

abia CA 125



REF DK.047.01.3

IVD



Note: Changes highlighted ★



abia

Intended use

Abia CA 125 is an enzyme immunoassay for the quantitative determination of CA 125 antigen concentration in human serum.

For professional use only.

Clinical value

CA 125 (MUC16) is a high-molecular weight mucin-type glycoprotein realized on the surface of ovarian tumor cells with restricted expression in normal adult tissues such as endocervix, endometrium, tubes, pleura, pericardium, peritoneum, ocular surface, breast and occasional expression in intestine, lung and kidney. It presents in various biological fluids: serum, cyst, peritoneal and amniotic fluids, human milk, seminal plasma, cervical mucus. CA 125 was first defined in 1981 by Bast et. al. The antigen has three antigenic domains which classifies the antibodies as OC125-like (group A), M-11-like (group B) and OV197-like (group C). CA 125 is a multifunctional glycoprotein with important role in signal transduction and potentiation of tumorigenicity and metastasis. It is involved in mediating both anti-adhesion and cell adhesion, and suppressing the immune system. The anti-adhesive property of MUC16 is suggested to provide a protective barrier for the epithelial surface from bacterial adherence and mechanical injury. At the same time CA 125 mediates cell adhesion by binding to another cell surface glycoproteins.

Serum concentration of CA 125 is raised in 80-90% of advanced-stage ovarian cancers. Serum concentration increases with disease progression, but decline after therapeutic intervention. CA 125 level assay is used to evaluate therapeutic status patients with documented disease and efficiency of therapy. High concentration of the marker are detected in other (pancreatic, breast, bladder, liver, and lung) cancers, benign diseases (diverticulitis, uterine fibroids, endometriosis, benign ovarian cysts, tubo-ovarian abscess, hyperstimulation syndrome, and ectopic pregnancy), and physiological conditions (pregnancy, menstruation).

Principle of the test

Abia CA 125 is a one-step immunoassay, based on the principle of the “sandwich” method.

The assay system utilizes high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact CA 125 molecule.

The test sample is allowed to react simultaneously with the two antibodies, resulting in the CA 125 molecules being sandwiched between the solid phase and enzyme-linked antibodies.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of CA 125 in the specimen and can be read at 450 nm.

Kit contents

	S	
CA 125 Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-CA 125 Ab
Conjugate	1 × 12 ml	ready to use; HRP-labeled monoclonal anti-CA 125; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 2.0 ml	protein based buffer not containing CA 125; pale yellow liquid
Calibrator 1	1 × 0.5 ml	protein based buffer containing CA 125 in concentration approx. 12.5 U/ml; pale yellow liquid
Calibrator 2	1 × 0.5 ml	protein based buffer containing CA 125 in concentration approx. 50 U/ml; pale yellow liquid
Calibrator 3	1 × 0.5 ml	protein based buffer containing CA 125 in concentration approx. 100 U/ml; pale yellow liquid
Calibrator 4	1 × 0.5 ml	protein based buffer containing CA 125 in concentration approx. 250 U/ml; pale yellow liquid
Calibrator 5	1 × 0.5 ml	protein based buffer containing CA 125 in concentration approx. 500 U/ml; pale yellow liquid
Control serum	1 × 0.5 ml	protein based control containing CA 125; colorless or pale yellow
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colorless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H ₂ O ₂ ; colorless liquid
Stopping reagent 0.2M H ₂ SO ₄	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.

Once opened, the components should be used within 2 months. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- as no known test method can offer complete assurance that infectious agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures. In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- repeat 5 times
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion Stability: 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia CA 125 for the quantitative determination of CA 125 antigen concentration in human serum

- 1 Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.
 - 2 Analyze each calibrator, control serum, and sample duplicate. Reserve one or two wells for TMB/substrate solution control (blank).
Add 25 µl of calibrators 0 - 5 into appropriate wells.
Add 25 µl of control serum into appropriate wells.
Add 25 µl of samples to be tested in rest of the wells.
The total time should not exceed 10 min.
 - 3 Add 100 µl conjugate into each well except blank.
Mix the contents of the wells for 30 seconds by careful tapping on the edge of the plate, then cover the plate with protective film.
 - 4 Incubate for 90 minutes at room temperature 20–25 °C.
 - 5 Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant).
Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
 - 6 Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 25 ± 5 minutes at 20–25 °C.
 - 7 Add 150 µl of stopping reagent into each well. Mix gently for 5–10 sec.
 - 8 Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.
-

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative. For the instrumentation without established validated protocol follow section “Test procedure” and ensure all requirements described in section “Precautions” are followed. All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 (approx. 500 IU/ml) should be greater than 1.300.
Calculated value of control serum should be within established range.

