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## CANCER ANTIGEN 125 (CA125) CHEMILUMINESCENCE

### IMMUNOASSAY KIT

Catalog No. CL0209-2

#### **INTENDED USE**

The Autobio cancer antigen 125 (CA125) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of CA125 concentration in human serum.

#### **INTRODUCTION**

Cancer Antigen 125 (CA125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA125 is associated with a high molecular weight glycoprotein. Published studies have indicated that elevated serum CA125 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma. Serum CA125 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.

Serum CA 125 assay values are useful for monitoring the course of disease in patients with invasive epithelial ovarian cancer.<sup>1</sup> In a review of nine published studies, the overall correlation reported between CA 125 serum levels and the course of the disease was 87%.<sup>2</sup> Serum levels of CA125 greater than 35 units per ml, combined with pelvic examination increases the test specificity. Serial determinations of serum CA125 further enhances the positive predictive value of the test for ovarian cancer. Serum CA125 concentration may be useful in monitoring patients with diagnosed ovarian cancer. Persistently rising CA 125 assay values may be associated with malignant disease and poor response to therapy, whereas decreasing CA 125 assay values may indicate a favorable response to therapy.<sup>2-10</sup>

In women with primary epithelial ovarian carcinoma who had undergone first-line therapy and were candidates for diagnostic second-look procedures, a CA 125 assay value greater than or equal to 35 U/ml was found to be indicative of the presence of residual tumor.<sup>2,5,7,9</sup> However, a CA125 assay value below 35 U/ml does not indicate the absence of residual ovarian cancer because patients with histopathologic evidence of ovarian carcinoma may have CA125 assay values within the range of normal individuals.<sup>3,4,6</sup>

Elevations of CA125 assay values have been reported in approximately 1-2% of healthy individuals,<sup>2,3</sup> and in individuals with nonmalignant conditions such as cirrhosis,<sup>11,12</sup> hepatitis,<sup>12,13</sup> endometriosis,<sup>14-19</sup> first trimester pregnancy,<sup>20-22</sup> ovarian cysts,<sup>6,23</sup> and pelvic inflammatory disease.<sup>6,20</sup> Elevations of CA125 assay values during the menstrual cycle have also been reported.<sup>18,24</sup> Non-ovarian malignancies in which elevated CA125 assay values have been reported include cervical,<sup>13,25</sup> liver,<sup>13</sup> pancreatic,<sup>13,26</sup> lung,<sup>13</sup> colon,<sup>13,26</sup> stomach,<sup>13,26</sup> biliary tract,<sup>13,26</sup> uterine,<sup>12</sup> fallopian tube,<sup>25</sup> breast,<sup>13</sup> and endometrial carcinomas.<sup>25,27</sup>

To date, CA125 is the most sensitive marker for residual epithelial ovarian cancer.

#### **PRINCIPLE OF THE TEST**

The CA125 CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes one anti-CA125 monoclonal antibody for solid phase (microtiter wells) immobilization and another anti-CA125 monoclonal antibody as antibody-enzyme (horseradish peroxidase) conjugate reagent. CA125 in the standards or in the patient's specimens binds to anti-CA125 MAB on the well and the anti-CA125 second antibody then binds to CA125. Unbound protein and HRP conjugate are removed by washing. Upon the addition of the substrate, the horseradish peroxidase activity bound on the wells is then assayed by a chemiluminescence reaction. The related light unit (RLU) of the reaction is proportional to the concentration of CA125 present in the specimen.

#### **MATERIALS PROVIDED**

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to cancer antigen 125 (anti-CA125 MAB) (1 plate, 48 wells/96wells)
1. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-CA125 MAB in Stabilizing Buffer (1 vial, 3.0ml/6.0 ml)
2. Reference Standards: 0, 15, 50, 100, 200, and 400U/ml CA125 in Stabilizing Buffer (6 vials, 0.5ml/ea)
3. Substrate A (1 vial, 3.5ml/6.0ml)
4. Substrate B (1 vial, 3.5ml/6.0ml)

**MATERIALS NOT PROVIDED**

The following materials are required but not provided in the kit.

1. Distilled water
2. Precision pipettes for delivery of 20-200 $\mu$ l (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent
5. Washer for microplate
6. Quality control specimens
7. Incubator
8. Absorbent paper

**STORAGE OF TEST KIT AND INSTRUMENTATION**

1. Unopened test kits should be stored at 2~8 $^{\circ}$ C upon receipt. The test kit may be used throughout the expiration date of the kit (6 months from the date of manufacture). Refer to the package label for the expiration date.
2. Liquid standards should be stored sealed at 2~8 $^{\circ}$ C. The remaining standards once opened should be used within 30 days and be frozen at -20 $^{\circ}$ C for long term storage. Avoid repeated freezing and thawing of the standards. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, provided it is stored as prescribed above.

