AB Diagnostic Systems

REF **DK.336.01.8** Σ **96** IVD **In vitro diagnostic medical device**

INSTRUCTIONS FOR USE
abia HBc Ab
Enzyme immunoassay for detection of total antibodies to Hepatitis B core antigen

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 strip) use of the kit or use of the kit on open type automated analyzer for enzyme immunoassay.

I. INTENDED USE

The abia HBc Ab kit is an enzyme immunoassay for the detection of total antibodies to Hepatitis B core antigen in human serum or plasma.

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

Detection of antibodies to Hepatitis B core antigen (HBc), in combination with antibodies against HBs is the most prevalent method of acute, chronic or past HBV infection diagnostics confirmation.

Antibodies against Hepatitis B core antigen appear at the onset of acute HBV infection, but may also be detectable at different stages of seroconversion and infection, indicating chronic HBV infection.

III. PRINCIPLE OF THE TEST

abia HBc Ab is a competitive solid-phase enzyme assay based on microwells coated with recombinant Hepatitis B core antigens. The conjugate contains HRP-labeled anti-HBc antibodies.

Serum or plasma samples and HRP-labeled anti-HBc antibodies (conjugate) are added to the wells. The specific anti-HBc antibodies present in a sample and the HRP-labeled anti-HBc antibodies (conjugate) compete on forming stable complexes with the HBc antigens immobilized on the wells.

The unbound components are removed by washing. After the addition of the solution containing TMB, the wells with bound conjugate develop a blue color, which is converted to yellow after the reaction has been stopped with sulfuric acid. The color intensity is inversely proportional to the concentration of antibodies to HBc antigen in the specimen and can be read at 450 nm or 450/620-680 nm filters.

IV. CONTENTS OF THE KIT ★

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
HBcore Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with purified recombinant Hepatitis B core antigen.	1 plate

Conjugate (concentrated 21-fold)	Antibodies against anti-HBcore, conjugated with HRP enzyme with addition of bovine serum albumin (1.7%) and Tween-20 (0.10%). Glycerol based solution. Transparent or slightly opalescent blue liquid. Preserving agents: 0.03% ProClin 300, 0.0009% gentamycin sulfate.	0.5 ml 1 vial
Positive Control	Control sample, containing anti-HBcore antibodies, with heat inactivated human plasma negative for anti-HIV-1,2 and anti-HCV. Transparent or slightly opalescent red liquid. Preserving agent: 0.02% gentamycin sulfate, 0.02% thimerosal.	2.0 ml 1 vial
Negative Control	Control sample with heat inactivated human plasma negative for anti-HBcore, anti-HIV-1,2, HBsAg and anti-HCV. Transparent or slightly opalescent green liquid. Preserving agent: 0.01% gentamycin sulfate, 0.01% thimerosal.	2.5 ml 1 vial
Washing Solution (concentrated 25-fold)	Phosphate saline buffer with Tween-20 (pH 7.4-7.7). Transparent or slightly opalescent liquid, colorless or pale yellow, sediment may form that dissolves at 35-39 °C and shaking.	50.0 ml 1 vial
Substrate Buffer	Citric acid (0.64%) solution, pH 4.1-4.3, containing H_2O_2 (0.02%). Transparent colorless liquid. Preserving agent: 0.04% ProClin 300.	25.0 ml 1 vial
TMB (concentrated 21-fold)	Solution containing 3,3',5,5'-Tetramethylbenzidine (TMB) (0.40%) and dimethyl sulfoxide (DMSO) (84.50%). Transparent colorless liquid.	2.5 ml 1 vial
Stopping Reagent	Sulfuric acid solution (H_2SO_4) 0.75M. Transparent colorless liquid.	25.0 ml 1 vial
Protective films for EIA plates		1
Polyethylene bag with a Zip-Lock		1
Disposable plastic dishes for liquid reagents		2
Disposable tips		16

V. PRECAUTIONS ★

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

- The temperature in the lab should be 18-25 °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 the kit reagents if the appearance of reagents is non-compliant (see section IV Content of the Kit).
- 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 purified 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's procedure.
- Use purified 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.
- Reagents are non-flammable. External packaging is not self-igniting, explosive.
- Do not misuse. Do not use for cosmetic and domestic purposes, do not eat.

