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For Professional Use Only

AmpliSens® *Enterovirus*-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***Enterovirus*-FEP** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Enterovirus* RNA in the clinical material (cerebrospinal fluid, blister exudates, pharyngeal swabs, and fecal samples), culture liquid and concentrated water samples by using end-point hybridization-fluorescence detection of amplified products.

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 the pathogen genome specific region using specific *Enterovirus* primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***Enterovirus*-FEP** PCR kit is a qualitative test that contains the Internal Control STI-87-rec (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*-FEP** 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 wax layer. Wax melts and reaction components mix only at 95 °C.

Enterovirus detection by the polymerase chain reaction (PCR) includes four steps: RNA isolation, reverse transcription, amplification and hybridization-fluorescence detection of amplified products.

3. CONTENT

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

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

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

AmpliSens® ***Enterovirus*-FEP** PCR kit includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FEP/FRT *Enterovirus* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.008	55 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
PCR-mix-Background	colorless clear liquid	0.5	1 tube
Mineral oil for PCR*	colorless viscous liquid	4.0	1 dropper bottle
Positive Control cDNA *Enterovirus* ($C+_{EV}$)	colorless clear liquid	0.1	1 tube
Positive Control STI (CS+)	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 STI-87-rec (IC)***	colorless clear liquid	0.12	5 tubes

* must be used for thermocyclers without constant-temperature lid (for example, Terzik (DNA-Technology)).

** must be used in the isolation procedure as Negative control of extraction.

*** add 10 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture (see RIBO-sorb, **REF** K2-1-Et-50-CE and RIBO-prep, **REF** K2-9-Et-50-CE protocols).

AmpliSens® *Enterovirus*-FEP PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA isolation kit.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research), GeneAmp PCR System 2700 (Applied Biosystems, USA), MaxyGene (Axygen, USA) or equivalent).

- Fluorometer (for example, ALA-1/4 (Biosan, Latvia) or equivalent).
- Disposable polypropylene microtubes for PCR (0.2- or 0.5-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 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 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 and then 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 are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

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

- *Cerebrospinal fluid;*
- *Blister exudates;*
- *Pharyngeal swabs;*
- *Fecal samples.*
- *Concentrated water samples.*

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA isolation

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

- RIBO-sorb, **REF** K2-1-Et-50-CE.
- RIBO-prep, **REF** K2-9-Et-50-CE.

Isolate RNA according to the manufacturer's instructions.
The volume of Internal Control STI-87-rec (IC) is 10 µl.

8.2. Reverse transcription

It is 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 according to the manufacturer's instructions.

8.3. Preparing PCR

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

8.3.1. Preparing tubes for PCR

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Enterovirus*** for amplification of cDNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Enterovirus***.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**) if a thermocycler without

constant-temperature cover is used.

4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Enterovirus*** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of the wax layer of each tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Enterovirus***. Add above **1 drop** of **mineral oil for PCR** if a thermocycler without constant-temperature lid is used.
5. Using tips with aerosol barrier, add **10 µl** of **cDNA samples** obtained in the RNA reverse transcription reaction.
6. Carry out the control amplification reactions:

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

$C+_{EV}$ - Add **10 µl** of **Positive Control cDNA *Enterovirus*** to the tube labeled $C+_{EV}$ (Positive Control of Amplification).

CS+ - Add **10 µl** of **Positive Control STI** to the tube labeled CS+ (Positive Control of Amplification of IC).

C– - Add **10 µl** of the sample extracted from **Negative Control** to the tube labeled C- (Negative control of extraction).

8.3.2. Amplification

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 °C (pause mode), insert tubes to cells of the thermocycler 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

Programming thermocyclers for *Enterovirus* cDNA amplification

	Thermocyclers with active temperature adjustment:					
	Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems), MaxyGene (Axygen)**		
Step	Temperature	Time	Cycles	Temperature	Time	Cycles
0	**95 °C**	pause		**95 °C**	pause	
1	**95 °C**	5 min	1	**95 °C**	5 min	1
2	**95 °C**	10 s	42	**95 °C**	10 s	42
	54 °C	10 s		**54 °C**	25 s	
	72 °C	10 s		**72 °C**	25 s	
3	**72 °C**	1 min	1	**72 °C**	1 min	1
4	**10 °C**	storage		**10 °C**	storage	

9. DATA ANALYSIS

Detection is conducted in a fluorescence detector.

