



For *in Vitro* Diagnostic Use

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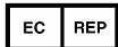
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AmpliSens® *Shigella* spp. and EIEC/*Salmonella*

spp./ *Campylobacter* spp.-FRT PCR kit

Instruction Manual

AmpliSens®



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1. INTENDED USE.

AmpliSens® Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FRT 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 real-time hybridization-fluorescence detection.

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 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® Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-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® Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-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® Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FRT PCR kit is produced in 2 forms:

AmpliSens® *Shigella* spp. and EIEC/Salmonella spp./ *Campylobacter* spp.-FRT PCR kit variant FRT (for use with RG) **REF** R-B44(RG)-CE.

AmpliSens® *Shigella* spp. and EIEC/Salmonella spp./ *Campylobacter* spp.-FRT PCR kit variant FRT (for use with iQ) **REF** R-B44(iQ)-CE.

AmpliSens Shigella spp. and EIEC/Salmonella spp./ Campylobacter spp.-FRT PCR kit, variant FRT 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 0.2 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 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
Positive Control DNA <i>Shigella sonnei</i> (C+_{Shigella})	colorless clear liquid	0.1	1 tube
Positive Control DNA <i>Salmonella</i> (C+_{Salmonella})	colorless clear liquid	0.1	1 tube
Positive Control DNA <i>Campylobacter jejuni</i> (C+_{Campylobacter})	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-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.-FRT 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, Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia); iQ5 or iQ iCycler (BioRad, USA) or equivalent)
- Disposable polypropylene 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 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.*-FRT 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 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 B during a year.
3. Precipitate is to be resuspended carefully, then it is to be centrifuged at maximum turn (~ 10000 g) during 5 minutes.
4. 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. 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.



No Positive Control of Extraction is used.



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*** and **PCR-mix-1-FEP/FRT *Campylobacter spp.* / IC** 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. 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.
4. 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

7.2.2.1. 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:

Program for «Rotor-Gene»

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**) on FAM/Green and JOE/Yellow channels.
4. 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:

Program for «iQ iCycler»

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	10 sec	–	42
	60	25 sec	FAM, HEX	
	72	10 sec	–	

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

8. DATA ANALYSIS.

The fluorescence curves are analyzed on FAM /Green and JOE /Yellow fluorescence channels (see Table 1).

Table 1

Specific detection on fluorescence channels

Reaction mix	«Shig/Sal»	«Csp/IC»
FAM/Green	Shigella spp. DNA	Campylobacter spp. DNA
JOE/Yellow/HEX	Salmonella spp. DNA	IC

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 *Campylobacter spp.* / IC

Control	Stage for control	Ct value on channel		Interpretation
		FAM/Green / FAM	JOE/Yellow / HEX	
C-	DNA isolation	Neg	Pos (< N*)	OK
NCA	Amplification	Neg	Neg	OK
C+ <i>Campylobacter</i>	Amplification	Pos (< Z*)	Neg	OK

Table 3

Results for controls for PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.

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>Shigella</i>	Amplification	Pos (< Y*)	Neg	OK
C+ <i>Salmonella</i>	Amplification	Neg	Pos (< X*)	OK

*For X, Y, 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 Z (for *Campylobacter* spp DNA), Y (for *Shigella* spp. DNA), X (for *Salmonella* spp. DNA).
2. The sample is considered to be negative for specified pathogen if Ct value on appropriate channel is absent or more than Y (for *Shigella* spp. DNA) or X (for *Salmonella* spp. DNA). The sample is considered to be negative for *Campylobacter* spp if Ct value on appropriate channel is absent or more than Z for *Campylobacter* spp DNA and if Ct value on appropriate channel is present and more than N for Internal Control in case of **PCR-mix-1-FEP/FRT *Campylobacter* spp. / IC** used.

9. TROUBLESHOOTING.

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

- If the Ct value is present for the Negative Control of Extraction (C-) on FAM/Green channel for tubes with **PCR-mix-1-FEP/FRT *Campylobacter* spp. / IC** and on FAM/Green and JOE/Yellow channels for tubes with **PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.** 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 N in the table for IC (JOE/Yellow channel) with **PCR-mix-1-FEP/FRT *Campylobacter* spp. / 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 *Shigella* spp. and EIEC/Salmonella spp./ *Campylobacter* spp.-FRT** 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.-FRT** 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.-FRT**

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.-FRT** 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.-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 *Shigella* spp. and EIEC/Salmonella spp./ *Campylobacter* spp.-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 *Shigella* spp. and EIEC/Salmonella spp./ *Campylobacter* spp.-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		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQiCycler
	Positive control		Negative control