

Instruction for use

EIA HSV 1+2 IgG

REF HSVG96



Kit for professional use

IVD **CE**

 TestLine Clinical Diagnostics s.r.o.
Křižíkova 68, 612 00 Brno, Czech Republic
Tel.: +420 541 248 311
FAX: +420 541 243 390
E-mail: info@testlinecd.com
www.testlinecd.cz
www.testlinecd.com



CONTENT

1	Introduction	3
2	Test Principle.....	4
3	Materials Provided	5
4	Other Material Required for Manual Test Performance	6
5	Storage and Stability	6
6	Preparation of Reagents.....	6
7	Preparation of Samples	7
8	Assay Procedure.....	7
9	Working Schedule	9
10	Quality Control.....	10
11	Results Interpretation	11
12	Safety Precautions	12
13	Procedural Notes.....	12
14	Index of Avidity	13
15	IFU Symbols	18

Enzyme immunoassay for the detection of IgG antibodies to Herpes simplex virus type 1+2 and IgG avidity in human serum, plasma or cerebrospinal fluid

1 Introduction

Herpes simplex virus exists in two types (HSV-1 and HSV-2) and belongs to the *Herpetoviridae* family. Both varieties have common antigens and cross-react during serological tests. The only host of the infection is humans. Herpes is transmitted by means of droplet infection or during close contact with an infected person. The virus replicates primarily in the mucous membranes of the eye, mouth, nose and genitals. Primary infection of HSV-1 generally occurs already during childhood. HSV-1 usually infects the bulbar conjunctiva or oral mucosa. The infection is frequently asymptomatic or may lead to the appearance of herpetic lesions.

HSV-2 infection is one of the most common venereal diseases which result in the formation of lesions in the genital mucosa. There are also rare cases of transplacental transmission of this disease. The infection of a child through cervical secretions during childbirth occurs more commonly. One of the most serious forms of HSV infection is herpetic encephalitis.

The infection tendency to persist in the organism is characteristic for HSV disease. It may also be reactivated under certain conditions (stress, reduced immunity).

Diagnosis of the disease is based on clinical manifestation, epidemiological anamnesis and laboratory tests. The primary infection is always accompanied by specific IgM antibodies which are already produced one week after the infection and persist for approximately 6 weeks. Specific IgA antibodies occur shortly after IgM and before an elevation of IgG class. Reactivation is accompanied by IgA antibodies production and by IgM antibodies production, respectively. IgA antibodies have significance for the confirmation of HSV reactivation provided they are simultaneously accompanied by IgG antibodies production. The significant elevation of IgG antibodies level is recorded in paired serum samples examined during primary infections as well as during recurrent ones. The specific IgG antibodies occur 2 or 3 weeks after primary infection, however, they may also appear after several months and they mostly remain in reduced levels throughout the entire life of the infected person.

2 Test Principle

The kit is intended for detection of specific IgG antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgG conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgG antibodies in the sample.

Antigen Used

Mixture of inactivated and purified HSV-1 and HSV-2 strains

3 Materials Provided

MICROPLATE	Microtitre Plate	1 pc
	coated with antigen, 12 x 8 wells in bag with desiccant	
CONTROL - CAL1	Negative Control (Calibrator 1) 5 U/ml	1 × 2 ml
	Solution containing no specific human antibodies, ready to use	
CUTOFF CAL2	CUT-OFF (Calibrator 2) 20 U/ml	1 × 3 ml
	Solution containing specific human antibodies in cut-off concentration, ready to use	
CONTROL + CAL3	Positive Control (Calibrator 3) 80 U/ml	1 × 2 ml
	Solution containing specific human antibodies, ready to use	
CAL4	Calibrator 4 (160 U/ml)	1 × 2 ml
	Solution containing specific human antibodies, ready to use	
CONJUGATE	Conjugate	1 × 15 ml
	Solution containing peroxidase labelled animal immunoglobulin to human IgG, ready to use	
DILUENT 2	Sample Diluent 2	1 × 105 ml
	Buffer with protein stabilisers, ready to use	
SUBSTRATE 2	TMB-Complete 2	1 × 15 ml
	Chromogenic substrate solution containing TMB/H ₂ O ₂ , ready to use	
WASH 20x	Wash Solution	1 × 75 ml
	20× concentrated buffer	
STOP	Stop Solution	1 × 15 ml
	1M sulphuric acid solution, ready to use	
AVIDITY 1	Avidity Solution 1	1 × 7 ml
	Stabilised urea solution	
	Instructions for use	1 pc