VI. HEALTH AND SAFETY INSTRUCTIONS ★

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Positive Control and Negative Control were prepared using inactivated human origin material not containing anti-HCV and anti-HIV-1,2.
- 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 Substrate Buffer, the TMB 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.

Danger!

Stopping Reagent contains 0.75M sulfuric acid.
Hazard statements:
H314: Causes severe skin burns and eye damage.
Safety precautions:
P260: Do not breathe vapors.
P264: Wash hands/face thoroughly after handling
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331: IF SHALLOWED: Rinse mouth. DO NOT induce vomiting!
P303+P361+P353: IF ON SKIN: 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.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE TEST ★

- Purified 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.
- Microplate incubator at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm or with 450 nm and 620-680 nm filters.
- Open type automated analyzer with 450 nm or with 450 nm and 620-680 nm filters (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS ★

Blood samples should be collected according to the current practices. The test should be performed on undiluted serum or plasma. Separate serum from red cells or plasma from the clot as soon as possible, to avoid haemolysis. Extensive haemolysis may affect test performance. Samples with observable particulate matter should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield falsely positive results. Thermo inactivation of samples is not recommended as it leads to the false results.

The samples can be stored at 2-8 °C if screening is performed within 48 hours or they may be deep-frozen at -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrozen more than 2 times cannot be used. Samples with expressed bacterial growing, haemolysis, hyperlipidemia must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready for use reagents:

- **HBcore Coated Strips.** 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 polyethylene bag. Do not remove dessicant.
- **Negative Control;**
- **Positive Control;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Working Washing Solution.** Thoroughly mix the content of the vial with concentrated Washing Solution (concentrated 25-fold). Dilute the required volume of concentrated Washing Solution with corresponding volume of purified water (see Table 2). Mix the solution thoroughly.
- **Working Solution of Conjugate.** The Working Solution of Conjugate should be prepared before use. Add the required volume of Working Washing Solution in a clean container and add the corresponding volume of Conjugate concentrate thoroughly mixed (see Table 2). Mix thoroughly, avoiding foaming (intensive mixing is not allowed).
- **Substrate Mixture.** Dilute the required volume of TMB (concentrated 21-fold) with the corresponding volume of Substrate Buffer (see Table 2). Mix thoroughly.
  Substrate Mixture should be colorless!

The required volume of reagents for the certain number of strips or plate represented in the Table 2.

Table 2

Reagent preparation

Number of used strips	Working Washing Solution		Working Solution of Conjugate		Substrate Mixture	
	Washing Solution (concentrated 25-fold) (ml)	Purified water (ml)	Conjugate (concentrated 21-fold) (ml)	Working Washing Solution (ml)	TMB (concentrated 21-fold) (ml)	Substrate Buffer (ml)
1	3.0	72.0	0.025	0.5	0.05	1.0
2	6.0	144.0	0.050	1.0	0.10	2.0
3	9.0	216.0	0.075	1.5	0.15	3.0
4	12.0	288.0	0.100	2.0	0.20	4.0
5	15.0	360.0	0.125	2.5	0.25	5.0
6	18.0	432.0	0.150	3.0	0.30	6.0
7	21.0	504.0	0.175	3.5	0.35	7.0
8	24.0	576.0	0.200	4.0	0.40	8.0
9	27.0	648.0	0.225	4.5	0.45	9.0
10	30.0	720.0	0.250	5.0	0.50	10.0
11	33.0	792.0	0.275	5.5	0.55	11.0
12	40.0	960.0	0.400	8.0	0.65	13.0

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18-25 °C) for 30 min.

Step	The assay procedure
1	Do not wash the coated strips before the assay. Add 50 µl of Positive Control into 1 well, and 50 µl of Negative Control into 3 wells. Add 50 µl of undiluted samples into the rest of the wells.
2	Add 50 µl of Working Solution of Conjugate into all wells. Mix the contents of the wells by careful tapping on the edge of the plate. Cover the plate protective film.
3	Incubate for **60 min** in a microplate incubator at (37.0 ± 1.0) °C.
4	Aspirate the contents of the wells and wash the plate **4 times** with the Working Washing Solution. To each well add not less than 380 µl of Working Washing Solution, wait for 40 seconds and remove Working Washing Solution into the container with disinfecting solution. Do not leave any liquid in the wells. It is strongly recommended that an automatic microplate washer should be used. Incomplete washing will adversely affect the assay precision.
5	Add 100 µl of Substrate Mixture to all the wells of the plate.
6	Incubate at 18-25 °C for a **30 min** in a dark place.
7	Add 50 µl of Stopping Reagent into each well to stop the reaction. Read the optical density at 450 nm with 620-680 nm as reference. Reading the absorbance at 450 nm only is possible.

