

ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-1820

Ovarian Cancer (CA125)

ELISA KIT Cat. No. 1820

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

For In Vitro Research Use Only



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**DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE
KIT FOR ANY LOT SPECIFIC CHANGES.**

Ovarian Cancer (CA125) ELISA KIT # 1820

Kit Contents: (reagents for 96 tests)

C o m p o n e n t s	Cat. #
Streptavidin coated microwell strip plate (96 wells), #1821	1 plate (96 wells)
CA125 Standard A (0 U/ml), 1 ml, #1822A	1 vial
CA125 Standard B (15 U/ml), 1 ml, #1822B	1 vial
CA125 Standard C (50 U/ml), 1 ml, #1822C	1 vial
CA125 Standard D (100 U/ml), 1 ml, #1822D	1 vial
CA125 Standard E (200 U/ml), 1 ml, #1822E	1 vial
CA125 Standard F (400 U/ml), 1 ml, #1822F	1 vial
Anti-CA125 antigen-HRP Conjugate; 13 ml #1803	1 bottle
HRP substrate Solution; 11 ml #TMB-1820	1 bottle
Wash buffer (20X), 25 ml (dilute 1:20 with distilled water) # WB-1820	1 bottle
Stop solution, 11 ml, # ST-1820	1 bottle
Complete Instruction Manual	M-1820

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 5.0 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.

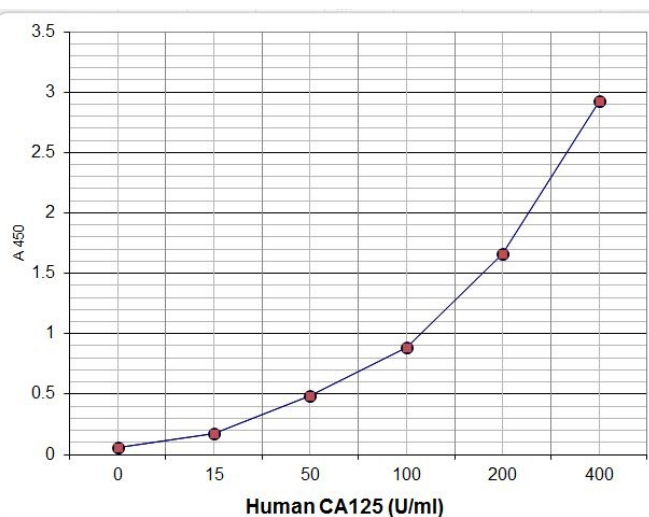
(2) Citations of ADI's CA125 ELISA kits (see web site for updated list)

Szeto CC (2007) Nephrol. Dial. Transplant., 22: 552 – 559; Bellone S (2009) Am J Obstet Gynecol. 200, 75.e1-75.e10; Pastuskovas C (2010) Drug Metab. Dispos., Dec 2010; 38: 2309 - 2319.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (U/ml)	Net Mean $A_{450\text{ nm}}$	Calculated Conc. (U/ml)
A1, A2	Std. A (0)	0.057	
B1, B2	Std. B (10 U/ml)	0.175	
C1, C2	Std. C (50 U/ml)	0.485	
D1, D2	Std. D (100 U/ml)	0.885	
E1, E2	Std. E (200 U/ml)	1.651	
F1, F2	Std. F (400 U/ml)	2.93	
G1, G2	Sample 1	0.890	100.05

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)

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.

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 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International 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 (20X) with distilled water (25 ml stock in total of 475 ml). 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 (1:20) with distilled water (25 ml stock in total of 475 ml).** Label or mark the microtiter well strips to be used on the plate.
2. Pipet **100 ul of standards, control, and serum** samples into appropriate wells in *duplicate*.
3. Add **100 ul of HRP conjugate** into each well. Mix gently for 5-10 seconds and incubate for **90 min at 37°C temp.**
4. Remove incubation mixture and **wash the wells 3X** 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.
5. Dispense **100 ul TMB substrate per well.** Mix gently for 5-10 seconds. Cover the plate and incubate for **20 minutes** at room temperature (blue color develops in standards and positive samples).
6. Stop the reaction by adding **100 ul of stop solution to all wells.** Mix gently for 5-10 seconds (blue color turns yellow).
7. 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 400 u/ml of CA125 should be diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

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.