



For *in Vitro* Diagnostic Use

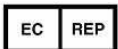
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# AmpliSens<sup>®</sup> EBV-EPh PCR kit

## Instruction Manual

# AmpliSens<sup>®</sup>



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## 1. INTENDED USE.

**AmpliSens® EBV-Eph PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of *Epstein-Barr virus* DNA 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.

*Epstein-Barr virus* detection by the polymerase chain reaction (PCR) detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Epstein-Bar virus* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® EBV-Eph PCR kit** is a qualitative test, which uses the principle of endogenous control – amplification of  $\beta$ -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® EBV-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. Wax melting and reaction mix components occur only at 95°C.

## 3. CONTENT.

**AmpliSens® EBV-Eph PCR kit** is produced in 3 forms:

AmpliSens® EBV-Eph PCR kit variant 100 R (tubes of 0.5 ml volume), [REF](#) V9-100-R0,5-CE.

AmpliSens® EBV-Eph PCR kit variant 100 R (tubes of 0.2 ml volume), [REF](#) V9-100-R0,2-CE.

AmpliSens® EBV-Eph PCR kit variant 200, [REF](#) V9-200-CE.

**AmpliSens® EBV-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 EBV 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-1 EBV	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 EBV 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	1 tube

\* must be used in the isolation procedure as Negative Control of Extraction (see “DNA-sorb-B” [REF](#) K1-2-100-CE, “DNA-sorb-AM” [REF](#) K1-12-100-CE, “DNA-sorb-C” [REF](#) K1-6-100-CE, “RIBO-prep” [REF](#) K1-2-Et-100-CE protocols).

AmpliSens® EBV-Eph PCR kit variant 100 R is intended for 110 reactions, including controls.

AmpliSens® EBV-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  $\mu$ l).
- Vortex mixer.
- PCR box.
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at temperature between 25°C and 100°C.
- Tube racks.
- Personal thermocycler (for example, “GeneAmp PCR System 2700” or equivalent)
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16 °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 to read this handbook before starting work

AmpliSens® EBV-Eph PCR kit is intended for analysis of DNA extracted by 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 into a tube with 6% EDTA solution and mixed; *cord blood* is obtained during cordocentesis.



Do not freeze the whole blood samples.

6.2. *White blood cells of peripheral or cord blood.* To obtain white blood cells add 1.0 ml of Hemolytic (produced 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 the supernatant by vacuum aspirator leaving 100 µl of liquid. The precipitate should be white or white with thin pink coat (destroyed erythrocytes remains) after washing.



Obtained leukocyte residue should be lysed immediately. The lysed residue can be frozen at the temperature not more than minus 68 °C for a long time.

6.3. *Biopsy or autopsy material* is taken from the proposed location of infection causative agent, from a damaged tissue or a zone borderline to the affected area. The biopsy material should be placed into sterile disposable 2.0 ml tube (for example, Eppendorf) contained 0.3 ml of transport medium.

For carrying out the analysis it's necessary to place the sample into a porcelain mortar, add equal amount of saline solution or PBS, and thoroughly homogenize with a pestle. Transfer 100 µl of aliquot in a sterile tube for DNA extraction. The suspension should be stored at the temperature not more than minus 16 °C.

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

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

6.6. *Throat washes and swabs.* Throat swab is obtained by a sterile cotton tampon from tonsillar area, palatine arches, and posterior oropharyngeal surface. Before sampling have a patient rinse the mouth with water. Place the working part of the probe with the tampon into the tube with 500 µl of transport medium, break the probe 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 the 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](#) K1-9-100-CE (saliva; throat washes and swabs; cerebrospinal fluid).
- "DNA-sorb-C", [REF](#) K1-6-50 (biopsy and autopsy material).
- "RIBO-prep", [REF](#) K-2-9-Et-100 (white blood cells).



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.



When using AmpliSens® *EBV*-Eph PCR kit variant 100 R steps 1 and 2 are not needed.

1. Place the tube with **Wax for PCR** into the heat block at 95 °C to melt the wax completely.
2. Prepare required quantity of the PCR tubes. Pipette 5 µl of **PCR-mix-1 EBV** 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 quantity of tubes prepared as described above or tubes with **PCR-mix-1-R EBV** 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 doesn't fall under the wax and mix with 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.

1. Use prepared tubes for PCR. Add **10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction, under or directly above the level of oil by tips with aerosol barrier.
2. 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 EBV and human DNA** to the tube for Positive Control of Amplification.
3. 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 short vortex (1–3 sec) before their insertion in thermocycler.

Table 1

Amplification program of *Epstein-Barr virus*

step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment "Uno-2", "MiniCycler", "PTC-100"		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700", "Gradient Palm Cycler"					
	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 c	42	95 °C	15 c	42	95 °C	1 min	42
	65 °C	10 c		65 °C	25 c		65 °C	1 min	
	72 °C	10 c		72 °C	25 c		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	

4. Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment — 1 h 50 min.
5. After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis. The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8 °C (be sure to heat the samples to room temperature before running electrophoresis).

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

## 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:

- **Epstein-Barr virus** - 290 bp
- **IC** - 723 bp



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

### Results interpretation

Table 2

Control	Which step of test is controlled	Results for controls		Interpretation
		290 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 *Epstein-Barr virus* DNA if the band of 290 bp is present in agarose gel regardless of the presence of IC band.
- The sample is considered to be negative for *Epstein-Barr virus* DNA if the band of 290 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 repeated. Remove any reagents that may be suspect.
- If both bands, 290 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 290 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.

All components of the AmpliSens® *EBV*-EPh PCR kit are to be stored at the temperature between 2 °C and 8 °C. All components of the PCR kit are to be stable until labeled expiration date.

## 11. SPECIFICATIONS.

### 11.1. Sensitivity.

Analytical Sensitivity of AmpliSens® *EBV*-EPh PCR kit is no less than  $1 \times 10^3$  genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of AmpliSens® *EBV*-EPh PCR kit are guaranteed only when additional kits of reagents "DNA-sorb-B", "DNA-sorb-AM" or "DNA-sorb-C" or "RIBO-prep" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), are used.

### 11.2. Specificity.

Specificity of AmpliSens® *EBV*-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® *EBV*-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 *in Vitro* Diagnostic Use



Version



Catalogue number



Negative Control of Amplification



Contains sufficient for <N> tests



Authorised representative in the European Community.



Consult instructions for use



Caution, consult accompanying documents

C+

Positive Control of Amplification

C-

Negative Control of Extraction

IC

Internal Control