AB Diagnostic Systems

REF **DK.049.01.9** Σ **96**

CE

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

INSTRUCTIONS FOR USE
abia HSV-1/2 IgG
Enzyme immunoassay
for the detection of IgG antibodies to
Herpes simplex virus
types 1 and 2 in human serum or plasma

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

I. INTENDED USE

The abia HSV-1/2 IgG kit is intended for the detection of IgG antibodies to *Herpes simplex virus* type 1 and 2 in human serum (plasma) by a microplate enzyme immunoassay.

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

Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) are two members of the alpha-Herpesviridae family that infect humans causing blisters in the skin or mucous membranes of the mouth, lips or genitals. HSV-1 is a highly contagious infection, which is common and endemic throughout the world. Most HSV-1 infections are acquired during childhood, and infection is lifelong. The vast majority of HSV-1 infections are oral herpes. HSV-2 infection is widespread throughout the world and is almost exclusively sexually transmitted, causing genital herpes. Infection with HSV-2 is lifelong and incurable [1].

Neonatal herpes is a very rare but serious viral infection with a high morbidity and mortality. Neonatal herpes may be caused by HSV-1 or HSV-2. Neonatal herpes occurs as the result of an infection at the time of birth. The risks are greatest when a woman acquires a new infection (primary genital herpes) in the third trimester, especially for 6 weeks before delivery [2].

Testing for HSV type-specific IgG antibodies can be used to diagnose HSV infection. The detection of HSV-1 IgG or HSV-2 IgG or both in a single sample represents HSV at some time [3].

III. PRINCIPLE OF THE TEST

The principle of the test is an indirect two-stage immunoassay. Microtiter strip wells precoated with the recombinant antigens of HSV-1,2 to bind corresponding antibodies. The antigen-antibody complex reacted with HRP-labeled anti-human-IgG antibodies. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in the TMB-Substrate. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The color intensity of the test sample is directly proportional to the concentration of anti-HSV-1,2 IgG.

IV. CONTENT OF THE KIT abia HSV-1/2 IgG

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
HSV-1,2-Ag Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with a mix of recombinant proteins, which represent the recombinant analogs of HSV-1,2 antigens. Store at 2-8 °C until expiration date.	1 plate
Conjugate	Antibodies against human IgG, conjugated with HRP enzyme with addition of 1M Tris HCl buffer (pH 7.4-7.6), Tween®20 (0.10%) and bovine serum albumin (3.81%). Preserving agent: 0.10% ProClin 300, 0.004% gentamicin sulfate, 0.10% phenol. Transparent or slightly opalescent yellow colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Positive Control, Inactivated	Control sample, containing IgG antibodies to HSV-1,2. Preserving agent: 0.20% sodium azide, 0.04% ProClin 300. Transparent or slightly opalescent red colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.2 ml
Negative Control, Inactivated	Control sample, not containing IgG antibodies to HSV-1,2. Preserving agent: 0.20% sodium azide, 0.04% ProClin 300, 0.001% gentamicin sulfate. Transparent or slightly opalescent green colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml
Preliminary Sample Diluent	Sample buffer that is used for preliminary dilution of samples. Preserving agent: 0.09% sodium azide. Transparent or slightly opalescent violet-blue colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Sample Diluent	Sample buffer that is used to dilute samples before analysis. Preserving agent: 0.10% sodium azide, 0.006% thimerosal. Transparent or slightly opalescent pale pink colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7). Transparent or slightly opalescent colorless or light yellow liquid, sediment may form that dissolves completely 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 in citric acid buffer, containing H_2O_2. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 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
Plate for preliminary dilution of sera	Polystyrene plate with transparent wells.	1 plate
Protective films for EIA plates		2
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 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. If labels are lost 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.
- 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's procedure.
- Use high quality water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

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 Positive Control and Negative Control has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2), antigen p24 HIV-1.
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious 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 contains 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!

Positive Control, Negative Control and Sample Diluent contain sodium azide.
H312: Harmful in contact with skin.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 IF ON SKIN: Wash with plenty of soap and water.
P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Danger!

