



For *in Vitro* Diagnostic Use

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AmpliSens[®] HHV VI-EPh PCR kit

Instruction Manual

AmpliSens[®]

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1. INTENDED USE.

AmpliSens® HHV VI-EPh PCR kit is an *in vitro* nucleic acid amplification test for a qualitative detection of *Human Herpes Virus* type 6 in the clinical material (whole peripheral or umbilical cord blood; white blood cells; biopsy or autopsy material; cerebrospinal fluid; saliva; throat washes and swabs) by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION.

Human Herpes Virus type 6 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen cDNA specific region using special *Human Herpes Virus* type 6 primers. **AmpliSens® HHV VI-EPh** PCR is a qualitative test, which uses the principle of endogenous control – amplification of β -globin gene. DNA-target selected as endogenous internal control 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® HHV VI-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. CONTENT.

AmpliSens® HHV VI-EPh PCR kit is produced in 3 forms:

AmpliSens® *HHV VI-EPh* PCR kit variant 100 R (tubes 0.5 ml), [REF](#) V10-100-R0,5-CE.

AmpliSens® *HHV VI-EPh* PCR kit variant 100 R (tubes 0.2 ml), [REF](#) V10-100-R0,2-CE.

AmpliSens® *HHV VI-EPh* PCR kit variant 200, [REF](#) V10-200-CE.

AmpliSens® HHV VI-EPh PCR kit variant 100 R or variant 200 includes:

Reagent	Description	variant 100 R		variant 200	
		Volume (ml)	Quantity	Volume (ml)	Quantity
PCR-mix-1-R HHV VI ready-to-use single-dose test tubes (<i>under wax</i>)	colorless, clear liquid	0.005	110 tubes of 0.5 or 0.2 ml	---	---
PCR-mix-1 HHV VI	colorless, clear liquid	---	---	1.1	1 tube
PCR-mix-2 blue	blue clear liquid	1.2	1 tube	1.2	2 tubes
Wax for PCR	white solid	---	---	1.7	2 tubes
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle	8.0	1 dropper bottle
Positive Control DNA HHV 6 and human DNA (C+)	colorless, clear liquid	0.2	1 tube	0.2	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube	1.2	2 tubes

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

(see “DNA-sorb-AM” [REF](#) K1-12-100-CE, “DNA-sorb-B”, [REF](#) K1-2-100-CE, “DNA-sorb-C”, [REF](#) K1-6-50-CE, “RIBO-prep” [REF](#) K1-2-Et-100-CE protocols).

AmpliSens® *HHV VI-EPh* PCR kit variant 100 R is intended for 110 reactions, including controls.

AmpliSens® *HHV VI-EPh* PCR kit variant 200 is intended for 220 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- Agarose gel detection kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barriers (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 minus16°C.
- Waste bin for used tips.
- Permanent pen for labeling
- Personal thermocyclers (for example, “Omn-E” (Hybaid), “GeneAmp PCR System 2400” (Applied Biosystems), “MiniCycler, “PTC-100” (MJ Research), “Terzik” (DNA-Technology);
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).

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 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 using a disinfectant such as 0.5% sodium hypochlorite solutions 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 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.

AmpliSens® HHV VI-Eph PCR kit is intended for analysis of DNA extracted with DNA isolation kits from:

- *Whole peripheral or umbilical cord blood*
- *White cells of peripheral or cord blood*
- *Biopsy or autopsy material*
- *Cerebrospinal fluid*
- *Saliva*
- *Throat washes and swabs*

6.1. *Whole peripheral blood* should be taken in the morning after overnight fasting; *cord blood* is obtained during cordocentesis. The blood is collected into a Vacuette® tube that contains 6% EDTA solution. After the tube is filled, it should be snaked carefully to ensure appropriate mixing.



Do not freeze the whole blood samples.

6.2. *White blood cells of peripheral or cord blood.* To obtain white blood cells add 1.5 ml of Hemolytic (produce of CRIE) and 0.25 ml of the blood into a 1.5 ml Eppendorph tube. Carefully vortex the tube then spine at 8,000 rpm for 2 min. Remove and discard the supernatant by vacuum aspirator leaving 100 µl of fluid above the pellet. The pellet should be white with thin pink coating (debris of the erythrocytes).



Collected leukocyte pellet should be lysed immediately. The lysed sample can be frozen at minus 68 °C for a long time.

6.3. *Biopsy or autopsy material* is taken from the zone of pathogen expected location, from a damaged tissue or form the site connected to the affected area. The biopsy material sample should be placed into sterile disposable 2.0 ml tube (for example, Eppendorph) that contains 0.3 ml of transport medium.

For examination: place the sample into a porcelain mortar, add equal amount of saline solution

or PBS, and thoroughly homogenize with a pestle. Transfer 100 µl aliquot in a sterile tube for DNA extraction. The suspension should be stored at minus 16 °C.

