

For *in Vitro* Diagnostic Use

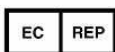
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AmpliSens[®] HSV I, II-EPh PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens® HSV I, II-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Herpes Simplex Virus* types I and II in the clinical material (scrapes and swabs from mucous membranes of urogenital tract; papules, vesicles, ulcers content; urine sediment; secret of the prostate gland) by using electrophoretic detection of the amplified products in agarose gel.

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 DNA specific region genome using special *Herpes Simplex Virus* types I and II primers. **AmpliSens® HSV I, II-EPh PCR kit** is a qualitative test, which uses the principle of endogenous control – amplification of β -globin gene. DNA-target selected as endogenous internal control (IC) is the fragment of human genome and must be present in a sample in sufficient quantity equivalent to that of cells in the sample. **AmpliSens® HSV I, II-EPh 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. CONTENTS

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

AmpliSens® HSV I, II-EPh PCR kit variant 100 R (0.5-ml tubes), **REF** V8-100-R0,5-CE.

AmpliSens® HSV I, II-EPh PCR kit variant 100 R (0.2-ml tubes), **REF** V8-100-R0,2-CE.

AmpliSens® HSV I, II-EPh PCR kit variant 100 R includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix -1-R HSV I, II ready-to-use single-dose test tubes (under wax)	colorless clear liquid	0.005	110 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	blue clear liquid	1.2	1 tube
Mineral oil for PCR	colorless clear liquid	4.0	1 dropper bottle
Positive Control DNA HSV I and human DNA (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

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

AmpliSens® HSV I, II-EPh PCR kit variant 100 R is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation kit
- Agarose gel detection kit
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile DNase-free pipette tips with aerosol filters (up to 200 μ l)
- Vortex mixer
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max. 16,000 x g)
- PCR box or Biological cabinet
- Vacuum aspirator with flask for removing supernatant
- Tube racks
- 1.5 ml polypropylene sterile tubes
- Refrigerator for 2–8 °C
- Deep-freezer with temperature not more than –16 °C.
- Waste bin for used tips.
- Permanent pen for labeling
- Thermostat for tube with controlled temperature and capable of incubating at 25 °C and 100 °C
- Personal thermocyclers (for example, Terzik (DNA-Technology, Russia), Gradient Palm Cycler (Corbett Research, Australia).

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile DNase-free pipette tips with aerosol filters 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 protective 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 biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills with 0.5% sodium hypochlorite solutions 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 where you carried out the previous step.



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-EPh PCR kit is intended for analysis of DNA extracted with DNA isolation kits from the clinical material (scrapes and swabs from mucous membranes of urogenital tract; papules, vesicles, ulcers content; urine sediment; secret of the prostate gland).

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.
- "DNA-sorb-B" (for secret of the prostate gland), REF K1-2-100-CE.



Carry the DNA isolation in compliance with 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.



When using AmpliSens® *HSV I*, II-EPh PCR kit variant 100 R steps 1 and 2 should be omitted.

1. Place the tube with **Wax for PCR** into the heat block at 95 °C to melt the wax completely.
2. Prepare the required number of the PCR tubes. Pipette 5 µl of **PCR-mix -1 *HSV I*, II** into the bottom of each tube. Add a drop (about 10-15 µl of melted wax above, so it covers completely the liquid, close the caps and mark each tube. The prepared tubes could be stored at 2 – 8 °C during 1 week.
3. Collect the required number of tubes prepared as describes above or tubes with **PCR-mix-1-R *HSV I*, II** and wax for amplification of DNA of study and control samples.
4. Add **10 µl of PCR-mix-2 blue** to the surface of wax of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-R *HSV I*, II**.
5. Add above 1 drop of **mineral oil for PCR** (about 25 µl). If thermocycler with heating cover is used, this step can be omitted.
6. Using tips with aerosol barrier add **10 µl cDNA samples** obtained from clinical or control samples.

7. Carry the control amplification reactions:

- NCA - Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
- C+ - Add 10 µl of **Positive Control DNA *HSV I* and human DNA (C+)** to the tube for 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 to cells of amplifier and press button to continue.

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

Table 1

Programming thermocyclers at DNA amplification of *Herpes Simplex Virus* types I and II

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)			"Biometra", "MiniCycler", "PTC-100" (MJ Research)		
	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	10 sec	42	95 °C	15 sec	42	95 °C	1 min	42
	65 °C	10 sec		65 °C	25 sec		65 °C	1 min	
	72 °C	10 sec		72 °C	25 sec		72 °C	1 min	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	4 °C	storage		4 °C	storage		10 °C	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment — 1 h 50 min.

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8°C and for a long time at minus 16°C (be sure to warm the samples to room temperature before running electrophoresis).

8. DATA ANALYSIS

It's recommended to use the following detection agarose kit:

- "EPh" variant 200, REF K5-200.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%). The length of specific amplified DNA fragments are:

- *HSV I*, II - 430 bp

- DNA of β -globin gene (Internal Control) - 723 bp



Put the protective mask or use the glass filter while watching and photographing the gel

Results interpretation

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		430 bp	723 bp	
C-	DNA isolation	No	No	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	Yes	OK

- The sample is considered to be positive for *Herpes Simplex Virus* types I and II DNA if the band of 430 bp is present in agarose gel. The band of IC (723 bp) could be absent in the samples with high concentration of *Herpes Simplex Virus* types I and II DNA.
- The sample is considered to be negative for *Herpes Simplex Virus* types I and II DNA if the band of 430 bp is absent and the band of 723 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

9. TROUBLESHOOTING

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

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests should be repeated.
- If in lanes none of bands of 430 and 723 nucleotide pairs is observed, result of analysis for this sample is irrelevant and the analysis of this sample should be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of DNA or inhibition of PCR.
- If in lanes nonspecific bands at different levels are presented, it may be caused by lack of «hot start» or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C-) specific band of 430 bp appears, it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis should be repeated and measures for detecting 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 AmpliSens® HSV I, II-Eph PCR kit are to be stored at 2-8 °C when not in use. They also must be stable until the expiry date stated on the label.

11. SPECIFICATIONS.

11.1. Sensitivity

Analytical Sensitivity of AmpliSens® HSV I, II-Eph PCR kit is no less than 5×10^3 genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of AmpliSens® HSV I, II-Eph PCR kit are guaranteed only when additional kits of reagents, “DNA-sorb-AM” or “DNA-sorb-B” (for secret of the prostate gland) and “EPh”, are used.

11.2. Specificity

Specificity of AmpliSens® HSV I, II-Eph PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.









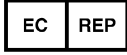


12. REFERENCES

1. Manual “Sampling, transportation and 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 Total Quality Management System, each lot of AmpliSens® HSV I, II-Eph PCR kit is tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS

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