CE

IVD

For Professional Use Only

AmpliSens® *Borrelia burgdorferi sensu lato*-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® *Borrelia burgdorferi sensu lato*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Borrelia burgdorferi sensu lato (B. burgdorferi sensu stricto, B. afzelii, B. garinii)* 16S rRNA in the biological material (ticks) 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

Borrelia burgdorferi sensu lato detection by the polymerase chain reaction (PCR) is based on the amplification of 16S rRNA specific region using special *Borrelia burgdorferi sensu lato* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit is a qualitative test which 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® *Borrelia burgdorferi sensu lato*-FRT** PCR kit uses “hot-start” which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using polymerase (TaqF). Chemically modified polymerase (TaqF) activates by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Borrelia burgdorferi sensu lato*-FRT PCR kit is produced in 1 form:

AmpliSens® *Borrelia burgdorferi sensu lato*-FRT PCR kit variant FRT (for use with RG) **REF** R-B37(RG)-CE.

AmpliSens® *Borrelia burgdorferi sensu lato*-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1-FEP/FRT *Borrelia burgdorferi sensu lato*	colorless clear liquid	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control cDNA *Borrelia burgdorferi sensu lato* (C+$_{B.\ burgdorferi\ sl}$)	colorless clear liquid	0.1	1 tube
Positive Control *Borrelia burgdorferi sensu lato*-rec*	colorless clear liquid	0.03	5 tubes
DNA-buffer	colorless clear liquid	0.5	1 tube
Internal Control STI-87-rec (IC)**	colorless clear liquid	0.12	5 tubes

* must be used in RNA extraction procedure as Positive Control of Extraction (PCE).

** add 10 µl of Internal Control STI-87-rec during the DNA extraction procedure directly to the sample/lysis mixture (see “RIBO-prep” **REF** K2-9-Et-50-CE protocols).

AmpliSens® *Borrelia burgdorferi sensu lato*-FRT PCR kit is intended for 60 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- Thermostat for microtubes.
- PCR box.
- Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia) Instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (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 extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens 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 sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a one directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. 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.

AmpliSens® *Borrelia burgdorferi sensu lato*-FRT PCR kit is intended for the analysis of RNA extracted with RNA extraction kits from biological material (ticks).

Number of ticks specimens in pool for analysis should not exceed 10. For Dermacentor ticks analysis of individual specimen is preferably. Place ticks in tubes like Eppendorf. Add 500 µl of 96 % ethanol and stir by vortex. Centrifuge the tube 3-5 sec at 5000 rpm to delete drops from internal surface of the tube cap. Remove liquid carefully by vacuum aspirator. Add in this tube with ticks 500 µl of 0.15 M sodium chloride solution, stir on vortex and centrifuge 5 sec at 5000 rpm to delete drops from internal surface of the tube cap. Remove liquid carefully by vacuum aspirator. Use sterile porcelain mortars and sterile pestles for ticks suspension preparation. Grind the ticks in 300 µl (if sample consist of 1 Ixodes tick), in 500 µl (if sample consist of 1 Dermacentor tick) or 1 ml (if pool of ticks is ground) of 0.15 M sodium chloride solution. Mix solution with ticks by two portions. Centrifuge obtained suspension 2 min at 5000 rpm. Take 100 µl of supernatant for RNA extraction from Ixodes ticks and 50 µl – from Dermacentor ticks. Add glycerol (10 % of volume) to residual part of suspension and freeze at the temperature not more than minus 16 °C for possible subsequent analysis.

It is acceptable to store material before analysis 1 month (live ticks) or 1 week at the temperature not more than minus 16 °C. Subsequent storage should be at the temperature not more than minus 68 °C.

Only one freeze-thawing cycle is allowed.

7. WORKING CONDITIONS

AmpliSens® *Borrelia burgdorferi sensu lato*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA Extraction

It's recommended to use the following nucleic acid extraction kits:

- "RIBO-prep" **REF** K2-9-Et-50-CE.

Carry out the RNA extraction according to the manufacturer's instructions.

Add **10 µl** of **Internal Control STI-87-rec** into each tube.

Add **100 µl** (for Ixodes) and **50 µl** (for Dermacentor) of **clinical samples** into each tibe with **Internal Control STI-87-rec.** Add only **10 µl** of **Internal Control STI-87-rec** to the tube for Negative Control of Extraction. **Add 10 µl** of **Positive Control *Borrelia burgdorferi sensu lato*-rec** to the tube for Positive Control of Extraction.

8.2. Reverse transcription

It's recommended to use the following reverse transcription reagents kits for complementary DNA (cDNA) synthesis from RNA.

- "REVERTA-L", form 1 REF К3-4-50-CE, which contains RT-G-mix-1.

Carry out the reverse transcription procedure according to the manufacturer's instruction.

8.3. Preparing the PCR

Total reaction volume is **25 µl**, the volume of cDNA sample is **10 µl**.

