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For Professional Use Only

AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1.INTENDED USE

AmpliSens® ***Rotavirus / Norovirus / Astrovirus*****-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *rotavirus* A, *norovirus* genotype 2, and *astrovirus* RNA in environmental samples (water sample concentrates) and clinical material (feces) 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

Detection of *rotavirus* A, *norovirus* genotype 2, and *astrovirus* RNA includes RNA extraction from test samples and reverse transcription of RNA into cDNA combined with real-time PCR amplification of cDNA (RT-PCR). 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®** ***Rotavirus / Norovirus / Astrovirus*****-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®** ***Rotavirus / Norovirus / Astrovirus*****-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.

3. CONTENT

AmpliSens® ***Rotavirus / Norovirus / Astrovirus*****-FRT** PCR kit is produced in 1 form:

AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT PCR kit variant FRT-50 F, **REF** R-V40(RG,iQ)-CE.

AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus*	colorless clear liquid	0.6	1 tube
RT-PCR-mix-1-FEP/FRT *Norovirus* / STI	colorless clear liquid	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.015	2 tubes
RT-G-mix-2	colorless clear liquid	0.015	2 tubes
Positive Control cDNA *Rotavirus*-Flu / *Astrovirus* (C+ *Rotavirus / Astrovirus*)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *Norovirus* genotype 2-Flu /STI (C+ *Norovirus* genotype 2 / STI)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.6	1 tube
Internal Control STI-87-rec (IC)**	colorless clear liquid	0.12	5 tubes
RNA-eluent***	colorless clear liquid	1.2	5 tubes

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

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

***must be used in the extraction procedure.

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

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction 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), or equivalent).
- Disposable polypropylene microtubes for PCR (0.1-ml 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 Areas. 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.

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit is intended to analyze RNA extracted with RNA/DNA extraction kits from

- water sample concentrates (pretreatment is not required),
- feces (pretreatment should be carried out as described in manufacturer's handbook [1]).

7. WORKING CONDITIONS

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

It is recommended that the following nucleic acid extraction kits are used:

- RIBO-prep **REF** K2-9-Et-50-CE;
- RIBO-sorb **REF** K2-1-Et-50-CE;
- Other nucleic acid extraction kits recommended by CRIE.

Extract RNA according to the manufacturer's instructions.

Use RNA-eluent included in this PCR kit during RNA extraction.

8.2. Preparing RT-PCR

8.2.1. Preparing tubes for RT-PCR

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

Mix the reaction mixture components just before use. Prepare the reaction mixture for the required number of reactions (including clinical and controls samples) as specified in Appendix 1. Carry out all control amplification reactions (positive (C+) and negative (NCA) for each RT-PCR-mix-1) for testing even one test or control sample. Prepare the reagent mixture for an even number of reactions to attain more precise dispensing.

1. Thaw the reagents, vortex the tubes thoroughly, and make sure that there are no drops on the walls of the tubes.
2. Prepare the required number of tubes for amplification of RNA from test and control samples.
3. To prepare the reaction mixture, mix one **RT-PCR-mix-1-FEP/FRT (RT-PCR-mix-1-**

FEP/FRT *Rotavirus* / *Astrovirus* or **RT-PCR-mix-1-FEP/FRT *Norovirus* / STI), RT-PCR-mix-2 FEP/FRT, polymerase (TaqF),** and **TM-Revertase (MMlv)** according to Appendix 1. Vortex the tubes thoroughly. Make sure that there are no drops on the walls of the tubes.

4. Transfer **15 µl** of the prepared mixture to the prepared tubes. Dispose of the unused reaction mixture.
5. Add **10 µl** of **RNA** obtained from clinical or control samples at the extraction stage to the prepared tubes using tips with aerosol barrier.

Avoid transferring sorbent together with the RNA sample in case of extraction with the RIBO-sorb 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+*Rotavirus / Astrovirus* -Add **10 µl** of **Positive Control cDNA *Rotavirus*-Flu / *Astrovirus*** (in case of using RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus)* to the tube labeled C+***Rotavirus / Astrovirus*** (Positive Control of Amplification)

C+*Norovirus* genotype 2 / STI Add **10 µl** of **Positive Control cDNA *Norovirus* genotype 2-Flu / STI** (in case of using RT-PCR-mix-1-FEP/FRT *Norovirus* / STI) to the tube labeled C+***Norovirus* genotype 2 / STI** (Positive Control of Amplification)

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***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**50**	30 min	1	**50**	30 min	1
2	**95**	15 min	1	**95**	15 min	1
3	**95**	10 s	45	**95**	10 s	45
	60	25 s *fluorescent signal detection*		**60**	25 s *fluorescent signal detection*	
	72	10 s		**72**	10 s	

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores.

