



For *in Vitro* Diagnostic Use

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AmpliSens® Poliovirus-FRT

PCR kit

Instruction Manual



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1. INTENDED USE

AmpliSens® Poliovirus-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Poliovirus* and *Enterovirus* group C (*HEV-C*) RNA with *Poliovirus* differentiation to strains (*Sabin 1*, *Sabin 2*, *Sabin 3*) in clinical materials and environmental samples by using real-time hybridization-fluorescence detection.



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

2. PRINCIPLE OF PCR DETECTION

Poliovirus detection by the polymerase chain reaction (PCR) is based on the multiplex amplification of the pathogen genome specific region in two tubes using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® Poliovirus-FRT** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® Poliovirus-FRT** 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 chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.



Use amplifiers with three and more channels for detection of 7 pathogens and IC.

3. CONTENT

AmpliSens® Poliovirus-FRT PCR kit is produced in 1 form:

AmpliSens® *Poliovirus-FRT* PCR kit variant FRT-50 F, **REF** R-V58(RG,iQ)-CE.

AmpliSens® Poliovirus-FRT PCR kit variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL <i>HEV-C</i> / <i>STI</i>	colorless clear liquid	0.6	1 tube
PCR-mix-1-FL <i>Sabin 1/2/3</i>	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Reagent	Description	Volume, ml	Quantity
DNA-buffer	colorless clear liquid	0.5	1 tube
Positive Control cDNA <i>HEV-C</i> (C ^{+HEV-C})	colorless clear liquid	0.1	1 tube
Positive Control cDNA <i>Sabin 1/2/3</i> (C ^{+Sabin 1/2/3})	colorless clear liquid	0.1	1 tube
Internal Control <i>STI-87</i> (IC)*	colorless clear liquid	0.6	1 tube
Negative Control (C-)**	colorless clear liquid	1.2	1 tube
Internal Control <i>STI-87-rec</i> (IC)***	colorless clear liquid	0.12	5 tubes

* Internal Control *STI-87* (IC) must be used for this kit as Positive control of amplification of Internal Control *STI-87-rec* and is indicated as CS+.

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

*** must be used in the extraction procedure as Internal Control and indicated as IC.

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

4. ADDITIONAL REQUIREMENTS

- RNA extraction 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); iCycler iQ or iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA), DT-96 (DNA-Technology, Russia) or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- 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 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 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 is described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.



The material must be analyzed according to rules and instructions.

AmpliSens® Poliovirus-FRT PCR kit is intended for the analysis of RNA extracted by RNA extraction kits from sterile and unsterile clinical material and concentrated water samples.

Cerebrospinal fluid and *water samples* are used without treatment.

Feces.

Transfer 0.4–1.0 g (≤ 1 ml) of feces to a sterile vial using a sterile spatula. Add 4.0 ml of saline to obtain 10–20 % suspension. Mix the vial on the vortex. Decolorize the suspension by centrifuging at 3000 rpm for 20 min. Use fecal decolorized extract (supernatant) for RNA extraction. Transfer the extract to a sterile tube for storing.



Liquid feces can be used without suspension preparing stage.

Store the fecal extract for 1 day at 2–8 °C, for 1 month (with addition of glycerol)

at ≤ -16 °C, and for a long time (with addition of glycerol) at ≤ -68 °C.



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

7. PROTOCOL

7.1. RNA extraction

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

- RIBO-prep, **REF** K2-9-Et-50-CE.
- RIBO-sorb, **REF** K2-1-Et-50-CE.
- Other nucleic acid extraction kits.



Extract RNA according to the manufacturer's instructions.

7.2. Reverse transcription

It is recommended that the following reverse transcription reagent kits are used:

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



Carry out the reverse transcription according to the manufacturer's instructions.



cDNA obtained with AmpliSens® *Enterovirus-FRT* PCR kit can be used as well.

7.3. Preparing PCR

7.3.1. Preparing tubes for PCR

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

1. Thaw the reagents and vortex the tubes thoroughly.
2. Prepare the required number of tubes including controls.
3. Mix **PCR-mix-1-FL HEV-C / STI** with **PCR-mix-2-FRT** and **polymerase (TaqF)** as well as **PCR-mix-1-FL Sabin 1/2/3** with **PCR-mix-2-FRT** and **polymerase (TaqF)** according to **Appendix 1**. Vortex the tubes thoroughly.
4. Transfer **15 μ l** of the prepared mixture to the prepared tubes.
5. Add **10 μ l** of **cDNA** obtained from clinical or control samples at the reverse transcription stage into the prepared tubes using tips with aerosol barrier.



