



For *in Vitro* Diagnostic Use

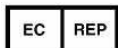
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AmpliSens[®] *Enterovirus-FEP* PCR kit

Instruction Manual

AmpliSens[®]



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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) and environmental samples (concentrated water samples) by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Enterovirus detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Enterovirus* primers. In **Fluorescent End-Point** PCR, the amplified product is detected by using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix 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, which contains 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-FEP** 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® Enterovirus-FEP PCR kit is produced in 2 forms:

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

AmpliSens® Enterovirus-FEP PCR kit (tubes of 0.5 ml), **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 (<i>under wax</i>) | 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+) | 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 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 rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, 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 with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for 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 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.*
- *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, from 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. PROTOCOL.

7.1. RNA Isolation.

It's 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.



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

7.2. 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 according to the manufacturer's instructions.

7.3. Preparing the PCR.

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

7.2.1. Preparing tubes for PCR.

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Enterovirus*** and wax 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**).
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**.
5. Using tips with aerosol barrier add **10 µl** of **cDNA samples** obtained in the RNA reverse transcription

reaction.

as well as Negative Control of extraction are passed (Table 2).

6. 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+ (Positive Control of Amplification).

7.2.2. 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 sec) before placing them in the thermocycler.

Table 1

Programming thermocyclers for *Enterovirus* cDNA amplification

| Step | Thermocyclers with active temperature adjustment: | | | | | |
|------|---------------------------------------------------|----------|--------|---------------------------------------------------------------------------|----------|--------|
| | "Terzik" (DNA-Technology) | | | "GeneAmp PCR System 2700" (Applied Biosystems), "Maxygene" (Axygen) | | |
| | 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 sec | 42 | 95 °C | 10 sec | 42 |
| | 54 °C | 10 sec | | 54 °C | 25 sec | |
| | 72 °C | 10 sec | | 72 °C | 25 sec | |
| 3 | 72 °C | 1 min | 1 | 72 °C | 1 min | 1 |
| 4 | 10 °C | хранение | | 10 °C | хранение | |

8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to the manufacturer's manual and Appendix 1.

8.1. Results interpretation.

1. When the analysis is complete the results are automatically shown in the table as follows:

pos – positive result;

neg – negative result;

eq – equivocal result (signal is in grey zone);

nd – invalid result (specific signal and IC signal are absent in the sample).

2. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification

Table 2

| Control | Stage for control | Results for controls | | Interpretation |
|---------|-------------------|------------------------------------|--------------------------|----------------|
| | | Result of automatic interpretation | | |
| | | FAM channel (IC) | HEX channel (samples) | |
| C- | RNA isolation | + | <i>Enterovirus</i> - neg | OK |
| NCA | Amplification | - | <i>Enterovirus</i> - nd | OK |
| C+ | Amplification | - | <i>Enterovirus</i> – pos | OK |

9. TROUBLESHOOTING.

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

- The analysis of the samples with result **nd** (except for NCA) are to be repeated from RNA extraction stage. Result nd for NCA is normal.
- The analysis of the samples with result **eq** are to be repeated from RNA extraction stage. In case of analogous result repeat the samples are considered to be positive.
- Positive signal in negative controls (C-, NCA) indicates the reagent or sample contamination. In such case results of analysis must be considered as inconclusive. The analyses must be repeated and measures taken to detect and eliminate the contamination source.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® Enterovirus-FEP** PCR kit are to be stored at the temperature between 2 and 8 °C, when not in use. All components of the **AmpliSens® Enterovirus-FEP** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Enterovirus-FEP** PCR kit is no less than 5×10^3 genome equivalents per 1 ml of sample (GE/ml).



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

11.2. Specificity.

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. Specificity of **AmpliSens® Enterovirus-FEP** PCR kit was confirmed in laboratory clinical trials.
















12. REFERENCES.

1. Handbook “Sampling, transportation, 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 Quality Management System, each lot of **AmpliSens® Enterovirus-FEP** PCR kit has been 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 |  | Negative Control of Amplification |
|  | Positive Control of Amplification |  | Negative control of Extraction |
|  | Internal control | | |