AB Diagnostic Systems

REF **DK.050.01.8**

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

INSTRUCTIONS FOR USE

abia HSV-2 IgG

Enzyme immunoassay for the detection of IgG antibodies to *Herpes simplex virus* type 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-2 IgG kit is intended for the detection of IgG antibodies to *Herpes simplex virus* type 2 in human serum (plasma) 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

Herpes simplex virus types 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-2 infection is widespread throughout the world and is almost exclusively sexually transmitted, causing genital herpes. According to WHO 11% people aged 15-49 worldwide had HSV-2 infection in 2012 [1]. Infection with HSV-2 is lifelong and incurable. Genital herpes infections often have no symptoms, or mild symptoms that go unrecognized. Most infected people are unaware that they have the infection. It was also shown HSV-2 infection to increase with age, though the highest numbers of people newly-infected were adolescents. More women are infected with HSV-2 than men.

However, HSV-1 can also be transmitted to the genitals through oral sex and is increasingly noted as a cause of genital HSV (infections in the genital or anal area), especially in high-income countries. But globally most symptomatic genital herpes is due to HSV-2 [2]. The first episode of symptoms of genital HSV-1 infection cannot be clinically differentiated from genital HSV-2 infection; it is only through laboratory tests that these infections can be differentiated [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 [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-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 colour change in the TMB-Substrate. The colour intensity is directly proportional to the concentration of specific antibodies in the specimen and can be read at 450/620-680 or 450 nm.

IV. CONTENT OF THE KIT abia HSV-2 IgG

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
HSV-2-Ag Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with a mix of recombinant proteins, which represent the recombinant analogs of antigens to HSV-2. 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 agents: 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-2. Preserving agents: 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-2. Preserving agents: 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. Phosphate-saline solution. 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. Water solution, containing casein bovine milk (0.96%). Preserving agents: 0.10% sodium azide, 0.006% thimerosal. Transparent or slightly opalescent 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
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
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
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.
- 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 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 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.
- Certain reagents contain biological material of animal origin.
- 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. 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 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, 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 water. Immediately call a poison center/doctor.
P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Danger!

Stopping Reagent contains 0.2 M 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.
- 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

Blood samples should be collected according to the current practices. Serum only may be used. Serum, plasma (citrate, heparin, EDTA) may be used. Separate serum or plasma from blood cells as soon as possible to avoid any hemolysis. 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 analyzed.

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 1 time cannot be used. Samples with expressed bacterial growing, hemolysis, hyperlipidemia must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **HSV-2-Ag 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 plastic bag. Do not remove desiccant.
- **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 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
	Distilled or deionized 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

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature 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 tested serum (plasma) samples. Carefully mix by pipetting. Violet-blue color should change to blue-green.
2	Add 100 µl of Positive and Negative Controls 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 room temperature 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 test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XIV). 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 0.6 at 450/620-680 nm.
2. **Negative Control**: the absorbance value should not be more than 0.2 at 450/620-680 nm.

Calculate Cut-Off value as:

Cut-Off = average OD value of Negative Control + A (A=0.250),

where **A** – is a coefficient defined by manufacturer during statistical processing for each lot. The coefficient is indicated for each lot in the Instructions for use which is enclosed in the package, and in the Certificate of Analysis.

Interpretation of Results (for qualitative assays)

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-2 IgG can be used for determining IgG antibodies in human serum (plasma).

Serum samples tested positive can be used for determining specific IgG to 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 the first well add 20 µl of the preliminary diluted sample containing antibodies to 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-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-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-2 IgG titer

OD/Cut-Off	Antibody titer
1.1 to 2.2	1/100
2.3 to 4.5	1/200
4.6 to 6.2	1/400
6.3 to 8.6	1/800
8.7 to 11.0	1/1600
> 11.0	> 1/1600

Interpretation of results

1. Negative result for IgG to HSV-2 does not prove the active HSV infection is absent.

2. The following is recommended in order to determine recent or current infection:

- Isolating and identifying virus, wherever possible.
- Testing human serum (plasma) samples for anti-HSV-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.
- Testing human serum (plasma) samples for avidity of anti-HSV-2 IgG. Low avidity IgG to HSV-2 detected indicate the primary herpes infection.

XII. PERFORMANCE CHARACTERISTICS

1. Interferences

Hemoglobin (up to 10 mg/ml), bilirubin (up to 0.3 mg/ml), lipids (up to 10 mg/ml) have no influence on the assay results.

2. Cross reactivity

The specificity for samples with anti-CMV IgG was 97.6% (95%CI: 87.7-99.6), for samples with anti-EBV antibody was 100% (95%CI: 87.1-100), for samples with anti-VZV IgG was 100% (95%CI: 91.6-100).

3. Diagnostic sensitivity

Diagnostic sensitivity of abia HSV-2 IgG with 54 anti-HSV-2 IgG positive samples is 100% (95%CI: 93.4-100).

4. Diagnostic specificity

Diagnostic specificity of abia HSV-2 IgG with 292 negative samples is 99.0% (95% CI: 97.0-99.6).

5. Trueness. Agreement with certified reference measurement procedure

The abia HSV-2 IgG was compared with the "SERION ELISA classic Herpes Simplex Virus (HSV) 2 IgG", Virion-Serion. 172 serum and plasma samples are tested.

	SERION ELISA classic Herpes Simplex Virus (HSV) 2 IgG			
abia HSV-2 IgG		Positive	Negative	Indeterminate
	Positive	71	7	17
	Negative	0	76	1

The abia HSV-2 IgG has not a borderline range, so indeterminate results were not included in the calculation. The agreement to comparative kit "SERION ELISA classic Herpes Simplex Virus (HSV) 2 IgG" is 95.5% (95%CI: 90.9-97.8%).

6. Precision

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

Intra-assay (within run) precision

Data	Sample 1	Sample 2	Sample 3
Mean (S/Co)	6.5	4.4	1.3
S_r	0.3	0.3	0.1
CV (%)≤ 8%	**4.9**	**6.5**	**4.8**

Inter-assay (between-run) precision

Data	Sample 1	Sample 2	Sample 3
Mean (S/Co)	6.5	4.4	1.3
S_{rr}	0.47	0.28	0.05
CV (%)≤ 8%	**7.2**	**6.3**	**3.6**

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.** Storage and transportation conditions for the kit, conditions and terms of storage for working solutions and unused reagents are specified in table 4.
- Transportation should be done by covered transport at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 4

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.		
	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.		
	HSV-2-Ag Coated Strips	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.
	Positive Control, Negative Control, Preliminary Sample Diluent, Sample Diluent, Washing Solution, Stopping Reagent	Close the vials tightly with screw caps and store them in the manufacturer's package.	Until the kit expiration date.
	Conjugate, TMB-Substrate	Close the vials tightly with screw caps and store them in the manufacturer's package.	For three months

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XV. REFERENCES

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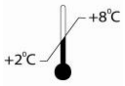
XVI. EXPLANATION OF SYMBOLS

	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM		For in vitro diagnostic use
	Expiry date CCYY-MM-DD		Sufficient for
	Batch code		Symbol “exclamation mark”
	Catalog number	**Warning!**	Signal word
	Fragile, handle with care		Symbol “health hazard”
	Keep away from sunlight		Symbol “corrosion”
	Keep dry	**Danger!**	Signal word
	Storage temperature limitation		Top

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