

# AmpliSens® Poliovirus-FRT PCR kit



For Professional Use Only

## Instruction Manual

### KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	Research Use Only		Use-by Date
	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
	Internal control	C+	Positive control of amplification

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

**NOTE:** For research use only. Not for diagnostic procedures.

### 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 2 forms:

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

variant FRT-50 F in bulk<sup>1</sup> R-V58(RG,iQ)-CE-B.

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 protocol or RIBO-prep protocol)).

Variant FRT-50 F 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.

<sup>1</sup> In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

## 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 biological 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 biological 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.
- RIBO-sorb.

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.

**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.

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 <sup>2</sup>	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

4 Transfer **15 µl** of the prepared mixture to each tube.

<sup>2</sup> 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).

5 Using tips with aerosol filter, add <b>10 µl</b> of <b>cDNA samples</b> at the RNA reverse transcription stage. Dispose of the unused reaction mixture.
<b>NOTE:</b> Avoid transferring of sorbent together with the cDNA samples extracted by <b>DNA-sorb-B</b> kit.
6 Carry out the control amplification reactions:
<b>NCA</b> – Add <b>10 µl</b> of <b>DNA-buffer</b> to the tube labeled NCA (Negative Control of Amplification).
<b>C+HEV-C</b> – Add <b>10 µl</b> of <b>Positive Control cDNA HEV-C</b> for PCR-mix-1-FL <b>HEV-C / STI</b> to the tube labeled <b>C+HEV-C</b> (Positive Control of Amplification).
<b>C+Sabin 1/2/3pylori</b> – Add <b>10 µl</b> of <b>Positive Control cDNA Sabin 1/2/3</b> for PCR-mix-1-FL <b>Sabin 1/2/3</b> to the tube labeled <b>C+Sabin 1/2/3</b> (Positive Control of Amplification).
<b>CS</b> – Add <b>10 µl</b> of <b>Internal Control STI-87 (IC)</b> (for PCR-mix-1-FL <b>HEV-C / STI</b> ) to the tube labeled <b>CS+</b> (Positive Control of Amplification of IC).
<b>C–</b> – Add <b>10 µl</b> of <b>cDNA</b> obtained by extraction and reverse transcription of the <b>Negative Control (C–) reagent</b> to the tube labeled <b>C–</b> (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 <sup>3</sup>			Plate-type Instruments <sup>4</sup>		
	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		54	20 s	
		fluorescent signal detection			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:

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

- 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.

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

- 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	<b>HEV-C</b> cDNA is <b>detected</b>
< boundary value	Absent or > boundary value	<b>HEV-C</b> cDNA is <b>not detected</b>
Absent or > boundary value	Absent or > boundary value	<b>Invalid result</b> 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	<b>Sabin 2</b> cDNA is <b>detected</b>
Absent or > boundary value	< boundary value	Absent or > boundary value	<b>Sabin 3</b> cDNA is <b>detected</b>
Absent or > boundary value	Absent or > boundary value	< boundary value	<b>Sabin 1</b> cDNA is <b>detected</b>
Absent or > boundary value	Absent or > boundary value	Absent or > boundary value	<b>Sabin 1/2/3</b> cDNA are <b>not detected</b> <sup>5</sup>

**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)

<sup>3</sup> For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.

<sup>4</sup> For example, iCycler, iQ5, Mx3000P, Mx3000, or equivalent.

<sup>5</sup> 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.

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+ <sub>HEV-C</sub>	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:

- 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.
- 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.

## 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 <sup>6</sup>
Concentrated water samples	RIBO-sorb	variant FRT-50 F	1x10 <sup>3</sup>
Feces	RIBO-prep	variant FRT-50 F	5x10 <sup>3</sup>

### 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 (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, Neisseria meningitidis, 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

- 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.
- 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 biological material 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.



<sup>6</sup> Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.