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For Professional Use Only

AmpliSens® *Enterovirus*-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens® *Enterovirus*-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Enterovirus* in the clinical material (cerebrospinal fluid) and environmental samples (concentrated water samples) 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

Enterovirus detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen cDNA specific region using special *Enterovirus* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® Enterovirus-EPh** 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® Enterovirus-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. CONTENTS

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

AmpliSens® *Enterovirus*-EPh PCR kit variant 50 R (0.5-ml tubes), **REF** V16-50-R0,5-CE.

AmpliSens® *Enterovirus* -EPh PCR kit variant 50 R (0.2-ml tubes), **REF** V16-50-R0,2-CE.

AmpliSens® *Enterovirus*-EPh PCR kit variant 50 R includes:

Reagent	***Description***	***variant 50 R***	
		Volume (ml)	***Amount***
PCR-mix-1-R *Enterovirus* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	clear liquid of blue color	0.6	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 dropper bottle
Positive Control cDNA *Enterovirus* (C+$_{Enterovirus}$)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control *Enterovirus*-rec**	colorless clear liquid	0.06	5 tubes

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

** add 5 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture (see RIBO-sorb, **REF** K2-1-Et-50-CE protocol).

AmpliSens® ***Enterovirus*-EPh** PCR kit variant 50 R is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- RNA isolation kit.
- Reverse transcription kit.
- Agarose gel detection kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- 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 for ≤ –16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.
- Thermostat for tube with controlled temperature and capable of incubating at 25-100 °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 RNase-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 are described in manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens® *Enterovirus*-EPh PCR kit is intended for analysis of RNA extracted with RNA isolation kits from:

- *Cerebrospinal fluid.*
- *Concentrated water samples (wastewater, drinking, from reservoir).*

6.1*Cerebrospinal fluid sample* (0.5 - 1.0 ml) is obtained by lumbar puncture procedure. Only disposable needles and tubes should be used.

6.2*Concentrated water samples* (1.0 - 2.0 ml) *(wastewater, drinking, form reservoir)* should be delivered in 1.5 ml autoclaved disposable plastic tubes.

Clinical material must be delivered into the laboratory in thermocontainer or in tank with ice within 6 hours at 2-8 °C and within 1 day in case of frozen material.

Only one freeze-thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA Isolation.

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

- RIBO-sorb, **REF** K2-1-Et-50-CE.

Carry the RNA isolation in compliance with the manufacturer protocol.
The volume of clinical sample is 100 µl.
The volume of Internal Control *Enterovirus*-rec (IC) is 5 µl.

8.2. Reverse transcription.

It's recommended to use the following kit for complementary DNA (cDNA) synthesis from RNA:

- REVERTA-L, **REF** K3-4-50-CE.

Carry out the reverse transcription in compliance with the manufacturer protocol. Dilute the cDNA sample **twofold** with DNA-buffer: add 20 µl of DNA-buffer to 20 µl of the sample and mix carefully.

8.3. Preparing the PCR.

Total reaction volume - 25 µl, volume of cDNA sample - 10 µl.

8.3.1. Preparing tubes for PCR.

1. Prepare the required number of tubes with **PCR-mix-1-R *Enterovirus*** and wax for amplification of cDNA from clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of the wax layer of each tubes ensuring that it does not fall under the wax and mix with PCR-mix-1-R *Enterovirus*.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).
4. Using tips with aerosol barrier add **10 µl cDNA samples** obtained from clinical or control samples.
5. Carry out 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 cDNA *Enterovirus*** to the tube labeled C+.

8.3.2. 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 the button to continue.

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

Table 1

Programming thermocyclers for *Enterovirus* cDNA amplification

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	GeneAmp PCR System 2400 (Applied Biosystems); Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems); Gradient Palm Cycler (Corbett Research): MaxyGene (Axygen)**			**Biometra, MiniCycler, PTC-100 (MJ Research)**		
step	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 s	42	**95 °C**	10 s	42	**95 °C**	1 min	42
	58 °C	10 s		**58 °C**	25 s		**58 °C**	1 min	
	72 °C	10 s		**72 °C**	25 s		**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	

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 cDNA fragments in agarose gel.

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

9. 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 cDNA in agarose gel (1.7%). The length of specific amplified cDNA fragments is:

- *Enterovirus* - 207 bp
- IC *Enterovirus* -rec - 440 bp

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

9.1. Results interpretation.

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		207 bp	440 bp	
C-	RNA isolation	No	Yes	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	No	OK

- The sample is considered to be positive for *Enterovirus* RNA if the band of 207 bp is present in agarose gel. The band of IC (440 bp) could be absent in the samples with high concentration of *Enterovirus* RNA.

- The sample is considered to be negative for *Enterovirus* RNA if the band of 207 bp is absent and the band of 440 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.

10. TROUBLESHOOTING

Analysis results 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 207 and 440 nucleotide pairs is observed, the test result of this sample is irrelevant and the analysis should be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lines nonspecific bands are presented at different levels, 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 207 bp appears, it means that reagents or samples contamination has taken place. In such cases analysis results 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 encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® ***Enterovirus*-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**® ***Enterovirus*-EPh** PCR kit are to be stored at 2–8 ºC when not in use. All components of the PCR kit are to be stable until labeled expiration date. 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**® ***Enterovirus*-EPh** PCR kit is no less than $5x10^3$ genome equivalents per 1 ml of sample.

The claimed analytical features of **AmpliSens® *Enterovirus*-EPh** PCR kit are guaranteed only when additional kits of reagents RIBO-sorb, REVERTA-L, and EPh, (manufactured by Federal Budget Institute of Science Central Research Institute of Epidemiology), are used.

13.2. Specificity.

Specificity of **AmpliSens®** ***Enterovirus*-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and 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 of Epidemiology, Moscow, 2008.

15. QUALITY CONTROL

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

16. KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	EC REP	Authorised representative in the European Community