

Product Information

CA125 ELISA Kit

Catalog Number **SE120017**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Cancer Antigen 125 (CA125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA125 is associated with a high molecular mass glycoprotein. Published studies have indicated that elevated serum CA125 levels can be found in individuals with serious endometroid, clear-cell, and undifferentiated ovarian carcinoma. The serum CA125 concentration is >35 units/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.

The CA125 ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA125 concentration in human serum. 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, for solid phase immobilization (on the multiwell plates). 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 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.

Components

Materials Provided	96 Tests
Microwells coated with Murine Monoclonal anti-CA125	12 x 8 x 1
CA125 reference standards: 6 vials (ready to use)	0.5 mL
Enzyme Conjugate Reagent	12 mL
TMB Reagent (One-Step)	12 mL
Stop Solution	12 mL
Wash Concentrate 20x: 1 Bottle	25 mL

Reagents and Equipment Required but Not Provided.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

Serum should be prepared from a whole blood specimen. This kit is only for use with serum samples without additives.

Reagent Preparation

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).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended serum samples be run in duplicate.

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.

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 100 µL of CA125 standards, specimens, and controls into the appropriate wells.
2. Dispense 100 µL 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 absorbent paper or paper towel.
7. Strike the multiwell plate sharply onto absorbent paper or paper towels to remove all residual liquid droplets.
8. Dispense 100 µL 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 100 µL 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.

Results

Calculations

1. Calculate the average absorbance values (A_{450}) 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 450 nm 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. A new standard curve must be set up each time the assay is run.

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

Note: Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the technical bulletin and with adherence to good laboratory practice. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 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.

Product Profile

Expected values and sensitivity

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

References

1. Engvall, E., Enzyme Immunoassay ELISA and EMIT. *Methods in Enzymology*, **70**, 419- 492 (1980).
2. Uotila, M. et al., Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. *J. Immunol. Methods*, **42**, 11-15 (1981).
3. Kenemans, P. et al., Ca125 in gynecological pathology a review. *Eur. J. Obstet. Gynecol.*, **49**, 115-124 (1993).
4. Saksela, F., Prognostic markers in epithelial ovarian cancer. *Intl. J. Gynecol. Pathol.*, **12**, 156-161 (1993).
5. Farghaly, S.A., Tumor markers in gynecologic cancer. *Gynecol. & Obstet. Invest.*, **34**, 65-72 (1992).
6. Welander, C.E., What do CA 125 and other antigens tell us about ovarian cancer biology? *Acta Obstet. Gynecol. Scand. Sup.*, **155**, 85-93 (1992).
7. McGowan, L., Pathology of the ovary. *Curr. Opin. on Obstet. Gynecol.*, **3**, 580-586 (1991).
8. Niloff, J.M., Ovarian malignancy. *Curr. Opin. on Obstet. Gynecol.*, **3**, 66-72 (1991).
9. Olt, G. et al., The role of tumor markers in gynecologic oncology. *Obstet. Gynecol. Survey*, **45**, 570-577 (1990).
10. Diez, M. et al., Evaluation of serum CA125 as a tumor marker in non-small cell lung cancer. *Cancer*, **67**, 150-154 (1991).
11. Niloff, J.M. et al., Elevation of serum CA125 in carcinomas of the fallopian tube, endometrium, and endocervix. *Am. J. Obstet. Gynecol.*, **148**, 1057 (1984).

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