



CA-125 ELISA

Catalog Number EA-0101

(For Research Use Only)

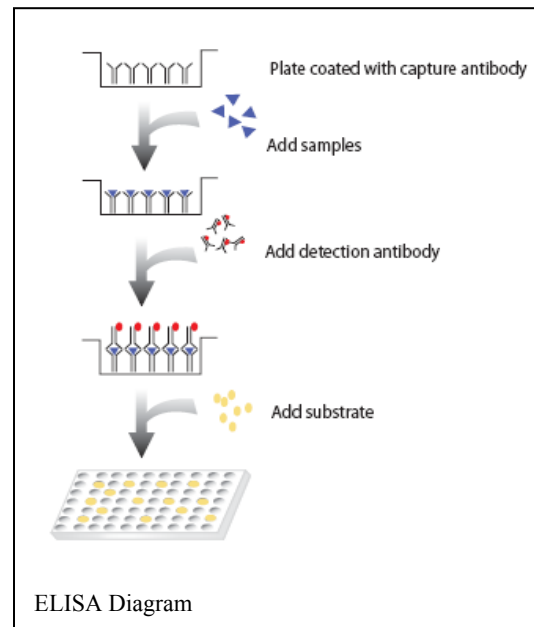
Introduction

Ovarian cancer Antigen (CA125) is a high molecular weight (>200kDa) mucin-like glycoprotein, CA-125 is produced on the surface of cells and is released in the blood stream (1). CA-125 is a well known marker for ovarian cancer, but it may also be elevated in other malignant cancers, including those originating in the endometrial, fallopian tubes, lungs, breast and gastrointestinal tract. 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. CA-125 is clinically approved for following the response to treatment and predicting prognosis after treatment. It is especially useful for detecting the recurrence of ovarian cancer. CA-125 as a prognostic indicator for ovarian cancer-after cytoreductive surgery and during chemotherapy the level of CA-125 can provide an early indicator of prognosis (3).

The Signosis CA-125 Immunoassay provides a rapid, sensitive, and reliable assay for the quantitative measurement of CA-125 level. The antibodies developed for the test will determine a minimal concentration of 1.0 ng/ml.

Principle of the assay

The CA-125 ELISA 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 (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with these antibodies, resulting in CA-125 being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA-125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Materials provided with the kits:

- Antibody-coated microtiter plate with 96 wells.
- CA125 standards containing: 0, 15, 50, 100, 200, and 400Unit/ml of CA125, 1 ml each, ready to use.
- Enzyme Conjugate Reagent, 13 ml.
- Wash Buffer (20X), 50 ml
- TMB Reagent, 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Warning and precautions

1. Caution: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens (8) or other appropriate national biohazard safety guidelines or regulations (9-11).
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

Preparation for Assay

- All reagents should be taken to room temperature (18-25 °C) before use.
- Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8 °C.

Assay Procedure

Samples of patient serum, plasma need to be diluted before use for best bet results

1. Add 20 µl of standard, specimens, and controls into appropriate wells.
2. Dispense 100 µl of Zero Buffer into each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at room temperature (18-25°C) for 30 minutes.
5. Remove the incubation mixture by flicking plate content into a waste container.
6. Rinse and flick the microtiter wells 5 times with Wash Buffer (1X). Strike the wells sharply onto paper towel to remove all residual water droplets.
7. Dispense 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
8. Incubated at room temperature for 30 minutes.
9. Remove the incubation mixture by flicking plate contents into a waster container.
10. Rinse and flick the microtiter wells 5 times with Wash Buffer (1X).
11. Strike the plate sharply onto paper towel to remove residual water droplets.
12. Dispense 100 µl TMB Reagent into each well, and mix gently for 10 seconds.

13. Incubate at room temperature in the dark for 20 minutes.
14. Add 100 µl Stop Solution to each well to stop the reaction.
15. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
16. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

References

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2. Uotila, M., Ruoslahti, E. and Engvall, E., J. Immunol. Methods, 42, 11-15 (1981).
3. Kenemans P, Yedema CA, Bon GG, von Mensdorff-Pouilly S. Ca125 in gynecological pathology a review. Eur J Obstet Gynecol 1993;49:115-124.
4. Saksela F. Prognostic markers in epithelial ovarian cancer. Intl J Gynecol Pathol 1993;12:156-161.
5. Farghaly SA. Tumor markers in gynecologic cancer. Gynecol & Obstet Invest 1992;34:65-72.
6. Welander CE. What do CA 125 and other antigens tell us about ovarian cancer biology. Acta Obstet Gynecol Scand Sup 1992;155:85-93.

Example of standard curve

| CA125 Values (U/ml) | Absorbance (450 nm) |
|---------------------|---------------------|
| 0 | 0.051 |
| 15 | 0.178 |
| 50 | 0.488 |
| 100 | 0.929 |
| 200 | 1.620 |
| 400 | 2.865 |