**SPECIMEN COLLECTION AND PREPARATION**

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2~8 $^{\circ}$ C. For longer storage, freeze the specimens at -20 $^{\circ}$ C. Thawed samples must be mixed prior to testing.

**PRECAUTIONS AND WARNINGS**

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

**REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18~25 $^{\circ}$ C) prior to use.

**IMPORTANT NOTES**

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder. Dispense 50µl of CA125 standards, specimens, and controls into appropriate wells.
2. Dispense 50µl of enzyme conjugate reagent to each well. Mix gently for 30 seconds.
3. Incubate at 37°C for 60 minutes.
4. At the end of incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the well is about 300µl.
5. Dispense 50µl of Substrate A, then 50µl of Substrate B into each well. Gently mix for 10 seconds.
6. Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

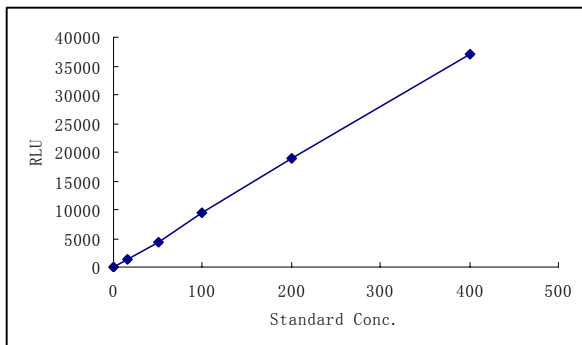
**CALCULATION OF RESULTS**

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the RLU (ordinate) obtained from each reference standard against the corresponding concentration of CA125 in U/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a point to point function curve fitting is recommended.
5. Any diluted specimens must be corrected by the appropriate dilution factor.

**EXAMPLE OF STANDARD CURVE**

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

CA125 (U/ml)	RLU
0	36.0
15	1326.7
50	4341.4
100	9499.5
200	18964.1
400	36989.7



**EXPECTED VALUES**

Each laboratory should establish its own normal range. Following information is given only for guidance. Approximately 95% of the normal healthy population has CA125 levels less than 35U/ml.

**PERFORMANCE**

**A. Sensitivity**

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio CA125 CLIA kit is not higher than 1.0U/ml.

**B. Specificity**

No interference was detected with the performance of Autobio CA125 CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration

Human Albumin	100mg/ml
AFP	500ng/ml
CEA	500ng/ml

### C. Precision

#### a. Intra-assay Precision

Intra-assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	34.26	2.21	6.45
High titer	20	130.59	5.26	4.03

#### b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	36.14	2.32	6.42
High titer	10	133.75	6.67	4.99

### D. High Dose Hook Effect

No hook effect occurred with CA125 concentration up to 5000U/ml.

### E. Accuracy

For 153 specimens in the range of 0U/ml to 400U/ml, the correlation between the Autobio CA125 CLIA kit and Roche Elecsys assay was as follows:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Roche® (ECLIA)	153	$y = 1.0176x - 0.9342$	0.951






