AB Diagnostic Systems

REF **DK.075.01.8** Σ **96**

REF **DK.075.05.8** Σ **480**

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

INSTRUCTIONS FOR USE
abia HIV AgAb express
Enzyme immunoassay for simultaneous detection of antibodies to human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2), HIV-1 group O and p24 HIV-1 antigen

The kit is available in two Formats[1]:

Format 2.1 contains sufficient reagents for 96 assays (one breakable plate), including controls, and are intended for both manual procedure and for automated ELISA open automated analyzers with a possible fractional use.

Format 2.2 contains sufficient reagents for 480 assays (five breakable plates), including controls; the kit is intended for manual testing with a possibility of fractional use of the kit or for the simultaneous 480 assays (96x5) on the open automated analyzers.

[1]*All formats are equivalent in their intended use, properties and performance.*

I. INTENDED USE

The abia HIV AgAb express kit intended for the simultaneous detection of antibodies to HIV-1, HIV-2, HIV-1 group O and p24 HIV-1 antigen in human serum or plasma.

Acquired immunodeficiency syndrome (AIDS) is a viral disease characterized by a weakening of the immune system.

Two types of viruses related to the Lentivirus group have been isolated from lymphocytes of patients suffering from AIDS or its early symptoms. The first one, named HIV-1, was isolated in France then in the United States. The second one, named HIV-2 was isolated from two patients living in Africa and has proved to be responsible for a new AIDS focus in West Africa. The HIV-1 viruses are divided into two groups: the M group, including 9 sub-types (A to I) and the O group. The HIV-2 virus includes five sub-types. Some HIV-1 variants have only 70% homology for the GAG and POL genes with the main isolates and only 50% for the ENV gene; these differences can explain the failure of the diagnosis of infection in some patients. The various HIV-2 isolates have less than 40% homology with the HIV-1 envelope proteins.

HIV antigens and antibodies appear and are detectable at different stages of seroconversion and of the infection. The usage of a kit for the simultaneous detection of antibodies and p24 HIV-1 Ag allows reducing the “window” period due to the detection of p24 HIV-1 antigen that is an early marker of the HIV infection. The detection of the HIV infection at early stage enables early consultation, specific antiretroviral treatment and timely epidemic control measures.

II. PRINCIPLE OF THE TEST

The abia HIV AgAb express kit is a solid-phase “sandwich” assay. The solid phase is coated with antibodies to HIV-1 p24 Ag and recombinant antigens identical to HIV-1, HIV-2 and HIV-1 group O proteins.

At the first stage, samples to be assayed and controls are incubated with Conjugate-1 (a mixture of biotinylated antibodies to p24 HIV-1 Ag and recombinant antigens of HIV-1, HIV-2 and HIV-1 group O). If present in the sample, anti-HIV simultaneously bind to HIV-1/2 and HIV-1 group O antigens contained in HIV AgAb Coated Strips and Conjugate-1, and p24 HIV-1 Ag, present in the sample, forms an immune complex with the antibodies bound to the solid phase and Conjugate-1.

At the second stage, after washing, the complexes formed are detected by horseradish peroxidase (HRP) streptavidin conjugate and HRP-labeled recombinant antigens of HIV-1/2. The unbound components are removed by washing. The peroxidase reacts with the substrate mixture by a change of color. Color intensity developed after the addition of Stopping Reagent is proportional to the quantity of HIV-1/2 antibodies or p24 HIV-1 Ag in the sample.