4 Other Material Required for Manual Test Performance

Single and multichannel pipettes

Disposable tips

Microplate washer

Timer

Incubator (37°C)

Microplate reader

5 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Samples Preparation and Storage

The following human body liquids can be used for testing: serum, citrate plasma and cerebrospinal fluid. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

6 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

The Controls (positive, negative and CUT-OFF) are ready to use, do not dilute further!

The Conjugate is ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

7 Preparation of Samples

Mix gently the Sample Diluent prior to use.

Dilution of sera and plasma samples

Dilute well mixed samples 1:101 with the Sample Diluent:

E.g.: 10 µl of sample + 1 ml of the Sample Diluent

Mix well.

Dilution of cerebrospinal fluid samples (CSF)

Dilute well mixed CSF 1:3 with the Sample Diluent:

E.g.: 50 µl of CSF + 100 µl of the Sample Diluent

Mix well.

8 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls (calibrators) and the diluted samples according to the working schedule.

Semiquantitative evaluation in Index of Positivity (IP)

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

Quantitative evaluation in Units U/ml

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 2 wells.
- Pipette 100 µl of the Calibrator 4 into 2 wells.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

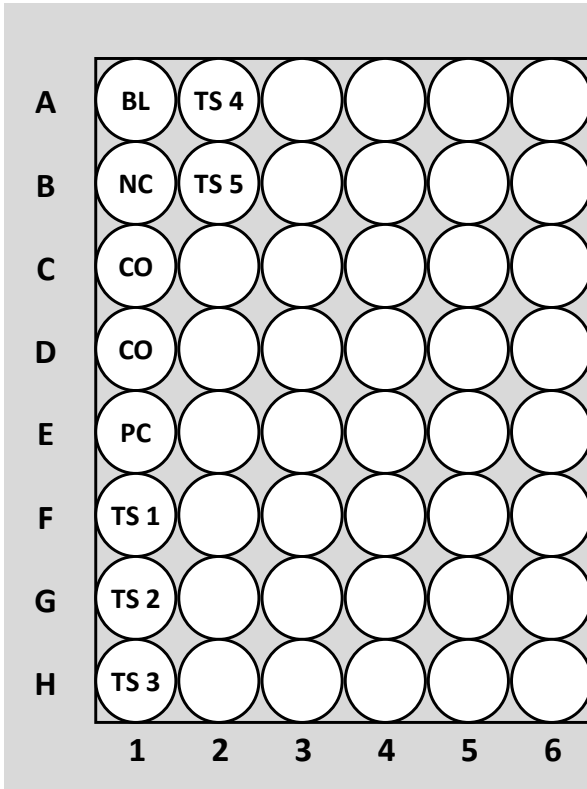
2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.

3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate it at 37°C for 30 minutes.
6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37°C for 30 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

9 Working Schedule

Semiquantitative evaluation

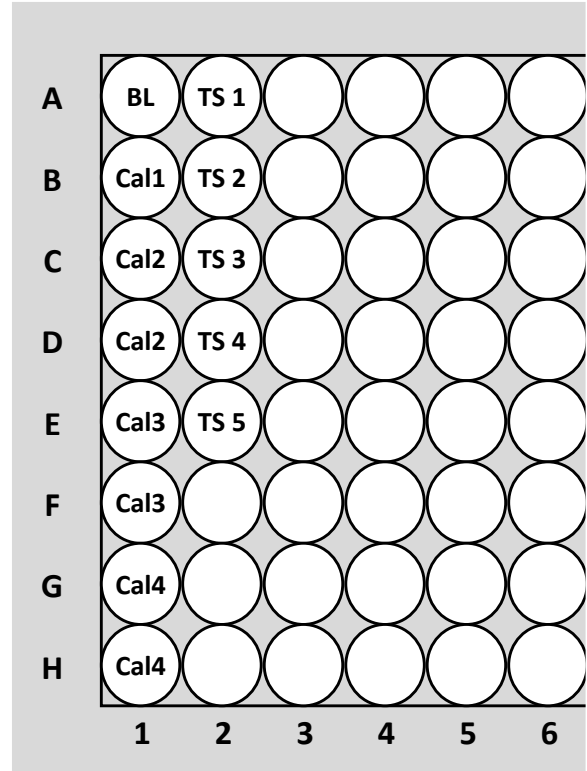
Index of Positivity (IP)