Calculation procedure

- 1 Calculate the mean optical density of each calibrator duplicate.
- 2 Calculate the mean optical density of each sample duplicate.
- 3 Subtract the mean absorbance value of the “blank” from the mean absorbance values of the calibrators, control and serum samples.
- 4 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
- 5 Read the values of the samples directly off the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads more than 500 U/ml then dilute it with calibrator 0. The result obtained should be multiplied by the dilution factor.

Example	OD 1	OD 2	Mean OD - blank (here 0.052)	Value, U/ml
Calibrator 0	0.051	0.052	0.000	0.00
Calibrator 1	0.117	0.114	0.064	12.50
Calibrator 2	0.352	0.340	0.295	50.00
Calibrator 3	0.613	0.610	0.560	100.00
Calibrator 4	1.374	1.365	1.318	250.00
Calibrator 5	2.448	2.470	2.408	500.00
Sample	0.295	0.278	0.235	40.70

This data is for illustration only and should **not be used** to calculate samples. Each user should obtain his or her own data and standard curve.

Performance characteristics

Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 30 analysis runs additional 2 x SD. Limit of detection defined at 1.00 IU/ml.

Specificity

There is no cross reactivity with CA 72-4 (10 U/ml), CA 19-9 (200 U/ml), CA 15-3 (30 U/ml), HE4 (3000 pM/l) antigens.

Precision	Mean, IU/ml	SD	CV, %
Intra-assay, sample 1	101.80	4.76	4.70
Intra-assay, sample 2	29.90	1.66	5.50
Inter-assay, sample 1	126.00	8.95	6.60
Inter-assay, sample 2	26.30	1.76	6.70

Accuracy

The assay was compared with an commercially available enzyme immunoassay as a reference test. The total number of specimens was 228. The values ranged from 1.40 to 1 001.40 U/ml. The least square regression equation and the correlation coefficient were computed for abia CA 125 in comparison with the reference method.

The least square regression analysis was $y = 1.07(x) + 16.36$ with correlation coefficient 0.98.

Expected normal value

Value, U/ml

Adult female population

< 35.00

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.













Limitations of test

- the assay was validated only for the determination of CA 125 in human serum
- only calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient
- the assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results
- no hook effect was observed at CA 125 concentrations up to 107 000 U/ml
- not intended for newborn screening

References

1. Singh A.P., Senapati Sh., Ponnusamy M.P., Jain M., Iele S.M., Davis J.S., Remmenda S., Batra S.K. "Clinical potential of mucins in diagnosis, prognosis, and therapy of ovarian cancer", *Lancet Oncol*, 9: 1076-1085 (2008).
2. Warren D.J., Nustad K., Beard J.B., O'Brien T. "Expression and epitope characterization of recombinant CA 125 repeat: fourth report from the ISOBM TD-1 Workshop". *Tumor Biol*, 30: 51-60 (2009).
3. Bast R.C., Feeney M., Lazarus H., Nadler L.M., Colvin R., Knapp R.C. "Reactivity of a monoclonal antibody with human ovarian carcinoma", *J. Clin. Invest*, Vol. 68, 1331-1337 (1981).
4. Davelaar E.M., Kamp G.J., Verstraeten R.A., Kenemans P. "Comparison of seven immunoassays for the quantification of CA 125 antigen in serum", *Clin. Chem*, 44:7 1417-1422 (1998).
5. Medeiros L.R., Rosa D.D., Rosa M.I., Bozetti M.C. "Accuracy of CA 125 in the diagnosis of ovarian tumors: A quantitative systematic review", *Eur. J. of Obstetrics & Gynecol. and Reprod. Biol*, 142: 99-105 (2009).
6. Markmann S, Gerber B., Briese V. "Prognostic value of CA 125 levels during primary therapy", *Anticancer Res*, 27: 1837-1839 (2007).

Key to symbols used

	Manufacturer
	For in vitro diagnostic use
	Catalogue number
	Batch code
 YYYY-MM-DD	Expiry date
 2°C — 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
 Σ_n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
	Changes highlighted

Hazard and precautionary statements for certain kit components

Stopping reagent



Warning

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Conjugate, calibrators 0 - 5, control serum



Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.

Attention!

For complete precautionary statements and detailed information see safety data sheets (SDS).



AB Diagnostic Systems GmbH
Sportfliegerstraße 4
12487 Berlin
Germany

☎ +49 30 208 987 160
☎ +49 30 208 987 199
✉ info@ab-ds.de
www.ab-ds.de

