



For *in Vitro* Diagnostic Use

**TABLE OF CONTENTS**

1. INTENDED USE .....3

2. PRINCIPLE OF PCR DETECTION .....3

3. CONTENT .....3

4. ADDITIONAL REQUIREMENTS .....4

5. GENERAL PRECAUTIONS .....4

6. SAMPLING AND HANDLING .....5

7. PROTOCOL .....5

8. DATA ANALYSIS .....7

9. TROUBLESHOOTING .....7

10. STABILITY AND STORAGE .....8

11. SPECIFICATIONS .....9

12. REFERENCES .....9

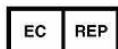
13. QUALITY CONTROL ..... 10

14. EXPLANATION OF SYMBOLS ..... 10

# AmpliSens® *Shigella spp.* and *EIEC-FRT*

PCR kit

## Instruction Manual



**Ecoli s.r.o.**, Studenohorská 12  
 841 03 Bratislava 47  
 Slovak Republic  
 Tel.: +421 2 6478 9336  
 Fax: +421 2 6478 9040  
[ecoli@ecoli.sk](mailto:ecoli@ecoli.sk)  
[www.ecoli.sk](http://www.ecoli.sk) [www.pcrdiagnostics.eu](http://www.pcrdiagnostics.eu)



Federal State Institution of Science  
 Central Research Institute of Epidemiology  
 3A Novogireevskaya Street  
 Moscow 111123 Russia

## 1. INTENDED USE

**AmpliSens<sup>®</sup> Shigella spp. and EIEC-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Shigella spp.* and enteroinvasive *E.coli* DNA in clinical material 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

*Shigella spp.* and enteroinvasive *E.coli* DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Shigella spp.* and enteroinvasive *E.coli* 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 PCR 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<sup>®</sup> Shigella spp. and EIEC-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<sup>®</sup> Shigella spp. and EIEC-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 a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

## 3. CONTENT

**AmpliSens<sup>®</sup> Shigella spp. and EIEC-FRT** PCR kit is produced in 1 form:

AmpliSens<sup>®</sup> *Shigella spp.* and *EIEC-FRT* PCR kit variant FRT-50 F (for use with RG, iQ) **REF** R-B12(RG,iQ)-CE.

**AmpliSens<sup>®</sup> Shigella spp. and EIEC-FRT** PCR kit variant FRT-50 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FL <i>Shigella spp.</i> / 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>Shigella sonnei</i> / STI (C+)	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 or RIBO-prep, **REF** K2-9-Et-50-CE).

AmpliSens<sup>®</sup> *Shigella spp.* and *EIEC-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); iCycler iQ or Q5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (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 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.



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

AmpliSens® *Shigella spp.* and *EIEC-FRT* PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from clinical material.

## 7. PROTOCOL

### 7.1. 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.

### 7.2. Preparing PCR

#### 7.2.1. 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 number of reactions (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). 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. 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-FL *Shigella 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. Transfer **15 µl** of the prepared reaction mixture to each PCR tube.
5. Add **10 µl** of **DNA samples** obtained from the clinical samples. Utilize the rest of reaction mixture.



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

6. Carry out the control amplification reactions:

- C+ -Add **10 µl** of **Positive Control DNA *Shigella sonnei* / 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).

### 7.2.2. 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

Step	Rotor-type instruments <sup>1</sup>			Plate-type instruments <sup>2</sup>		
	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 <i>fluorescent signal detection</i>		60	25 s <i>fluorescent signal detection</i>	
	72	10 s		72	10 s	

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

2. Adjust the fluorescence channel sensitivity according to the *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.

<sup>1</sup> For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.

<sup>2</sup> For example, iCycler, iQ5, Mx3000P, Mx3000 or equivalent.

## 8. DATA ANALYSIS

Internal Control DNA is detected in the FAM/Green fluorescence channel, *Shigella spp.* DNA is detected in the JOE/Yellow/HEX fluorescence channel.

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

### 8.1. Interpretation of results

The results are interpreted by the software of the used instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

The principle of interpretation is given in Table 2.

