



For *in Vitro* Diagnostic Use



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AmpliSens® *Rotavirus/Norovirus/*

Astrovirus-FRT PCR kit

Instruction Manual

AmpliSens®



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1. INTENDED USE.

AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of rotaviruses of group A (*Rotavirus A*), noroviruses of genotype 2 (*Norovirus* genotype 2), and astroviruses (*Astrovirus*) RNA in environmental compartments and clinical material by using real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

Rotaviruses of group A (*Rotavirus A*), noroviruses of genotype 2 (*Norovirus* genotype 2), and astroviruses (*Astrovirus*) detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special Rotaviruses of group A (*Rotavirus A*), noroviruses of genotype 2 (*Norovirus* genotype 2), and astroviruses (*Astrovirus*) primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT** PCR kit is a qualitative test, which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. Wax melting and reaction mix components occur only at 95°C.

3. CONTENT.

AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT PCR kit is produced in 2 forms:

AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT PCR kit variant FRT (for use with RG) **REF** R-V40(RG)-CE.

AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT PCR kit variant FRT (for use with iQ) **REF** R-V40(iQ)-CE.

AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT Rotavirus /Astrovirus ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.008	55 tubes
PCR-mix-1-FEP/FRT Norovirus /IC ready-to-use single-dose test tubes	colorless, clear liquid	0.008	55 tubes
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
Positive Control cDNA Rotavirus-Flu (C+Rotavirus)	colorless, clear liquid	0.1	1 tube
Positive Control cDNA Norovirus genotype 2-Flu (C+Norovirus)	colorless, clear liquid	0.1	1 tube
Positive Control cDNA Astrovirus (C+Astrovirus)	colorless, clear liquid	0.1	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)	colorless, clear liquid	1.6	3 tubes
Internal Control STI-87-rec (IC)	colorless, clear liquid	0.12	5 tubes

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

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see “RIBO-sorb” **REF** K2-1-Et-50-CE, “RIBO-prep” **REF** K2-9-Et-50-CE, “REVERTA-L”, **REF** K3-4-50-CE protocols).



RNA-eluent reagent **REF** 1197 is additionally needed for DNA isolation. It is used instead of RNA-buffer in case of RIBO-sorb or RIBO-prep reagent kit application.

AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT PCR kit is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- RNA isolation kit
- Reverse transcription kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile RNase and DNase-free pipette tips with aerosol barriers (up to 200 µl)
- Tube racks
- Vortex mixer
- PCR box
- Personal thermocyclers (for example, Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia); iQ5 or iQ iCycler (BioRad, USA) or equivalent)
- Disposable polypropylene RNase and DNase-free microtubes for PCR with 0.2 ml capacity (for example, “Axygen”, USA)

- Refrigerator for temperature between 2 and 8 °C
- Deep-freezer with temperature not more than minus16°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 other 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 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Shigella spp. and EIEC/Salmonella spp./Campylobacter spp.*-FEP PCR kit is intended for the analysis of DNA extracted by using DNA isolation kits from the environmental compartments and clinical material.

6.1. The following material is used for analysis:

- concentrated water probes (sampled from wastewater, water body, drinking water) 1,0- 2,0 ml;
- faeces probes(0,4–1,0 g) is taken from diaper (infants) and disposable plastic sachet or plastic container (Petri dish) placed into a chamber-pot or bedpan (adults). About 1,0 g is transferred into sterile container.



Container with clinical material in tank with ice must be delivered into the laboratory within 1 day

6.2. Material pretreatment:

- concentrated water probes. Used without pretreatment.

- faeces:

A. Prepare the 10-20 % fecal suspension (aqueous faeces don't need dilution).

1. Take 5 ml tube with tightly sealed cap, add into each tube 4 ml of saline solution (0,9 % sodium chloride solution).
2. Transfer 0.4–1.0 g (0.4 - 0.1 ml) of fecal sample with a spatula into prepared tube. Stir well to ensure homogenous suspension. Add 20 % glycerin and store at minus 16 °C for 1 month if necessary.