BL	Blank (empty well)			
NC	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>-</td><td>CAL1</td></tr></table>	CONTROL	-	CAL1
CONTROL	-	CAL1		
CO	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CUTOFF</td><td>CAL2</td></tr></table>	CUTOFF	CAL2	
CUTOFF	CAL2			
PC	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>+</td><td>CAL3</td></tr></table>	CONTROL	+	CAL3
CONTROL	+	CAL3		
TS 1-x	100 µl diluted tested sample			

Quantitative evaluation

Units U/ml



BL	Blank (empty well)			
Cal1	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>-</td><td>CAL1</td></tr></table>	CONTROL	-	CAL1
CONTROL	-	CAL1		
Cal2	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CUTOFF</td><td>CAL2</td></tr></table>	CUTOFF	CAL2	
CUTOFF	CAL2			
Cal3	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>+</td><td>CAL3</td></tr></table>	CONTROL	+	CAL3
CONTROL	+	CAL3		
Cal4	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CAL4</td></tr></table>	CAL4		
CAL4				
TS 1-x	100 µl diluted tested sample			

10 Quality Control

The test is valid if:

The absorbance of blank is lower than 0.150.

$$\text{BLANK} < 0.150$$

The absorbance of the Negative Control (Calibrator 1) is lower than half of the mean absorbance of CUT-OFF (Calibrator 2).

$$\boxed{\text{CONTROL}} - \boxed{\text{CAL1}} < 0.5 \times \boxed{\text{CUTOFF}} \boxed{\text{CAL2}}$$

The mean absorbance of CUT-OFF (Calibrator 2) is within a range of 0.150 – 0.900.

$$0.150 < \boxed{\text{CUTOFF}} \boxed{\text{CAL2}} < 0.900$$

The absorbance of the Positive Control (Calibrator 3) is 1.5-fold higher than the mean absorbance of CUT-OFF (Calibrator 2).

$$\boxed{\text{CONTROL}} + \boxed{\text{CAL3}} > 1.5 \times \boxed{\text{CUTOFF}} \boxed{\text{CAL2}}$$

The absorbance of the Calibrator 4 is higher than the absorbance of the Positive Control (Calibrator 3).

$$\boxed{\text{CAL4}} > \boxed{\text{CONTROL}} + \boxed{\text{CAL3}}$$

11 Results Interpretation

Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$IP = \frac{\text{Absorbance of sample}}{\text{Mean absorbance of CUT-OFF}}$$

Interpretation of the test results is described in Table 1.

Table 1 Interpretation of test results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Quantitative evaluation in Units (U/ml)

Construct a calibration curve by plotting the concentration (X) of the calibrators in U/ml against the corresponding absorbance (Y). Construct the calibration curve by single point cross connection. Read the values of antibody level (U/ml) in samples from the calibration curve. Interpretation of the quantitative test results is described in Table 2.

Table 2 Quantitative interpretation in Units (U/ml)

Antibody level (U/ml)	Evaluation
lower than 18	negative
18 to 22	borderline
higher than 22	positive

Examination of borderline samples should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

12 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

13 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

Microtitre Plate – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

Wash Solution – use high quality distilled water for preparing the working strength Wash Solution.

Washing procedure – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

TMB-Complete – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

The kit might be used in all types of automatic EIA analysers.

