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For *in Vitro* Diagnostic Use

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AmpliSens[®] Rotavirus-EPh PCR kit

Instruction Manual

AmpliSens[®]

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1. INTENDED USE.

AmpliSens® Rotavirus-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Rotavirus* RNA in the clinical material (feces) and environmental samples (concentrated water samples) by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION.

Rotavirus detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of cDNA of pathogen genome using special *Rotavirus* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® Rotavirus-EPh** PCR kit is 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-EPh** 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-EPh PCR kit is produced in 2 forms:

AmpliSens® *Rotavirus-EPh* PCR kit variant 50 R (tubes 0.5 ml), **REF** V15-50-R0,5-CE.

AmpliSens® *Rotavirus-EPh* PCR kit variant 50 R (tubes 0.2 ml), **REF** V15-50-R0,2-CE.

AmpliSens® Rotavirus-EPh PCR kit variant 50 R includes:

Reagent	Description	variant 50 R	
		Volume (ml)	Amount
PCR-mix -1-R Rotavirus ready-to-use single-dose test tubes (<i>under wax</i>)	colorless, clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	clear liquid of blue color	0.6	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 dropper bottle
Positive Control cDNA Rotavirus (C+)	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 Rotavirus-rec**	colorless, clear liquid	0.06	5 tubes

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

** add 5 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture (see “RIBO-sorb”, **REF** K2-1-Et-50-CE protocol).

AmpliSens® Rotavirus-EPh PCR kit variant 50 R is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- RNA isolation kit.
- Reverse transcription kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- PCR box.
- Tube racks.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2400, GeneAmp PCR System 2700 (Applied Biosystems, USA), Biometra, MiniCycler, PTC-100 (MJ Research, USA), Terzik (DNA-Technology, Russia), Maxygene (Axygen, USA)).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator with 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 [2]. It is recommended to read this handbook before starting work.

AmpliSens® Rotavirus-EPh PCR kit is intended to analyze RNA extracted with RNA isolation kits from:

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

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



Deliver fecal sample 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 sample 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 sample 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.

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

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



Carry the RNA isolation in compliance with the manufacturer protocol.



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 with sample/lysis solution/IC mixture.



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

7.2. Reverse transcription.

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

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



Carry the reverse transcription in compliance with the manufacturer protocol.

7.3. Preparing the PCR.

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

7.3.1. Preparing tubes for PCR.

1. Prepare the required number of tubes with **PCR-mix-1-R Rotavirus** with wax for amplification of cDNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2 blue** 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-R Rotavirus**.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).
4. Using tips with aerosol barrier add **10 µl** of **cDNA samples** obtained from clinical or control samples.
5. Carry out the control amplification reactions:

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

7.3.2. Amplification.

Run the following program on the thermocycler (see table 1). When the temperature reaches 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 for *Rotavirus* cDNA amplification

Thermocyclers with active temperature adjustment							Thermocyclers with block temperature adjustment					
"GeneAmp PCR System 2400" (Applied Biosystems), "Terzik" (DNA-technology)							"GeneAmp PCR System 2700" (Applied Biosystems), "Gradient Palm Cycler" (Corbett Research), "Maxygene" (Axygen)			"Biometra", "MiniCycler", "PTC-100" (MJ Research)		
Step	Temperature	Time	Cycle	Temperature	Time	Cycle	Temperature	Time	Cycle			
0	95°C	pause		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			
	55°C	10 sec		55°C	25 sec		55°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 cDNA 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.

It's recommended to use the following detection agarose kit:

- "EPh" variant 200, **REF** K5-200-CE.

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 are:

- Rotavirus* - 290 bp

- IC *Rotavirus*-rec - 514 bp



Put the protective mask or use the glass barrier 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
		290 bp	514 bp	
C-	RNA isolation	No	Yes	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	No	OK

- The sample is considered to be positive for *Rotavirus* RNA if the band of 290 bp is present in agarose gel. The band of IC (514 bp) could be absent in the samples with high concentration of *Rotavirus* RNA.
- The sample is considered to be negative for *Rotavirus* RNA if the band of 290 bp is absent and the band of 514 bp is present.

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.

Analysis results are not obtained as per the following examples:

- 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 none of bands of 290 and 514 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- 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 290 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.

All components of **AmpliSens® Rotavirus-EPh** PCR kit are to be stored at the temperature between 2

and 8 °C when not in use. All components of the PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Rotavirus-EPh** PCR kit is no less than 1×10^4 genome equivalents per 1 ml of sample.



The claimed analytical features of **AmpliSens® Rotavirus-EPh** PCR kit are guaranteed only when additional kits of reagents, "RIBO-sorb", "REVERTA-L", and "EPh" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), are used.

11.2. Specificity.

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
















12. REFERENCES.

1. Sen A, Kobayashi N, Das S, Krishnan T, Bhattacharya SK, Urasawa S, Naik TN. Amplification of various genes of human group B rotavirus from stool specimens by RT-PCR. J. Clin. Virol. 2000 Sep 1; 17(3):177-81.
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 compliance with Federal State Institution of Science Central Research Institute of Epidemiology ISO 13485 – certified Total Quality Management System, each lot of **AmpliSens® Rotavirus-EPh** PCR kit is 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		Internal Control
	Contains sufficient for <N> tests		Authorised representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	Positive control of Amplification		Negative control of Extraction
	Negative control of Amplification		