III. CONTENTS OF THE abia HIV AgAb express

NATURE OF THE REAGENTS		PRESENTATION	
		Format 2.1 Σ **96**	**Format 2.2** Σ **480**
HIV AGAB Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with mixture of recombinant antigens identical to HIV-1, HIV-2 and HIV-1 group O proteins, and antibodies to p24 HIV-1 antigen. Store at 2-8 °C until expiration date.	1 plate	5 plates
Conjugate-1	Mixture of biotinylated recombinant antigens of HIV-1, HIV-1 group O, HIV-2 and antibodies to p24 HIV-1 Ag. Transparent or slightly opalescent, crimson-red liquid. Preserving agent: 0.19% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 5.0 ml	1 vial 20.0 ml or 3 vials 8.0 ml or 5 vials 5.0 ml
Conjugate-2	Peroxidase labelled streptavidin and recombinant antigens of HIV-1/HIV-2. Transparent or slightly opalescent, violet liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml	3 vials 23.0 ml or 5 vials 12.0 ml
AB Positive Control, Inactivated	Control sample positive for HIV antibodies. Transparent or slightly opalescent, orange liquid. Preserving agent: 0.10% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.0 ml	1 vial 4.0 ml or 2 vials 2.0 ml
AG Positive Control, Inactivated	Control sample positive for recombinant p24 HIV-1 Ag. Transparent or slightly opalescent, red liquid. Preserving agent: 0.10% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.0 ml	1 vial 4.0 ml or 2 vials 2.0 ml
Negative Control, Inactivated	Control sample negative for HIV antibodies and p24 HIV-1 antigen. Transparent or slightly opalescent, green liquid. Preserving agent: 0.04% thimerosal; 0.02% sodium azide; 0.01% gentamycin sulfate. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml	1 vial 5.0 ml or 2 vials 2.5 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7). Contains Tween-20 (2.8%). 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	2 vials 120.0 ml
Stopping Reagent	Sulfuric acid solution (H_2SO_4) 0.2M. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml	2 vials 50.0 ml or 4 vials 25.0 ml

TMB-Substrate	Tetramethylbenzidine in citric acid buffer, containing H_2O_2. Transparent colorless liquid, coloration is possible. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml	3 vials 23.0 ml or 5 vials 12.0 ml
Disposable tips		16	80
Disposable plastic dishes for liquid reagents		2	10
Protective films for EIA plates		2	10

Accessories (upon request):

Name	PRESENTATION	
	Format 2.1 Σ **96**	**Format 2.2** Σ **480**
Polyethylene bag with a Zip-Lock	1	3

IV. PRECAUTIONS

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

- The temperature in the laboratory should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. If labels are lost or labels/vials are damaged, 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 avoid any contamination.
- Do not carry out the test in the presence of reactive vapours (acid, alkaline, aldehyde vapours) 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 metallic ions. Consequently, do not allow any metal element to come into contact with various conjugate or substrate solutions.
- Do not reuse HIV AGAB Coated Strips.
- Do not reuse protective films for EIA plates.
- Once the assay has been started, all subsequent steps should be performed without interruption.
- Use a new distribution tip for each specimen.
- 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.
- 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.

V. 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 the Negative Control has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), p24 HIV-1 antigen, antibodies to HIV-1,2 and antibodies to hepatitis C virus.
- Human origin material used in the preparation of the AB Positive Control has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), p24 HIV-1 antigen and antibodies to hepatitis C virus.
- Human origin material used in the preparation of the AG Positive Control has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), p24 antigen HIV-1, antibodies to HIV-1,2 and antibodies to hepatitis C virus.
- 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 specimens and thoroughly wash your hands after handling them.
- Avoid spilling specimens or solutions containing specimens. Wipe spills immediately and decontaminate affected surfaces.
- 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-1, AB Positive Control, AG Positive Control contain ProClin 300.
H317: May cause an allergic skin reaction.
P261: Avoid breathing vapors.
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.

VI. 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;
- Microplate incubator or shaker thermostatically set at (37.0 ± 1.0) °C;
- Automatic microplate washer;
- Microplate reader equipped with 450 nm or 450 nm and 620-680 nm filters;
- Any model of automated open automated analyzer (for automated procedure);
- Laboratory clock.

VII. COLLECTING AND HANDLING OF SAMPLES

Blood samples should be collected according to the current practices. Use only native (undiluted) human serum or plasma (heparin, EDTA, citrate) for the assay. Separate the serum or plasma from the clot or red cells as soon as possible to avoid any haemolysis. Samples with observable particulate matter should be clarified by centrifugation.

