

CANCER ANTIGEN CA125 ENZYME IMMUNOASSAY TEST KIT

Catalog Number: BC-1013

Enzyme Immunoassay for the Quantitative Determination of Ovarian Cancer Antigen CA125 in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

CA125 Enzyme Immunoassay

INTENDED USE

For the quantitative determination of the Cancer Antigen 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. 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 a 3-hour 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.

REAGENTS

Materials provided with the kit:

- Murine Monoclonal anti-CA125 coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml.
- CA125 reference standards containing; 0, 15, 50, 100, 200, and 400 Unit/ml of CA125, 1 ml each, ready to use.
- TMB Reagent (One-Step), 11ml.
- Stop Solution (1N HCl), 11ml.

Materials required but not provided:

- Precision pipettes and tips, 0.1ml, 0.2ml, 1ml, and 5ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.

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.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or

greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.

ASSAY PROCEDURE

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 3 hours.
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Rinse and empty the microtiter plate 5 times with distilled or deionized water. (Please do not use tap water.)
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water 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.***

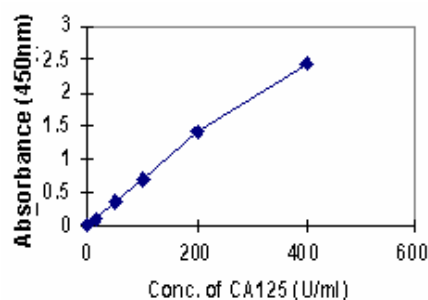
CALCULATION OF RESULTS

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.

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

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