



For Professional Use Only

AmpliSens[®] *Salmonella* spp.-FEP

PCR kit

Instruction Manual

AmpliSens[®]



Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040



Federal Budget Institute of
Science "Central Research
Institute for Epidemiology"
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens[®] Salmonella spp.-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Salmonella* spp. DNA in clinical material by using end-point hybridization-fluorescence detection of amplified products.



The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Salmonella spp. DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Salmonella* spp. primers. In **Fluorescent End-Point** PCR, the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in a reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens[®] Salmonella spp.-FEP** 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[®] Salmonella spp.-FEP** 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). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens[®] Salmonella spp.-FEP PCR kit is produced in 1 form:

AmpliSens[®] *Salmonella* spp.-FEP PCR kit variant FEP-50 F **REF** B11-FEP-CE.

AmpliSens® *Salmonella* spp.-FEP PCR kit variant FEP-50 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FL <i>Salmonella</i> spp. / STI	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control DNA <i>Salmonella</i> spp. / STI (C+<i>Salmonella</i> spp. / STI)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube
RNA-eluent	colorless clear liquid	1.2	4 tubes

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

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

AmpliSens® *Salmonella* spp.-FEP PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- 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 a rotor for 2-ml tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), MaxyGene (Axygen, USA), or equivalent).
- Fluorometer (for example, ALA-1/4 (Biosan, Latvia), or equivalent).
- Personal computer.
- Disposable polypropylene microtubes for PCR (0.5- 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, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- 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 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.



The clinical material must be taken according to state and local authorities' requirements.

AmpliSens® *Salmonella* spp.-FEP PCR kit is intended to analyze DNA extracted with DNA extraction kits from samples of primary enrichment media (selective liquid media used for detection of *Salmonella* spp., such as selenite F broth, magnesium medium) prepared in accordance with effective regulatory documents.

7. WORKING CONDITIONS

AmpliSens® *Salmonella* spp.-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- DNA-sorb-B, **REF** K1-2-50-CE.
- RIBO-prep, **REF** K2-9-Et-50-CE.



Extract DNA according to the manufacturer's instructions.



Only RNA-eluent from **AmpliSens® *Salmonella* spp.-FEP** PCR kit can be used for DNA extraction from clinical material.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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



Reaction mixture components should be mixed just before analysis with calculating for the required reaction number (test and control samples) according to Appendix 1. Note that even for analysis of one test or control DNA sample it is necessary to run all controls of the PCR amplification stage: positive control (C+), negative control of amplification (NCA), and two Background tubes. It is recommended to mix the reagents for an even reaction number to ensure more exact dosage.

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Prepare the required number of tubes including controls and Background samples. The

type of tubes depends on the PCR instrument used for analysis.

3. To prepare the reaction mixture and mixture for two Background tubes mix **PCR-mix-1-FL *Salmonella* spp. / STI, PCR-mix-2-FRT** and **Polymerase (TaqF)** in a new sterile tube (see Appendix 1). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.
4. Prepare two **Background** samples. To do this, transfer **15 µl** of **reaction mixture** (without **polymerase (TaqF)**) to two tubes **Background**, add **10 µl** of **DNA-buffer**, mix by pipeting. Add above **1 drop** of **mineral oil for PCR (~25 µl)**.
5. Add the necessary quantity of polymerase (TaqF) into the remaining reaction mixture (see Appendix 1). Vortex thoroughly.
6. Transfer **15 µl** of the prepared reaction mixture to each PCR tube. Add above **1 drop** of **mineral oil for PCR (~25 µl)**.
7. Add **10 µl** of **DNA samples** obtained from the clinical samples. Dispose of the unused reaction mixture.



Avoid transferring sorbent beads together with the DNA sample in case of extraction with DNA-sorb-B reagents kit.

8. Carry out the control amplification reactions:

C+	-Add 10 µl of Positive Control DNA <i>Salmonella</i> spp. / STI to the tube labeled C+ (Positive Control of Amplification).
NCA	-Add 10 µl of DNA-buffer to the tube labeled NCA (Negative Control of Amplification).
Background samples	- Add 10 µl of DNA-buffer to the tubes labeled Background



Insert tubes and run amplification program immediately (10–15 min after mixing the reaction mixture with DNA and controls).

8.2.2. Amplification

Run the following program in the thermocycler (see Table 1). When the temperature reaches 95 °C (pause mode), insert tubes into the thermocycler cells and press the button to continue.

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

Amplification program

Step	Thermocyclers with active temperature adjustment ¹			Thermocyclers with active temperature adjustment ²			Thermocyclers with block temperature adjustment ³		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	15 min	1	95 °C	15 min	1	95 °C	15 min	1
2	95 °C	10 s	42	95 °C	10 s	42	95 °C	1 min	42
	60 °C	10 s		60 °C	25 s		60 °C	1 min	
	72 °C	10 s		72 °C	25 s		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	

9. DATA ANALYSIS

Detection is performed using a fluorescence detector.



Please read the fluorescence detector Operating Manual before using this kit.



Detection can be conducted within 1 day after completion of amplification only if the tubes with the amplified product were stored at 2-8 °C in a light-free area.

