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For Professional Use Only

AmpliSens® *Borrelia burgdorferi sensu lato*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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 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 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 that contains the Internal Control (Internal Control STI-87-rec (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 the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated 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 **REF** R-B37(RG)-CE.

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

Reagent	*Description*	*Volume, ml*	*Quantity*
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 RNA 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 a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2 ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia).
- Disposable polypropylene PCR tubes (0.2 or 0.5 ml).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.

- Reservoir 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 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 Eppendorf tubes. Add 500 µl of 96 % ethanol and stir by vortex. Centrifuge the tube 3-5 sec at 5,000 rpm to sediment 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 5,000 rpm to sediment 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 5,000 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 kit:

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

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

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

Add **100 µl** (for *Ixodes*) and **50 µl** (for *Dermacentor*) of **biological samples** into each tube with **Internal Control STI-87-rec (IC)** and **Solution for Lysis.** In case

of Negative Control of Extraction (C–) add only **10 µl** of **Internal Control STI-87-rec (IC)** to the tube with **Solution for Lysis**. **Add 10 µl** of **Positive Control *Borrelia burgdorferi sensu lato*-rec** to the tube for Positive Control of Extraction (PCE).

After each washing use a new one **200-µl** tip for each sample.

8.2. Reverse transcription

It's recommended to use the following kit for complementary DNA (cDNA) synthesis from the RNA.

- "REVERTA-L", REF К3-4-50-CE.

Carry out the reverse transcription according to the manufacturer's protocol.

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 test and control samples.
2. Prepare the **reaction mixture** 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)**

   Take into account that for analysis of even 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 mixture** into each prepared tube. **Do not store prepared mix!**
4. Using filter tips add **10 µl** of **cDNA samples** obtained at the RNA reverse transcription stage into the tubes with reaction mixture. Mix it carefully by pippeting.
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* (C+$_{B.\ burgdorferi\ sl}$)** to the tube labeled C+ (Positive Control of Amplification).

C– - Add **10 µl of cDNA obtained by extraction and reverse transcription of the Negative control of Extraction (containing the Internal Control STI-87-rec (IC) reagent only)** to the tube labeled C–.

PCE - Add **10 µl of cDNA obtained by extraction and reverse transcription of the Positive Control *Borrelia burgdorferi sensu lato*-rec reagent** to the tube labeled PCE (Positive control of Extraction).

8.3.2. Amplification

1. Create a temperature profile on your 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 s	-	10
	63 °C	50 s	-	
	72 °C	20 s	-	
3	95 °C	15 s	-	40
	58 °C	50 s	FAM, JOE	
	72 °C	20 s	-	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores.

2. Adjust the fluorescence channel sensitivity according to the *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 two channels:

– The signal of the *Borrelia burgdorferi sensu lato* cDNA amplification product is detected in the channel for the FAM fluorophore.

– The signal of the IC cDNA amplification product is detected in the channel for the JOE fluorophore.

Principle of interpretation is the following:

– *Borrelia burgdorferi sensu lato* RNA is **detected** if the *Ct* value determined in the results grid in the channel for the FAM fluorophore is less than the boundary *Ct* value.

– *Borrelia burgdorferi sensu lato* RNA is **not detected** in a sample if the *Ct* value

determined in the results grid in the channel for the FAM fluorophore is greater than the boundary *Ct* value, whereas the *Ct* value determined in the channel for the JOE fluorophore is less than the boundary *Ct* value.

– The result is **invalid** if the *Ct* value determined in the results grid in the channel for the FAM fluorophore is greater than the boundary *Ct* value, whereas the *Ct* value determined in the channel for the JOE fluorophore is also greater than the boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the RNA extraction stage.

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 Positive and Negative Control of extraction are correct (seeTable 2).

Table 2

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	RNA extraction	Absent	**<**boundary value
PCE	RNA extraction	**<**boundary value	**<**boundary value
NCA	RT-PCR	Absent	Absent
C+	RT-PCR	**<**boundary value	Absent

10. TROUBLESHOOTING

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

1. If any *Ct* value is determined for the Negative Control of extraction (C–) in the channel for the FAM fluorophore and/or for the 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 samples, and also to take measures to detect and eliminate the source of contamination.
2. If the *Ct* values are absent for the Negative control of Extraction (C–) in the channel for the JOE fluorophore and/or for the Positive Control of Extraction (PCE) in the channels for the FAM and JOE fluorophores, results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all samples from extraction stage.

3. If the *Ct* value is absent for the Positive Control of Amplification (C+) in the channel for the FAM fluorophore, results of the analysis for all samples are considered invalid. It is necessary to repeat the amplification and detection of all samples.

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 are to be stored at 2–8 °C, when not in use (except for PCR-mix-1-FEP/FRT *Borrelia burgdorferi sensu lato*, RT-PCR-mix-2-FEP/FRT and polymerase (TaqF)). All components of the **AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit are stable until the expiry date stated on the label. 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 have been 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, 2010.
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 by the 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 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
		IC	Internal control
		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”
01.06.15 ME	Text	Corrections according to the template
	8.1. RNA Extraction	The phrase: “After each washing use a new one 200-µl tip for each sample” was added
	8.3.1 Preparing tubes for PCR	Information about carrying out the positive and negative controls of extraction was added
	9. Data analysis	The section was rewritten