AB Diagnostic Systems

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CE

IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia CA 125
Enzyme immunoassay for the quantitative determination of cancer antigen 125 (CA 125) concentration in human serum

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 assays (one breakable plate) including controls; the kit is intended for manual testing with a possibility of fractional (one well) use of the kit or use of the kit on open type automated analyzer for enzyme immunoassay.

I. INTENDED USE

The abia CA 125 kit is intended for the quantitative determination of CA 125 antigen concentration in human serum by a microplate immunoenzymometric assay.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

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. all. 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 tumorigenecity 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).

III. PRINCIPLE OF THE TEST

The abia CA 125 is a one-step immunoassay, based on principle of "sandwich" method. The assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the 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. After incubation, the wells are washed with Washing Solution to remove unbound labeled antibodies. A solution of TMB-Substrate is added and incubated, resulting in the development of a blue color. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The concentration of CA 125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

IV. CONTENT OF THE KIT abia CA 125

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-CA 125-coated microtiter wells	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal antibodies to CA 125. Store at 2-8 °C until expiration date.	1 plate
Conjugate	Monoclonal anti-CA 125 antibodies conjugated to horseradish peroxidase. Prepared with addition of bovine serum albumin (2.89%), and Tween-20 (0.11%). Preserving agent: 0.1% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml
Calibrator 0 Calibrator 1 Calibrator 2 Calibrator 3 Calibrator 4 Calibrator 5	Six vials of reference calibrators containing CA 125, bovine serum albumin (1.96%) and 1M Tris-HCl (pH 7.4-7.6). The CA 125 concentration levels in Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis.* Preserving agents: 0.1% ProClin 300, 0.004% gentamicin sulfate, 0.1% phenol. Transparent or slightly opalescent liquids, pale yellow. Store at 2-8 °C until expiration date in a tightly sealed vial.	5 vials 0.5 ml. Calibrator 0 – 2.0 ml
Control Serum	Control Serum with a defined quantity of CA 125, bovine serum albumin (1.96 %) and 1M Tris-HCl (pH 7.4-7.6). The CA 125 concentration level in Control Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Preserving agents: 0.1% ProClin 300, 0.004% gentamicin sulfate, 0.1% phenol. Transparent or opalescent liquid, pale yellow. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 0.5 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 6.9-7.7). Transparent or slightly opalescent liquid, colorless or pale yellow, sediment may form that dissolves at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric acid buffer, containing H_2O_2 (0.01%). Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulfuric acid solution. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml
Protective films for EIA plates		2
Disposable tips		16
Disposable plastic dishes for liquid reagents		2
Polyethylene bag with a Zip-Lock		1

* Nominal values of Calibrators are traceable to a collection of serum samples certified using the test of comparison - "CanAg CA 125 EIA" in accordance with EN ISO 17511:2021 In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples.

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. In case of label loss or labels/vials damage, vials should be disposed and **kit cannot be used.**

- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of Calibrators and Control Serum has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Certain reagents contain biological material of animal origin.
- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patients samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.

- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Calibrators 0-5, Control Serum contain ProClin 300.

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

Danger!

Stopping Reagent contains 0.2M sulfuric acid.

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Automatic microplate washer.
- Microplate reader equipped with 450 and 405-415 nm filter.
- Open type automated analyzer with 450 and 405-415 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Blood samples should be collected according to the current practices. Only serum may be used. Separate serum as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Samples can be stored at 2-8 °C not more than for 48 hours; they should be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed bacterial growing, hemolysis, hyperlipidemia and which were preserved by sodium azide, thimerosal or phenol must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-CA 125-coated microtiter wells.** Each plate containing 12 strips (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of strips/wells required for the assay. Place the unused strips/wells back into the foil-lined bag; reseal the foil-lined bag in Zip-Lock plastic bag. Do not remove desiccant.
- **Calibrators 0-5;**
- **Control Serum;**
- **Conjugate;**
- **TMB-Substrate;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial. Thoroughly mix the solution.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature for 30 min.

1. To the wells add 25 µl of Calibrators and Control Serum in duplicate. Leave two wells for OD control of TMB-Substrate (blank).

