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For Professional Use Only

AmpliSens® *HSV* I, II-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***HSV*** **I, II-FEP PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of *herpes simplex virus* types I and II (*HSV* I, II) DNA in the clinical materials (urogenital, rectal, and oral swabs; exudate of blisters and erosive-ulcerative lesions of skin and mucosa; whole blood and cerebrospinal fluid) by using end-point hybridization-fluorescence detection of amplified products.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Herpes simplex virus types I and II detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *HSV* I, II primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in a reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***HSV*** **I, II-FEP** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens®** ***HSV*** **I, II-FEP** PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction components mix only at 95 °C.

3. CONTENT

AmpliSens® ***HSV*** **I, II-FEP** PCR kit is produced in 2 forms:

AmpliSens® *HSV* I, II-FEP PCR kit variant FEP (0.5-ml tubes),

REF V8-100-R0,5-FEP-CE.

AmpliSens® *HSV* I, II-FEP PCR kit variant FEP (0.2-ml tubes),

REF V8-100-R0,2-FEP-CE.

AmpliSens® *HSV* I, II-FEP PCR kit includes:

Reagent	***Description***	***Volume (ml)***	***Amount***
PCR-mix-1-FL *HSV* I, II ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
PCR-mix-Background-red*	red clear liquid	0.6	1 tube
Mineral oil for PCR**	colorless viscous liquid	4.0	1 tube
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)***	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)******	colorless clear liquid	1.0	1 tube

* used to analyze DNA samples extracted with DNA-sorb-AM and DNA-sorb-B extraction kits.

** must be used for thermocyclers without constant-temperature cover (for example, Terzik (DNA-Technology)).

*** must be used in the extraction procedure as Negative Control of Extraction.

**** add 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see the DNA-sorb-AM **REF** K1-12-100-CE protocol).

AmpliSens® *HSV* I, II-FEP PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit
- Transport medium.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml tubes.
- PCR box.
- Personal thermocyclers (for example, Terzik (DNA-Technolohy, Russia), Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), MaxyGene (Axygen, USA), or equivalent).
- Fluorometer (for example, ALA-1/4 (Biosan, Latvia) or equivalent).

- Personal computer.
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml) (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *HSV* I, II-FEP PCR kit is intended to analyze DNA extracted with DNA extraction kits from:

- *urogenital, rectal, and oral swabs,*
- *exudate of blisters and erosive-ulcerative lesions of skin and mucosa,*
- *whole blood,*
- *cerebrospinal fluid.*

7. WORKING CONDITIONS

AmpliSens® *HSV* I, II-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It's recommended that the following nucleic acid extraction kits are used:

- DNA-sorb-AM, **REF** K1-12-100-CE,
- DNA-sorb-B, **REF** K1-2-100-CE (for blood and cerebrospinal fluid samples),
- Other nucleic acid extraction kits recommended by FBIS CRIE.

Extract DNA according to the manufacturer's instructions.

8.2. Preparing PCR

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

8.2.1 Preparing tubes for PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FL *HSV* I, II** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of wax layer of each tube, ensuring that it does not fall under the wax and mix with PCR-mix-1-FL *HSV* I, II.
3. Add above **1** drop of **mineral oil for PCR** (about **25 µl**) if a thermocycler without constant-temperature lid is used.
4. Prepare one **Background** sample. To do this, mark one **PCR-mix-1-FL *HSV* I, II** tube as **Background** and add **20 µl** of **PCR-mix-Background-red** above the wax layer surface ensuring that it does not fall under the wax and mix with PCR-mix-1-FL *HSV* I, II. Add above **1** drop of **mineral oil for PCR** (if a thermocycler without a constant-temperature lid is used).

PCR-mix-Background-red is used if DNA was extracted using DNA-sorb-AM (**REF** K1-12-100-CE) or DNA-sorb-B (**REF** K1-2-100-CE). If any other nucleic acid extraction kit (recommended by FBIS CRIE) was used, follow the instructions provided by the manufacturer.

5. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage using tips with aerosol barrier.

6. Carry out control reactions:

NCA -Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ -Add **10 µl** of **Positive Control complex** (to the tube labeled C+ (Positive Control of Amplification).

C– -Add **10 µl** of a sample extracted from the **Negative Control** to the tube labeled C- (Negative Control of Extraction).

8.2.2 Amplification

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 ºC (pause mode), insert the tubes into the cells of the thermocycler and press the button to continue.

