



For *in Vitro* Diagnostic Use

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AmpliSens® All screen-FEP PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE.

AmpliSens® All screen-FEP PCR kit is an in vitro nucleic acid amplification test for qualitative detection and differentiation of *Shigella* species and enteroinvasive *E. coli* (EIEC) DNA, *Salmonella* species and *Campylobacter* species DNA, group F of *Adenoviruses* DNA and group A of *Rotaviruses* RNA, *Norovirus* genotype 2 and *Astroviruses* RNA in the clinical material (feces) and environmental samples (concentrated water samples) by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Acute intestine infections (All) detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special primers. In **Fluorescent End-Point** PCR, the amplified product is detected by using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® All screen-FEP** PCR kit is a qualitative test, which contains 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® All screen-FEP** 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® All screen-FEP PCR kit is produced in 2 forms:

AmpliSens® All screen-FEP PCR kit (tubes 0.2 ml), **REF** B45-50-R0,2-FEP-CE.

AmpliSens® All screen-FEP PCR kit (tubes 0.5 ml), **REF** B45-50-R0,5-FEP-CE.

AmpliSens® All screen-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT Shigella spp. / Salmonella spp. ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.008	55 tubes
PCR-mix-1-FEP/FRT Campylobacter spp. / Adenovirus ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.008	55 tubes
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 (under wax)	colorless, clear liquid	0.008	55 tubes
PCR-mix-2-FL	colorless, clear liquid	0.77	2 tubes
PCR-mix-Background	colorless, clear liquid	0.5	2 tubes
Mineral oil for PCR	colorless viscous liquid	8.0	1 dropper bottle
Positive Control DNA Shigella sonnei	colorless, clear liquid	0.1	1 tube
Positive Control DNA Salmonella	colorless, clear liquid	0.1	1 tube
Positive Control DNA Campylobacter jejuni	colorless, clear liquid	0.1	1 tube
Positive Control DNA Adenovirus F-Flu	colorless, clear liquid	0.1	1 tube
Positive Control cDNA Rotavirus-Flu	colorless, clear liquid	0.1	1 tube
Positive Control cDNA Norovirus genotype 2-Flu	colorless, clear liquid	0.1	1 tube
Positive Control cDNA Astrovirus	colorless, clear liquid	0.1	1 tube
Internal Control STI-87-rec (IC)**	colorless, clear liquid	0.12	5 tubes
Negative Control (C-)*	colorless, clear liquid	1.6	3 tubes
DNA-buffer	colorless, clear liquid	0.5	1 tube

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

** add 10 µl of Internal Control STI-87-rec during the DNA/RNA isolation procedure directly to the sample/lysis mixture (see “RIBO-sorb”, **REF** K2-1-Et-50-CE protocol).

AmpliSens® All screen-FEP PCR kit is intended for 55 reactions, including controls.



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.

4. ADDITIONAL REQUIREMENTS.

- DNA/RNA isolation kit.
- Reverse transcription 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, Palm Cycler (Corbett Research, Australia), Maxygene (Axygen, USA) or equivalent).
- Fluorometer (for example, ALA-1/4 (Biosan, Latvia) or equivalent).
- 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® All screen-FEP PCR kit is intended for analysis of DNA/RNA extracted with DNA/RNA isolation kits from:

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

6.1. *Fecal (0.4–1.0 g) sample.* Feces are taken from disposable plastic sachet or plastic container (Petri dish) placed into a chamber-pot or bedpan; feces of infants are obtained from diaper. Approximately 1.0 g of feces should be transferred into a special sterile container.

Deliver sample within 1 day in a container with an icepack.

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

1. Collect 5 ml tube 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 tube. Stir well to ensure homogenous suspension. Add 20 % glycerine and store at minus 16 °C for 1 month if necessary.

