



For *in Vitro* Diagnostic Use

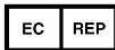


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AmpliSens[®] HSV I, II-FEP PCR kit

Instruction Manual



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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 (*HSV1, II*) DNA in the biological material (scrapes (swabs) of urogenital tract mucous membranes; papules, vesicles, or ulcers fluid; urine sediment) by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Herpes simplex virus types I, II detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *HSV I, II* primers. In Fluorescent End-Point PCR, the amplified product is detected by using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® HSV I, II-FEP** PCR kit is a qualitative test, which contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® HSV I, II-FEP** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer or chemically modified polymerase (TaqF). Wax melting and reaction mix components occur only at 95°C. Chemically modified polymerase (TaqF) is activated by heating at 95°C for 15 min.

3. CONTENT.

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

AmpliSens® HSV I, II-FEP PCR kit (tubes of 0.5 ml volume), [REF](#) V8-100-R0,5-FEP-CE.

AmpliSens® HSV I, II-FEP PCR kit (tubes of 0.2 ml volume), [REF](#) V8-100-R0,2-FEP-CE.

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

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT HSV I, II ready-to-use single-dose test tubes (<i>under wax</i>)	colorless clear liquid	0.008	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
PCR-mix-Background	colorless clear liquid	0.5	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

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

** add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (see “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 isolation kit
- 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 rotor.
- PCR box.
- Personal thermocycler (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), Uno-2 (Biometra, Germany) or equivalent
- Fluorometer ALA-1/4 (“Biosan”, Latvia) or equivalent
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus 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, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 move to the Amplification and Detection Area. 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 is 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 for analysis of DNA extracted by using DNA isolation kits from urogenital tract mucous membranes scrapes (swabs); fluid of papules, vesicles, ulcers; urine sediment (first portion of the morning specimen).

7. PROTOCOL.

7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-AM", **REF** K1-12-100-CE.



Please carry out the DNA isolation according to the manufacturer instruction.

7.2. Preparing the PCR.

Total reaction volume - 25 µl, volume of DNA sample - 10 µl.

7.2.1 Preparing tubes for PCR.

- Prepare the required number of tubes with **PCR-mix-1-FEP/FRT HSV I, II** and wax for amplification of DNA from clinical and control samples.
- Add **7 µl** of **PCR-mix-2-FL** to the surface of wax layer of each tube, so that it wouldn't fall under the wax and mix with **PCR-mix-1-FEP/FRT HSV I, II**.
- Add above **1** drop of **mineral oil for PCR** (about **25 µl**).
- Prepare 2 tubes with **PCR-mix-1-FEP/FRT HSV I, II** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of wax layer of each tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT HSV I, II**. Add above **1** drop of **mineral oil for PCR**.



Background samples, that have once passed thermo cycling, can be used for further runs without thermo cycling. Multiple use of Background samples is permitted only if the PCR kit of the same lot is applied. Store the Background tubes at 2-25 °C for 1 week. Keep away from light.

- Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction.



The tubes with **PCR-mix-1-FEP/FRT HSV I, II** that are not used at the moment should be kept away from light.

- Carry out control amplification 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).

7.2.2 Amplification.

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

It is recommended to precipitate drops from walls of tubes by short vortex (1–3 sec) before their insertion in a thermocycler.

Table 1

Amplification program of *Herpes simplex virus* types I, II (**65-60-45**)

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment		
	"GeneAmp PCR System 2700" (Applied Biosystems)			"Gradient Palm Cycler" (Corbett Research),			"Uno-2" (Biometra)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	20 sec	20	95 °C	2 sec	24	95 °C	25 sec	20
	65 °C	25 sec		65 °C	10 sec		65 °C	40 sec	
	72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
3	95 °C	20 sec	24	95 °C	2 sec	19	95 °C	25 sec	24
	60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
	72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
4	95 °C	20 sec	1	95 °C	2 sec	1	95 °C	25 sec	1
	60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
5	10 °C	storage		10 °C	storage		10 °C	storage	

8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Appendix 1.



Detection can be conducted within 1 day from the end of the amplification only if the tubes with amplified product were stored at 28°C and below in a light-protected place.

Results interpretation

- When the analysis is complete the results are automatically shown in the table in the manner of

following indications:

pos – positive result;

neg – negative result;

eq – equivocal result (signal is in grey zone);

nd – invalid result (specific signal and IC signal are absent in the sample).

- Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2).

Table 2

Results for controls

Control	Stage for control	Results		Interpretation
		FAM channel (samples)	HEX channel (IC)	
C-	DNA isolation	HSV I, II - neg	+	OK
NCA	Amplification	HSV I, II - nd	-	OK
C+	Amplification	HSV I, II - pos	-	OK

9. TROUBLESHOOTING.

Results of analysis are not being registered in the following cases:

- No positive signal with positive control of PCR (C+) can indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components did not comply with manufacturer instruction, or reagents kit has expired. It is necessary to check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- If no signal was detected on both channel for detection of pathogen DNA and for detection of Internal Control, the sample should be examined repeatedly (PCR and detection). The same procedures should be applied to the samples with equivocal result, that is, the specific signal exceeds the background not enough to consider the sample as positive. If equivocal result is registered in the second run, the analysis should be repeated starting from the stage of DNA extraction.
- Positive signal in negative controls indicates the reagents or samples contamination. In such case results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting of contamination source must be undertaken.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® HSV I, II-FEP** PCR kit are to be stored at the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® HSV I, II-FEP** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® HSV I, II-FEP** PCR kit is no less than 1x10³ genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of **AmpliSens® HSV I, II-FEP** PCR kit are guaranteed only when additional reagents kit “DNA-sorb-AM” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) is used.

11.2. Specificity.

Specificity of **AmpliSens® HSV I, II-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® HSV I, II-FEP** PCR kit was confirmed in laboratory clinical trials.







12. REFERENCES.

- Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science “Central Research Institute of 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.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by	LOT	Batch code
IVD	For <i>in Vitro</i> Diagnostic Use	VER	Version
REF	Catalogue number	EC REP	Authorised representative in the European Community.
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use	NCA	Negative Control of Amplification
C+	Positive Control of Amplification	C-	Negative control of Extraction
IC	Internal Control		