If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

14 Index of Avidity

14.1 Introduction

Antibody avidity expresses the strength of bond between antigen and antibody. Low avidity antibodies are produced in the early stages of a primary infection. As the infection progresses, immune response of organism matures and avidity of antibodies increases. Antibodies show high avidity in the latent phase of the disease. High avidity IgG antibodies are produced by memory B-cells from the beginning of a secondary infection or reactivation.

Determination of IgG antibodies avidity enables differentiation of various stages of infection and is a useful addition to serological diagnostics.

14.2 Test Principle

Avidity determination is based on dissociation of antigen-antibody bond by means of Avidity Solution (urea solution). After Avidity Solution treatment, low avidity antibodies are released and washed out while high avidity antibodies remain bound to the antigen. The binding strength is expressed by the Index of Avidity (IAv). IAv

determines the portion of IgG antibodies that remains bound to antigen after incubation with Avidity Solution. IAv assay procedure is a modification of the standard ELISA procedure using Avidity Solution.

The IgG avidity is determined only in IgG positive samples.

14.3 Dilution of Samples

Dilution of sera and plasma samples (see Chapter Preparation of Samples).

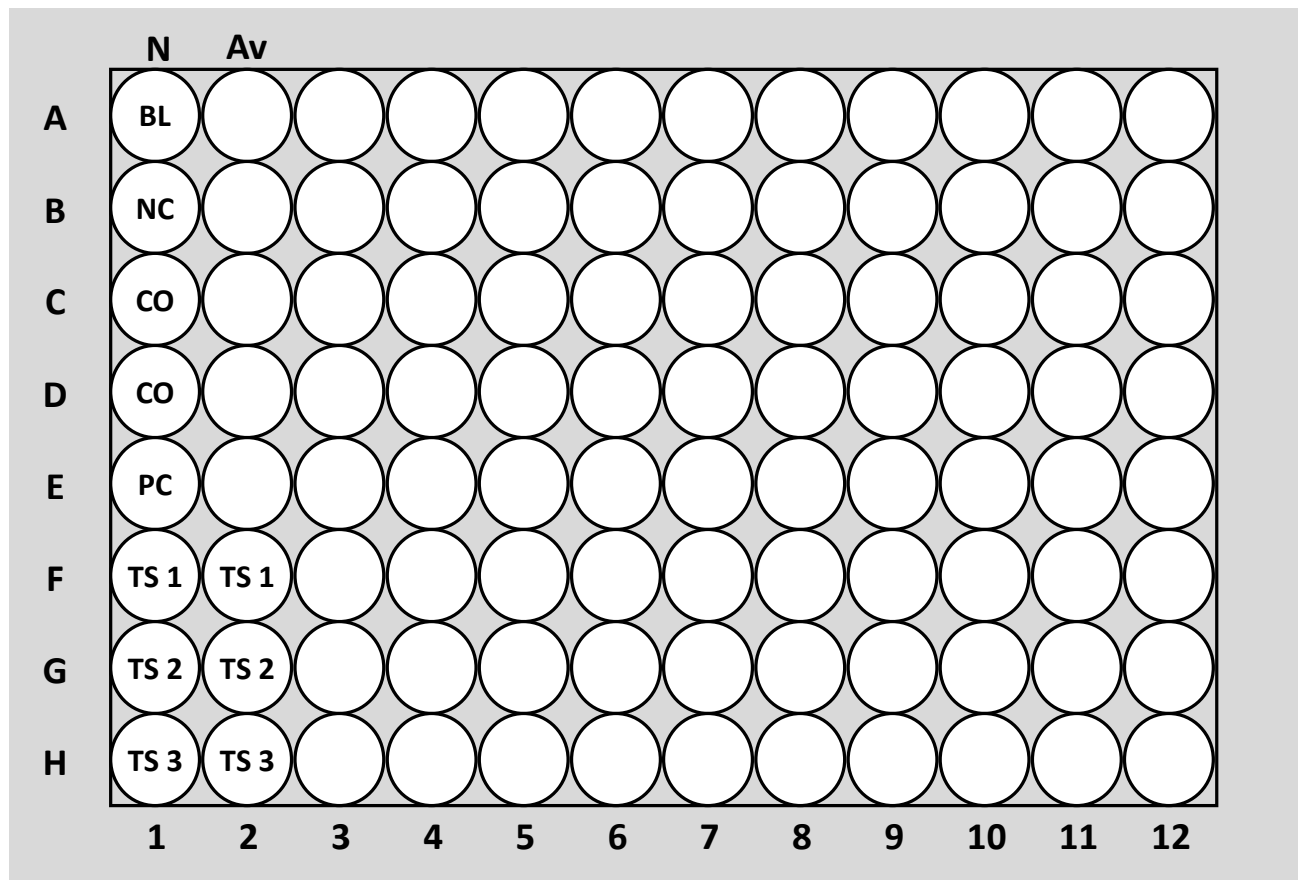
14.4 Assay Procedure

Allow all reagents including the Avidity Solution to come to room temperature and mix well. Crystals might develop in the vial with the Avidity Solution. Prior to use, it is necessary to dissolve the crystals by short-time warming up. The functionality of the Avidity Solution is indicated by yellow colour. The solution is thermolabile. The solution is deteriorated, if it changes its colour from yellow to red. A red-coloured Avidity Solution cannot be used further!

1. Dispense the controls and the diluted samples according to the working schedule.
 - Leave A1 well empty (blank).
 - Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
 - Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
 - Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
 - Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into two adjacent wells of N and Av strips.
2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Avidity Solution into all wells of Av strips.
5. Pipette 100 µl of the working strength Wash Solution into all wells of N strip.
6. Cover the microplate with the lid and incubate at room temperature for 5 minutes.
7. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