Samples with expressed bacterial growing, haemolysis, hyperlipidemia should not be tested. Samples containing up to 10 mg/L bilirubin, up to 10 g/L hemoglobin, lipemic samples containing up to 25 g/L equivalent of triglyceride do not affect the results and can be assayed.

Store/transport samples as requested by the current regulations. Long storage is acceptable at temperatures not exceeding -18 °C (not more than 3 cycles of freeze/thaw).

VIII. PREPARATION OF REAGENTS

1. Ready-to-use reagents:

- **HIV AGAB Coated Strips.** Each 12-strips plate (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 a Zip-Lock polyethylene bag. Do not remove desiccant;
- **Conjugate-1;**
- **Conjugate-2;**
- **AB Positive Control;**
- **AG Positive Control;**
- **Negative Control;**
- **Stopping Reagent (0.2M);**
- **TMB-Substrate.**

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.
  The required volumes of Working Washing Solution for the certain number of strips or plate are tabulated in Table 1.

Table 1

Reagent preparation

Number of strips to be used		1	2	3	4	5	6	7	8	9	10	11	12
Working Washing Solution	Washing Solution (×25), 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
	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

IX. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18 – 24 °C) for 30 min.

Attention! Incubation is possible as two alternative procedures. It is very important that the following assay step should be carried out in the same incubation mode. The combination of incubation modes is not supposed.

9.1. Manual test:

<table>
<tr><th rowspan="2">Step</th><th colspan="2">Test procedure</th></tr>
<tr><th>Procedure 1</th><th>Procedure 2</th></tr>
<tr><td>1</td><td colspan="2">Add 30 µl of Conjugate-1 in each well. Conjugate-1 should be added to the wells immediately before dispensing controls and samples. Conjugate-1 may change its color.</td></tr>
<tr><td>2</td><td colspan="2">Add 70 µl of Controls per well as follows:
1-2 strips– AB Positive Control into 1 well, AG Positive Control into 1 well, Negative Control into 2 wells;
3 strips and more– AB Positive Control into 1 well, AG Positive Control into 1 well, Negative Control into 3 wells.
Add 70 µl of undiluted samples into the rest of the wells. Color of Conjugate-1 should change after the samples have been added. The time of dispensing samples should not exceed 15 min.</td></tr>
<tr><td rowspan="2">3</td><td rowspan="2">Incubate for 30 min in a thermoshaker at 500 rpm at (37.0 ± 1.0) °C.</td><td>Mix by careful tapping on the edge of the plate.</td></tr>
<tr><td>Cover the plate with a protective film and incubate in a microplate incubator for 60 min at (37.0 ± 1.0) °C.</td></tr>
<tr><td>4</td><td colspan="2">Remove the content of the wells into the container with disinfectant solution and wash the plate one time with the Working Washing Solution. To each well add not less than 360 µl of Working Washing Solution and remove Working Washing Solution into the container with disinfecting solution.
Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision.</td></tr>
<tr><td>5</td><td colspan="2">Add 100 µl of Conjugate-2 into each well.</td></tr>
<tr><td>6</td><td>Incubate for 10 min in a thermoshaker at 500 rpm at (37.0 ± 1.0) °C.</td><td>Cover the plate with a protective film and incubate in a microplate incubator for 10 min at (37.0 ± 1.0) °C.</td></tr>
<tr><td>7</td><td colspan="2">Remove the contents of the wells and wash the plate 6 times with Working Washing Solution as described in step 4.</td></tr>
<tr><td>8</td><td colspan="2">Add 100 µl of TMB-Substrate into each well.</td></tr>
<tr><td>9</td><td colspan="2">Incubate the plate for 10 min in a dark place at (18-24) ºC or at (37.0 ± 1.0) ºC.</td></tr>
<tr><td>10</td><td colspan="2">Add 150 µl of Stopping Reagent into each well and read the absorbance after 2-3 min at 450/620-680 nm. Reading the absorbance at 450 nm only is possible.
Results can be read within 15 min.</td></tr>
</table>

Scheme of the assay is represented in Annex.