Stopping Reagent contains 0.2 M sulphuric 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.
- Microplate incubator at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm or with 450 and 620-680 nm filters.
- Laboratory clock.
- Open type automated analyzer with 450 nm or with 450 and 620-680 nm filters (for automated procedure).

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collection of blood samples should be implemented according to the current practices. Serum, plasma (citrate, heparin, EDTA) may be used. Separate serum or plasma from blood cells 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. The samples after heat inactivation cannot be analysed.

Samples can be stored at 2-8 °C not more than for 48 hours; they may be deep-frozen at -20 °C. Samples that have been frozen and defrosted more than one time cannot be used. Samples with expressed bacterial growing, hemolysis and hyperlipidemia must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **HSV-1,2-Ag Coated Strips.** Strips are wrapped in a sealed foil-lined bag. Open the bag and remove the tray. Select the number of Coated Strips required for the assay. Return unused strips in the bag. After the bag has been opened the Coated Strips are stable during the shelf life of the kit at 2-8 °C, provided that the foil-lined bag is resealed in Zip-Locked plastic bag. The silica gel bag should not be removed from the foil packaging.
- **Conjugate;**
- **Positive Control;**
- **Negative Control;**
- **Preliminary Sample Diluent;**
- **Sample Diluent;**
- **Stopping Reagent;**
- **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. Thoroughly mix the solution. The prepared Working Washing Solution is stable for 14 days at 18-24 °C or for 28 days at 2-8 °C.

The required volumes of Working Washing Solution for the certain number of strips or plate are tabulated in Table 2.

Table 2

Number of strips to be used		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1 well**
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	0.2
	High quality 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	4.8

3. Storage of unused reagents

After opening the vials the unused components of the kit: Positive Control, Negative Control, Preliminary Sample Diluent, Sample Diluent, Washing Solution (concentrated 25-fold), Stopping Reagent can be stored in tightly sealed vials until the kit expiration date at 2-8 °C. Coated Strips can be stored until the kit expiration date at 2-8 °C.

Conjugate, TMB-Substrate can be stored in tightly sealed vials within 3 months at 2-8 °C.

X. TEST PROCEDURE

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

Step	The assay procedure
1	Add 90 µl of Preliminary Sample Diluent into the wells of the plate for preliminary samples dilution and 10 µl of the serum (plasma) samples. Carefully mix by pipetting. Violet-blue color should change to blue-green.
2	Add 100 µl of Positive Control, Negative Control into the wells. 1 strip – Positive Control to 1 well, Negative Control to 2 wells; 2 strips and more – Positive Control to 1 well, Negative Control to 3 wells.
3	Add 90 µl of Sample Diluent and 10 µl of the preliminary diluted samples to the rest of the wells (the final serum dilution ratio is 1:100). Carefully mix fluid in wells by gentle pipetting. Cover the strips with a protective film.
4	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
5	Aspirate the contents of the wells and wash the plate **4 times** with the Working Washing Solution. Add into each well not less than 380 µl of Working Washing Solution and remove Washing Solution into the container with disinfecting solution. Do not leave any fluid in the wells. Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect the assay precision.
6	Add 100 µl of Conjugate to all the wells of the plate. Cover the plate with a protective film.
7	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C. Remove fluid from wells, wash the plate **4 times** as described in step 5.
8	Add 100 µl of TMB-Substrate into all the wells.
9	Incubate at 18-24 °C for a **20 min** in a dark place.
10	Add 150 µl of Stopping Reagent into wells to stop the reaction results are read by microplate plate reader at wavelength of 450 nm, with reference filter at 620-680 nm. Reading of the absorbance at 450 nm only is possible.

Scheme of the assay is represented in Annex.

Automated analyzer

Validated protocols for automated analyzers can be obtained from your representative. 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. RESULTS

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. **Positive Control**: the absorbance value should not be less than 1.9 at 450/620-680 nm.
2. **Negative Control**: the absorbance value should not be more than 0.2 at 450/620 nm.