6.1.2. *Preparation of fecal fraction.*

1. Spin the tube with prepared suspension or liquid feces at 10,000 rpm for 5 min.
2. Transfer 50 µl of bacterial fecal fraction (upper white-yellowish phase of the sediment) and clarified supernatant in a clean tube and use for RNA extraction.

6.2. *Concentrated water samples: wastewater, from reservoir, drinking (1.0 – 2.0 ml).* Collected water sample should be centrifuged at maximum speed for 10 min. For nucleic acid extraction use 100 µl of the pellet.



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

7. PROTOCOL.

7.1. DNA/RNA Isolation.

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

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



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



IC volume is 10 µl.



Add 50 µl of Negative Control (C-) directly to the IC/lysis solution mixture.



Volume of clinical sample used for RNA/DNA extraction should be 50 µl (instead of 100 µl).



Into the tube for Negative Control of extraction add 50 µl of Negative Control (C-) (instead of a clinical sample).



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.

7.2. Reverse transcription.

For detection of virus pathogens containing RNA (PCR-mix-1-FEP/FRT Rotavirus/Astrovirus, and PCR-mix-1-FEP/FRT Norovirus/IC) reverse transcription reaction should be performed.



For bacterial pathogens and adenoviruses (PCR-mix-1-FEP/FRT *Shigella spp./Salmonella spp.* and PCR-mix-1-FEP/FRT *Campylobacter spp./Adenovirus*) reverse transcription is not performed.

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 procedure according to the manufacturer's instructions.

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 the tubes with:

PCR-mix-1 FEP/FRT *Shigella spp./Salmonella spp.*,

PCR-mix-1 FEP/FRT *Campylobacter spp./ Adenovirus,*

PCR-mix-1 FEP/FRT *Rotavirus/Astrovirus,* and

PCR-mix-1 FEP/FRT *Norovirus/IC*

with wax for amplification of DNA/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. Add above 1 drop of **mineral oil for PCR** (about 25 µl).
4. Prepare 4 tubes with **PCR-mix-1-FEP/FRT** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** 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**. Add above 1 drop of **mineral oil for PCR**.
5. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction or reverse transcription into prepared tubes.
6. Carry the control amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).

C⁺*Shigella* - Add **10 µl** of **Positive Control DNA *Shigella sonnei*** to the tube labeled C⁺*Shigella*.

C⁺*Salmonella* - Add **10 µl** of **Positive Control DNA *Salmonella*** to the tube labeled C⁺*Salmonella*.

C⁺*Campylobacter* - Add **10 µl** of **Positive Control DNA *Campylobacter jejuni*** to the tube labeled C⁺*Campylobacter*.

C⁺*Adenovirus* - Add **10 µl** of **Positive Control DNA *Adenovirus* F-Flu** to the tube labeled C⁺*Adenovirus*.

C⁺*Rotavirus* - Add **10 µl** of **Positive Control DNA *Rotavirus*-Flu** to the tube labeled C⁺*Rotavirus*.

C⁺*Norovirus* - Add **10 µl** of **Positive Control DNA *Norovirus* genotype 2-Flu** to the tube labeled C⁺*Norovirus*.

C⁺*Astrovirus* - Add **10 µl** of **Positive Control DNA *Astrovirus*** to the tube labeled C⁺*Astrovirus*.

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 the button to continue.

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

Programming thermocyclers for DNA/cDNA amplification

Step	Thermocyclers with active temperature adjustment:			Thermocyclers with block temperature adjustment:			Temperature	Time	Cycles
	Temperature	Time	Cycles	Temperature	Time	Cycles			
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
	60 °C	10 sec		60 °C	25 sec		60 °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	10 °C	storage		10 °C	storage		10 °C	storage	

8. DATA ANALYSIS.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to the manufacturer's manual and Appendix 1.

8.1. Results interpretation.

- When the analysis is complete the results are automatically shown in the table in the manner of following indications:
 - pos** – positive result;
 - neg** – negative result;
 - eq** – equivocal result (signal is in grey zone);
 - nd** – invalid result (specific signal and IC signal are absent in the sample).
- 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. Results for controls for Noro/IC test listed in table 2.