B. Preparation of fecal bacteria fraction.

1. Spin the tube with prepared suspension or aqueous feces at 10,000 r/min for 5 min.
2. Transfer 50 µl of bacterial fecal fraction (upper white-yellowish phase of precipitate). In case of precipitate's absence or white-yellowish boundary layer between precipitate and supernatant 100 µl is sampled from the tube's bottom or from border between precipitate and supernatant respectively. Transfer a part of the sample which contains the high bacterial concentration into new tube with 800 µl of phosphate buffer. The phosphate buffer consists of sodium chloride, 137 mM, potassium chloride, 2,7 mM, sodium monophosphate, 10 mM, potassium diphosphate, 2 mM; pH 7,5±0,2. The phosphate buffer is stored at polypropylene bottle with tightly sealed cap at temperature between 2 and 8 °C during a year.
3. Precipitate is to be resuspended carefully, then it is to be centrifuged at maximum turn (~ 10000 g) during 5 minutes.

Supernatant is deleted; precipitate is resuspended in 300 µl of phosphate buffer. DNA is isolated from 100 µl of bacterial suspension

7. PROTOCOL.

7.1. RNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "RIBO-sorb" **REF** K2-1-Et-50-CE,
- "RIBO-prep" **REF** K2-9-Et-50-CE.



Carry the RNA isolation according to the manufacturer's instructions.



RNA-eluent reagent **REF** 1197 is additionally needed for DNA isolation. It is used instead of RNA-buffer in case of RIBO-sorb or RIBO-prep reagent kit application.



Tubes for RNA isolation are to be prepared by following way:

450 µl of **Lysis Solution**, **10 µl** of **Internal Control STI-87-rec** and **50 µl** of **Negative Control** are to be added into each tube;

50 µl of **sample** are to be added into tubes with Lysis Solution, Internal Control STI-87-rec and Negative Control.

Add **50 µl** of **Negative Control** to the tube labeled **C-**.

7.2. Reverse transcription

It's recommended to use the following RT reagents kits for complementary DNA (cDNA) synthesis from RNA.

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



Carry the reverse transcription procedure according to the manufacturer instruction.

7.3 Preparing tubes for PCR.

Total reaction volume is **25 µl**; the volume of cDNA sample is **10 µl**.

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT Rotavirus/Astrovirus** and **PCR-mix-1-FEP/FRT Norovirus/IC** for amplification of cDNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT**.
3. Using tips with aerosol barrier add **10 µl** of **cDNA** obtained at the stage of RNA reverse transcription reaction into prepared tubes.
4. Carry out the control amplification reactions:

- NCA** -Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
- C⁺Rotavirus** -Add **10 µl** of **Positive Control cDNA Rotavirus-Flu (C⁺Rotavirus)** to the tube labeled **C⁺Rotavirus** (Positive Control of Amplification).
- C⁺Norovirus** -Add **10 µl** of **Positive Control cDNA Norovirus genotype 2-Flu (C⁺Norovirus)** to the tube labeled **C⁺Norovirus** (Positive Control of Amplification).
- C⁺Astrovirus** -Add **10 µl** of **Positive Control cDNA Astrovirus (C⁺Astrovirus)** to the tube labeled **C⁺Astrovirus** (Positive Control of Amplification).

7.4. Amplification

7.4.1. RG

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

Amplification program for «Noro/IC» test

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	10 sec	–	45
	60	25 sec	FAM/Green, JOE/Yellow	
	72	10 sec	–	

3. Fluorescence detection is on the 2-nd pass (**60°C**) in FAM/Green and JOE/Yellow fluorometer channels.

Amplification program for «Rota/Astro» test

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	10 sec	–	42
	54	25 sec	FAM/Green, JOE/Yellow	
	72	10 sec	–	

4. Fluorescence detection is on the 2-nd pass (**54°C**) in FAM/Green and JOE/Yellow fluorometer channels
5. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

7.2.2.2. iQ

1. Program the iQ™ according to manufacturer's manual and Appendix 2.
2. Create a temperature profile on your iQ™ instrument as follows:

Amplification program «All Rota Noro Astro»

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	42
	60	20 sec	FAM, HEX	
	72	15 sec	–	

3. Fluorescence detection is on the 2-nd pass (**60°C**) in FAM and HEX fluorometer channels.
4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

8. DATA ANALYSIS.

The results of *Rotavirus A*, *Astrovirus*, *Norovirus 2* genotype and IC cDNA amplification are analyzed.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

See **Appendix 2** for data analysis settings for iQ5 or iQiCycler.