It is recommended that drops are removed from walls of the tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

Table 1

AmpliSens-1-FEP amplification program

	Terzik			**GeneAmp PCR System 2700**			**Gradient Palm Cycler, MaxyGene**		
Step	Tempera ture, °C	Time	Cycles	Tempera ture, °C	Time	Cycles	Tempera ture, °C	Time	Cycles
0	**95**	Pause		**95**	Pause		**95**	Pause	
1	**95**	5 min	1	**95**	5 min	1	**95**	5 min	1
2	**95**	2 s	35	**95**	20 s	20	**95**	2 sec	24
	65	5 s		**65**	25 s		**65**	10 s	
	72	5 s		**72**	30 s		**72**	10 s	
3	**95**	2 s	9	**95**	20 s	24	**95**	2 s	20
	60	10 s		**60**	30 s		**60**	15 s	
	72	5 s		**72**	30 s		**72**	10 s	
4	**95**	2 s	1	**95**	20 s	1	**95**	2 s	1
	60	10 s		**60**	30 s		**60**	15 s	
5	**10**	Storage		**10**	Storage		**10**	Storage	

Amplification programs for different thermocycler models are described in **Guidelines** "**End-Point PCR Detection of STIs and Other Reproductive Tract Infections**" [2].

9. DATA ANALYSIS

Detection is performed using a fluorescence detector.

Please read the fluorescence detector Operating Manual before using this kit.

Detection can be conducted within 1 day after completion of amplification only if the tubes with the amplified product have been stored at or below 28 °C in a light-free area.

The fluorescent signal intensity is detected in two channels:

– The signal from the *HSV* I, II DNA amplification product is detected in the FAM channel (or analogous, depending on the detector model);

– The signal from the Internal Control amplification product is detected in the HEX channel (or analogous, depending on the detector model).

Prior to detection, all settings should be entered and saved. Refer to the **Guidelines [2]** and the **Important Product Information Bulletin** for settings.

Result interpretation

Principle of interpretation:

– *HSV* I, II DNA is **detected** in a sample if its signal in the FAM channel is greater than the defined threshold value of the positive result.

– *HSV* I, II DNA is **not detected** in a sample if its signal in the FAM channel is less than the defined threshold value of the negative result whereas the signal in the HEX channel is greater than the specified threshold value.

– The result is **invalid** if the signal of a sample in the FAM channel is less than the defined threshold value of the negative result and the signal in the HEX channel is less than the specified threshold value as well.

– The result is **equivocal** if the signal of a sample in the FAM channel is greater than the defined threshold value of the negative result but less than the threshold value of the positive result (the signal is between thresholds).

If the result is invalid or equivocal, the PCR should be repeated once again.

1. Result of the analysis is considered reliable only if the results obtained for both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 2).

Table 2

Results for controls

Control	Stage for control	Signal in the channel		Interpretation
		FAM	HEX	
C–	DNA extraction	**<** threshold value of negative result	**>** threshold value	"–" or OK
NCA	Amplification	**<** threshold value of negative result	**<** threshold value	"nd"
C+	Amplification	**>** threshold value of positive result	**>** threshold value	"+" or OK

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The absence of positive signal for the positive control of amplification (C+) may indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of PCR, noncompliance of the storage conditions for kit components with the manufacturer's instruction, or expiration of the reagent kit. Check programming of the thermocycler (see 8.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR once again for all samples.
2. If no signal was detected either in the channel for detection of the pathogen DNA or in the channel for detection of the Internal Control, the sample should be examined once again (PCR and detection). The same applies to the samples with equivocal results, because the fact that the specific signal does not exceed the threshold value is not sufficient to consider a sample as positive. If equivocal results are obtained in the second run, the analysis should be repeated starting from the DNA extraction stage.
3. Positive signal in negative controls (C– and NCA) indicates reagent or sample contamination. In this case, the results of analysis must be considered as invalid. The analyses must be repeated and measures for detecting and eliminating the contamination source must be taken.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *HSV* I, II-FEP PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HSV* I, II-FEP** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *HSV* I, II-FEP** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the

same, unless otherwise stated.

PCR-mix-1-FL *HSV* I, II is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *HSV* I, II-FEP** PCR kit is specified in the table below.

Clinical material	Transport medium	DNA extraction kit	Analytical sensitivity, GE/ml*
Urogenital swabs	Transport Medium for Swabs (REF 956-CE, REF 987-CE) or Transport Medium with Mucolytic (REF 952-CE, REF 953-CE)	DNA-sorb-AM	1×10^3

* Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

13.2. Specificity

The analytical specificity of **AmpliSens® *HSV* I, II-FEP** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. There were no nonspecific test responses during examination of human DNA as well as a DNA panel of the following microorganisms: *CMV, EBV, HHV* types 6 and 7, *HPV*, *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Candida albicans, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Neisseria flava, Neisseria subflava, Neisseria sicca, Neisseria mucosa, Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Trichomonas vaginalis,* and *Toxoplasma gondii.*

The clinical specificity of **AmpliSens® *HSV* I, II-FEP** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines “End-Point PCR Detection of STIs and Other Reproductive Tract

Infections", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-certified Quality Management System, each lot of **AmpliSens®** ***HSV*** **I, II-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
22.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”