### LIMITATIONS

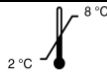


- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

### QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

### SYMBOLS

	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE

	<b>TEMPERATURE LIMITATION</b>
	<b>CATALOGUE NUMBER</b>
	<b>CONSULT INSTRUCTIONS FOR USE</b>

**REFERENCES:**

1. NIH Consensus Conference, Ovarian Cancer: Screening, Treatment and Follow-Up. JAMA 1995;273:491.
2. Kenemans P, Yedema CA, Bon CG, et al. CA 125 in Gynecologic Pathology-A Review. Eur J Obstet Gynecol and Reprod Biol 1993;49:115.
3. Bast RC Jr, Klug TL, St. John E, et al. A Radio-Immunoassay Using a Monoclonal Antibody to Monitor the Course of Epithelial Ovarian Cancer. N Engl J Med 1983;309:883.
4. Bast RC Jr, Klug TL, Schaetzl E, et al. Monitoring Human Ovarian Carcinoma with a Combination of CA 125, CA 19-9 and Carcinoembryonic Antigen. Am J Obstet Gynecol 1984;149:553.
5. Niloff JM, Bast RC Jr, Schaetzl EM, et al. Predictive Value of CA 125 Antigen Levels in Second-Look Procedures for Ovarian Cancer. Am J Obstet Gynecol 1985;151:981.
6. Niloff JM, Knapp RC, Schaetzl E, et al. CA 125 Antigen Levels in Obstetric and Gynecologic Patients. Obstet Gynecol 1984;64:703.
7. Atack DB, Nisker JA, Allen HH, et al. CA 125 Surveillance and Second-Look Laparotomy in Ovarian Carcinoma. Am J Obstet Gynecol 1986;154:287.
8. Schilthuis MS, Aalders JG, Bouma J, et al. Serum CA 125 Levels in Epithelial Ovarian Cancer: Relation with Findings at Second-Look Operations and Their Role in the Detection of Tumor Recurrence. Br J Obstet Gynecol 1987;94:202.
9. Berek JS, Knapp RC, Malkasian GD, et al. CA 125 Serum Levels Correlated with Second-Look Operations Among Ovarian Cancer Patients. Obstet Gynecol 1986;67:685.
10. Crombach G, Zippel HH, Wrz H. Clinical Significance of Cancer Antigen 125 (CA 125) in Ovarian Cancer. Cancer Detect Prev 1985;8:135.
11. Bergmann JF, Beaugrand M, Labadie H, et al. CA 125 (Ovarian Tumour-Associated Antigen) in Ascitic Liver Diseases. Clin Chim Acta 1986;155:163.
12. Molina R, Ballesta AM, Casals E, et al. Value of CA 125 Antigen as Tumor Marker: Preliminary Results. In: Peeters H, editor. Protides of the Biological Fluids. Brussels:Proc. of the 32nd Colloquium, 1984;613.
13. Ruibal A, Encabo G, Miralles EM, et al. CA 125 Seric Levels in Non Ovarian Pathologies. In: Peeters H, editor. Protides of the Biological Fluids. Brussels:Proc. of the 32nd Colloquium, 1984;605.
14. Barbieri RL, Niloff JM, Bast RC Jr, et al. Elevated Serum Concentrations of CA 125 in Patients with Advanced Endometriosis. Fertil Steril 1986;45(5):630.
15. Pittaway DE. CA 125 in Women with Endometriosis. Obstet Gynecol Clin North Am 1989;16(1):237.
16. Takahashi K, Kijima S, Yoshino K, et al. Serum CA 125 as a Marker for Patients with External Endometriosis. Int J Fertil 1989;34(2):143.
17. Giudice LC, Jacobs A, Pineda J, et al. Serum Levels of CA 125 in Patients with Endometriosis: A Preliminary Report. Fertil Steril 1986;45(6):876.
18. Masahashi T, Matsuzawa K, Ohsawa M, et al. Serum CA 125 Levels in Patients with Endometriosis: Changes of CA 125 Levels During Menstruation. Obstet Gynecol 1988;72(3):328.
19. Malkasian GD Jr, Podratz KC, Stanhope CR, et al. CA 125 in Gynecologic Practice. Am J Obstet Gynecol 1986;155:515.
20. Halila H, Stenman UH, Seppala M. Ovarian Cancer Antigen CA 125 Levels in Pelvic Inflammatory Disease and Pregnancy. Cancer 1986;57:1327.
21. Haga Y, Sakamoto K, Egami H, et al. Evaluation of Serum CA 125 Values in Healthy Individuals and Pregnant Women. Am J Med Sci 1986;292:25.
22. Takahashi K, Yamane Y, Yoshino K, et al. Studies on Serum CA 125 Levels in Pregnant Women. Acta Obstet Gynaec Jpn 1985;37:1931.
23. Pittaway DE, Fayez JA, Douglas JW. Serum CA 125 in the Evaluation of Benign Adnexal Cysts. Am J Obstet Gynecol 1987;157:1426.
24. Pittaway DE, Fayez JA. Serum CA 125 Antigen Levels Increase During Menses. Am J Obstet Gynecol 1987;156:75.
25. Niloff JM, Klug TL, Schaetzl E, et al. Elevation of Serum CA 125 in Carcinomas of the Fallopian Tube, Endometrium and Endocervix. Am J Obstet Gynecol 1984;148:1057.

26. Haga Y, Sakamoto K, Egami H, et al. Clinical Significance of Serum CA125 Values in Patients with Cancers of the Digestive System. Am J Med Sci 1986;292:30.
27. Duk JM, Aalders JG, Fleuren GJ, et al. CA 125: A Useful Marker in Endometrial Carcinoma. Am J Obstet Gynecol 1986;155:1097.

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