Please read the operating manual for the detector before using this kit.

Program the detector according to the manufacturer's manual.

1. Principle of interpretation:

   - *Enterovirus* cDNA is **detected** in a sample if its signal in the HEX channel is greater than the specified threshold value of the positive result.
   - *Enterovirus* cDNA is **not detected** in a sample if the signal in the HEX channel is less than the specified threshold value of the negative result whereas the signal in the FAM channel is greater than the specified threshold value.
   - The result is **invalid** in a sample if the signal in the FAM channel is less than the specified threshold value of the negative result and the signal in the HEX channel is less than the specified threshold value.
   - The result is **equivocal** if the signal of a sample in the HEX channel is greater than the specified threshold value of the negative result but less than the threshold value of the positive result (the signal is between thresholds).

If the result is invalid or equivocal, PCR should be repeated once again.

2. The result of the analysis is considered reliable only if the results obtained for both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 2).

Table 2

Results for controls

Control	**Stage for control**	**Result of automatic interpretation**		**Interpretation**
		FAM channel (IC)	**HEX channel** (samples)	
C–	RNA isolation	**>** threshold	**<** threshold of negative result	OK
NCA	Amplification	**<** threshold	**<** threshold of negative result	OK
C+$_{EV}$	Amplification	**<** threshold	**>** threshold of positive result	OK
CS+	Amplification	**>** threshold	**<** threshold of negative result	OK

10. TROUBLESHOOTING

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

1. If the signal of C+$_{EV}$ in the HEX channel is less than the threshold of the positive result, run PCR and detection for all samples in which *Enterovirus* cDNA was not found.
2. If the signal of C– and/or NCA in the HEX channel is greater than the threshold of the positive result, run PCR test starting from the RNA extraction stage for all samples in which *Enterovirus* cDNA was found.

3. If no signal was detected either in the channel for detection of the pathogen cDNA or in the channel for detection of IC, the sample should be examined once again (PCR and detection). The same applies to the samples with equivocal results, because the fact that the specific signal does not exceed the threshold value is not sufficient to consider a sample as positive. If equivocal results are obtained in the second run, the analysis should be repeated starting from the RNA extraction stage.

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

11. TRANSPORTATION

AmpliSens® *Enterovirus*-FEP PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Enterovirus*-FEP** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Enterovirus*-FEP** PCR kit are stable until the labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FEP/FRT *Enterovirus* is to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Nucleic acid extraction kit	PCR kit	Sensitivity, GE/ml
Cerebrospinal fluid	RIBO-sorb	PCR kit variant FEP	$5x10^3$
	RIBO-prep		

13.2. Specificity

Analytical specificity of **AmpliSens® *Enterovirus*-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Clinical specificity of **AmpliSens® *Enterovirus*-FEP** PCR kit was confirmed in laboratory clinical trials.

Nonspecific responses were absent in tests of DNA samples of the following microorganisms: *Enterovirus* strains (Coxsackie B1, B2, B3, B4, B5, and B6; Polio (Sabin)

I, II, and III); *Influenza virus A* (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, and H5N1), *Influenza virus B*, *Rhinovirus*, *RS* viruses, human *Adenovirus* strains 3, 5, 7, 37, and 40; *N.meningitidis*, *St.pneumoniae*, *H.influenzae*, *Clebsiella* K 65 SW4, *Listeria monocytogenes* USHI 19, *Listeria monocytogenes* USHI 52, *Proteus vulgaris* 115/98, *Pseudomonas aeruginosa* DN c1, *Staphilococcus aureus* 653, *Staphilococcus aureus* 29112, *Morganella morganii* 619 c 01, and *Enterobacter faecalis* 356.

14. REFERENCES

1. Handbook “Sampling, Transportation, Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institute of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers” Rights Protection and Human Well-Being, 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**® ***Enterovirus*-FEP** PCR kit has been 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+$_{EV}$, CS+**	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
25.01.11	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” 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
	Through the text	For Positive Control cDNA *Enterovirus* the abbreviation was changed into $C+_{EV}$
	Intended use	Concentrated water samples were added
	Sampling and handling	
	Troubleshooting	The item was changed
	Content	The reagent Positive Control STI (CS+) was added
18.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
24.10.11 VV	Intended Use	Culture liquid was added as a test material