



For *in Vitro* Diagnostic Use

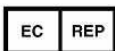
AmpliSens® HSV-typing-FEP PCR kit

Instruction Manual



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1. INTENDED USE.

AmpliSens® HSV-typing-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and typing of *Herpes Simplex virus* types I and II (*HSV I* and *HSV 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 end point PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product in ordinary thermocycler. 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-typing-FEP PCR kit is a qualitative test, which contain 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-typing-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. The wax melting and reaction mix component occurs only at 95°C.

3. CONTENT.

AmpliSens® HSV-typing-FEP PCR kit is produced in 2 forms:

AmpliSens® HSV-typing-FEP PCR kit (vials 0.5 ml), REF V38-100-R0,5-FEP-CE.

AmpliSens® HSV-typing-FEP PCR kit (vials 0.2 ml), REF V38-100-R0,2-FEP-CE.

AmpliSens® HSV-typing-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix -1-FEP/FRT HSV typing ready-to-use single-dose test tubes (under wax)	colorless, clear fluid	0.008	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless, clear fluid	0.77	1 tube
PCR-mix-Background	colorless, clear fluid	0.5	1 tube
Mineral oil for PCR	colorless, viscous fluid	4.0	1 vial
Positive Control complex (C+)	colorless, clear fluid	0.2	1 tube
DNA-buffer	colorless, clear fluid	0.5	1 tube
Negative Control (C-)*	colorless, clear fluid	1.2	1 tube
Internal Control-FL (IC)**	colorless, clear fluid	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 protocols).

AmpliSens® HSV-typing-FEP PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters (up to 200 µl)
- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 or GeneAmp PCR System 2400 (Applied Biosystems), Uno-2 (Biometra), MiniCycler, PTC-100 (MJ Research);
- Fluorometer ALA-1/4 ("Biosan", Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity .

- Refrigerator for 2–8 °C
- Deep-freezer with temperature not more than –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 filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 compliance 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 mucose membranes. If skin, eyes and mucose membranes 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* -typing-FEP PCR kit is intended for analysis 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

Different manufacturers offer DNA isolation kits. We recommend following nucleic acid extraction kits:

- "DNA-sorb-AM", [REF](#) K1-12-100-CE.
- "DNA-sorb-B", [REF](#) K1-2-100-CE (for extraction DNA from whole blood and cerebrospinal fluid).



Carry the DNA isolation according to the manufacturer's instruction.

7.2. Preparing the PCR.

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

7.2.1 Preparing tubes for PCR.

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT HSV typing** and wax for amplification of DNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** 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
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT HSV typing** 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 I, II. Add above **1 drop** of **mineral oil for PCR**.



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

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



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

6. Carry the 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 will reach 95°C (pause regimen), insert tubes into the cells of amplifier and press the button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before placing them in the thermocycler.

Table 1

Programming thermocyclers at DNA amplification of

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

Step	Thermocyclers with active temperature adjustment:									Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems)			"Gradient Palm Cycler" (Corbett Research),			"Uno-2" (Biometra)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		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	95 °C	5 min	1
2	95 °C	2 sec	35	95 °C	20 sec	20	95 °C	2 sec	24	95 °C	25 sec	20
	65 °C	5 sec		65 °C	25 sec		65 °C	10 sec		65 °C	40 sec	
	72 °C	5 sec		72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
3	95 °C	2 sec	9	95 °C	20 sec	24	95 °C	2 sec	19	95 °C	25 sec	24
	60 °C	10 sec		60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
	72 °C	5 sec		72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
4	95 °C	2 sec	1	95 °C	20 sec	1	95 °C	2 sec	1	95 °C	25 sec	1
	60 °C	10 sec		60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
5	10 °C	storage		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 have been stored at 28°C or below in a light-free area.

8.1. Results interpretation

1. When the analysis is complete the results are automatically shown in the table as follows:

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).

2. 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	Displayed results			Interpretation
		1 (FAM)	2 (HEX)	3 (ROX)	
C-	DNA isolation	<i>HSV</i> II - neg	<i>HSV</i> I - neg	IC +	negative
NCA	Amplification	<i>HSV</i> II - nd	<i>HSV</i> I - nd	IC -	negative
C+	Amplification	<i>HSV</i> II - pos	<i>HSV</i> I - pos	IC -	positive for <i>HSV</i> types I and II DNA

9. TROUBLESHOOTING.

Results of analysis are not obtained as per the following examples:

- 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 not complying 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.
- Positive signal in negative controls indicates the reagents or samples contamination. In such case results of analysis must be considered as inconclusive. It is required to repeat the analysis of all samples and take measures to detect and eliminate the source of contamination.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens[®] HSV-typing-FEP** PCR kit should be stored from 2°C to 8°C. They must be stable until the expiry date stated on the label.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens[®] HSV-typing-FEP** PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens[®] Mycoplasma genitalium-FEP** PCR kit are guaranteed only when additional reagent kits "DNA-sorb-AM" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) is used.

11.2. Specificity.

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

12. REFERENCES.

1. 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-typing-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For in Vitro Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with IQ5, iQ.iCycler
	Positive control		Negative control