Qualitative results

Calculate Cut-Off value as:

Cut-Off = average OD value of Negative Control + A (A=0.230)

where **A** – is a coefficient defined by manufacturer during statistical processing for each lot.

Interpretation of results

Sample is positive, if the OD value is ≥ Cut-Off.
Sample is negative, if the OD value is < Cut-Off.

Determining antibody titers

The reagent kit abia HSV-1/2 IgG can be used for determining IgG antibodies in human serum (plasma).

Serum samples tested positive can be used for determining specific IgG antibodies to HSV-1 and HSV-2. There are two methods to calculate the antibody titer.

Method 1

1. Dilute test samples with Preliminary Sample Diluent 10 times. To accomplish this, to the wells of a preliminary dilution plate add 90 µl of Preliminary Sample Diluent and 10 µl of test sample.
2. Add 180 µl of Sample Diluent to the first well of coated strips and 100 µl of Sample Diluent to the rest of the wells.
3. To well 1 add 20 µl of the preliminary diluted sample containing antibodies to HSV-1 and HSV-2 (final dilution of test samples is 1:100). Mix the content of the well by accurate pipetting and take 100 µl of the diluted sample to the next well of the row. Then mix the content of this well thoroughly and repeat this procedure for all wells of the row. When the mixing procedure is done, take 100 µl of the content of the last well and transfer to a biomaterial disinfection container.

Conduct follow-up operations in accordance with section X. Consider the antibody titer to HSV-1,2 as the maximum sample dilution giving positive result (OD > Cut-Off).

Method 2

The titer of IgG antibodies to HSV is calculated using the table of correspondence of OD/Cut-off to the anti-HSV-1,2 IgG titer (see Table 3).

If the OD/Cut-off value exceeds 11, the sample should be diluted four times with Sample Diluent and retested. When determining the final titer, additional sample dilution should be considered.

Note: according to the correspondence table, the measurement error of determining antibody titer is ± 1 titer.

Table 3

Correspondence of OD/Cut-Off to the anti-HSV-1,2 IgG titer

OD/Cut-Off	Antibody titer
1.1 to 2.2	1/100
2.2 to 4.5	1/200
4.5 to 6.2	1/400
6.2 to 8.6	1/800
8.6 to 11.0	1/1600
≥ 11.0	≥ 1/1600

Interpretation of results

1. Negative result for IgG to HSV-1 and HSV-2 does not prove the active HSV infection is absent.
2. To determine recent or current infection we recommend:

- Isolating and identifying virus, wherever possible.
- Testing human serum (plasma) samples for anti-HSV-1,2 IgG and IgM. Positive result for IgM or significant increase in the IgG antibody titer in paired samples may be indicative of the acute infection.
- Low avidity IgG to HSV-1 and HSV-2 detected indicate the primary herpes infection.

XII. PERFORMANCE CHARACTERISTICS

1. Interferences

Hemoglobin (up to 6 mg/ml), bilirubin (up to 0.3 mg/ml), lipids (up to 20 mg/ml) have no influence on the assay results.

2. Cross reactivity

No cross reactivity was found for samples with antibody to *Epstein-Barr virus, Cytomegalovirus, Varicella zoster* virus.

3. Diagnostic sensitivity

Diagnostic sensitivity of abia HSV-1/2 IgG with 93 anti-HSV-1,2 IgG positive samples is 98.9% (95%CI: 94.2-99.8%).

4. Diagnostic specificity

Diagnostic specificity of abia HSV-1/2 IgG with 55 negative samples is 100.0% (95% CI:92.9-100.0%).

5. Trueness. Agreement with certified reference measurement procedure

The abia HSV-1/2 IgG was compared with the "Anti-HSV-1/2 Pool ELISA (IgG)", EUROIMMUN. 172 serum and plasma samples are tested.

