CE

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For Professional Use Only

AmpliSens® Escherichioses-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® Escherichioses-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of diarrheagenic *E.coli* (*EPEC, ETEC, EIEC, EHEC*, and *EAgEC*) DNA in environmental compartments and 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

E.coli (*EPEC, ETEC, EIEC, EHEC,* and *EAgEC*) detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *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 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® Escherichioses-FRT** PCR kit is a qualitative test that contains 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® Escherichioses-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). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® Escherichioses-FRT PCR kit is produced in 1 form:

AmpliSens® Escherichioses-FRT PCR kit variant FRT-50 F, **REF** R-B62(RG,iQ)-CE.

AmpliSens® Escherichioses–FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FEP/FRT *EIEC* / *EHEC* / STI	colorless clear liquid	0.6	1 tube
PCR-mix-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control DNA *EIEC* / *EHEC* / STI (C+ $_{EIEC / EHEC / STI}$)	colorless clear liquid	0.1	1 tube
Positive Control DNA *EPEC* / *ETEC* / *EAgEC* (C+ $_{EPEC / ETEC / EAgEC}$)	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-FL during the DNA extraction 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).

AmpliSens® Escherichioses-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 iQ5 (Bio-Rad, USA), or equivalent).
- 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, 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 are described in the manufacturer’s handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® Escherichioses-FRT PCR kit is intended for the analysis of DNA extracted

with DNA extraction kits from environmental compartments (water samples) and clinical material (feaces).

Water samples are used without treatment.

Liquid faeces can be used without the suspension preparation stage.

Store the faecal extract for 1 day at 2-8 °C, for 1 month (with addition of glycerol) at ≤ –16 °C, and for a long time (with addition of glycerol) at ≤ –68 °C.

7. WORKING CONDITIONS

AmpliSens® Escherichioses-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-sorb, **REF** K2-1-Et-50-CE.
- RIBO-prep, **REF** K2-9-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**.

1. Thaw the reagents and vortex the tubes thoroughly. Make sure that there are no drops on the tube caps.
2. Prepare the required number of tubes (including controls).
   Mix **PCR-mix-1-FEP/FRT *EIEC* / *EHEC* / STI** with **PCR-mix-2-FRT** and **polymerase (TaqF)** as well as **PCR-mix-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*** with **PCR-mix-2-FRT** and **polymerase (TaqF)** (see Appendix 1). Vortex the tubes thoroughly. Make sure that there are no drops on the tube caps.
3. Transfer **15 µl** of the resultant mixture to the prepared tubes.
4. Add **10 µl** of **DNA** obtained from clinical or control samples using tips with aerosol barrier.

Dispose the unused reaction mixture.

5. Carry out the control amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ ***EIEC / EHEC*** **/ STI** - Add **10 µl** of **Positive Control DNA *EIEC / EHEC* / STI** to the tube labeled C+ *EIEC / EHEC / STI* (Positive Control of Amplification) for **PCR-mix-1 *EIEC / EHEC* / STI.**

C+*EPEC / ETEC / EAgEC* - Add **10 µl** of **Positive Control DNA *EPEC / ETEC / EAgEC*** to the tube labeled C+ *EPEC / ETEC / EAgEC* (Positive Control of Amplification) for **PCR-mix-1 *EPEC / ETEC / EAgEC*.**

8.2.2. Amplification

1. Program the real-time amplification instrument according to manufacturer's manual.

Create a temperature profile on your instrument as follows:

Table 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 1	**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, JOE and ROX fluorophores on the 2nd step of stage Cycling 1.

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.

9. DATA ANALYSIS

9.1 Results interpretation

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

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[2] For example, iCycler iQ, iQ5, Mx3000P, Mx3000, DT-96, or equivalent.

Table 2

Interpretation of results for PCR-analysis

PCR-mix-1	Ct value in channel			Interpretation
	FAM	**HEX**	**ROX**	
PCR-mix-1-FEP/FRT *EIEC / EHEC* / STI	Pos (< boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)	*EIEC* and *EHEC* DNA is not detected
	Neg (> or < boundary value*)	Pos (< boundary value*)	Neg (> or < boundary value*)	*EHEC* DNA is detected
	Neg (> or < boundary value*)	Neg (> or < boundary value*)	Pos (< boundary value*)	*EIEC* DNA is detected
	Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)	invalid
PCR-mix-1-FEP/FRT *EPEC / ETEC /EAgEC*	Pos (< boundary value*)	Neg (> or < boundary value*)	Neg (> or < boundary value*)	*EAgEC* DNA is detected
	Neg (> or < boundary value*)	Pos (< boundary value*)	Neg (> or < boundary value*)	*EPEC* DNA is detected
	Neg (> or < boundary value*)	Neg (> or < boundary value*)	Pos (< boundary value*)	*ETEC* DNA is detected
	Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)	*EPEC* / *ETEC* / *EAgEC*[3] DNA are not detected

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

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

[3] If the result is positive for PCR-mix-1-FEP/FRT *EIEC* / *EHEC* / STI in the FAM channel.

