



IVD For *in Vitro* Diagnostic Use

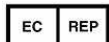
# AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit

## Instruction Manual

AmpliSens®

### TABLE OF CONTENTS

1. INTENDED USE	2
2. PRINCIPLE OF PCR ASSAY	2
3. CONTENTS OF THE KIT	2
4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES	2
5. GENERAL PRECAUTIONS	2
6. SPECIMEN COLLECTION AND HANDLING	3
7. PROTOCOL	3
8. DATA ANALYSIS	5
9. TROUBLESHOOTING	5
10. STABILITY AND STORAGE	6
11. SPECIFICATIONS	6
12. REFERENCES	6
13. QUALITY CONTROL	6
14. EXPLANATION OF SYMBOLS	6



Ecoli s.r.o., Studenohorská 12  
841 03 Bratislava 47  
Slovak Republic  
Tel.: +421 2 6478 9336  
Fax: +421 2 6478 9040  
[ecoli@ecoli.sk](mailto:ecoli@ecoli.sk)  
[www.ecoli.sk](http://www.ecoli.sk)  
[www.pcrdiagnostics.eu](http://www.pcrdiagnostics.eu)



Federal State Institution of Science  
"Central Research Institute of Epidemiology"  
3A Novogireevskaya Street  
Moscow 111123  
Russia

### 1. INTENDED USE

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Norovirus* genotypes 1, 2 RNA in the clinical material (feces) and environmental samples (concentrated water samples) by means of detection of the amplified products by agarose gel electrophoresis.

### 2. PRINCIPLE OF PCR ASSAY

*Norovirus* genotype 1, 2 detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of cDNA of pathogen genome using specific *Norovirus* primers. After PCR the amplified product is detected in agarose gel.

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit uses "hot-start", that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Melting of wax and mix of reaction components occur only at 95°C, which greatly diminish frequency of nonspecifically primed reactions.

### 3. CONTENTS OF THE KIT

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit is produced in 2 forms:

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit variant 50 R (vials 0.5 ml), REF V27-50-R0,5.

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit variant 50 R (vials 0.2 ml), REF V27-50-R0,2.

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit variant 50 R includes:

Reagent	Description	variant 50 R	
		Volume (ml)	Amount
PCR-mix -1-R <i>Norovirus</i> genotype 1 ready-to-use single-dose test tubes (under wax)	colorless, clear fluid	0.005	55 vials of 0.5 or 0.2 ml
PCR-mix -1-R <i>Norovirus</i> genotype 2 ready-to-use single-dose test tubes (under wax)	colorless, clear fluid	0.005	55 vials of 0.5 or 0.2 ml
PCR-mix-2 blue	clear fluid of blue color	1.2	1 vial
Mineral oil for PCR	colorless viscous fluid	4.0	1 vial
Positive Control cDNA <i>Norovirus</i> genotype 1 (C <sub>1</sub> +)	colorless, clear fluid	0.1	1 vial
Positive Control cDNA <i>Norovirus</i> genotype 2 (C <sub>2</sub> +)	colorless, clear fluid	0.1	1 vial
DNA-buffer	colorless, clear fluid	0.5	1 vial
Negative Control (C-)*	colorless, clear fluid	1.6	3 vials
Positive Control <i>Norovirus</i> genotype 2-rec	colorless, clear fluid	0.03	5 vials

\* must be used in the isolation procedure as Negative Control of Extraction.

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit variant 50 R is sufficient for 55 reactions, including controls.

### 4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES

- Disposable powder-free gloves
- RNA isolation kit
- Detection agarose kit
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters ( up to 200 µl)
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Personal thermocyclers
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8 °C with deep-freezer with temperature no less than –16°C
- Reservoir for disposed tips

### 5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and put the new tip for every procedure.
- Store and handle amplicons separately from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.

REF V27-50-R0,5; or V27-50-R0,2

- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.



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

## 6. SPECIMEN COLLECTION AND HANDLING



In detail, sampling biological materials for PCR-analysis, transportation and storage is described in handbook of the manufacture [2]. It is recommended to read this handbook before beginning of the work.

AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit is intended to analyze RNA extracted with RNA isolation kits from:

- *Feces*
- *Concentrated water samples (wastewater, drinking, from reservoir)*

**6.1. Feces** (0.4–1.0 g). Fecal sample is obtained from disposable plastic sachet or plastic container placed into a chamber-pot or bedpan or from diaper in infants. About 1.0 g of specimen should be transferred into special sterile container.



Deliver fecal specimen in a lab within 1 day in a container with an icepack.

6.1.1 *Preparation of 10-20% fecal suspension* (omit for liquid feces).

1. Collect tubes with tightly sealed cap and pipette 4 ml of saline solution.
2. Transfer 0.4–1.0 g (0.4 - 0.1 ml) of fecal specimen with a spatula into prepared tubes. Stir well to ensure homogenous suspension.

6.1.2. *Preparation of clarified fecal suspension.*

1. Spin the tube with prepared suspension or liquid feces at 3,000 r/min for 20 min.
2. Use required volume of supernatant for RNA extraction. The rest of obtained specimen should be transferred into a disposable tube and stored frozen for further use.

**6.2. Concentrated water samples:** *wastewater, drinking, from reservoir* (1.0 – 2.0 ml). Additional treatment is not required.



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

## 7. PROTOCOL

### 7.1. RNA Isolation

Different manufacturers offer RNA isolation kits. We recommend following nucleic acid extraction kits:

- "RIBO-sorb", [REF](#) K2-1-50.



Please carry out the RNA isolation according to the manufacturer instruction.



Volume of clinical sample used for RNA extraction from feces should be 50 µl.



Add 50 µl of Negative Control (C-) directly into **each** tube containing sample/lysis mixture.



Into the tube of Negative Control of extraction add 50 µl of Negative Control (C-)



Into the tube of Positive Control of extraction add:  
10 µl of Positive Control *Norovirus* genotype 2-rec  
40 µl of Negative Control (C-)

### 7.2. Reverse transcription

Different manufacturers offer Reverse Transcription kits. We recommend following kit for complementary DNA (cDNA) synthesis from RNA:

- "REVERTA-L", [REF](#) K3-4-50.



Please carry out the reverse transcription procedure according to the manufacturer instruction.

### 7.3. Preparing the PCR

Total reaction volume - 25 µl, volume of cDNA sample - 10 µl.

#### 7.3.1 Preparing tubes for PCR

1. Collect the required quantity of tubes with **PCR-mix-1-R *Norovirus* genotype 1** and **PCR-mix-1-R *Norovirus* genotype 2** with wax for amplification of cDNA of study and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, so that it wouldn't fall under the wax and mix with PCR-mix-1-R.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).

#### 7.3.2 Amplification

Use prepared tubes for PCR. Under or immediately above the level of oil, using tips with aerosol barrier, **add 10 µl of cDNA samples**, obtained from clinical or control samples at the stage of reverse transcription.

Perform **control amplification reactions**:

<b>NCA</b>	Add 10 µl of <b>DNA-buffer</b> to the tube for Negative Control of Amplification (NCA).
<b>C<sub>1+</sub></b>	Add 10 µl of <b>Positive Control cDNA <i>Norovirus</i> genotype 1</b> to the tube for Positive Control of Amplification of <i>Norovirus</i> genotype 1 (tube with PCR-mix-1-R <i>Norovirus</i> genotype 1).
<b>C<sub>2+</sub></b>	Add 10 µl of <b>Positive Control cDNA <i>Norovirus</i> genotype 2</b> to the tube for Positive Control of Amplification of <i>Norovirus</i> genotype 2 (tube with PCR-mix-1-R <i>Norovirus</i> genotype 2).

Run the following program on the thermocycler (see table 1). When the temperature will reach 95°C (pause regimen), insert tubes to cells of amplifier and press button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before their insertion in thermocycler.

Table 1.