9.2. Automated test procedure

For automated test procedure, using a protocol submitted by the manufacturer is recommended. When creating the protocol independently, follow the procedure specified in section IX. TEST PROCEDURE, and comply with the requirements provided in sections IV. PRECAUTIONS, V. HEALTH AND SAFETY INSTRUCTIONS.

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.

Fractional use is acceptable in no more than 3 assays for Format 2.1. For Format 2.2 plate fractional use is not possible.

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

Time needed to run an assay with two abia HIV AgAb express plates on EVOLIS is 2 h 07 min (Procedure 1).

Spectrophotometric verification of reagent pipetting on EIA analyzers is possible. For the verification criteria, contact the manufacturer.

X. RESULTS

The presence or absence of detectable HIV-1 p24 antigen or antibodies to HIV-1/2 is determined by comparing the absorbance measured for each sample to the calculated Cut-Off value.

For the assay to be valid, mean OD (optical density) in two or three wells with Negative Control (average OD of NC) should be not greater than 0.200 and in wells with AB Positive Control and AG Positive Control – not less than 0.800.

Calculate the Cut-Off value:

Cut-Off = average OD value of Negative Control + A

(**$A_{(Procedure\ 1)}$ = 0.150**, **$A_{(Procedure\ 2)}$ = 0.250**),

where **A** is a coefficient defined by the manufacturer by statistical processing. The coefficient may be different for each assay procedure (thermoshaker and microplate incubator).

Samples should be deemed positive: if OD ≥ Cut-Off.
Samples should be deemed negative: if OD < Cut-Off.

Samples with OD values that are equal to or more than Cut-Off should be re-tested in two wells. If a positive result is obtained for at least one of the two wells, the samples should be regarded as positive.

XI. PERFORMANCE CHARACTERISTICS

11.1. Analytical sensitivity

The sensitivity of the abia HIV AgAb express kit to detect p24 HIV-1 Ag has been determined by using different concentration levels of HIV-1 p24 ANTIGEN 1st International Reference Reagent (NIBSC Code: 90/636), "Standard HIV-1 AG p24(+)" (Medical Biological Union Ltd, Russia). The least detectable concentration of p24 HIV-1 Ag is ≤ 20 pg/ml for both procedures.

11.2. Diagnostic sensitivity

Diagnostic sensitivity of the abia HIV AgAb express was evaluated by testing:

- **Confirmed HIV-positive samples**
  Sensitivity was determined on:
  - 966 confirmed HIV-1 human serum (plasma), sensitivity was 100% (95% CI: 99.6-100.0%) (966/966);
  - 13 confirmed HIV-2 human serum (plasma), sensitivity was 100% (95% CI: 77.19-100.0%) (13/13);
  - 8 confirmed serum (plasma) samples from patients positive for antibodies to HIV-1 group O, sensitivity was 100% (95% CI: 67.6-100.0%) (8/8);

– 20 HIV-1 group M positive samples, different subtypes (A, B, C, D, E, F, G), and was 100% (95% CI: 83.89-100.0%) (20/20).

- **Fresh native samples**
  – 47 serum (plasma) samples with the confirmed presence of anti-HIV-1 were assayed on the day of bleed (fresh samples), sensitivity was 100% (95% CI: 92.44-100.0%) (47/47).
- **Commercial seroconversion panels**

The ability of the assay to diagnose early HIV infection was determined on 14 commercial seroconversion panels (BBI and ZeptoMetrix, USA). The results of testing are provided in Table 2. The abia HIV AgAb express kit determined 79 samples as positive out of 162 samples. The 4th generation reference assays identified 75 samples as positive out of 162. The abia HIV AgAb express identified HIV infection on average on day 37.4 after the first bleed (for this set of panels), that is 4 days later than PCR test and 8 days earlier than by kits designed only for the detection of HIV antibodies. The 4th generation assays detected HIV markers on average on day 39 after the first bleed. It should be noted that HIV markers were detected simultaneously by the abia HIV AgAb express kit and a PCR on three seroconconversion panels (ZMC - HIV9077, ZMC - HIV9079, BBI - PRB933).

