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For Professional Use Only

AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of DNA of virulent and avirulent *Yersinia enterocolitica* strains (virulence is assessed by genes encoding enterotoxin (*Yst*), attachment invasion locus (*ail*), and plasmid pYV adhesion (*yadA*)) and *Yersinia pseudotuberculosis* strains in environmental samples (concentrated water samples) and clinical material (feces) using real-time 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

Detection of virulent and avirulent strains of *Yersinia enterocolitica* and strains of *Yersinia pseudotuberculosis* by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the real-time PCR, the amplified product is detected with the use of 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® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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® *Yersinia enterocolitica / Y.pseudotuberculosis*-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 using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT PCR kit is produced in 1 form:

AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT PCR kit variant FRT-50 F, **REF** R-B64(RG,iQ)-CE.

AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FEP/FRT *Y.enterocolitica* / *Y.pseudotuberculosis*	colorless clear liquid	0.6	1 tube
PCR-mix-1-FEP/FRT *Yersinia enterocolitica* type	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 *Y.enterocolitica* / *Y.pseudotuberculosis* / STI (C+$_{Y.e./Y.p./STI}$)	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 Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see the 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 protocols).

AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-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.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia), iCycler iQ or iQ5 (Bio-Rad, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (for example, Axygen, USA).
  a) 0.2-ml PCR tubes with optical transparent domed caps if a plate-type instrument is

used;

b) 0.2-ml PCR tubes with flat caps Rotor-Gene PCR tubes if a rotor-type instrument is used.

- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir 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 a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where 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® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT PCR kit is intended for analysis of the DNA extracted with the use of DNA extraction kits from:

- concentrated water samples (pretreatment is not required),
- feces (pretreatment should be carried out as described in manufacturer's handbook [1]).

7. WORKING CONDITIONS

AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended that the following nucleic acid extraction kits are used:

- DNA-sorb-B **REF** K1-2-50-CE;
- RIBO-prep **REF** K2-9-Et-50-CE;
- RIBO-sorb **REF** K2-1-Et-50-CE;

The DNA extraction of each test sample is carried out in the presence of **Internal Control STI-FL (IC).** The **Negative Control (C–)** reagent is used as the Negative control of extraction (C–).

Extract DNA according to the manufacturer's protocol

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

Reaction mixture components should be mixed just before analysis with calculating for the required number of reactions (test and control samples) according to Table 1. Note that even for analysis of one test cDNA sample, it is necessary to carry out all control of the PCR stage (Positive Control of Amplification (C+) and Negative Control of Amplification (NCA) for each PCR-mix-1). 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 test and control samples. The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix one **PCR-mix-1 (PCR-mix-1-FEP/FRT *Y.enterocolitica / Y.pseudotuberculosis* or PCR-mix-1-FEP/FRT *Yersinia enterocolitica* type), PCR-mix-2 FRT,** and **polymerase (TaqF)** according to Table 1. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 1

Scheme of reaction mixture preparation

	Reagent volume for the specified number of reactions (µl)		
Reagent volume per one reaction (µl)	**10.00**	**5.00**	**0.50**
Number of reactions[1]	**PCR-mix-1-FEP/FRT**	**PCR-mix-2-FRT**	**Polymerase (TaqF)**
4	**40**	**20**	**2.0**
6	**60**	**30**	**3.0**
8	**80**	**40**	**4.0**
10	**100**	**50**	**5.0**
12	**120**	**60**	**6.0**
14	**140**	**70**	**7.0**
16	**160**	**80**	**8.0**
18	**180**	**90**	**9.0**
20	**200**	**100**	**10.0**
22	**220**	**110**	**11.0**
24	**240**	**120**	**12.0**
26	**260**	**130**	**13.0**
28	**280**	**140**	**14.0**
30	**300**	**150**	**15.0**
32	**320**	**160**	**16.0**

4. Transfer **15 µl** of the prepared mixture to the prepared tubes. Dispose the unused reaction mixture.
5. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage into the prepared tubes.

Avoid transferring sorbent together with the DNA samples extracted by DNA-sorb-B or RIBO-sorb kits.

6. Carry out the control amplification reactions:

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

[1] Number of test samples including the control of extraction stage (N), controls of amplification, and one extra reaction (N+2+1).

C+ – Add **10 µl** of **Positive Control DNA *Y.enterocolitica* / *Y.pseudotuberculosis* / STI** to the tube labeled C+ (Positive Control of Amplification).

C– – Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2. Amplification

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

Table 2

Amplification program

	Rotor-type Instruments[2]			Plate-type Instruments[3]		
Step	***Temperature, °C***	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
1	**95**	15 min	1	**95**	15 min	1
2	**95**	10 s	45	**95**	10 s	45
3	**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 for the FAM, JOE and ROX fluorophores.

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin* and Guidelines [2].
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

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels: FAM, JOE, ROX.

Correspondence table for detection channels and pathogens

Channel for fluorophore	PCR-mix-1-FEP/FRT *Y.enterocolitica* / *Y.pseudotuberculosis*	PCR-mix-1-FEP/FRT *Yersinia enterocolitica* type
FAM	Internal Control-FL DNA	*Y.enterocolitica* DNA (*Ail*-positive)
JOE	*Y.pseudotuberculosis* DNA (all strains)	*Y.enterocolitica* DNA (*Yst*-positive)
ROX	*Y. enterocolitica* DNA (all strains)	*Y.enterocolitica* DNA (*yadA*-positive)

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

[2] Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[3] iCycler, iQ5, Mx3000P, Mx3000, or equivalent.

Principle of interpretation is specified in the Table 3 and *Important Product Information Bulletin*.

