



## CA 125 ELISA

Catalog No. BQ 1013T (96 tests)

### INTENDED USE

The CA 125 ELISA used 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 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 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.	Microwell 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

### 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.

**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. 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 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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**Warning**

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