Table 3

Results for controls

PCR-mix-1	Control	Stage for control	Ct value in channel		
			FAM	HEX	ROX
PCR-mix-1-FEP/FRT *EIEC / EHEC /* STI	C-	DNA extraction	Pos (< boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)
	NCA	Amplification	Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)
	C+ *EIEC / EHEC* / STI	Amplification	Pos (< boundary value*)	Pos (< boundary value*)	Pos (< boundary value*)
PCR-mix-1-FEP/FRT *EPEC / ETEC / EAgEC*	C-	DNA extraction	Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)
	NCA	Amplification	Neg (> boundary value*)	Neg (> boundary value*)	Neg (> boundary value*)
	C+*EPEC / ETEC / EAgEC*	Amplification	Pos (< boundary value*)	Pos (< boundary value*)	Pos (< boundary value*)

10. TROUBLESHOOTING

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

- If the signal for C– (except for C– in FAM channel for PCR-mix-1-FEP/FRT *EIEC* / *EHEC* / STI) and/or for NCA is less than the boundary value, analysis should be repeated starting from the DNA extraction stage.
- 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 did not comply with the manufacturer's instruction, or that the reagent kit 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 you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® Escherichioses-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® Escherichioses-FRT** PCR kit (except for PCR-mix-1-FEP/FRT *EIEC* / *EHEC* / STI, PCR-mix-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*, PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® Escherichioses-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 *EIEC* / *EHEC* / STI, PCR-mix-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*, PCR-mix-2-FRT and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

PCR-mix-1-FEP/FRT *EIEC* / *EHEC* / STI and PCR-mix-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC* are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Pathogene	Clinical material	Kit for DNA extration	Kit for amplification and detection	Sensitivity, GE/ml [4]
EPEC	feaces	RIBO-prep	PCR kit variant FRT-50 F	$1x10^3$
ETEC				
EIEC				
EHEC				
EAgEC				

The claimed analytical performance characteristics of **AmpliSens® Escherichioses-FRT** PCR kit are guaranteed only when additional reagent kits DNA-sorb-B, RIBO-sorb, or RIBO-prep (manufactured by FBIS CRIE) are used.

13.2. Specificity

The analytical specificity of **AmpliSens® Escherichioses-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

[4] Genome equivalents of microorganism per 1 ml of the sample from transport medium.

banks by sequence comparison analysis. Nonspecific responses were absent during examination of human DNA as well as a DNA panel of the following microorganisms:

- *E.coli* strains: O157H7 No. 4, O157H7 No. 23, O157H7 No. 212, O157H7 No. 214, O157H7 No. 1330, O143, O124 No. 227, O144, O86 No. 990, O125 Carioni, O85, O61 No. 10167в/41, O59 No. 9095/41, No. 409 (O34), K12, 3912/41, Krym No. 56, O148H28 B7a, O6 No. 3091, 113/3, 675, O111 No. 153, O62 10524/41, O126 No. 611, M17, Krym No. 1274, 168/59, O57 8198/41, Krym No. 14169, O48, NCTC 9001.
- Strains of other microorganisms: *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* USHCH 19 and *L.monocytogenes* USHCH 52; *Proteus vulgaris* 115/98; *Pseudomonas aeruginosa* DH c1; *Staphylococcus aureus* 653 and *S. aureus* 29112; *Morganella morganii* 619 c 01; *Enterococcus faecalis* 356, 12 strains of *Yersinia enterocolitica,* and 6 strains of *Yersinia pseudotuberculosis.*

The specificity of diarrheagenic *E.coli* strains was confirmed by sequence analysis of the studied genome fragments.

The clinical specificity of **AmpliSens® Escherichioses-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 diarrheagenic *E.coli* 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® Escherichioses-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+** ***EIEC / EHEC / STI*****,** **C+** ***EPEC / ETEC / EAgEC***	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control
ETEC	Enterotoxigenic *E.coli*	***EPEC***	Enteropathogenic *E.coli*
EHEC	Enterohemorrhagic *E.coli*	***EIEC***	Enteroinvasive *E.coli*
EAgEC	Enteroaggregative *E.coli*		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
18.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”