CE

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For Professional Use Only

AmpliSens® *EBV*-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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 clinical material (whole peripheral or umbilical cord blood; white blood cells; biopsy or autopsy material; cerebrospinal fluid; saliva; and oropharyngeal washes and swabs) by using electrophoretic detection of the amplified products in agarose gel.

The results of PCR analysis are taken into account in complex diagnostics of disease.

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 specific *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 β-globin gene. DNA-target selected as an endogenous internal control is a human genome fragment; it must be present in a sample in sufficient quantity equivalent to the quantity of cells in the sample. **AmpliSens® *EBV*-EPh PCR kit** uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction mix components mix only at 95 °C.

3. CONTENT

AmpliSens® *EBV*-EPh PCR kit is produced in 2 forms:

AmpliSens® *EBV*-EPh PCR kit variant 100 R (0.5-ml tubes), **REF** V9-100-R0,5-CE.

AmpliSens® *EBV*-EPh PCR kit variant 100 R (0.2-ml tubes), **REF** V9-100-R0,2-CE.

AmpliSens® *EBV*-EPh PCR kit variant 100 R includes:

Reagent	***Description***	***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-2 blue	blue clear liquid	1.2	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Positive Control DNA *EBV* and human DNA (C+$_{EBV\ /\ h.DNA}$)	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 extraction 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).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- PCR box.
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at 25–100 °C.
- Tube racks.
- Personal thermocycler (for example, GeneAmp PCR System 2700 (Applied Biosystems, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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 another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water and seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area move to the Amplification and Detection Areas. 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 are 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 extraction kits from:

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

6.1. *Whole peripheral blood* should be taken in the morning after overnight fasting to a tube with 6% EDTA 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 by CRIE) and 0.25 ml of blood to a 1.5-ml Eppendorf tube. Carefully vortex the tube and spin it at 8,000 rpm for 2 min. Remove the supernatant with a vacuum aspirator leaving 100 µl of liquid. After washing, the pellet should be white or white with a thin pink film (erythrocyte debris).

The obtained leukocyte pellet should be lysed immediately. The lysed pellet can be stored frozen at ≤ –68 ºC for a long time.

6.3. *Biopsy or autopsy material* is taken from the area of presumable pathogen location. The biopsy material should be placed to a sterile disposable 2.0-ml tube (for example, Eppendorf) containing 0.3 ml of transport medium.

To prepare the sample for analysis, place it to a porcelain mortar, add an equal amount of saline or PBS, and thoroughly homogenize with a pestle. Transfer a 100-µl aliquot to a sterile tube for DNA extraction. The suspension should be stored at ≤ –16 ºC.

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

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

6.6. *Oropharyngeal washes and swabs.* Oropharyngeal swab is taken with a sterile probe with a cotton tampon from the 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 a tube with 500 µl of transport medium, break the probe so that it does not protrude above the rim of the tube, and close the tube.

7. WORKING CONDITIONS

AmpliSens® *EBV*-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

It is recommended to use the following nucleic acid extraction kits:

Nucleic acid extraction kit	REF	Test material
DNA-sorb-B	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	K1-9-100-CE	– saliva – throat washes and swabs – cerebrospinal fluid
DNA-sorb-C	K1-6-50-CE	– biopsy and autopsy material
RIBO-prep	K-2-9-Et-100-CE	– white blood cells

Extract DNA according to the manufacturer's instructions.

8.2. Preparing the PCR

The total reaction volume is 25 µl, the volume of DNA sample is 10 µl.

8.2.1 Preparing tubes for PCR

1. Prepare the required number of tubes with **PCR-mix-1-R *EBV*** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-R *EBV*.**
3. Add above 1 drop of **mineral oil for PCR** (~ 25 µl).
4. Use prepared tubes for PCR. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage using tips with aerosol barrier.
5. Carry out the **control amplification reactions:**

NCA – Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).

