



For *in Vitro* Diagnostic Use

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AmpliSens® *Borrelia burgdorferi sensu lato*-EPh

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE.

AmpliSens® Borrelia burgdorferi sensu lato-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of 16S rRNA *Borrelia burgdorferi sensu lato* (*B. burgdorferi sensu stricto*, *B. afzelii*, *B. garinii*) in the biological material (suspension of ticks) by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION.

Borrelia burgdorferi sensu lato detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® Borrelia burgdorferi sensu lato-EPh PCR kit** is a qualitative test, which contain 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® Borrelia burgdorferi sensu lato-EPh 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 wax layer. Wax melting and reaction mix components occur only at 95°C.

3. CONTENT.

AmpliSens® Borrelia burgdorferi sensu lato -EPh PCR kit is produced in 2 forms:

AmpliSens® *Borrelens® Borrelia burgdorferi sensu lato-EPh PCR kit variant 50 R* (tubes of 0.2 ml volume),

REF B37-50-R0,2-CE.

AmpliSens® Borrelia burgdorferi sensu lato-EPh PCR kit variant 50 R includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-R <i>Borrelia burgdorferi sensu lato</i> ready-to-use single-dose test tubes (under wax)	colorless clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	blue clear liquid	0.6	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 tube
Positive Control cDNA <i>Borrelia burgdorferi</i>	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.6	3 tubes
Positive Control <i>Borrelia burgdorferi sensu lato-rec</i>	colorless clear liquid	0.03	5 tubes
Internal Control <i>Borrelia burgdorferi sensu lato-rec**</i>	colorless clear liquid	0.06	5 tubes

* must be used in the isolation procedure as Negative Control of Extraction (only for “RIBO-sorb” extraction kit).

** add 5 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture (see “RIBO-sorb”, **REF** K2-1-50-CE or “RIBO-prep”, **REF** K2-9-50-CE protocols).

AmpliSens® *Borrelia burgdorferi sensu lato-EPh PCR kit variant 50 R* is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- RNA isolation kit
- Reverse transcription kit
- Agarose gel detection kit
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barriers (up to 200 µl)
- Vortex mixer
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at temperature between 25 °C and 100 °C
- Tube racks.
- PCR box
- Personal thermocycler (for example, «GeneAmp PCR System 2700» («Applied Biosystems», USA) or equivalent)
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, «Axygen», USA).
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16 °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 other 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 move 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 to read this handbook before starting work

AmpliSens® *Borrelia burgdorferi sensu lato*-EPh PCR kit is intended for analysis of RNA extracted by RNA isolation kits from:

- *Tick suspension*

6.1. *Tick suspension*. Ticks are placed into tubes (single ticks and pools of not more than 10 specimens can be used). Add 1 ml of 96% ethanol and vortex. Spin the tube with ticks at 5,000 r/min for 3-5 sec then remove liquid by vacuum aspirator. Add 1 ml of saline solution (0.15 M sodium chloride), vortex, and spin at 5,000 r/min for 5 sec. Remove liquid by vacuum aspirator.

If a large number of ticks is intended to be used for RNA extraction then store prepared (as described above) ticks at 2-8 °C for no longer than 1 hour until homogenization.

For tick suspension use sterile porcelain mortar and a pestle. A single tick should be homogenized in 300 µl of 0.15 M sodium chloride then centrifuged at 5,000 r/min for 2 min. Remove 100 µl of supernatant for RNA extraction from Ixodes ticks and 50 µl of supernatant if RNA is extracted from Dermacentor ticks.

Add glycerol (10% by volume) into the tube with remained suspension, stir, and froze at the temperature not more than minus 16 °C for possible further use.

7. PROTOCOL.

7.1. RNA Isolation

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

- "RIBO-sorb", REF K2-1-50-CE.
- "RIBO-prep", REF K2-9-50-CE.



Please carry out the RNA isolation according to the manufacturer instruction.



Volume of Ixodes specimen used for RNA extraction should be 100 µl; volume of Dermacentor specimen should be 50 µl.



"RIBO-prep" extraction kit

Into the tube of Negative Control of extraction add 5 µl of Internal Control *Borrelia burgdorferi sensu lato-rec* 300 µl of lysis solution only.



Into the tube of Positive Control of Extraction add 10 µl of Positive Control *Borrelia burgdorferi sensu lato-rec* 5 µl of Internal Control *Borrelia burgdorferi sensu lato-rec* 300 µl of lysis solution



"RIBO-sorb" extraction kit

Add 50 µl of Negative Control (C-) directly into **each** IC/ lysis solution/ sample mixture

7.2. Reverse transcription

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

- "REVERTA-L", REF K3-4-50-CE.



Please carry out the reverse transcription procedure according to the manufacturer instruction.

7.3. Preparing the PCR.

Total reaction volume - 25 µl, volume of cDNA sample - 10 µl.