2. To the rest of the wells, add 25 µl of samples in duplicate. Pipetting of samples should not extend beyond ten (10) minutes.

3. Add 100 µl of Conjugate to all wells except for the wells for OD control of TMB-Substrate.

4. Swirl the microplate gently for 20 seconds after adding of samples and Conjugate to mix, cover the strips with a protective film and incubate for 90 minutes at room temperature (here 20-25 °C).

5. Aspirate the contents of the wells into the container with disinfecting solution. Wash the plate 5 times with 300 µl of Working Washing Solution per well and remove Working Washing Solution using a washer into the container with disinfecting solution. Tap the plate firmly against absorbent paper to ensure that it is dry – the residual volume must be lower than 10 µl (the use of a washer is recommended). Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.

6. Pipette 100 µl of TMB-Substrate into each well.

7. Incubate for 20-30 minutes at room temperature in a dark place.

8. Stop the reaction by adding 150 µl of Stopping Reagent to the wells, shake the strips for 5-10 seconds and read the results. The time between stopping the reaction and measuring OD should not exceed 20 min.

9. Read the plate on microplate reader at 450 nm. In case of overflow absorbance values, read at 405-415 nm. Reading must be completed within 20 minutes after addition of the Stopping Reagent.

Scheme of the assay is represented in Annex.

Spectrophotometric verification of reagent pipetting

The presence of conjugate + sample in the well can be verified by automatic reading at 492 nm. Each well containing sample and conjugate must have an OD higher than 0.150.

10. Automated analyzers

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XV). For the instrumentation without established validated protocol follow the section "TEST PROCEDURE" and ensure all requirements described in the section "PRECAUTIONS" are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider "dead" volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. CALCULATION OF RESULTS

1. Calculate the mean optical density of each Calibrator duplicate.
2. Calculate the mean optical density of each unknown 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. If immunoassay software is being used, a 4-parameter curve is recommended.
5. In case of overflow absorbance values at 450 nm, read the results at 405-415 nm.
6. If a sample reads more than the value of Calibrator 5, then dilute it with Calibrator 0. The result obtained should be multiplied by the dilution factor.

Typical tabulated data (450 nm):

Calibrator	OD1	OD2	Mean OD-blank	Value (U/ml)
0	0.051	0.052	0	0
1	0.117	0.114	0.064	12.5
2	0.352	0.340	0.295	50
3	0.613	0.610	0.560	100
4	1.374	1.365	1.318	250
5	2.448	2.470	2.408	500
Unknown	0.295	0.278	0.235	40.7

This data 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.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Blank OD:** The absorbance value should not be more than 0.1 at 450 nm.
2. The absorbance (OD) of **Calibrator 5** should not be less than 1.3 at 450 nm.
3. Calculated Value of **Control Serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia CA 125

1. Assay Dynamic Range

The range of the assay is between 0-500 U/ml.

2. Analytical sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator 0 (based on 30 replicate analyses) plus 2 SD.

The sensitivity of the abia CA 125 kit does not exceed **1.0 U/ml.**

3. Specificity (cross reactivity)

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.

4. Precision

Intra-assay precision

The within assay variability is shown below:

Sample	n	Mean, U/ml	SD	CV,%
1	30	101.8	4.76	4.7
2	30	29.9	1.66	5.5

Inter-Assay precision

The between assay variability is shown below:

Sample	Mean, U/ml	SD	CV,%
1	26.3	1.76	6.7
2	126.0	8.95	6.6

5. Recovery

Samples were prepared by adding defined amount of CA 125 (Calibrator 2) to Control Serum. The results (in U/ml) are tabulated below:

Sample	Number of repeats	Measured Concentration, U/ml	Expected Concentration, U/ml	Recovery, %
Serum sample	9	45.2	-	-
Calibrator 2	3	51.0	-	-
Serum sample + Calibrator 2	9	50.3	48.1	104.6%

6. Linearity

Patient sample (H) was serially diluted with Calibrator 0 (L) and analyzed. The obtained values were 100±10% of the expected values.