6.4. *Cerebrospinal fluid* sample is obtained by lumbar, occipital, or ventricular standard puncture procedure.

6.5. *Saliva* (0.2 – 1.0 ml) is collected in a 1.5 ml sterile tube (for example, Eppendorph). Make the patient rinse his mouth with water three times before sampling.

6.6. *Throat washes and swabs*. Throat swabs are obtained by a sterile cotton tampon. Before sampling make a patient rinse the mouth with water. Swab tonsillar area, palatine arches, and posterior oropharyngeal surface. Place the effective part of the tampon into the tube with 500 µl of transport medium, break the shaft so that it doesn't protrude above the rim of the tube, and close the tube.

7. PROTOCOL.

7.1. DNA Isolation

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

- “DNA-sorb-B”, **REF** K1-2-100-CE (whole peripheral and cord blood; white blood cells; biopsy and autopsy material; saliva; throat washes and swabs; cerebrospinal fluid)
- “DNA-sorb-AM”, **REF** K1-12-100-CE (saliva; throat washes and swabs; cerebrospinal fluid).
- “DNA-sorb-C”, **REF** K1-6-50-CE.
- “RIBO-prep”, **REF** K-2-9-Et-100-CE (white blood cells).



Carry out 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® *HHV VI*-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 PCR tubes. Add 5 µl of **PCR-mix-1 *HHV VI*** into the bottom of

each tube. Add a drop (about 10-15 µl of melted wax above, to cover 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 quantity of tubes prepared as describes above or tubes with **PCR-mix-1-*HHV VI*** with wax for amplification of DNA from clinical and control samples.
4. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, ensuring that it does not fall under the wax and mix with the reagents in the tube.
5. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.

7.2.2 Amplification.

Use prepared tubes for PCR. Under or immediately above the level of oil, using tips with aerosol barrier, **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction.

Carry out 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 *HHV 6* and human DNA** to the tube for Positive Control of 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 precipitate drops from walls of tubes by using vortex shortly (1–3 sec) before their insertion in thermocycler.

Table 1.

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), "MiniCycler", "PTC-100" (MJ Research)		
	Tempe rature, °C	Time	Cycles	Tempe rature, °C	Time	Cycles	Tempe rature, °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	10 c	42	95	15 c	42	95	1 min	42
	65	10 c		65	25 c		65	1 min	
	72	10 c		72	25 c		72	1 min	
3	72	1 min	1	72	1 min	1	72	1 min	1
4	4	storage		4	storage		10	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h, in thermocycler with active temperature adjustment — 1 h 30 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 carried out 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 of electrophoresis).

8. DATA ANALYSIS.

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

- "EPH" variant 200, REF K5-200-CE.

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 is:

- *Human Herpes Virus* type VI - 380 bp
- *Internal Control* - 723 bp



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

Results interpretation

Results for controls

Control	Controlled stage	Specific bands in the agarose gel		Interpretation
		380 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 *Human Herpes Virus* type VI DNA if the band of 380 bp is present in agarose gel regardless of the band of Internal Control.
- The sample is considered to be negative for *Human Herpes Virus* type VI DNA if the band of 380 bp is absent and the band of 723 bp is present in agarose gel.
- 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.



Internal Control band, 723 bp, can be absent in the cerebrospinal fluid samples due to a little amount of cells.

9. TROUBLESHOOTING.

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

- If results of control points of analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If both bands, 380 bp and 723 bp, are not observed in a lane corresponding to any of studying samples the result of analysis is considered invalid for this sample. The sample should be re-examined starting from the stage of DNA extraction. It can be caused by mistake in clinical processing that provoked loss of DNA or inhibition of PCR.
- If in lines nonspecific bands at different levels are present, 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 380 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 must be repeated and measures for detecting contamination source must be undertaken.

10. STABILITY AND STORAGE.

The all components of the AmpliSens® *Human Herpes Virus* type VI-EPh PCR kit are to be stored between 2°C and 8°C, when not in use. All components of the AmpliSens® *Human Herpes Virus* type VI-EPh PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of AmpliSens® *Human Herpes Virus* type VI-EPh PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of AmpliSens® *Human Herpes Virus* type VI-EPh PCR kit are guaranteed only when additional kits of reagents “DNA-sorb-AM”, “DNA-sorb-B”, “DNA-sorb-C” or “RIBO-prep” and “EPh” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology are used.

11.2. Specificity.

Specificity of AmpliSens® *Human Herpes Virus* type VI-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 accordance with Federal State Institution of Science Central Research Institute of Epidemiology ISO 13485 –certified Total Quality Management System, each lot of AmpliSens® *HHV VI-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		Authorised representative in the European Community.
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use		Positive Control of Amplification
	Negative control of Extraction		Negative Control of Amplification