



For *in Vitro* Diagnostic Use

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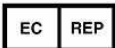
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AmpliSens® *Enterovirus-FRT*

PCR kit

Instruction Manual



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1. INTENDED USE.

AmpliSens® Enterovirus-FRT 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 real-time hybridization-fluorescence detection.

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 real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® Enterovirus-FRT** 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-FRT** 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-FRT PCR kit is produced in 1 form:

AmpliSens® Enterovirus-FRT PCR kit variant FRT (for use with RG), **REF** R-V16(RG)-CE.

AmpliSens® Enterovirus-FRT 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
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
Positive Control cDNA Enterovirus (C+)	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 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-FRT 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, Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia).
- 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-FRT* 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**.

7.3.1. Preparing tubes for PCR.

1. Prepare the required number of the 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. Using tips with aerosol barrier add **10 µl** of **cDNA samples**, obtained in RNA reverse transcription reaction.
4. Carry 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* (C+)** to the tube labeled C+ (Positive Control of Amplification).

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

7.3.2. Amplification.

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

Table 1

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	10 sec	–	45
	54	20 sec	FAM/Green, JOE/Yellow	
	72	10 sec	–	

3. Fluorescence detection is on Fam/Green and Joe/Yellow channels on the **54 °C** step.
4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

8. DATA ANALYSIS.

Internal Control is detected on the FAM/Green fluorescence channel, *Enterovirus* cDNA is detected on the JOE/Yellow fluorescence channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

8.1. Results interpretation.

The results are interpreted by the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument by the crossing (or not) of the fluorescence curve with the threshold line.

Table 2

Results for controls

Control	Stage for control	Ct channel FAM (Green)	Ct channel JOE (Yellow)	Interpretation
C-	RNA isolation	Pos (< X*)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Neg	Pos (< Z*)	OK
CS+	Amplification	Pos (< Y*)	Neg	OK

* For X, Y, Z values see Appendix 1.

- The sample is considered to be positive for *Enterovirus* if its Ct value is less than Z on JOE /Yellow channel.
- The sample is considered to be negative for *Enterovirus* if its Ct value is absent on JOE/Yellow channel while Ct value on FAM/Green channel doesn't exceed X.

9. TROUBLESHOOTING.

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

- If Ct value of the sample on JOE/Yellow channel is more than Z, while Ct value on FAM/Green channel does not exceed X, PCR is to be repeated. If in the second run the result is the same or Ct value on JOE/Yellow channel is less than Z the result is considered to be positive.
- If fluorescent signal is absent on both JOE/Yellow and FAM/Green channels or Ct value on FAM/Green channel is more than X, PCR is to be repeated. If repeatedly received result is the same, it is required to repeat the analysis of the sample from the extraction stage.
- If signals are detected in negative control of RNA isolation (C-) on JOE/Yellow channel and in negative control of amplification (NCA) on any of channels, it indicates contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is required to repeat the analysis of all samples and take measures to detect and eliminate the source of contamination.

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

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens[®] Enterovirus-FRT** PCR kit is no less than 5×10^3 genomes equivalents in 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens[®] Enterovirus-FRT** 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-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens[®] Enterovirus-FRT** PCR kit was confirmed in laboratory clinical trials.

















12. REFERENCES.

- 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-FRT** 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		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control