Scheme of the assay is represented in Annex.

Automated analyzer

For automated test procedure, it is advisable to use protocol submitted by the manufacturer. When creating the protocol independently, follow the procedure specified in section X. TEST PROCEDURE, and comply with the requirements provided in section V. PRECAUTIONS.

When preparing working solutions of reagents for the automated test procedure, dead volume of vials or containers used to place the solutions onboard should be taken into account.

Validated test protocols and dilution tables of working solutions for different models of EIA analyzers can be obtained upon request from the manufacturer (see section XV.).

XI. RESULTS ★

The presence or absence of antibodies to Hepatitis B core antigen is determined by comparing the absorbance measured for each sample to the calculated Cut-Off value.

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. If obtained repeat results do not meet the criteria, please contact the manufacturer.

1. **Positive Control:** the absorbance value should not be more than 0.200.
2. **Negative Control:** the absorbance value should not be less than 1.400.

Calculate Cut-Off value as:

Cut-Off = (average OD value of Negative Control x 0.5) – 0.500,

where **0.5** and **0.500** – are a coefficients defined by manufacturer during statistical processing.

Negative: if the OD value is > Cut-Off
Positive: if the OD value is ≤ Cut-Off

XII. PERFORMANCE CHARACTERISTICS ★

1. Equivalence of serum and plasma

Studies of anti-HBc positive (n=25) and negative (n=25) paired samples of human serum and plasma containing different anticoagulants (EDTA, heparin, sodium citrate) demonstrated their equivalence, which allows us to apply the parameters of diagnostic sensitivity and specificity to both types of samples.

2. Precision

Repeatability and Reproducibility

Within-run, between-run, between-days, between-operator (laboratory) precision of two lots were evaluated by testing 3 positive samples 2 times each during 20 days in two laboratories. The variation coefficient did not exceed 10%.

Multisite precision (between days, between-laboratories) of one lot was evaluated by testing 3 positive samples and 2 negative samples 5 times each during 5 days in three laboratories. The variation coefficient did not exceed 10%.

3. Analytical sensitivity

The analytical sensitivity of abia HBc Ab, determined using the WHO First International Standard for anti-Hepatitis B core antigen (anti-HBc), NIBSC code 95/522, is ≤ 1.0 IU/ml.

4. Analytical specificity

Endogenous interfering substances

Samples, containing up to 0.2 mg/ml bilirubin, lipemic samples containing up to 30 mg/ml intralipid, haemolysed samples containing up to 5 mg/ml hemoglobin and samples, containing up to 60 mg/ml human albumin do not affect the results.

Cross reactivity

No cross-reactivity was detected when testing 106 serum and plasma samples from patients with infectious diseases caused by the following pathogens: HIV, hepatitis C, E viruses, Treponema pallidum, herpes simplex virus types 1 and 2, cytomegalovirus, Epstein–Barr virus, rubella virus in the abia HBc Ab kit.

Influence of physiological and clinical conditions

No influence of the tested physiological or clinical conditions is observed. Testing of 179 serum samples from pregnant women and hospitalised patients shows qualitative results concordant with the comparator method.

7. Diagnostic sensitivity

Testing of clinical samples

A total of 193 samples are tested using the abia HBc Ab assay, including 60 samples from acute HBV cases, 66 from recovered individuals, 2 from chronic HBV cases, and 65 samples with an indeterminate status. Comparison with the CE-marked comparator assay shows concordant results, with a positive percent agreement (PPA) of 99.0% (95% CI: 96.3–99.7%).

Testing of performance panels

Sensitivity was assessed using 2 BBI Diagnostics performance panels:

- "Anti-HBc IgM Mixed Titer Performance Panel" PHE203;
- "Anti-HBc/HBs Mixed Titer Performance Panel (Modified)" PHG203 (M).

The results of the abia HBc Ab assay were compared with data from comparator assays provided in the panel package inserts and are summarized in the table below.