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

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

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

The fluorescent signal intensity is detected in two channels (see below).

Table 2

Correspondence table of detection channels and pathogens

Detection channel	**RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus***	**RT-PCR-mix-1-FEP/FRT *Norovirus* / STI**
FAM/Green	*Rotavirus* grA cDNA	Internal Control STI-87-rec
JOE/Yellow/HEX	*Astrovirus* cDNA	*Norovirus* G2 cDNA

Result interpretation

The results are interpreted by the software of the instrument by the crossing (or not-crossing) of the fluorescence curve with a threshold line and shown as the presence (or absence) of the Ct (threshold cycle) value in the result grid.

Results should be interpreted in accordance with Table 3 and *Important Product Information Bulletin*.

Table 3

Interpretation of results

Detection channel	**RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus***	**RT-PCR-mix-1-FEP/FRT *Norovirus* / STI**
FAM/Green	**< boundary value** *Rotavirus* grA RNA **is detected**	**< boundary value** The result for the IC sample is valid
	Absent or > boundary value *Rotavirus* grA RNA **is not detected**[3]	Absent or > boundary value **Invalid result**[4]
JOE/Yellow/HEX	**< boundary value** *Astrovirus* RNA **is detected**	**< boundary value** *Norovirus* G2 RNA **is detected**
	Absent or > boundary value *Astrovirus* RNA **is not detected**[3]	Absent or > boundary value *Norovirus* G2 RNA **is not detected**[3]

* For boundary values, see the *Important Product Information Bulletin*.

[3] Only if the Ct value for RT-PCR-mix-FEP/FRT *Norovirus* / STI in the FAM/Green channel is less than the boundary value.

[4] If Ct value for RT-PCR-mix-FEP/FRT *Norovirus* / STI in the FAM/Green channel is absent or greater than the boundary value, the negative result obtained with the other PCR-mix-1 is considered invalid; therefore, the sample should be examined once again starting from RNA extraction.

Result of the analysis is considered reliable only if the results for both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (Table 4).

Table 4

Results for controls

RT-PCR-mix-1	Control	Stage for control	Ct value in the channel	
			FAM/Green	JOE/Yellow/ HEX
RT-PCR-mix-1-FEP/FRT *Norovirus* / STI	**C–**	RNA extraction	≤ boundary value	Absent or > boundary value
	NCA	Amplification	Absent or > boundary value	Absent or > boundary value
	C+ *Norovirus* genotype 2 / STI	Amplification	< boundary value	< boundary value
RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*	**C–**	RNA extraction	Absent or > boundary value	Absent or > boundary value
	NCA	Amplification	Absent or > boundary value	Absent or > boundary value
	C+ *Rotavirus / Astrovirus*	Amplification	< boundary value	< boundary value

*For boundary values, see the *Important Product Information Bulletin* enclosed to the PCR kit.

10. TROUBLESHOOTING

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

- If the signal for C– (except for C– in JOE/Yellow/HEX channel for RT-PCR-mix-1-FEP/FRT *Norovirus* / STI) and/or the signal for NCA in the JOE/Yellow/HEX and/or FAM/Green channel is less than the boundary value, analysis should be repeated starting from the DNA extraction stage.
- If no signal is detected for the positive controls of amplification, 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 did not comply with the manufacturer's instruction, or that the reagent kit 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 iCycler 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® *Rotavirus / Norovirus / Astrovirus*-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit are to be stored at 2–8 °C (except for RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus*, RT-PCR-mix-1-FEP/FRT *Norovirus* / STI, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), TM-Revertase (MMlv), and RT-G-mix-2) when not in use. All components of the **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-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.

RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus*, RT-PCR-mix-1-FEP/FRT *Norovirus* / STI, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), TM-Revertase (MMlv), and RT-G-mix-2 are to be stored at temperature from minus 24 to minus 16 °C when not in use.

RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus* and RT-PCR-mix-1-FEP/FRT *Norovirus* / STI are to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit is specified in the table below.

Pathogen	Test material	RNA/DNA extraction kit	PCR kit	Analytical sensitivity, GE/ml*
Rotavirus A	Feces	RIBO-prep	PCR kit variant FRT-50 F	1×10^4
Norovirus genotype 2	Feces	RIBO-prep	PCR kit variant FRT-50 F	5×10^3
Astrovirus	Feces	RIBO-prep	PCR kit variant FRT-50 F	1×10^4

* Genome equivalents (GE) of the microorganism per 1 ml of a sample.