Programming thermocyclers at cDNA amplification of *Norovirus* genotypes 1, 2

	Thermocyclers with active temperature adjustment						Thermocyclers with block temperature adjustment		
	"GeneAmp PCR System 2400" (ABI), "Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (ABI), "Gradient Palm Cycler" (Corbett Research), "Maxygene" (Axygen)			"Biometra", "MiniCycler", "PTC-100" (MJ Research)		
step	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles
<b>a) amplification program of <i>Norovirus</i> genotype 1 cDNA</b>									
0	95°C			95°C	pause		95°C	pause	
1	95°C	5 min	1	95°C	5 min	1	95°C	5 min	1
2	95°C	10 sec	42	95°C	10 sec	42	95°C	1 min	42
	61°C	10 sec		61°C	25 sec		61°C	1 min	
	72°C	10 sec		72°C	25 sec		72°C	1 min	
3	72°C	1 min	1	72°C	1 min	1	72°C	1 min	1
4	4°C		storage	4°C		storage	10°C		storage
<b>b) amplification program of <i>Norovirus</i> genotype 2 cDNA</b>									
0	95°C			95°C	pause		95°C	pause	
1	95°C	5 min	1	95°C	5 min	1	95°C	5 min	1
2	95°C	10 sec	42	95°C	10 sec	42	95°C	1 min	42
	63°C	10 sec		63°C	25 sec		63°C	1 min	
	72°C	10 sec		72°C	25 sec		72°C	1 min	
3	72°C	1 min	1	72°C	1 min	1	72°C	1 min	1
4	4°C		storage	4°C		storage	10°C		storage

Amplification in thermocycler with block temperature adjustment lasts 2 h, in thermocycler with active temperature adjustment — 1 h 30 min.


After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8 °C (be sure to warm the samples to room temperature before running electrophoresis).

## 8. DATA ANALYSIS

We recommend the following detection agarose kit:

- “EPh” variant 200,  K5-200.

Analysis of results is based on the presence or absence of specific bands of amplified cDNA in agarose gel (1.7%). The length of specific amplified cDNA fragments is:

- **Norovirus genotype1** - 333 bp
- **Norovirus genotype 2** - 322 bp



Put the protective mask or use the glass filter while watching and photographing the gel

### 8.1. Results interpretation

Table 2.

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		333 bp	322 bp	
PC	RNA isolation	No	Yes	Valid result
C-	RNA isolation	No	No	Valid result
NCA	Amplification	No	No	Valid result
C <sub>1</sub> +	Amplification	Yes	–	Valid result
C <sub>2</sub> +	Amplification	–	Yes	Valid result

- The sample is considered to be positive for *Norovirus* genotypes 1, 2 RNA if the band of 333 bp or 322 bp is present in agarose gel.
- The sample is considered to be negative for *Norovirus* genotype 1, 2 RNA if the band of 333 bp or 322 bp is absent.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

## 9. TROUBLESHOOTING

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

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lanes corresponding to positive controls of amplification (C<sub>1</sub>+, C<sub>2</sub>+) specific band of 333 bp or 322 bp is not observed, it can be caused by mistake in PCR conducting.
- If in lines nonspecific bands at different levels are presented, it may be caused by lack of “hot start” or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C–) specific band of 333 bp or 322 bp appears, it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

## 10. STABILITY AND STORAGE

The all components of the AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit should be stored from 2 °C to 8 °C and are stable until the expiry date stated on the label.

## 11. SPECIFICATIONS

### 11.1. Sensitivity

Analytical Sensitivity of AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit is no less than 1x10<sup>4</sup> genome equivalents per 1 ml of sample.



Claimed analytical features of AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit are guaranteed only when additional kits of reagents, “RIBO-sorb”, “REVERTA-L”, and “EPh”, are used.

### 11.2. Specificity

Specificity of AmpliSens® *Norovirus* genotypes 1, 2-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

## 12. REFERENCES

1. Mukhina AA, Shipulin GA, Bokovoĭ AG, Iatsyshina SB. Pilot experience of studying calicivirus infection in Moscow children. *Vopr Virusol.* 2002 Nov-Dec; 47(6): 33-7.
2. 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 accordance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 –certified Total Quality Management System, each lot of AmpliSens® *Norovirus* genotypes 1, 2 -EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

## 14. EXPLANATION OF SYMBOLS



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ*i*Cycler



Negative control