Dilution	Expected concentration (U/ml)	Replicates	Mean measured concentration (U/ml)	% Recovered
L		3	0.00	
0.975L+0.025H	13.57	3	12.30	90.67
0.95L+0.05H	27.13	3	26.87	99.04
0.9L+0.1H	54.27	3	49.98	92.10
0.8L+0.2H	108.53	3	100.88	92.95
0.7L+0.3H	162.80	3	167.37	102.80
0.6L+0.4H	217.07	3	221.94	102.25
0.5L+0.5H	271.33	3	268.29	98.88
0.4L+0.6H	325.60	3	331.01	101.66
0.3L+0.7H	379.87	3	359.98	94.77
0.2L+0.8H	434.13	3	412.41	95.00
0.1L+0.9H	488.40	3	499.40	102.25
H		3	542.67	

7. Expected normal Value

CA 125 was measured in 132 healthy blood female donors. The median value was 7.71 U/ml, range 1.93-28.3 U/ml. 100% of the healthy women had assay values below 35 U/ml.

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

8. Accuracy

The abia CA 125 test system was compared with a reference assay "Roche ELEXIS CA 125". The total number of specimens was 228. The values ranged from 1.4 to 1001.4 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.

XIII. LIMITS OF THE TEST

- All the reagents within the kit are calibrated for the direct determination of CA 125 in human serum. The kit is not calibrated for the determination of CA 125 in saliva, plasma or other specimens of human or animal origin.
- Any improper handling of samples or modification of this test might influence the results.
- 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 kit 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.
- Some individuals may have heterophilic antibodies to mouse or other animal proteins that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.
- No hook effect was observed in this test.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

- **Expiry date is indicated on the packaging**.
  Storage and transportation conditions for the kit, conditions and terms of storage for working solutions and unused reagents are specified in table 2.
- Transportation should be done at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 2

1	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited.		
2	**Transportation conditions**		
	at 2-8 °C		
	at 9-20 °C	not more than during ten (10) days	
3	**Conditions and terms of storage for working solutions** Keep in a dark dry place and in a chemically neutral vial.		
	Working Washing Solution	at 2-8 °C	For up to 28 days
		at 18-24 °C	For up to 14 days
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C.		
	Anti-CA 125-coated microtiter wells	Place the unused strips/wells back into the bag, reseal the foil-lined package in Zip-Lock plastic bag. Do not remove desiccant.	Until the kit expiration date
	Washing Solution, Stopping Reagent	Close the vials tightly with screw caps and stored them in the manufacturer's package.	Until the kit expiration date
	Calibrators 0-5, Control Serum, Conjugate, TMB-Substrate	Close the vials tightly with screw caps and stored them in the manufacturer's package.	For two months

XV. GUARANTEE

- Manufacturer guarantees conformity of the product to the requirements of regulatory and technical documentation.
- Quality and safety of the kit is guaranteed within established shelf life.
- Please contact Manufacturer or Authorized Representative if you have any questions.

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XVI. REFERENCES

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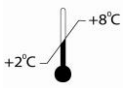
XVII. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)		Storage temperature limitation
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM	IVD	For in vitro diagnostic use
	Expiry date CCYY-MM-DD		Sufficient for
LOT	Batch code		Symbol “exclamation mark”
REF	Catalog number	**Warning!**	Signal word
	Fragile, handle with care		Symbol “corrosion”
	Keep away from sunlight	**Danger!**	Signal word
	Keep dry		Top

Annex

Scheme of the assay

1	**Add**	25 µl of Calibrators, Control Serum in duplicates; 25 µl of samples in duplicates; two wells for TMB control (blank)
2	**Add**	100 µl of Conjugate into each well, except blank
3	**Mix**	20 seconds
4	**Incubate**	90 min, at 20-25 °C
5	**Wash the plate**	**Working Washing Solution, 300 µl, 5 times**
6	**Add**	100 µl of TMB-Substrate into all wells
7	**Incubate**	20-30 min, at room temperature in a dark place
8	**Add**	150 µl of Stopping Reagent into all wells
9	**Mix**	5-10 seconds
10	**Read the optical density**	450 nm, in case overflow OD values – 405-415 nm