AmpliSens® *Poliovirus*-FRT PCR kit

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

Symbol	Meaning	Symbol	Meaning
REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
EC REP	Authorized representative in the European Community	C+	Positive control of amplification
		IC	Internal control

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 material and environmental samples using real-time hybridization-fluorescence detection of amplified products.

NOTE: 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 simultaneous 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 (Internal Control STI-87 (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.

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

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	Internal Control STI-87 (IC) DNA	*HEV-C* cDNA
Target gene	Artificially synthesized sequence	3'-UTR

3. CONTENT

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

variant FRT-50 F REF R-V58(RG,iQ)-CE.

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	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
DNA-buffer	colorless clear liquid	0.5	1 tube
Positive Control cDNA *HEV-C* ($C+_{HEV-C}$)	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

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

Variant FRT is intended for 55 reactions (including controls).

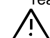
4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Reverse transcription kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 100 and 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); iCycler iQ or iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips..

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.



Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

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6. SAMPLING AND HANDLING

NOTE: 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.

NOTE: The material must be analyzed according to rules and instructions.

AmpliSens® *Poliovirus*-FRT PCR kit is intended for analysis of RNA extracted with 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 3,000 rpm for 20 min. Use fecal decolorized extract (supernatant) for RNA extraction. Transfer the extract to a sterile tube for storing.

NOTE: 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 the temperature below minus 16 °C, and for a long time (with addition of glycerol) at the temperature below minus 68 °C.

NOTE: 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.

The RNA extraction of each test sample is carried out in the presence of **Internal Control STI-87-rec (IC).** The **Negative Control (C–)** reagent is used as the Negative control of extraction (C–).

NOTE: Extract RNA according to the manufacturer's protocols.

NOTE: In case of extracting with RIBO-sorb reagent kit, make sure that the volume of **Internal Control STI-87-rec (IC)** reagent added to each tube is **10 µl**..

8.2. Reverse transcription

It is recommended to use the following kit for the complementary DNA (cDNA) synthesis from the RNA:

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

NOTE: Carry out the reverse transcription according to the manufacturer's protocol.

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

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.

Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

NOTE: Reaction mixture components should be mixed just before analysis with calculating for the required number of reactions (test and control samples) according to Table 2. Note that even for analysis of one test cDNA sample, it is necessary to carry out all controls of the PCR stage (Positive Controls of Amplification (C+, CS+) and Negative Control of Amplification (NCA)). It is recommended to mix the reagents for an even reaction number to ensure more exact dosage.

1 Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2 Take the required number of tubes for amplification for the test and control cDNA samples. The type of tubes/strips depends on the PCR instrument used for analysis.
3 To prepare the reaction mixture, mix reagents: **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 **Table 2**. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Scheme of reaction mixture preparation

Reagent volume for 1 reaction (µl)	10.00	5.00	0.50
The number of reactions [1]	**PCR-mix-1-FL**	**PCR-mix-2-FRT**	**Polymerase (TaqF)**
8	80	40	4.0
10	100	50	5.0
12	120	60	6.0
14	140	70	7.0
16	160	80	8.0
18	180	90	9.0
20	200	100	10.0
22	220	110	11.0
24	240	120	12.0
26	260	130	13.0
28	280	140	14.0
30	300	150	15.0
32	320	160	16.0

[1] for **PCR-mix-1-FL *HEV-C* / STI** – the number of samples and IC (N), controls of amplification, one extra sample (N+3+1);
for **PCR-mix-1-FL Sabin 1/2/3** – the number of samples and IC (N), controls of amplification, one extra sample. (N+2+1).

4 Transfer **15 µl** of the prepared mixture to each tube.
5 Using tips with aerosol filter, add **10 µl** of **cDNA samples** at the RNA reverse transcription stage. Dispose of the unused reaction mixture.

NOTE: Avoid transferring of sorbent together with the cDNA samples extracted by **DNA-sorb-B** kit.

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+$_{Sabin\ 1/2/3pylori}$** – 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 (IC)** (for PCR-mix-1-FL *HEV-C* / STI) to the tube labeled CS+ (Positive Control of Amplification of IC).
- **C–** Add **10 µl** of **cDNA** obtained by extraction and reverse transcription of the **Negative Control (C-) reagent** to the tube labeled C– (Negative control of Extraction)

8.3.2. Amplification

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

Table 3

AmpliSens-1 amplification program

Step	Rotor-type Instruments[2]			Plate-type Instruments[3]		
	Temperature, °C	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	15 min	1	**95**	15 min	1
2	**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 for the FAM, JOE and ROX fluorophores.
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

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels:

<u>**For PCR-mix-1-FL *HEV-C* / STI:**</u>

- The signal of the **IC** DNA amplification product is detected in the channel for the FAM fluorophore
- The signal of the ***HEV-C*** cDNA amplification product is detected in the channel for the JOE fluorophore.