Discharge the mixture that is not in use.

6. Carry out the control amplification reactions:

NCA	- Add 10 μl of DNA-buffer to the tube labeled NCA (Negative Control of Amplification).
C⁺_{HEV-C}	- Add 10 μl of Positive Control cDNA HEV-C for PCR-mix-1-FL <i>HEV-C</i> / STI to the tube labeled C ⁺ _{HEV-C} (Positive Control of Amplification).
C⁺_{Sabin 1/2/3}	- Add 10 μl of Positive Control cDNA Sabin 1/2/3 for PCR-mix-1-FL <i>Sabin 1/2/3</i> to the tube labeled C ⁺ _{Sabin 1/2/3} (Positive Control of Amplification).
CS⁺	- Add 10 μ l of Internal Control STI-87 (for PCR-mix-1-FL <i>HEV-C</i> / STI) to the tube labeled CS ⁺ (Positive Control of Amplification of IC).

7.3.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual.

1. Create a temperature profile on your instrument as follows:

Table 1

Amplification program

Step	Rotor-type Instruments ¹			Plate-type Instruments ²		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling	95	10 s	45	95	10 s	45
	54	20 s <i>fluorescent signal detection</i>		54	20 s <i>fluorescent signal detection</i>	
	72	10 s		72	10 s	

Fluorescent signal is detected in the channels designed for the FAM/Green, JOE/Yellow/HEX and ROX/Orange fluorophores on the 2nd step of stage Cycling.

- Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
- Insert tubes into the reaction module of the device.
- Run the amplification program with fluorescence detection.
- Analyze results after the amplification program is completed.

8. DATA ANALYSIS

PCR-mix-1-FL HEV-C / STI:

IC is detected in the JOE/Yellow/HEX fluorescence channel,
HEV-C cDNA is detected in the FAM/Green fluorescence channel.

PCR-mix-1-FL Sabin 1/2/3:

Sabin 1 cDNA is detected in the ROX/Orange fluorescence channel,
Sabin 2 cDNA is detected in the FAM/Green fluorescence channel,
Sabin 3 cDNA is detected in the JOE/Yellow/HEX fluorescence channel.
See *Guidelines* for data analysis settings for the instrument.

8.1. Interpretation of results

The results are interpreted by the software of the PCR instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

Table 2

Interpretation of results for PCR-mix-1-FL HEV-C / STI

Ct value in channel		Interpretation
FAM/Green	JOE/Yellow/HEX	
Pos (≤ boundary value*) or Neg (> boundary value*)	Pos (≤ boundary value*)	HEV-C cDNA is detected
Pos (< boundary value*)	Neg (> boundary value*)	HEV-C cDNA is not detected
Neg (> boundary value*)	Neg (> boundary value*)	Invalid result

¹ For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.

² For example, iCycler, iQ5, Mx3000P, Mx3000, or equivalent.

Table 3

Interpretation of results for PCR-mix-1-FL Sabin 1/2/3

Ct value in channel			Interpretation
FAM/Green	JOE/Yellow/HEX	ROX/Orange	
Pos (≤ boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)	Sabin 2 cDNA is detected
Neg (> boundary value*)	Pos (≤ boundary value*)	Neg (> boundary value*)	Sabin 3 cDNA is detected
Neg (> boundary value*)	Neg (> boundary value*)	Pos (≤ boundary value*)	Sabin 1 cDNA is detected
Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)	Sabin 1/2/3 cDNA are not detected **