Table 3

Interpretation of results

PCR-mix-1	*Ct* value in the channel for fluorophore			Result
	FAM/	**JOE**	**ROX**	
PCR-mix-1-FEP/FRT *Y.enterocolitica* / *Y.pseudotuberculosis*	**< boundary value**	> boundary value or absent	> boundary value or absent	*Yersinia enterocolitica* and *Yersinia pseudotuberculosis* DNA **are not detected**
	> boundary value or < boundary value	**< boundary value**	> boundary value or < boundary value	*Y. pseudotuberculosis* DNA **is detected**
	> boundary value or < boundary value	> boundary value or < boundary value	**< boundary value**	*Y. enterocolitica* DNA **is detected**
	> boundary value	> boundary value	> boundary value	**Invalid result** Repeat extraction and PCR
PCR-mix-1-FEP/FRT *Yersinia enterocolitica* type	**< boundary value**	> boundary value or < boundary value	> boundary value or < boundary value	*Y. enterocolitica* virulence factor (attachment invasion locus *Ail*) **is detected**
	> boundary value or < boundary value	**< boundary value**	> boundary value or < boundary value	*Y. enterocolitica* virulence factor (*Yst* enterotoxin) **is detected**
	> boundary value or < boundary value	> boundary value or < boundary value	**< boundary value**	*Y. enterocolitica* virulence factor (plasmid pYV adhesin *yadA*) **is detected**
	> boundary value	> boundary value	> boundary value	*Y. enterocolitica* virulence factors **are not detected**

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

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 (see Table 4).

Table 4

Results for controls

PCR-mix-1	Control	Stage for control	*Ct* value in the channel for fluorophore		
			FAM	**JOE**	**ROX**
PCR-mix-1-FEP/FRT *Y.enterocolitica* / *Y.pseudotu-berculosis*	**C–**	DNA extraction	**< boundary value**	> boundary value or absent	> boundary value or absent
	NCA	PCR	> boundary value or absent	> boundary value or absent	> boundary value or absent
	C+	PCR	**< boundary value**	**< boundary value**	**< boundary value**
PCR-mix-1-FEP/FRT *Yersinia enterocolitica* type	**C–**	DNA extraction	> boundary value or absent	> boundary value or absent	> boundary value or absent
	NCA	PCR	> boundary value or absent	> boundary value or absent	> boundary value or absent
	C+	PCR	**< boundary value**	**< boundary value**	**< boundary value**

10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Controls of Amplification (C+) in the channels for the FAM, JOE or ROX fluorophores is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples in which *Ct* value is greater than the boundary value in the channels for the FAM, JOE, or ROX fluorophore for appropriate PCR-mix-1.
2. If the *Ct* value determined for the Negative Control of Extraction (C–) (except for **PCR-mix-1-FEP/FRT *Y.enterocolitica* / *Y.pseudotuberculosis*** in the channel for the FAM fluorophore) and/or Negative Control of Amplification (NCA) in all channels is less than the boundary value, the PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which DNA of corresponding pathogens was detected.

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

11. TRANSPORTATION

AmpliSens® *Yersinia enterocolitica* / *Y.pseudotuberculosis*-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® *Yersinia enterocolitica* / *Y.pseudotuberculosis*-FRT** PCR kit are to be stored at 2–8 °C (except for PCR-mix-1-FEP/FRT *Y.enterocolitica* / *Y.pseudotuberculosis*, PCR-mix-1-FEP/FRT *Y.enterocolitica* type, PCR-mix-2-FRT, and

polymerase (TaqF)) when not in use. All components of the **AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-FRT** PCR kit are stable until the expiry 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 *Y.enterocolitica / Y.pseudotuberculosis*, PCR-mix-1-FEP/FRT *Yersinia enterocolitica* type, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

PCR-mix-1-FEP/FRT *Y.enterocolitica / Y.pseudotuberculosis* and PCR-mix-1-FEP/FRT *Yersinia enterocolitica* type are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Pathogen	Clinical material	Nucleic acid extraction kit	PCR kit	Analytical sensitivity, GE/ml[4]
Y.enterocolitica	Feces	RIBO-prep	PCR kit variant FRT-50 F	1 x 10^3
Y.pseudotuberculosis	Feces	RIBO-prep	PCR kit variant FRT-50 F	1 x 10^3

13.2. Specificity

The analytical specificity of **AmpliSens® *Yersinia enterocolitica / Y.pseudotuberculosis*-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.

Specificity was confirmed using the following microorganism strains:

- Strains from the 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.
- Strains from the CRIE collection: *Yersinia enterocolitica* (115 strains) and *Y.pseudotuberculosis* (84 strains).
- Vaccination strains of *Yersinia pestis*.

There were no nonspecific responses in tests with human DNA and a DNA panel of the above-mentioned microorganisms.

[4] Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.

The clinical specificity of **AmpliSens®** ***Yersinia enterocolitica / Y.pseudotuberculosis*-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, 2010.
2. Guidelines to the **AmpliSens®** ***Yersinia enterocolitica / Y.pseudotuberculosis*-FRT** PCR kit for qualitative detection and differentiation of DNA of virulent and avirulent strains of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* strains in environmental samples and clinical material by polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens®** ***Yersinia enterocolitica / Y.pseudotuberculosis*-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	Authorized 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
30.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
29.10.15 PM	Through the text	Corrections according the template
	8.1. DNA extraction	Additions about carrying out the control of extraction
	8.2.1. Preparing tubes for PCR	Scheme of reaction mixture preparation was added from Appendix 1
	10. Troubleshooting	The section was rewritten
	14. References	The name of the Guidelines was specified