Table 2

**Interpretation of amplification results**

Ct value in channel		Interpretation
FAM	HEX	
Pos (< boundary value*) or defined	Pos (< boundary value*)	<i>Shigella spp.</i> DNA is <b>detected</b>
Pos (< boundary value*)	Neg (> boundary value*) or undefined	<i>Shigella spp.</i> DNA is <b>not detected</b>
Neg (> boundary value*) or undefined	Neg (> boundary value*) or undefined	<b>Invalid result</b>

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/Green channel	JOE/Yellow/HEX channel	
C-	DNA extraction	Pos ( $\leq$ boundary value*)	Neg (> boundary value*) or undefined	<b>OK</b>
NCA	Amplification	Neg (> boundary value*) or undefined	Neg (> boundary value*) or undefined	<b>OK</b>
C+	Amplification	Pos (< boundary value*)	Pos (< boundary value*)	<b>OK</b>

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

## 9. TROUBLESHOOTING

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

- If Ct value of the Positive Controls of PCR (C+) is greater than boundary value in the JOE/Yellow/HEX channel the PCR and detection should be repeated for all samples in which *Shigella spp.* DNA was not detected.

- If Ct value of the Negative Control of extraction (C-) and/or Negative Control of amplification (NCA) JOE/Yellow/HEX channel is less than boundary value, analysis should be repeated (starting from DNA extraction) for all samples in which *Shigella spp.* DNA was detected.
- Positive result obtained for Negative Control of extraction (C-), that is a sterile sample of a culture medium, can 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 food primary enrichment with non-contaminated media and perform additional negative control extraction reaction using Negative Control reagent (see section 3. Content).
- If the Ct value is absent in both JOE/Yellow/HEX and FAM/Green channels or the Ct value in the JOE/Yellow/HEX channel is higher than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (if iCycler iQ or iQ5 instruments are used).
- If the Ct value is present for C- in the FAM/Green channel and/or for NCA in the FAM/Green, JOE/Yellow/HEX channels 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).

## 10. STABILITY AND STORAGE

All components of the **AmpliSens® *Shigella spp.* and EIEC-FRT** PCR kit (except for PCR-mix-1-FL *Shigella 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® *Shigella spp.* and EIEC-FRT** PCR kit are stable until the

expiration date on the label.



PCR-mix-1-FL *Shigella spp.* / STI, Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at  $\leq -16^{\circ}\text{C}$ .



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

## 11. SPECIFICATIONS

### 11.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Shigella spp.* and EIEC-FRT** PCR kit is the following:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml <sup>3</sup>
Selenite F Broth <sup>4</sup>	DNA-sorb-B	1x10 <sup>3</sup>
	RIBO-prep	1x10 <sup>3</sup>

### 11.2. Specificity

The analytical specificity of **AmpliSens® *Shigella spp.* and EIEC-FRT** 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: 12 strains of different species and serogroups of *Shigella spp.*, 31 strains of different serogroups of *Escherichia coli* (including *EHEC*, *EPEC*, *ETEC*, *EAggEC* and *EIEC*), 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*), 18 strains of different serogroups of *Salmomella spp.*, 22 strains of different species and serogroups of *Yersinia spp.*, *Citrobacter freundii*, *Clostridium perfringens*, *Klebsiella pneumonia*, *Listeria monocytogene*, *Protrus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcessens*. The clinical specificity of **AmpliSens® *Shigella spp.* and EIEC-FRT** PCR kit was confirmed in laboratory clinical trials.

## 12. REFERENCES

- Handbook "Sampling, Transportation, and 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.

- Guidelines "Real-Time PCR Detection of *Shigella spp.* DNA", 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.

## 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-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 *in Vitro* Diagnostic Use



Version



Catalogue number



Caution, consult accompanying documents



Contains sufficient for <n> tests



Negative Control of Amplification



Consult instructions for use



Negative control of extraction



Positive control of amplification



Internal Control



Central Research Institute of Epidemiology (Moscow, Russia)

<sup>3</sup> Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

<sup>4</sup> Pretreatment is not required.