<u>**For PCR-mix-1-FL Sabin 1/2/3:**</u>

- The signal of the **Sabin 1** cDNA amplification product is detected in the channel for the ROX fluorophore,
- The signal of the **Sabin 2** cDNA amplification product is detected in the channel for the FAM fluorophore,
- The signal of the **Sabin 3** cDNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.
Before the detection run, the required settings of the detector software should be adjusted according to the *Important Product Information Bulletin* enclosed to the PCR kit and Guidelines [2].
Principle of interpretation is specified in the Tables 4 and 5.

Table 4

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

***Ct* value in the channel for fluorophore**		**Result**
FAM	**JOE**	
< boundary value or >boundary value	< boundary value	***HEV-C*** cDNA is **detected**
< boundary value	Absent or > boundary value	***HEV-C*** cDNA is **not detected**
Absent or > boundary value	Absent or > boundary value	**Invalid result** Repeat analysis of the sample beginning from the cDNA extraction stage

Table 5

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

***Ct* value in the channel for fluorophore**			**Result**
FAM	**JOE**	**ROX**	
< boundary value	Absent or > boundary value	Absent or > boundary value	**Sabin 2** cDNA is **detected**
Absent or > boundary value	< boundary value	Absent or > boundary value	**Sabin 3** cDNA is **detected**
Absent or > boundary value	Absent or > boundary value	< boundary value	**Sabin 1** cDNA is **detected**
Absent or > boundary value	Absent or > boundary value	Absent or > boundary value	**Sabin 1/2/3** cDNA are **not detected[4]**

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2].

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 6)

[2] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[3] For example, iCycler, iQ5, Mx3000P, Mx3000, or equivalent.
[4] If the *Ct* value determined for PCR-mix-1-FL *HEV-C* / STI in the channel for FAM fluorophore is less than the boundary Ct value

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Table 6

Results for controls

PCR-mix-1-FL	Control	Stage for control	*Ct* value in the channel for fluorophore		
			FAM	JOE	ROX
***HEV-C* / STI**	C–	RNA extraction	<boundary value	> boundary value or Absent	–
	C+*HEV-C*	PCR	> boundary value or Absent	<boundary value	–
	CS+	PCR	<boundary value	> boundary value or Absent	–
	NCA	PCR	> boundary value or Absent	> boundary value or Absent	–
Sabin 1/2/3	C–	RNA extraction	> boundary value or Absent	> boundary value or Absent	> boundary value or Absent
	C+Sabin 1/2/3	PCR	<boundary value	<boundary value	<boundary value
	NCA	PCR	> boundary value or Absent	> boundary value or Absent	> boundary value or Absent

10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the relevant channel is greater than the boundary *Ct* value, the amplification and detection should be repeated for all samples in which required cDNA was not detected in the relevant channel.
2. If the *Ct* value determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the relevant channel is less than the boundary value, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which required cDNA was detected in the relevant channel.

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 are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FL *HEV-C* / STI, PCR-mix-1-FL Sabin 1/2/3, polymerase (TaqF), and PCR-mix-2-FRT). All components of the **AmpliSens®** ***Poliovirus*****-FRT** PCR kit are stable until the expiry date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: 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 the temperature from minus 24 to minus 16 °C.

NOTE: 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

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

13.2. 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.

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, *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.

The clinical specificity of **AmpliSens®** ***Poliovirus*****-FRT** PCR kit was confirmed in laboratory clinical trials.

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.
2. Guidelines to the **AmpliSens®** ***Poliovirus*****-FRT** PCR kit for qualitative detection of *Poliovirus* and *Enterovirus* group C (*HEV-C*) RNA with *Poliovirus* differentiation to strains (Sabin 1, Sabin 2, Sabin 3) in environmental samples and clinical materials by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

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.

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"
22.09.15 PM	Text	Corrections according to the template.
	8.1. DNA extraction	Additions about carrying out the control of extraction
	8.2.1. Preparing tubes for PCR	Scheme of reaction mixture preparation was added from Appendix 1
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
	14. References	The reference to the Guidelines was added
21.05.18 PM	3. Content	The color of the reagent was specified
08.05.20 KK	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added.
	Footer	The phrase "Not for use in the Russian Federation" was added
11.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

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[5] Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.