	Anti-HSV-1/2 Pool ELISA (IgG)			
abia HSV-1/2 IgG		Positive	Negative	Indeterminate
	Positive	111	1	0
	Negative	1	59	1

The abia HSV-1/2 IgG has not a borderline range, so indeterminate results were not included in the calculation. The agreement to comparative kit "Anti-HSV-1/2 Pool ELISA (IgG)" is 98.8% (95%CI: 95.9-99.7%).

6. Precision

The precision of the abia HSV-1/2 IgG was determined by 20 days × 3 samples ×2 replicates covering the measuring range.

Intra-assay (within run) precision

Data	Sample Pool #1	Sample Pool #2	Sample Pool #3
Mean (S/Co)	9.8	6.4	1.4
S_r	0.43	0.22	0.08
CV (%) ≤ 8%	4.3	3.4	6.1

Inter-assay (between-run) precision

Data	Sample Pool #1	Sample Pool #2	Sample Pool #3
Mean (S/Co)	9.8	6.4	1.4
S_{rr}	0.15	0.26	0.06
CV (%)≤ 8%	1.6	4.0	4.6

XIII. LIMITS OF THE TEST

It is inadmissible to make a diagnosis only on the basis of the anti-HSV IgG testing results. The diagnosis of acute HSV infection is possible only in the presence of clinical manifestations and a complex of laboratory studies (detection of an increase in the level of anti-HSV IgM, the detection of high levels of anti-HSV IgG, the isolation of the virus in urine or positive PCR result in serum).

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging. Keep in dark dry place at 2-8 °C.

Transportation should be done at 2-8 °C. Transportation at 9-25 °C is allowed for not more than ten (10) days. Freezing is prohibited.

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. REFERENCES

1. WHO. *Herpes simplex* virus. 31 January 2017, http://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus.

2. Foley E., Clarke E., Beckett V.A., Harrison S. et al. Management of genital herpes in pregnancy. - British Association for Sexual Health and HIV (BASHH) and the Royal College of Obstetricians and Gynecologists (RCOG). October 2014.

3. Patel R., Green J., Clarke E., Seneviratne K. et al. 2014 UK national guideline for the management of anogenital herpes. International Journal of STD & AIDS. 0(0) 1–14, https://www.bashhguidelines.org/media/1019/hsv_2014-ijstda.pdf.

4. Van Rooijen M.S., Roest W., Hansen G., Kwa D., de Vries H.J. False-negative type-specific glycoprotein G antibody responses in STI clinic patients with recurrent HSV-1 or HSV-2 DNA positive genital herpes, The Netherlands. Sex Transm Infect. 2016 Jun; 92(4): 257-60.

5. Liermann K., Schäfler A., Henke A., Sauerbrei A. Evaluation of commercial *Herpes simplex* virus IgG and IgM enzyme immunoassays. J Virol Methods. 2014 Apr; 199: 29-34.

6. Zahariadis G., Severini A. Evaluation of a novel serology algorithm to detect *Herpes simplex* virus 1 or 2 antibodies. Sex Transm Dis. 2010 Nov; 37(11): 696-9.

XVI. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)	IVD	For in vitro diagnostic use
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM		Symbol “exclamation mark”
REF	Catalog number		Symbol “corrosion”
Σ	Sufficient for		Symbol “health hazard”
LOT	Batch code	**Danger! Warning!**	Signal words
+2°C +8°C	Storage temperature limitation		Keep away from sunlight
	Expiry date CCYY-MM-DD		Keep dry
	Top		Fragile, handle with care

Annex

Scheme of the assay

1	**Add**	90 µl of Preliminary Sample Diluent and 10 µl of the samples (conduct on the plate for preliminary dilution of samples)
2	**Add**	100 µl of Positive Control, Negative Control
3	**Add**	90 µl of Sample Diluent
4	**Add**	10 µl of preliminary diluted samples
5	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
6	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
7	**Add**	100 µl of Conjugate
8	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
9	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
10	**Add**	100 µl of TMB-Substrate
11	**Incubate**	20 min, 18-24 °C in a dark place
12	**Add**	150 µl of Stopping Reagent
13	**Read the optical density**	450 nm/620-680 nm or 450 nm