7.3.1 Preparing tubes for PCR.

1. Collect the required quantity of tubes with **PCR-mix-1-R *Borrelia burgdorferi sensu lato*** and wax for amplification of cDNA from clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, so that it wouldn't fall under the wax and mix with PCR-mix-1-R *Borrelia burgdorferi sensu lato*.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).

7.3.2 Amplification.

- Use prepared tubes for PCR. Add **10 µl** of **DNA samples**, obtained from clinical or control samples at the stage of DNA extraction, under or directly above the level of oil by tips with aerosol barrier.
- Carry out the **control amplification reactions**:
 NCA -Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
 C+ -Add 10 µl of **Positive Control cDNA *Borrelia burgdorferi sensu lato*** to the tube for Positive Control of Amplification.
- Run the following program on the thermocycler (see table 1). When the temperature reaches 95°C (pause regimen), insert tubes to cells of amplifier and press button to continue. It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before their insertion in thermocycler.

Table 1

Amplification program of *Borrelia burgdorferi sensu lato*

step	Thermocyclers with active temperature adjustment:											
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems)			"Palm Cycler"(Corbett Research)			«Maxygene» («Axygen»), CLJA		
	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles
0	95 °C	pause		93 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	93 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	94 °C	20 sec	42	95 °C	30 sec	42	95 °C	30 sec	42
	67 °C	25 sec		67 °C	40 sec		67 °C	60 sec		67 °C	60 sec	
	72 °C	10 sec		72 °C	30 sec		72 °C	40 sec		72 °C	40 sec	
3	72 °C	2 min	1	72 °C	2 min	1	72 °C	2 min	1	72 °C	2 min	1
4	10 °C	storage		10 °C	storage		10 °C	storage		4 °C	storage	

Table 2

Amplification program of *Borrelia burgdorferi sensu lato*

step	Thermocyclers with block temperature adjustment: "Biometra"		
	temperature	time	cycles
0	95 °C	pause	
1	95 °C	5 min	1
2	95 °C	30 sec	42
	67 °C	60 sec	
	72 °C	40 sec	
3	72 °C	2 min	1
4	10 °C	storage	

- After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis. Analysis of amplification products is performed by separation of cDNA fragments in agarose gel. The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8 °C (be sure to warm the samples to room temperature before running electrophoresis).

8. DATA ANALYSIS.

It's recommended to use the following detection agarose kit:

- "EPh" variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified cDNA in agarose gel (1.7%). The length of specific amplified cDNA fragments is:

- Borrelia burgdorferi sensu lato* - 370 bp
- IC *Borrelia burgdorferi sensu lato*-rec - 571 bp



Put the protective mask or use the glass filter while watching and photographing the gel

Results interpretation

Table 3

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		370 bp	571 bp	
PCE	RNA isolation	Yes	Yes	OK
C-	RNA isolation	No	Yes	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	No	OK

- The sample is considered to be positive for *Borrelia burgdorferi sensu lato* rRNA if the band of 370 bp is present in agarose gel. The band of IC (571 bp) could be absent in the samples with high concentration of *Borrelia burgdorferi sensu lato* rRNA.
- The sample is considered to be negative for *Borrelia burgdorferi sensu lato* rRNA if the band of 370 bp is absent and the band of 571 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

9. TROUBLESHOOTING.

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

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lanes none of bands of 370 and 571 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lines nonspecific bands at different levels are presented, it may be caused by lack of "hot start" or false temperature regimen in thermocycler.

- If in lanes corresponding to negative control (NCA, C-) specific band of 370 bp appears, it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

10. STABILITY AND STORAGE.

All components of the AmpliSens® *Borrelia burgdorferi sensu lato*-EPh PCR kit are to be stored at the temperature between 2 °C and 8 °C. All components of the PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of AmpliSens® *Borrelia burgdorferi sensu lato*-EPh PCR kit is no less than 1×10^4 genome equivalents per 1 ml of sample.



Claimed analytical features of AmpliSens® *Borrelia burgdorferi sensu lato*-EPh PCR kit are guaranteed only when additional kits of reagents, "RIBO-sorb" or "RIBO-prep", "REVERTA-L" and "EPh" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

11.2. Specificity.

Specificity of AmpliSens® *Borrelia burgdorferi sensu lato*-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.
















12. REFERENCES.

1. Korotkov IuS, Kislenko GS, Burenkova LA, Rudnikova NA, Karan' LS. Spatial and temporal variability of Ixodes ricinus and Ixodes persulcatus infection with the Lyme disease agent in Moscow Region. Parazitologiya. 2008 Nov-Dec;42(6):441-51.
2. Handbook "Sampling, transportation, 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.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 –certified Total Quality Management System, each lot of AmpliSens® *Borrelia burgdorferi sensu lato*-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Authorised representative in the European Community.
	Contains sufficient for <N> tests		Caution, consult accompanying documents
	Consult instructions for use		Negative Control
	Positive Control		Positive Control of Extraction
	Negative Control of Amplification		