$C+_{EBV\ /\ h.DNA}$ – Add 10 µl of **Positive Control DNA *EBV* and human DNA** to the tube for Positive Control of Amplification.

8.2.2 Amplification

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

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

Table 1

Amplification program of *Epstein-Barr virus*

	Thermocyclers with active temperature adjustment:			Thermocyclers with block temperature adjustment		
	GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)			**Uno-2 (Biometra), MiniCycler, PTC-100 (MJ Research)**		
step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
0	**95**	pause		**95**	pause	
1	**95**	5 min	1	**95**	5 min	1
2	**95**	15 s	42	**95**	1 min	42
	65	25 s		**65**	1 min	
	72	25 s		**72**	1 min	
3	**72**	1 min	1	**72**	1 min	1
4	**4**	storage		**10**	storage	

The run takes approximately 2 h to complete in a thermocycler with block temperature adjustment or 1 h 30 min in a thermocycler with active temperature adjustment.

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

The amplified samples can be stored at room temperature for 16 h or at 2–8 °C for 1 week (make sure that the samples are warmed up to room temperature before running electrophoresis).

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

9. DATA ANALYSIS

It is 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**

Use a protective mask or a glass filter when looking at the gel or taking photos

Results interpretation

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		290 bp	723 bp	
C-	DNA extraction	No	No	OK
NCA	Amplification	No	No	OK
C+$_{EBV\ /\ h.DNA}$	Amplification	Yes	Yes	OK

- The sample is considered positive for *Epstein-Barr virus* DNA if the 290-bp band is present in agarose gel regardless of the presence of IC band.
- The sample is considered negative for *Epstein-Barr virus* DNA if the 290-bp band is absent and the 723-bp band is present in agarose gel.

Besides the specific products the fuzzy bands of primer dimers may appear in the lanes below the 100-bp level.

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

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

- If the results of the controls do not match with the listed above (Table 2), then the appropriate step of the test should be repeated.
- If none of the 290- and 723-bp bands is observed in the lane of the clinical sample, the result of analysis for this sample is invalid. It can be caused by errors in sample processing that led to the loss of DNA or inhibition of PCR. The test should be repeated from DNA extraction stage. **The 723-bp band corresponding to Internal Control may be absent in the cerebrospinal fluid samples because they contain a small number of cells.**
- If nonspecific bands are seen at different levels in the lanes, the result of analysis is invalid. It may be caused by lack of “hot start” or incorrect temperature profile of the thermocycler. The PCR should be repeated.
- The appearance of the specific 290-bp band appears in lanes corresponding to negative controls (NCA, C–), suggests contamination of reagents or samples. In such cases, the results of analysis are considered to be invalid. Analysis of all samples must be repeated and measures to detect and eliminate the source of contamination must be taken.

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

11. TRANSPORTATION

AmpliSens® *EBV*-EPh PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the AmpliSens® *EBV*-EPh PCR kit are to be stored at 2–8 ºC when not in use. All components of the PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of AmpliSens® *EBV*-EPh PCR kit is no less than $1x10^3$ genome equivalents per 1 ml of a 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, DNA-sorb-C or RIBO-prep and EPh (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology"), are used.

13.2. Specificity

The analytical specificity of AmpliSens® *EBV*-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions.

The clinical specificity of AmpliSens® *EBV*-EPh PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Manual "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology", Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of AmpliSens® *EBV*-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**$_{EBV\ /\ h.DNA}$	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
08.12.10	7.2.1 Preparing tubes for PCR	Items 1 and 2 were deleted in the 7.2.1 item
	Intended Use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added
	Through the text	The abbreviation of Positive Control DNA *EBV* and human DNA was changed into $C+_{EBV\ /\ h.DNA}$
	Amplification	Terzik instrument was deleted
	Data Analysis	The length of specific *EBV* DNA was corrected from 500 to 290 bp
	Troubleshooting	
	Cover page	The phrase «For Professional Use Only» was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
17.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”