Results for controls for Noro/IC test

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (IC)	HEX channel (samples)	
C-	DNA/RNA isolation	+	<i>Norovirus</i> – neg	OK
NCA	Amplification	-	<i>Norovirus</i> – nd	OK
C+ <i>Norovirus</i>	Amplification	-	<i>Norovirus</i> – pos	OK

Results for controls for Shig/Salm, Camp/Adeno, Rota/Astro tests listed in table 3. Results analysis is performed only for samples, which have result + on FAM channel in Noro/IC test.

Table 3

Results for controls for Shig/Salm, Camp/Adeno, Rota/Astro tests

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (samples)	HEX channel (samples)	
C-	DNA/RNA isolation	neg (for all tests)	neg (for all tests)	OK
NCA	Amplification	neg (for all tests)	neg (for all tests)	OK
C+ <i>Shigella</i>	Amplification	pos	neg	OK
C+ <i>Salmonella</i>	Amplification	neg	pos	OK
C+ <i>Campylobacter</i>	Amplification	pos	neg	OK
C+ <i>Adenovirus</i>	Amplification	neg	pos	OK
C+ <i>Rotavirus</i>	Amplification	pos	neg	OK
C+ <i>Astrovirus</i>	Amplification	neg	pos	OK

9. TROUBLESHOOTING.

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

- The samples with result **nd** are to be repeated starting from the stage of DNA/RNA extraction. Invalid result is normal for NCA sample.
- The samples with result **eq** are to be repeated starting from the stage of DNA/RNA extraction. If **eq** result is detected again the sample should be considered positive.
- If in negative control samples (NCA, C-) positive signal is detected it means that samples or reagents contamination has taken place. In such case results of analysis must be considered as inconclusive. The analyses must be repeated and measures taken detect and eliminate the contamination source. 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® All screen-FEP** PCR kit are to be stored at the temperature between 2 and 8 °C, when not in use. All components of the **AmpliSens® All screen-FEP** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® All screen-FEP** PCR kit is no less than:

1x10³ genome equivalents per 1 ml of sample (GE/ml) for *Shigella sonnei* DNA;

1x10³ GE/ml for *Salmonella* species DNA;

1x10³ GE/ml for *Campylobacter jejuni* DNA;

1x10⁴ GE/ml for *Adenovirus* DNA;

1x10⁴ GE/ml for *Rotavirus* cDNA;

5x10³ GE/ml for *Norovirus* genotype 2 cDNA;

1x10⁴ GE/ml for *Astrovirus* cDNA.



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

11.2. Specificity.

Specificity of **AmpliSens® All screen-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all in gene banks published sequences by sequence comparison analysis.

Specificity of **AmpliSens® All screen-FEP** PCR kit was confirmed in laboratory clinical trials.












12. REFERENCES.

- 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® All screen-FEP** 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		Contains sufficient for <n> tests
	Authorised representative in the European Community.		Consult instructions for use
	Caution, consult accompanying documents	C-	Negative control of Extraction
NCA	Negative Control of Amplification	C+ <i>Shigella</i>	Positive Control DNA <i>Shigella sonnei</i>
C+ <i>Salmonella</i>	Positive Control DNA <i>Salmonella</i>	C+ <i>Campylobacter</i>	Positive Control DNA <i>Campylobacter jejuni</i>
C+ <i>Adenovirus</i>	Positive Control DNA <i>Adenovirus</i> F-Flu	C+ <i>Rotavirus</i>	Positive Control DNA <i>Rotavirus</i> -Flu
C+ <i>Norovirus</i>	Positive Control DNA <i>Norovirus</i> genotype 2-Flu	C+ <i>Astrovirus</i>	Positive Control DNA <i>Astrovirus</i>
IC	Internal control		