

Ovarian Cancer (CA125)

**For Quantitative Determination of CA125
In Human Serum**

For In Vitro Research Use Only

Ovarian Cancer (CA125) ELISA KIT # 1820

Kit Contents: (reagents for 96 tests)

Components	Cat. #
Streptavidin coated microwell strip plate (96 wells), Ready-to-use	1 8 2 1
CA125 sample diluent or Standard A (0 U/ml), 11 ml	1 8 2 2
CA125 Standard B (10 U/ml), 0.5 ml	1 8 2 3
CA125 Standard C (50 U/ml), 0.5 ml	1 8 2 4
CA125 Standard D (100 U/ml), 0.5 ml	1 8 2 5
CA125 Standard E (200 U/ml), 0.5 ml	1 8 2 6
CA125 Standard F (500 U/ml), 0.5 ml	1 8 2 7
Anti-EOC antigen-HRP Conjugate ; 11 ml	1 8 2 8
Biotinylated capture antibody 11 ml	1 8 2 9
HRP substrate Solution ; 11 ml	T M B - 1 8 2 0
Wash buffer (100X), 10 ml (dilute 1:100 with distilled water)	W - 1 0 0
Stop solution, 10 ml	T - 1 0
Complete Instruction Manual	M - 1 8 2 0

Introduction

Ovarian cancer Antigen (CA125) is a high molecular weight(>200kDa) mucin-like glycoprotein and is expressed by greater than 80% of nonmucinous epithelial ovarian carcinomas (EOC). This EOC is found in the most serious, endometrioid and clear cell carcinomas of ovary(1). Epithelial ovarian cancer (EOC) is the most common cause of death from gynecologic malignancy in the United States and has an over-all 5 year survival rates of less than 30% stages I diseases, 5-year survival rates of 80% to 90% are achieved. The ovarian cancer assay may have the following clinical application:

1. EOC for monitoring tumor growth serum EOC level correlate with clinical disease status in over 90% of cases in which EOC is elevated in preoperative(2) serum sample a rising serum EOC 125 level is therefore, an extremely reliable indicator of recurrent or progressive disease.
2. EOC 125 as a prognostic indicator for ovarian cancer-after cytoreductive surgery and during chemotherapy the level of EOC can provide an early indicator of prognosis(3).
3. EOC might have role as a screening test for the early detection of ovarian cancer.

ADI's CA125 ELISA kit provides for the measurement of CA125 in serum for monitoring patients with ovarian cancer.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of human CA125 detected using this assay is 5 U/ml. The detection limit is defined as the value deviating by 3 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Two serum samples (20 and 77 U/ml) were run in ten replicates in an assay. The samples showed good intra-assay precision (5-10%CV). The actual values were: mean 22.71 U/ml, SD 1.15 and 77.7 U/ml, SD 8.0.

Inter-assay precision:

Two serum samples were run in duplicate in eight independent assays. The samples showed good inter-assay precision (8-10 %CV). The actual values were: mean 23.5 U/ml, SD 2.5 U/ml, %CV 8.0; mean 69.8 U/ml, SD 5.8 U/ml, %CV 10.6.

3. RECOVERY

A known amount of CA125 (250 U/ml) was added to three samples with initial CA125 of 10, 50, 100, 200, and 500 U/ml and the total CA125 concentrations measured. The assay showed excellent mean recoveries of about 81-101%).

General References:

1. Jacob et al (1989) Human Reprod. 4, 1-12; Bast RC et al (1990) In Identification of markers for epithelial ovarian cancer, p 265-272; Hawkins RE et al (1990) Br. J. Obstet. Gynecol. 96, 1395-1399; Noel R et al (1992) In manual of Lab. Immunology 4th ed. ASM, p812.