13.2. Specificity

The analytical specificity of **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-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 confirmed on the following microorganism strains:

GISK collection: *Enterovirus* strains (Coxsakie B1, B2, B3, B4, B5, and B6; Polio (Sabin) I, II, and III). *Adenovirus* serogroups 5 and 7; *influenza virus* A (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, and H5N1) and B; *rhinovirus*; *RS viruses*; and human *adenovirus* types 3, 5, 7, 37, and 40.

VGNKI collection: *Salmonella enteritidis* S-6, *S.choleraesuis* 370, *S.typhimurium* 371, *S.dublin* 373, *S.typhi* C1, *S.abortusovis 372*, and *S.gallinarum-pullorum;*, *Shigella flexneri* 851b; *Campylobacter fetus* ssp. *fetus* 25936 and *C.jejuni* ssp. *jejuni* 43435; *Clebsiella* K 65 SW4; *Listeria monocitogenes* USKHCH 19 and *L.monocitogenes* USKHCH 52; *Proteus vulgaris* 115/98; *Pseudomonas aeruginosa* DN c1; *Staphilococcus aureus* 653 and *S. aureus* 29112; *Morganella morganii* 619 c 01; and *Enterobacter faecalis* 356.

Center for Disease Control and Prevention (CDC, USA) collection: 44 isolates of *norovirus* genotype 1 and 2 different genetic clusters; 40 strains of different *rotavirus* [P]G types, 19 strains of *astrovirus* serotypes 1, 2, 4, 5, and 8; and 15 strains of different *adenovirus* types and the following bacterial strains (see table 5).

Table 5

The panel of bacterial pathogens
Center for Disease Control and Prevention (CDC, USA)

Strain ID	Organism	Strain ID	Organism
K2033	*Salmonella* ser. Grumpensis	K2015	*Salmonella* ser. Oranienburg
K1806	*Salmonella* ser. Newport	AM01144	*Salmonella* ser. Newport
K2077	*Salmonella* ser. Enteriditis	K1810	*Salmonella* ser. Anatum
83-99	*Salmonella* ser. Typhimurium	K1991	*Salmonella* ser. Typhimurium
PS505	*Shigella boydii*	K1898	*Salmonella* ser. Heidelberg
PS408	*Shigella sonnei*	PS555	*Shigella boydii*
B4003	*Shigella sonnei*	F6446	*Shigella dysenteriae*
PS801	*Shigella dysenteriae*	S821X1	*Shigella dysenteriae* type 1
C898	*Shigella dysenteriae* type1	S177X1	*Shigella dysenteriae* type 1
F2035	*Shigella flexneri*	S3314	*Shigella dysenteriae* type 2
E2539-C1	Enterotoxigenic *Escherichia coli* (ETEC)	PS071	*Shigella flexneri*
H10407	Enterotoxigenic *Escherichia coli* (ETEC)	PS050	*Shigella flexneri*
F1008	Enterotoxigenic *Escherichia coli* (ETEC)	F7862	*Shigella flexneri*
EDL 933	Shiga-toxin *E. coli* (STEC)	TX1	Enterotoxigenic *Escherichia coli* (ETEC)
3543-01	Shiga-toxin *E. coli* (STEC)	3525-01	Shiga-toxin *Escherichia coli* (STEC)
4752-71	*Proteus vulgaris*	25922	*Escherichia coli* O6:H1
QA/QC	*Citrobacter freundii*	621-64	*Citrobacter freundii*
QA/QC	*Aeromonas*	3910-68	*Aeromonas spp.*
3043-74	*Serratia marcescens*	E9113	*Vibrio cholerae*
QA/QC	*Serratia marcescens*	501-83	*Edwardsiella spp.*
F7894	*Vibrio vulnificus*	587-82	*Providencia stuartii*
F8515	*Yersinia enterocolitica*	27853	*Pseudomonas aeruginosa*
F8510	*Yersinia enterocolitica*	D4989	*Helicobacter cineadi*
K4299	*Vibrio parahaemolyticus*	D6827	*Helicobacter pullorum*
F9835	*Vibrio parahaemolyticus*	D5127	*Helicobacter pylori*
K2023	*Salmonella* ser. Kentucky	D2686	*Arcobacter butzleri*
K1684	*Salmonella* O-1, 4, 12 gr. B		

There were no nonspecific test responses during examination of human DNA as well as a DNA panel of the above-mentioned microorganisms.

The clinical specificity of **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-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 State Institute of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In accordance with Federal Budget Institution of Science “Central Research Institute for 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.

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+*Rotavirus / Astrovirus*** **C+*Norovirus* genotype 2 / STI**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control
RT	Reverse Transcription		

List of Changes Made in the Instruction Manual

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