Results interpretation

The results are interpreted by the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 or iQ5 or iQiCycler Instrument by the crossing (or not) of the fluorescence curve with the threshold line.

Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2 and Table 3).

Table 2

Results for controls for PCR-mix-1-FEP/FRT <i>Norovirus</i> /IC				
Control	Stage for control	Ct value on channel		Interpretation
		FAM/Green / FAM	JOE/Yellow / HEX	
C-	DNA isolation	Pos (< K*)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+ <i>Norovirus</i>	Amplification	Neg	Pos (< X*)	OK

Table 3

Results for controls for PCR-mix-1-FEP/FRT <i>Rotavirus</i> / <i>Astrovirus</i>				
Control	Stage for control	Ct value on channel		Interpretation
		FAM/Green / FAM	JOE/Yellow / HEX	
C-	DNA isolation	Neg	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+ <i>Rotavirus</i>	Amplification	Pos (< Y*)	Neg	OK
C+ <i>Astrovirus</i>	Amplification	Neg	Pos (< Z*)	OK

*For K, X, Y and Z values see Appendix 1 in case of using Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument or Appendix 2 in case of using iQ5 or iQiCycler Instrument.

1. The sample is considered to be positive for specified pathogen if Ct value on appropriate channel is less than X (for *Norovirus* 2 genotype), Y (for *Rotavirus* A), Z (for *Astrovirus*).
2. The sample is considered to be negative for specified pathogen if Ct value on appropriate channel is absent or more than Y (for *Rotavirus* A) or Z (for *Astrovirus*). The sample is considered to be negative for *Norovirus* 2 genotype if Ct value on appropriate channel is absent or more than X for *Norovirus* 2 genotype and if Ct value on appropriate channel is present and more than K for Internal Control in case of PCR-mix-1-FEP/FRT *Norovirus* /IC is used.

9. TROUBLESHOOTING.

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

- If the Ct value is present for the Negative Control of Extraction (C-) on JOE/Yellow/HEX channel for tubes with PCR-mix-1-FEP/FRT *Norovirus* /IC and on FAM/Green and JOE/Yellow/HEX channels for tubes with PCR-mix-1-FEP/FRT *Rotavirus* /*Astrovirus* and for Negative Control of amplification (NCA) on any channel, it indicates the contamination of reagent or samples. In this case the results of analysis are considered to be irrelevant. Test analysis must be repeated and measures to detect and eliminate the source of contamination are to be taken.

- If the Ct value exceeds K in the table for IC (FAM/Green channel) with PCR-mix-1-FEP/FRT *Norovirus* /IC the analysis should be repeated from the stage of DNA extraction.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® *Rotavirus/Norovirus/Astrovirus*-FRT** are to be stored at the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® *Rotavirus/Norovirus/Astrovirus*-FRT** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® *Rotavirus/Norovirus/Astrovirus*-FRT** PCR kit is following:

Rotaviruses of group A (<i>Rotavirus</i> A)	1x10 ⁴ GE/ml
Noroviruses of genotype 2 (<i>Norovirus</i> genotype 2)	5x10 ³ GE/ml
Astroviruses (<i>Astrovirus</i>)	1x10 ⁴ GE/ml



The claimed analytical features of **AmpliSens® *Rotavirus/Norovirus/Astrovirus*-FRT** PCR kit are guaranteed only when additional reagents kits “RIBO-sorb”, or “RIBO-prep” and “REVERTA-L” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

11.2. Specificity.

Specificity of **AmpliSens® *Rotavirus/Norovirus/Astrovirus*-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® *Rotavirus/Norovirus/Astrovirus*-FRT** PCR kit was confirmed in laboratory clinical trials.












12. REFERENCES.

1. 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 compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of **AmpliSens® Rotavirus/Norovirus/Astrovirus-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Authorised representative in the European Community.
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use	NCA	Negative Control of Amplification
C-	Negative control of Extraction	C+<i>Rotavirus</i>	Positive Control cDNA Rotavirus-Flu (C+Rotavirus)
C+<i>Norovirus</i>	Positive Control cDNA Norovirus genotype 2-Flu (C+Norovirus)	C+<i>Astrovirus</i>	Positive Control cDNA Astrovirus (C+Astrovirus)