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For Professional Use Only

AmpliSens® *Poliovirus*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

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 *HEV-C* / STI	colorless clear liquid	0.6	1 tube
PCR-mix-1-FL Sabin 1/2/3	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 *HEV-C* ($C+_{HEV\text{-}C}$)	colorless clear liquid	0.1	2 tubes
Positive Control cDNA Sabin 1/2/3 ($C+_{Sabin\ 1/2/3}$)	colorless clear liquid	0.1	2 tubes
Internal Control STI-87 (IC)*	colorless clear liquid	0.6	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

* 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 are 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. WORKING CONDITIONS

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

8. PROTOCOL

8.1. 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.

8.2. 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.

8.3. Preparing PCR

8.3.1. Preparing tubes for PCR

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

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 µl** of the prepared mixture to the prepared tubes.
5. Add **10 µl** 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 *HEV-C* / 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 Sabin 1/2/3 to the tube labeled C+Sabin 1/2/3 (Positive Control of Amplification).

CS+ - Add 10 µl of **Internal Control STI-87** (for PCR-mix-1-FL *HEV-C* / STI) to the tube labeled CS+ (Positive Control of Amplification of IC).

8.3.2. 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

	Rotor-type Instruments[1]			**Plate-type Instruments**[2]		
Step	***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 *fluorescent signal detection*		**54**	20 s *fluorescent signal detection*	
	72	10 s		**72**	10 s	

Fluorescent signal is detected in the channels designed for the FAM/Green,

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[2] For example, iCycler, iQ5, Mx3000P, Mx3000, or equivalent.

JOE/Yellow/HEX and ROX/Orange fluorophores on the 2nd step of stage Cycling.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin.*

3. Insert tubes into the reaction module of the device.

4. Run the amplification program with fluorescence detection.

5. Analyze results after the amplification program is completed.

9. 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.

9.1. 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**

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+$_{HEV\text{-}C}$ (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+$_{\text{Sabin 1/2/3}}$ (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.

10. 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+$_{HEV\text{-}C}$ and C+$_{\text{Sabin 1/2/3}}$, 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).

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

11. TRANSPORTATION

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

12. 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. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

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 temperature from minus 24 to minus 16 °C when not in use.

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

13. SPECIFICATIONS

13.1. Sensitivity

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

13.2. Specificity

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

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* (*Coxsakie* 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 Clebsiella* 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.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for 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® *Poliovirus*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
24.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”