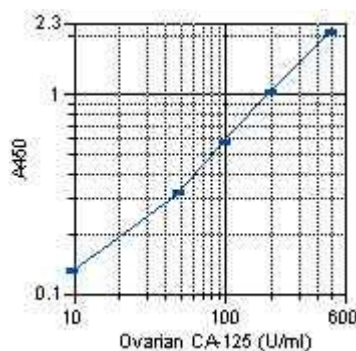
(2) Citations of ADI's CA125 ELISA kits (see web site for updated list)

Szeto C-C 2006 Clinical biocompatibility of a neutral peritoneal dialysis solution with minimal glucose-degradation products--A 1-year randomized control trial, Nephrol. Dial. Transplant., Sep 2006 in press

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (U/ml)	Net Mean $A_{450\text{ nm}}$	Calculated Conc. (U/ml)
A1, A2	Std. A (0)	0.070	
B1, B2	Std. B (10 U/ml)	0.135	
C1, C2	Std. C (50 U/ml)	0.432	
D1, D2	Std. D (100 U/ml)	0.590	
E1, E2	Std. E (200 U/ml)	1.050	
F1, F2	Std. F (500 U/ml)	2.150	
G1, G2	Sample 1	0.197	19.5

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

CA125 ELISA kit is a solid phase ELISA. The wells are coated with Streptavidin. The samples, std., and controls, and biotinylated anti-EOC antibody are allowed to bind to Streptavidin-coated plates. During the incubation, EOC antigen is bound to anti-EOC antigen antibodies on the wells. Unbound EOC antigen is removed by washing the wells with buffer. Enzyme conjugate is then added to all wells. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (blue color) is directly proportional to the amount of CA125 present in the sample. Adding stopping solution terminates the reaction and converts blue color into yellow. Absorbance is then measured on an ELISA reader at 450 nm. and the concentration of CA125 in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μ l) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The CA125 ELISA test is intended for *in vitro* research use only. The reagents contain proclin-300 (0.1% v/v in standards and antibody conjugates and) as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

MSDS for following chemicals may be obtained at the web site or requested from ADI if they are not already on file.

TMB (substrate).

H₂SO₄ (stop solution).

Proclin-300 (preservative)

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).
Store at 4°C.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. HRP substrate buffer (solution A) and HRP substrate (solution B) should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. Reconstituted control serum is stable for one week at 2-8°C. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

1. **Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).** Label or mark the microtiter well strips to be used on the plate. Dispense 200-300 ul of wash buffer or water to all wells. **Mix for 5** seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.
2. Pipet **25 ul of standards, control, and serum** samples into appropriate wells in *duplicate*.
3. Add **100 ul of biotinylated capture antibody** into each well and incubate for **120 min** at room temp.
4. Remove incubation mixture and **wash the wells 5X** with wash buffer.
5. Add **100 ul of anti-EOC-HRP conjugate** into **each well**. Mix gently. Cover the plate and incubate for **60 minutes** at room temperature.
6. Remove reaction mixture and **wash 5X** with wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
7. Dispense **100 ul TMB substrate per well**. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temperature (blue color develops in standards and positive samples).
8. Stop the reaction by adding **50 ul of stop solution to all wells**. Mix gently for 5-10 seconds (blue color turns yellow).
9. Measure the absorbance at 450 nm using an ELISA reader within 30 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples containing more than 500 U/ml of CA125 should be diluted with the zero standard (standard A), reassayed, and the results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate CA125 concentrations. Read off the CA125 concentrations of the control and patient samples.

EXPECTED VALUES

It is recommended that each laboratory must determine its own normal and abnormal ranges. EOC is not ovarian carcinoma specific. It can also be detected in the cancer of fallopian tube, endometrium, endocervix, pancreas, liver, as well as lung. The elevation of EOC 125 during menstruation is slight (35-80 U/ml) (4). EOC 125 levels associated with stage I ovarian cancer are lower (0-500 U/ml) than levels in advanced stage disease (0-100, 000 U/ml) (4).

LIMITATIONS

ADI's CA125 ELISA kit should be used in conjunction with other data available to the physicians. This kit is designed to avoid high dose hook effect.