



IVD

For in Vitro Diagnostic Use

AmpliSens® *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-FEP PCR kit

Instruction Manual



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1. INTENDED USE.

AmpliSens® *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *Shigella* species (*Shigella* spp.) and enteroinvasive *E. coli* (EIEC), *Salmonella* species (*Salmonella* spp.), and *Campylobacter* species (*Campylobacter* spp.) DNA in environmental compartments and clinical material by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Shigella spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp. detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Shigella* spp. and EIEC, *Salmonella* spp., *Campylobacter* spp. primers. In Fluorescent End-Point 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. 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® *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-FEP 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® *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-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® *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-FEP PCR kit is produced in 2 forms:
 AmpliSens® *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-FEP PCR kit variant FEP (0.5 ml volume tubes) **REF** B44-50-R0,5-FEP-CE.
 AmpliSens® *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-FEP PCR kit variant FEP (0.2 ml volume tubes) **REF** B44-50-R0,2-FEP-CE.
 AmpliSens *Shigella* spp. and EIEC/*Salmonella* spp./ *Campylobacter* spp.-FEP PCR kit, variant FEP includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>Shigella</i> spp. / <i>Salmonella</i> spp. ready-to-use single-dose test tubes (under wax)	colorless clear liquid	0.008	55 tubes of 0.5 or 0.2 ml
PCR-mix-1-FEP/FRT <i>Campylobacter</i> spp. / IC ready-to-use single-dose test tubes (under wax)	colorless clear liquid	0.008	55 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
PCR-mix-Background	colorless clear liquid	0.5	1 tube
Mineral oil for PCR	colorless, viscous liquid	4.0	1 dropper bottle

Positive Control DNA <i>Shigella sonnei</i> (C+ <i>Shigella</i>)	colorless clear liquid	0.1	1 tube
Positive Control DNA <i>Salmonella</i> (C+ <i>Salmonella</i>)	colorless clear liquid	0.1	1 tube
Positive Control DNA <i>Campylobacter jejuni</i> (C+ <i>Campylobacter</i>)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless, straw liquid	1.6	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

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

** add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (DNA-sorb-B, [REF](#) K1-2-50-CE, RIBO-sorb [REF](#) K1-1-Et-50-CE or RIBO-prep [REF](#) K2-9-Et-50-CE).



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® *Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.*-FEP PCR kit is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation 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, Gradient Palm Cycler (Corbett Research, Australia) or equivalent)
- Fluorometer (for example, ALA-1/4 ("Biosan", Latvia) or equivalent).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- 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 extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- 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:

- Prepare the 10-20 % fecal suspension (aqueous faeces don't need dilution).
 - Take 5 ml tube with tightly sealed cap, add into each tube 4 ml of saline solution (0,9 % sodium chloride solution).
 - 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.
- Preparation of fecal bacterial fraction.
 - Spin the tube with prepared suspension or aqueous feces at 10,000 r/min for 5 min.
 - 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 is consist 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.
 - 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.
 - Transfer 50 µl of bacterial fecal fraction (upper white-yellowish phase of the deposit) and clarified supernatant in a clean tube and use for RNA extraction.

7. PROTOCOL.

7.1. DNA Isolation

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

- "DNA-sorb-B", [REF](#) K1-2-50-CE.
- "RIBO-sorb", [REF](#) K2-1-Et-50-CE.
- "RIBO-prep", [REF](#) K2-9-Et-50-CE.



Carry the DNA 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.

7.2. Preparing the PCR.

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

7.2.1 Preparing tubes for PCR.

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Shigella spp.* / *Salmonella spp.*** (colorless wax) and **PCR-mix-1-FEP/FRT *Campylobacter spp.* / IC** (colorless wax) for amplification of DNA 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 2 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 DNA extraction stage into prepared tubes.
6. Carry out the control amplification reactions:

- NCA Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
- C+ *Shigella* Add **10 µl** of **Positive Control DNA *Shigella sonnei* (C+ *Shigella*)** to the tube labeled C+ *Shigella* (Positive Control of Amplification).
- C+ *Salmonella* Add **10 µl** of **Positive Control DNA *Salmonella* (C+ *Salmonella*)** to the tube labeled C+ *Salmonella* (Positive Control of Amplification).
- C+ *Campylobacter* Add **10 µl** **Positive Control DNA *Campylobacter jejuni* (C+ *Campylobacter*)** to the tube labeled C+ *Campylobacter* (Positive Control of Amplification).

7.2.2. 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.

Table 1

Amplification program of DNA *Shigella spp.* and EIEC/*Salmonella spp./Campylobacter*

spp.

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	«GeneAmp PCR System 2400» («Perkin Elmer»), Terzik® (DNA-Technology)			«GeneAmp PCR System 2700» («Applied Biosystems»), «Gradient Palm Cycler» («Corbett Research»), «Maxygene» («Axygen»)			«Uno-2» («Biometra»), «MiniCycler», «PTC-100» («MJ Research»)		
	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.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

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

Results interpretation

1. When the analysis is complete the results are automatically shown in the table as follows:
 - 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).
2. 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 test «Camp/IC»

Control	Stage for control	Results		Interpretation
		FAM channel	HEX channel	
C-	DNA isolation	neg	+	OK
NCA	Amplification	nd	-	OK
C+ <i>Campylobacter</i>	Amplification	pos	-	OK

Table 3

Results for controls for test «Shig/Salm»

Control	Stage for control	Results		Interpretation
		FAM channel	HEX channel	
C-	DNA 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

9. TROUBLESHOOTING.

If analysis results are not obtained as per the following examples:

- If the invalid result was obtained (except for NCA) then the analysis should be repeated from the stage of DNA extraction. For NCA such result is normal.
- If the equivocal result was obtained then the analysis should be repeated from the stage of DNA extraction. In case of the same result the samples are considered to be positive.
- Positive signal in negative controls (C-, NCA) indicates the reagent or sample contamination. In such case results of analysis must be considered to be invalid. The analyses must be repeated and measures are to be taken to 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 Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FEP** are to be stored at the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FEP** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FEP** PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FEP** PCR kit are guaranteed only when additional reagents kits “DNA-sorb-B”, “RIBO-sorb”, or “RIBO-prep”(manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used..

11.2. Specificity.

Specificity of **AmpliSens Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FEP** 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 Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FEP** 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 Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FEP** PCR kit has been 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 iCycler



Negative control