Table 4

Results for controls

PCR-mix-1-FL	Control	Stage for control	Ct value in channel		
			FAM/Green	JOE/Yellow/HEX	ROX/Orange
HEV-C / STI	C-	RNA extraction	Pos (≤ boundary value*)	Neg (> boundary value*)	-
HEV-C / STI	C ⁺ _{HEV-C} (Positive Control cDNA HEV-C)	Amplification	Neg (> boundary value*)	Pos (≤ boundary value*)	-
HEV-C / STI	CS+	Amplification	Pos (≤ boundary value*)	Neg (> boundary value*)	-
HEV-C / STI	NCA	Amplification	Neg (> boundary value*)	Neg (> boundary value*)	-
Sabin 1/2/3	C-	RNA extraction	Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)
Sabin 1/2/3	C ⁺ _{Sabin 1/2/3} (Positive Control cDNA Sabin 1/2/3)	Amplification	Pos (≤ boundary value*)	Pos (≤ boundary value*)	Pos (≤ boundary value*)
Sabin 1/2/3	NCA	Amplification	Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)

* For boundary values, see the *Important Product Information Bulletin*.

** If the result is positive for PCR-mix-1-FL HEV-C / STI in the FAM/Green channel.

9. TROUBLESHOOTING

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

- If a Ct value is present for C- and/or for NCA (except for C- in FAM/Green channel for PCR-mix-1-FL HEV-C / STI) in the results grid, it indicates contamination of reagents or samples. In such

cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.

- If no signal is detected for C⁺_{HEV-C} and C⁺_{Sabin 1/2/3}, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer’s instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if Cycler iQ or iQ5 instruments are used).

10. STABILITY AND STORAGE

All components of the **AmpliSens[®] Poliovirus-FRT** PCR kit (except for PCR-mix-1-FL *HEV-C*/STI, PCR-mix-1-FL *Sabin 1/2/3*, polymerase (TaqF), and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens[®] Poliovirus-FRT** PCR kit are stable until the expiration date on the label.



PCR-mix-1-FL *HEV-C*/STI, PCR-mix-1-FL *Sabin 1/2/3*, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at ≤ -16 °C.



PCR-mix-1-FL *HEV-C*/STI and PCR-mix-1-FL *Sabin 1/2/3* are to be kept away from light.

11. SPECIFICATIONS

11.1. Sensitivity

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml ³
Concentrated water samples	RIBO-sorb	1x10 ³
Feces	RIBO-prep	5x10 ³

11.2. Specificity

The analytical specificity of **AmpliSens[®] Poliovirus-FRT** PCR kit is ensured by selection of specific

primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. The clinical specificity of **AmpliSens[®] Poliovirus-FRT** PCR kit was confirmed in laboratory clinical trials.

Specificity was checked while testing DNA samples of following microorganisms: *Enterovirus* (*Coxsackie B1, B2, B3, B4, B5, B6; Polio (Sabin) I, II, III*); *Influenza virus A* (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), *Influenza virus B, Rhinovirus, RS virus, human Adenovirus – 3, 5, 7, 37, 40*.

Specificity was estimated while testing DNA samples of following microorganisms: *Neisseria meningitides, Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella K 65 SW4, Listeria monocytogenes* USHC 19, *Listeria monocytogenes* USHC 52, *Proteus vulgaris* 115/98, *Pseudomonas aeruginosa* DN c1, *Staphylococcus aureus* 653, *Staphylococcus aureus* 29112, *Morganella morganii* 619 c 01, *Enterobacter faecalis* 356.

12. REFERENCES











1. Handbook “Sampling, Transportation, and 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[®] Poliovirus-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

³ The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.

14. EXPLANATION OF SYMBOLS

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Caution, consult accompanying documents
	Contains sufficient for <n> tests	NCA	Negative Control of Amplification
	Consult instructions for use	C-	Negative control of extraction
C⁺_{HEV-C}, C⁺_{Sabin 1/2/3}, CS⁺	Positive controls of amplification	IC	Internal Control
CRIE	Central Research Institute of Epidemiology (Moscow, Russia)		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
30.08.10	7.3 Preparing PCR	The scheme or reaction mixture preparation is relocated to Appendix 1
13.10.10	Through the text	Abbreviations C ₁ ⁺ and C ₂ ⁺ for Positive Control cDNA <i>HEV-C</i> and Positive Control cDNA <i>Sabin 1/2/3</i> are added
20.10.10	Through the text	Abbreviations C ⁺ _{HEV-C} and C ⁺ _{Sabin 1/2/3} for Positive Control cDNA <i>HEV-C</i> and Positive Control cDNA <i>Sabin 1/2/3</i> are added