Table 3

Panel ID	Tested samples	Reactive	Relative sensitivity to comparator assays
PHE203	25	24	100%
PHG203 (M)	13	12	100%

Testing of seroconversion panels

Seroconversion sensitivity was assessed using 4 commercially available seroconversion panels. The results of the abia HBc Ab assay were compared with those of comparator assays and are summarized in the tables below.

Table 4

Panel ID	abia HBc Ab		Comparator assay #1		Comparator assay #2	
	Reactive on bleed	Day of first reactivity	Reactive on bleed	Day of first reactivity	Reactive on bleed	Day of first reactivity
HBV 6278	10	37	11	41	10	37
HBV 6281	9	41	9	41	9	41

Table 5

Panel ID	abia HBc Ab		Comparator assay #1		Comparator assay #3	
	Reactive on bleed	Day of first reactivity	Reactive on bleed	Day of first reactivity	Reactive on bleed	Day of first reactivity
HBV 9092	18	78	ND	ND	19	85
HBV 9093	11	49	11	49	10	42

8. Diagnostic specificity

A total of 972 samples are evaluated, including 793 serum samples from blood donors, 90 samples from hospitalised patients, and 89 samples from pregnant women. Of these, 73 samples classified as positive by the abia HBc Ab assay are concordant with the results of the CE-marked comparator assay. Samples with a final anti-HBc–positive status are excluded from the specificity analysis.

Testing of 740 serum samples from blood donors shows a negative percent agreement (NPA) of 99.7% (95% CI: 99.0%–99.9%).

The total negative percent agreement across all 899 negative samples is 99.8% (95% CI: 99.2%–99.9%).

XIII. LIMITS OF THE TEST ★

- In the early stage of infection, anti-HBc antibody levels may be below the detection limit of the assay; therefore, a non-reactive result does not exclude HBV infection.
- The presence of anti-HBc antibodies alone is not sufficient for diagnosis. Results should be interpreted in conjunction with other hepatitis B virus markers and clinical information.

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 6.
- 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 6

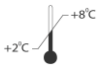
1	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited.		
2	**Transportation conditions**		
	at 2-8 °C		
	at 9-25 °C	not more than during ten (10) days	
3	**Conditions and terms of storage for working solutions** Keep in a dark dry place and in a chemically neutral vial or in reagent container used in open automatic EIA analyzer		
	Working Washing Solution	at 2-30 °C	For up to 42 days
	Working Solution of Conjugate	at 18-25 °C	For up to 12 hours
	Substrate Mixture	at 18-25 °C	For up to 10 hours
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C.		
	HBcore Coated Strips	Return unused strips/wells to the foil bag and reseal in a plastic Zip-lock bag.	Until expiration date of the kit
	Conjugate, Positive Control, Negative Control, Washing Solution, Substrate Buffer, TMB, Stopping Reagent	Close the vials tightly with screw caps and store them in the original packaging.	Until expiration date of the kit

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 if you have any questions.
- Any serious incident occurred in relation to the kit shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

AB Diagnostic Systems GmbH
Sportfliegerstraße 4, Berlin, 12487, Germany
Tel. +49 30 208987160, Fax: +49 30 208987199
E-Mail: info@ab-ds.de, www.ab-ds.de

XVI. EXPLANATION OF SYMBOLS ★

	Manufacturer		
	Date of manufacture YYYY-MM		Keep dry
	Expiry date YYYY-MM-DD		Temperature limit
	Batch code		Consult Instruction for use
	Catalogue number	**Danger!**	Signal word
	Do not use if package is damaged and consult instructions for use		Symbol “corrosion”
	Fragile, handle with care		In vitro diagnostic medical device
	Top		Contains sufficient for <n> tests
	Keep away from sunlight	★	Changes highlighted

Annex

Scheme of the assay

1	**Add**	50 µl of Positive Control into 1 well, Negative Control into 3 wells
2	**Add**	50 µl of samples
3	**Add**	50 µl of Working Solution of Conjugate into all wells
4	**Incubate**	60 min, (37.0 ± 1.0) °C, microplate incubator
5	**Wash the plate**	**Working Washing Solution, not less than 380 µl, 4 times**
6	**Add**	100 µl of Substrate Mixture
7	**Incubate**	30 min, 18-25 °C in a dark place
8	**Add**	50 µl of Stopping Reagent
9	**Read the optical density**	450 nm/620-680 nm or 450 nm