

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

1. Hall, E., Methods in Enzymology, Volume 70, Van Vunakis, H. and Langone, J. J. (eds.), Academic Press, New York, 419- 492 (1980).
1. Tila, M., Ruoslahti, E. and Engvall, E., J. Immunol. Methods, 42, 11-15 (1981).
2. Kenemans P, Yedema CA, Bon GG, von Mensdorff-Pouilly S. Ca125 in gynecological pathology a review. Eur J Obstet Gynecol 1993;49:115-124.
3. Saksela F. Prognostic markers in epithelial ovarian cancer. Intl J Gynecol Pathol 1993;12:156-161.
4. Farghaly SA. Tumor markers in gynecologic cancer. Gynecol & Obstet Invest 1992;34:65-72.
5. Welandar CE. What do CA 125 and other antigens tell us about ovarian cancer biology. Acta Obstet Gynecol Scand Sup 1992;155:85-93.
6. McGowan L. Pathology of the ovary. Curr Opin on Obstet Gynecol 1991;3:580-586.
7. Niloff JM. Ovarian malignancy. Curr Opin on Obstet Gynecol 1991;3:66-72.
8. Olt G, Berchuck A, Bast RC. The role of tumor markers in gynecologic oncology. Obstet Gynecol Survey 1990;45:570-577.
9. Diez M, Cerdan FJ, Ortega MD, Torres A, Picardo A, Balibrea JL. Evaluation of serum CA125 as a tumor marker in non-small cell lung cancer. Cancer 1991;67:150-154.
10. Niloff JM, Klug TL, Schaeztl E. Elevation of serum CA125 in carcinomas of the fallopian tube, endometrium, and endocervix. AM J. Obstet Gynecol 1984; 148:1057

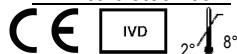
2009-03-25(Mfg.: 040203)



For Research Use Only. Not for use in Diagnostic Procedures.

Cat#: CA125T (96 Tests)

For Order and Inquiries, please contact

 Calbiotech Inc.,
10461 Austin Dr, Spring Valley, CA, 91978
Tel (619) 660-6162, Fax (619) 660-6970,
www.calbiotech.com



  CEpartner4U, 3951DB; 13. NL.
tel: +31 (0)6.516.536.26)

**CA125 ELISA**

Catalog No. CA125T (96 Tests)

INTENDED USE

The Calbiotech CA125 ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA125 concentration in human serum.

SUMMARY AND EXPLANATION

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 endometrioid, clear-cell and undifferentiated ovarian carcinoma. The serum CA125 concentration is greater than 35 units per ml in 60% of women with ovarian cancer and >80% of patients with disseminated ovarian cancer. The serum CA125 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 salpingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serial determinations of serum CA125 as well as pelvic examination increase the test specificity. Serum CA125 concentration may be useful in monitoring treatment and distinguishing between good response to treatment and progressive malignant disease with poor therapeutic response. To date, CA125 is the most sensitive marker for residual epithelial ovarian cancer. CA125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

PRINCIPLE OF THE TEST

The CA125 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA125 molecule is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA125 antibody conjugated to horseradish peroxidase (HRPO) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CA125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 90 minutes of incubation at 37°C, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Murine Monoclonal anti-CA125	12x8x1
2. CA125 reference standards: 6 vials (ready to use)	1.0ml
3. Enzyme Conjugate Reagent	13ml
4. TMB Reagent (One-Step)	11ml
5. Stop Solution	11ml
6. Wash Concentrate 20x: 1 Bottle	25 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for Research Use Only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

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.

REAGENT PREPARATION

1. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Secure the desired number of coated wells in the holder. Dispense 100µl of CA125 standards, specimens, and controls into the appropriate wells.
2. Dispense 100µl Enzyme Conjugate Reagent into each well.
3. Mix gently for 30 seconds. It is very important to have complete mixing in this setup.
4. Incubate at 37°C for 90 minutes.
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual liquid droplets.
8. Dispense 100µl of TMB Reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.
9. Stop the reaction by adding 100µl of Stop Solution to each well.
10. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

CALCULATION RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance 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 CA125 in U/ml from the standard curve.

Example of Standard Curve

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against CA125 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

CA125 Values (U/ml)	Absorbance (450nm)
0	0.010
15	0.105
50	0.347
100	0.703
200	1.411
400	2.437

EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA125 assay values below 35 U/ml. The minimum detectable concentration of CA125 in this assay is estimated to be 5 U/ml.