative Light Units (RLU) (10^5)
0	0.20
15	1.00
50	2.90
100	5.47
200	11.64
	19.28



EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA-125 assay values below 35 U/ml. The minimum detectable concentration of CA-125 in this assay is estimated to be 5 U/ml.

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

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