Table 2

Panel	**abia HIV AgAb express**	**4th generation HIV AgAb assays**
	Qty of positive results / qty of samples assayed	
BBI - PRB914	5/5	5/5
BBI - PRB926	4/6	4/6
BBI - PRB933	2/3	2/3
BBI - PRB944	5/6	4/6
BBI - PRB955	4/5	4/5
BBI - PRB965	5/6	5/6
BBI - PRB966	3/10	3/10
BBI - PRB968	4/10	4/10
BBI - PRB969	5/10	3/10
ZMC - HIV9021	4/17	4/17
ZMC - HIV9030	3/16	3/16
ZMC - HIV9031	5/19	4/19
ZMC - HIV9077	13/24	13/24
ZMC - HIV9079	17/25	17/25
Total	**79/162**	**75/162**

11.3. Sensitivity of the abia HIV AgAb express kit was additionally determined by testing:

- "Standard HIV-1 AB(+)" Standard biological material positive for antibodies to human immunodeficiency virus of type 1 (HIV-1) in different concentrations, and "Standard HIV-2 AB(+)" Standard biological material positive for antibodies to human immunodeficiency virus of type 2 (HIV-2) (RPC "Diagnostic Systems" Ltd, Russia). Sensitivity was 100%.
- Performance panel of human sera containing anti-HIV-1 antibodies "Standard AB (+) HIV-1" and performance panel of human sera containing anti-HIV-2 antibodies "Standard AB (+)HIV-2" (Medical Biological Union Ltd., Russia). Sensitivity was 100%.

- Commercial panels: 1st International Reference Panel for anti-HIV (NIBSC, UK, code: 02/210), Anti-HIV 1 Mixed Titer Performance Panel PRB205(M) (BBI, USA), Anti-HIV-1 Low Titer Performance Panel Modified PRB 109(M) (BBI, USA), HIV p24 Antigen Mixed Titer Performance Panel Modified PRB 204(M) (BBI, USA), HIV 1 Incidence/Prevalence Performance panel, PRB601 (BBI, USA), Mixed Subtype Panel MSP-HIV-001 (BioMex, Germany). Sensitivity of the kit on these panels was 100%.
- Commercial panel - HIV Subtype Infectivity Panel PRD320 (BBI, USA), subtypes of cell culture supernatants: A, B, C, D, F, G, H HIV-1 group M, HIV-1 group O. Sensitivity of the kit on the panel samples was 100%.

11.4. Diagnostic specificity

Diagnostic specificity of the abia HIV AgAb express kit was determined by testing:

- 5175 unselected donor serum (plasma) samples and was 99.85% (95% CI: 99.7-99.92%);
- 538 serum (plasma) samples from individuals with various somatic diseases and was 99.81% (95% CI: 98.95-99.97%);
- 863 cross-reacting serum (plasma) samples (pregnant women, samples positive for rheumatoid factor, serum positive for antibodies to Hepatitis A, B, C, flu, herpes simplex virus, cytomegalovirus, *Treponema pallidum*, *Chlamydia trachomatis*) and was 99.9% (95% CI: 99.35-99.98%);
- "Standard HIV-1,2 AB(-), HIV-1 AG p24(-)" Standard panel of samples negative for antibodies to human immunodeficiency viruses of 1 and 2 types (HIV-1 and HIV-2) and antigen p24 HIV-1 (RPC "Diagnostic Systems" Ltd, Russia); specificity was 100%;
- Performance panel of human sera negative for anti-HIV-1 and anti-HIV-2 antibodies and p24 HIV-1 antigen "Standard AB(-) HIV" (Medical Biological Union Ltd., Russia), specificity was 100%.

If the procedure of obtaining and preparing samples to be assayed is not compliant with the requirements described in section VII of the IFU, specificity values may differ.

