CE

IVD

For Professional Use Only

AmpliSens® *Salmonella typhi*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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

TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *Salmonella typhi*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Salmonella typhi* DNA (detection is performed with respect to Vi antigen genes and the first phase of flagellar H antigen *d* (phase H1 of flagellar antigen *d*), *Salmonella* spp.*)*, which makes it possible to distinguish *S.typhi* from *S.paratyphi C* and *S.dublin*, which possess the Vi antigen, and from *S.stanley, S.isangi, S.muenchen, S.gaminara,* and *S.utrecht,* which have the H1 phase of flagellar antigen *d*, in clinical material and environmental samples by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Salmonella typhi DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Salmonella typhi* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Salmonella typhi*-FRT** 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 typhi*-FRT** 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 typhi*-FRT PCR kit is produced in 1 form:

AmpliSens® *Salmonella typhi*-FRT PCR kit variant FRT-50 F (for use with RG, iQ) **REF** R-B63(RG,iQ)-CE.

AmpliSens® *Salmonella typhi*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FEP/FRT *S.typhi* / 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 *S.typhi* / STI (C+ *S.typhi* / STI)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

* 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, RIBO-sorb, **REF** K2-1-Et-50-CE or RIBO-prep, **REF** K2-9-Et-50-CE).

AmpliSens® *Salmonella typhi*-FRT 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 rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- 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, 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 another 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 clinical 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 and environment samples must be taken according to state and local authorities’ requirements.

AmpliSens® *Salmonella typhi*-FRT PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from clinical material and environment samples.

7. WORKING CONDITIONS

AmpliSens® *Salmonella typhi*-FRT 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,
- RIBO-sorb, **REF** K2-1-Et-50-CE.

Extract DNA according to the manufacturer's instructions.

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+) and negative control of amplification (NCA) for each type of mixture. It is recommended to mix the reagents for an even reaction number to ensure more precise 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. Take the required number of tubes for amplification for the clinical and control samples. The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix **PCR-mix-1-FEP/FRT *S.typhi / STI***, **PCR-mix-2-FRT**, and **Polymerase (TaqF)** in a new sterile tube (see Appendix 1). Thoroughly vortex the mixture and make sure that there are no drops on the caps of the tubes.
4. Transfer **15 µl** of the prepared reaction mixture to each PCR tube.
5. Add **10 µl** of **DNA samples** obtained from the test samples. Dispose of the unused reaction mixture.

Avoid transferring sorbent beads together with the DNA sample in case of extraction with DNA-sorb-B or RIBO-sorb reagent kits.

6. Carry out the control amplification reactions:

C+ -Add **10 µl** of **Positive Control DNA *S.typhi* / 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).

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

1. Create a temperature profile on your instrument as follows:

Table 1

AmpliSens-1 amplification program

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	***Temperature, °C***	***Time***	**Cycles**	***Temperature, °C***	***Time***	**Cycles**
Hold	**95**	15 min	1	**95**	15 min	1
Cycling	**95**	10 s	45	**95**	10 s	45
	60	25 s *fluorescent signal detection*		**60**	25 s *fluorescent signal detection*	
	72	10 s		**72**	10 s	

Fluorescent signal is detected in the channels designed for the FAM/Green, JOE/Yellow/HEX, and ROX/Orange fluorophores on the 2nd step (60°C) of stage Cycling.

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin.*
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Interpretation of results

The results are interpreted by the software of used instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line. FAM/Green, JOE/Yellow/HEX и ROX/Orange fluorescence channels are used.

See **Guidelines** for data analysis settings for the instrument.

The principle of interpretation is given in Table 2.

[1] For example,Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, iQ5, Mx3000P, Mx3000 or equivalent.

Table 2

Interpretation of amplification results

PCR-mix-1	Ct value in channel			Interpretation
	FAM channel	HEX channel	ROX channel	
PCR-mix-1-FEP/FRT *S.typhi* / *STI*	< boundary value*	> boundary value*	> boundary value*	*S.typhi* DNA is **detected**
	> or < boundary value*	< boundary value*	< boundary value*	*S.typhi* DNA is **not detected**
	> or < boundary value*	> boundary value*	< boundary value*	DNA of *S.stanley, S.isangi, S muenchen, S.gaminara, S.utrecht* (or other serovars of salmonellas, having H1-phase of d flagellant antigen) is **detected**
	> or < boundary value*	< boundary value*	> boundary value*	*S. paratyphi C* or *S. dublin* DNA is **detected**
	> boundary value*	> boundary value*	> boundary value*	**Invalid result**, PCR should be repeated

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

PCR-mix-1	Control	Stage for control	FAM channel	HEX channel	ROX channel
PCR-mix-1-FEP/FRT *S.typhi* / *STI*	**C–**	DNA extraction	< boundary value*	> boundary value*	> boundary value*
	NCA	Amplification	> boundary value*	> boundary value*	> boundary value*
	C+	Amplification	< boundary value*	< boundary value*	< boundary value*

*For boundary values, see the *Important Product Information Bulletin.*

10. TROUBLESHOOTING

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

- If Ct value for the Positive Control of PCR (C+) is greater than the boundary value in the FAM/Green, JOE/Yellow/HEX, or ROX/Orange channels, the PCR and detection should be repeated for all samples in which Ct value is greater than the boundary value in respective channel.
- If a Ct value of the Negative Control of extraction (C–) (except for the FAM/Green channel) and/or Negative Control of amplification (NCA) in all channels is less than the boundary value, analysis should be repeated starting from the DNA extraction stage for

all samples in which DNA of corresponding pathogen was detected.

- If a Ct value is present for C– in the FAM/Green channel and/or for NCA in all in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer's instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® *Salmonella typhi*-FRT 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 typhi*-FRT** PCR kit (except for PCR-mix-1-FEP/FRT *S.typhi* / 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 typhi*-FRT** 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-FEP/FRT *S.typhi* / 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-FEP/FRT *S.typhi* / STI is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Salmonella typhi*-FRT** PCR kit is the following:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml [3]
Faeces	RIBO-sorb	$1x10^3$
	RIBO-prep	$1x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® *Salmonella typhi*-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent 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 in tests with human DNA samples and a DNA panel of the following microorganisms:

- Strains from FSI VGNKI collection: *Salmonella enteritidis* S-6, *S.choleraesuis* 370, *S.typhimurium* 371, *S.dublin* 373, *S.typhi* C1, *S.abortusovis* 372 and *S.gallinarum-pullorum*; *Shigella flexneri* 851b; *Campylobacter fetus* ssp*. fetus* 25936 and *C.jejuni ssp. jejuni* 43435; *Klebsiella K* 65 SW4; *Listeria monocytogenes* USKhCh 19 and *L.monocytogenes* USKhCh 52; *Proteus vulgaris* 115/98; *Pseudomonas aeruginosa* DN c1; *Staphylococcus aureus* 653 and *S.aureus* 29112; *Morganella morganii* 619 c 01; and *Enterococcus faecalis* 356;
- *Salmonella typhi* strains of different phagotypes (18 strains);
- Strains from the CRIE collection: *Yersinia enterocolitica* strains (14 strains) and *Yersinia pseudotuberculosis* strains (12 strains).

The clinical specificity of **AmpliSens® *Salmonella typhi*-FRT** 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.
2. Guidelines “Real-Time PCR Detection of *Salmonella typhi* 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,

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

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 typhi*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	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
EC REP	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”