8.3.1 Preparing tubes for PCR

1. Prepare the required number of the tubes for amplification of cDNA from clinical and control samples.
2. Prepare the **reaction mix** for necessary number of reactions – mix in a new tube **PCR-mix-1-FEP/FRT *Borrelia burgdorferi sensu lato,* RT-PCR-mix-2-FEP/FRT** and **Polymerase (TaqF)**. For each reaction add:
   - **10 µl of PCR-mix-1-FEP/FRT *Borrelia burgdorferi sensu lato***
   - **5 µl of RT-PCR-mix-2-FEP/FRT**
   - **0.5 µl of polymerase (TaqF)**

   It should be taken into account that for analysis of one sample 4 control reactions are to be carried out (positive and negative controls of extraction (PCE and C-), positive and negative control of amplification(C+ and NCA)).
3. Add **15 µl** of **reaction mix** into each prepared tube. **Do not store prepared mix!**
4. Using tips with aerosol barrier add **10 µl** of **cDNA samples** obtained from clinical or control samples at the RNA extraction stage into tubes with reaction mix. Mix it carefully.
5. Carry out the control amplification reactions:

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

C+ - Add **10 µl** of **Positive Control cDNA *Borrelia burgdorferi sensu lato*** to the

tube labeled C+ (Positive Control of Amplification).

8.3.2. Amplification

1. Program the Rotor-Gene according to manufacturer's manual and Guidelines [2].
2. Create a temperature profile on your Rotor-Gene instrument as follows:

Table 1

Amplification program of *Borrelia burgdorferi sensu lato* cDNA

Step	Temperature	Time	Fluorescence detection	Cycles
1	95 °C	15 min	-	1
2	95 °C	15 sec	-	10
	63 °C	50 sec	-	
	72 °C	20 sec	-	
3	95 °C	15 sec	-	40
	58 °C	50 sec	FAM/Green, JOE/Yellow	
	72 °C	20 sec	-	

3. Fluorescence detection is on the 3-nd pass **(58°C)** in FAM/Green and JOE/Yellow fluorometer channels.
4. Make the adjustment of the fluorescence channel sensitivity according to Guidelines.

9. DATA ANALYSIS

Internal Control is detected on the JOE/Yellow fluorescence channel, *Borrelia burgdorferi sensu lato* cDNA is detected on the FAM/Green fluorescence channel.

See Guidelines for data analysis settings for Rotor-Gene 3000 or Rotor-Gene 6000.

9.1. Results interpretation

The results are interpreted by the software of Rotor-Gene 3000 or Rotor-Gene 6000 Instrument by the crossing (or not) of the fluorescence curve with the threshold line.

Table 2

Results for controls

Control	Stage for control	Ct channel FAM/Green	Ct channel JOE/Yellow	Interpretation
C-	DNA extraction	Neg	Pos (< Z*)	OK
PCE	DNA extraction	Pos (< X*)	Pos (< Z*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< Y*)	Neg	OK

*For X, Y, Z values see Guidelines.

1. The sample is considered positive for *Borrelia burgdorferi sensu lato* if in FAM/Green channel its Ct value doesn't exceed H (see Guidelines) and in the results grid in the JOE/Yellow channel the Ct value doesn't exceed Z.
2. The sample is considered negative for *Borrelia burgdorferi sensu lato* if its Ct value

exceed H in FAM/Green channel and in the results grid in the JOE/Yellow channel the Ct value doesn't exceed Z.

Results are accepted as relevant if both positive and negative controls of amplification along with positive and negative control of extraction are passed.

10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

1. If the signal is registered in Negative Control of extraction (C-) on FAM/Green channel and/or in Negative Control of amplification (NCA) in any of the channels, it indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.
2. If Ct value is absent for Negative control of Extraction (C-) in JOE/Yellow channel and/or for Positive Control in FAM/Green and JOE/Yellow channels, results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests from extraction stage.
3. If Ct value is absent for Positive control of Amplification (C+) in FAM/Green channel, results of the analysis for all samples are considered invalid. It is necessary to repeat the amplification and detection of all tests.

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

11. TRANSPORTATION

AmpliSens® *Borrelia burgdorferi sensu lato*-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® *Borrelia burgdorferi sensu lato*-FRT** PCR kit (except for PCR-mix-1-FEP/FRT *Borrelia burgdorferi sensu lato*, RT-PCR-mix-2-FEP/FRT and polymerase (TaqF)) are to be stored at 2–8 °C, when not in use. All components of the **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit are stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FEP/FRT *Borrelia burgdorferi sensu lato*, RT-PCR-mix-2-FEP/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 *Borrelia burgdorferi sensu lato* is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit is no less than $1x10^4$ genome equivalents per 1 ml of sample (GE/ml).

The claimed analytical features of **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit are guaranteed only when additional reagents kits “RIBO-prep” and “REVERTA-L” (manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”) are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *Borrelia burgdorferi sensu lato*-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. The clinical specificity of **AmpliSens® *Borrelia burgdorferi sensu lato*-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 to **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit for qualitative detection of *Borrelia burgdorferi sensu lato (B. burgdorferi sensu stricto, B. afzelii, B. garinii)* 16S rRNA in the biological material (ticks) by using real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

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® *Borrelia burgdorferi sensu lato*-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
RG	For working with Rotor-Gene 3000/6000 (Corbett Research)	**IC**	Internal control
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**PCE**	Positive Control of Extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
21.12.10 LA	Cover page	The phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that PCR-mix-1-FEP/FRT *Borrelia burgdorferi sensu lato* is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
21.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”