11.5. Precision

- The coefficient of variation did not exceed 10%.
- A multiple study of three samples with the absorbance close to Cut-Off was carried out to analyze risks of false negative results when retesting weak positives. A stable result with a 92.6% probability was obtained when testing samples with OD = ±20% from Cut-off in the abia HIV AgAb express kit.

11.6. Equivalence of human serum and plasma

Studies of HIV positive (n=25) and negative (n=25) paired human serum and plasma demonstrated their equivalence. Therefore, the parameters of diagnostic sensitivity and specificity can apply to both types of samples.

XII. LIMITS OF THE TEST

- At the early stage of the disease, the concentration of p24 HIV-1 antigen and anti-HIV can be below the limit of detection of the assay. A negative result caused by non-detectable concentration of HIV markers does not exclude the infection.

- Variability of HIV-1 (Groups M, O) and HIV-2 does not exclude false negative results. No method can guarantee that the human immunodeficiency virus is absent in the sample.
- Highly sensitive EIA assays may cause false positives. All samples positive with abia HIV AgAb express should be retested in confirmatory assays (p24 HIV-1 neutralization test and immunoblotting to confirm the presence of antibodies).

XIII. SHELF LIFE. CONDITIONS OF STORAGE AND TRANSPORTATION

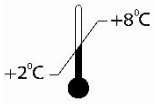
13.1	**Expiry date is indicated on the packaging. Do not use expired kits.**		
13.2	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited. Do not use kits that have been stored not respecting the storage requirements.		
13.3	**Transportation conditions**		
	at 2 to 8 °C		
	at 9 to 24 °C	not more than during ten (10) days	
	at 26 to 30 °C	not more than during five (5) days	
13.4	**Conditions and terms of storage for working solutions** *(Keep in a dark dry place or in a special container intended for the automated test procedure)*		
	Working Washing Solution	at 2 to 8 °C	For up to 28 days
		at 18 to 24 °C	For up to 14 days
13.5	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited.		
	HIV AGAB Coated Strips	Place the unused strips/wells back into the bag, reseal the foil-lined package in Zip-Lock polyethylene bag. Do not remove desiccant	Until the kit expiration date
	Conjugate-1, Conjugate-2, AB Positive Control, AG Positive Control, Negative Control, Washing Solution, Stopping Reagent, TMB-Substrate	Close the vials tightly with screw caps and store them in the manufacturer's package.	Until the kit expiration date

XIV. 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.

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

XV. EXPLANATION OF SYMBOLS

	Manufacturer		Storage temperature limitation
	Date of manufacture CCYY-MM		Consult Instruction for use
	Expiry date CCYY-MM-DD		For *in vitro* diagnostic use
	Batch code		Sufficient for
	Catalog number		Symbol “exclamation mark”
	Do not use if package is damaged	**Warning!**	Signal word
	Fragile, handle with care		Symbol “corrosion”
	Keep away from sunlight	**Danger!**	Signal word
	Keep dry		Top

SCHEME OF THE ASSAY

Add	30 µl of Conjugate-1
Add	70 µl of AG Positive Control, AB Positive Control, Negative Control
Add	70 µl of samples
Incubate	**Procedure 1** 30 min, (37.0 ± 1.0) °C, 500 rpm, thermoshaker **Procedure 2** 60 min, (37.0 ± 1.0) °C, microplate incubator
Wash the plate	**Working Washing Solution,** at least 360 µl, **1 time**
Add	100 µl of Conjugate-2
Incubate	**Procedure 1** 10 min, (37.0 ± 1.0) °C, 500 rpm, thermoshaker **Procedure 2** 10 min, (37.0 ± 1.0) °C, microplate incubator
Wash the plate	**Working Washing Solution,** at least 360 µl, **6 times**
Add	100 µl of TMB-Substrate
Incubate	10 min at 18-24 °C or at (37.0 ± 1.0) °C in a dark place
Add	150 µl of Stopping Reagent
Read the optical density	450 nm/620-680 nm or 450 nm