8. Pipette 100 µl of the Conjugate into all wells except A1 well.
9. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
10. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.

11. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
12. Cover the microplate with the lid and incubate at 37°C for 30 minutes. Keep out of light.
13. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
14. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

14.5 Working Schedule



N strip for ELISA (without Avidity Solution)

Av strip for avidity test (incubation with Avidity Solution)

BL Blank (empty well)

NC 100 µl

CONTROL	-	CAL1
---------	---	------

CO 100 µl

CUTOFF	CAL2
--------	------

PC 100 µl

CONTROL	+	CAL3
---------	---	------

TS 1-x 100 µl diluted tested sample

14.6 Quality Control

Quality control of the test is performed in N strips (see Chapter Quality Control).

14.7 Results Interpretation

Calculation of Index of Avidity (IAv)

Divide the absorbance of a tested sample in Av strip by the absorbance of the tested sample in N strip measured in the same test run:

$$\text{IAv} = \frac{\text{Absorbance of sample in Av strip}}{\text{Absorbance of sample in N strip}} \times 100 [\%]$$

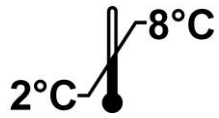
Interpretation of the test results is described in Table 3.

Table 3 Interpretation of test results

Index of Avidity (IAv) in %	Evaluation of avidity	Interpretation of results
< 40	low	primary infection
40 - 45	borderline	repeat examination
> 45	high	latent infection reactivation of latent infection or reinfection, if IgM antibodies are detected at the same time

Examination should be repeated in case of borderline results. Collect a second sample after more than seven days after the first collection and examine both samples in the same test run.

15 IFU Symbols



Temperature limitation



Keep dry



Expiry date



Lot number



Manufactured by



Consult instructions



Catalogue number














Number of tests



In vitro diagnostic medical device

Notes

Summary of EIA HSV 1+2 IgG Protocol

Step No.	Symbol	Test steps
1		Dilute samples serum/plasma 1:101 (10 µl + 1 ml) cerebrospinal fluids 1:3 (50 µl + 100 µl)
2		Pipette Controls (Calibrators) and diluted samples – 100 µl Blank = empty well
3		Incubate at 37°C for 30 min
4		Aspirate and wash the wells 5x
5		Pipette Conjugate – 100 µl Blank = empty well
6		Incubate at 37°C for 30 min
7		Aspirate and wash the wells 5x
8		Pipette Substrate (TMB-Complete) – 100 µl Including blank
9		Incubate at 37°C for 30 min
10		Pipette Stop Solution – 100 µl Including blank
11		Read colour intensity at 450 nm