Program the detector according to the manufacturer's manual and Guidelines [2].

The fluorescent signal intensity is detected in two channels:

- the signal from the **Salmonella spp.** DNA amplification product is detected in the HEX channel (or analogous, depending on the detector model);
- the signal from the **IC** amplification product is detected in the FAM channel (or analogous, depending on the detector model).



Prior to detection, all settings should be entered and saved. Refer to the **Guidelines** and the **Important Product Information Bulletin** for settings.

¹ For example, GeneAmp PCR System 2400 (Perkin-Elmer div of Applied Biosystems).

² For example, GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research).

³ For example, Uno-2 (Biometra), MiniCycler, PTC-100 (MJ Research).

Interpretation of results

Table 2

Interpretation of amplification results

Ct value in channel		Interpretation
FAM	HEX	
> threshold or < threshold	> threshold of positive result	<i>Salmonella</i> spp. DNA is detected
> threshold	< threshold of negative result	<i>Salmonella</i> spp. DNA is not detected
< threshold	< threshold of negative result	Invalid result
> threshold	> threshold of negative result or < threshold of positive result	Equivocal result



If the result is invalid or equivocal, the PCR should be repeated once again.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 3).

Table 3

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel	JOE channel	
C-	DNA extraction	> threshold*	< threshold* of negative result	OK
NCA	Amplification	> threshold*	> threshold* of positive result	OK
C+	Amplification	< threshold*	< threshold* of negative result *	OK

*For boundary values, see the Important Product Information Bulletin

10. TROUBLESHOOTING

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

1. If the signal of the Positive Control of amplification (C+) in the HEX channel is less than the threshold of positive result, PCR and detection should be repeated for all samples in which *Salmonella* spp. DNA was not detected.
2. If the signal of the Negative Control of extraction (C-) and/or amplification (NCA) detected in the HEX channel is greater than the threshold of positive signal, PCR analysis should be repeated (starting from DNA extraction) for all samples in which *Salmonella* spp. DNA was detected.

3. Positive result obtained for Negative Control of extraction (C-), that is a sterile sample of the culture medium, may indicate contamination of the primary enrichment medium with the genetic material of the examined microorganism. In this case, the analysis should be repeated. To do this, start from primary enrichment of food with non-contaminated media and perform an additional negative control extraction reaction using the Negative Control (C-) reagent (see Section 3. Content).
4. No positive signal in C+ may indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of PCR, noncompliance of the storage conditions for kit components with the manufacturer's instruction, or the expiration of the reagent kit. Check programming of the thermocycler (see 8.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR once again for all samples.
5. If no signal was detected either in the channel for detection of the pathogen DNA or in the channel for detection of IC, the sample should be examined once again (PCR and detection). The same applies to the samples with equivocal results, because the fact that the specific signal does not exceed the threshold value is not sufficient to consider a sample as positive. If equivocal results are obtained in the second run, the analysis should be repeated starting from the DNA extraction stage.
6. Positive signal in C- and NCA indicates reagent or sample contamination. In this case, the results of analysis must be considered as invalid. The analyses must be repeated and measures for detecting and eliminating the contamination source must be taken.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Salmonella* spp.-FEP PCR kit should be transported at 2–8 °C for no longer than 5 days

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Salmonella* spp.-FEP** PCR kit (except for PCR-mix-1-FL *Salmonella* spp. / STI, polymerase (TaqF), and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Salmonella* spp.-FEP** 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.



PCR-mix-1-FL *Salmonella* spp. / STI, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.



PCR-mix-1-FL *Salmonella* spp. / STI is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens[®] *Salmonella* spp.-FEP** PCR kit is the following:

Test material	Nucleic acid extraction kit	Sensitivity, GE/ml ⁴
Selenite F Broth ⁵	DNA-sorb-B	1x10 ³
	RIBO-prep	1x10 ³

13.2. Specificity

The analytical specificity of **AmpliSens[®] *Salmonella* spp.-FEP** 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. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: 18 strains of different serogroups of *Salmonella* spp., 3 strains of *Cronobacter sakazakii*, 4 strains of *Enterobacter cloacae*, 2 strains of *Enterobacter aerogenes*, 2 strains of *Pantoea agglomerans*, 8 strains of *Campylobacter* spp (*C. jejuni*, *C. coli* and *C. fetus fetus*), 31 strains of different serogroups of *Escherichia coli* (including *EHEC*, *EPEC*, *ETEC*, *EAggEC* and *EIEC*), 12 strains of different species and serogroups of *Shigella* spp., 22 strains of different species and serogroups of *Yersinia* spp., *Citrobacter freundii*, *Clostridium perfringens*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Protrus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcessens*. The clinical specificity of **AmpliSens[®] *Salmonella* spp.-FEP** 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 Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

⁴ Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.














⁵ Pretreatment is not required.

2. Guidelines “End-Point PCR Detection of *Salmonella spp.* DNA”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens®** ***Salmonella spp.-FEP*** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	<i>In vitro</i> diagnostic medical device		Expiration Date
	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
	Authorised representative in the European Community	C+	Positive control of Amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	IC	Internal control

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 Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”