

GeneProof®

Borrelia burgdorferi

PCR kit



in vitro Diagnostics

The kit is designed for professional use in specialized clinical and research laboratories.

Method principles

The kit is designed for the detection of *B. afzelii*, *B. garini* and *B. burgdorferi* sensu stricto sp. from the "Borrelia burgdorferi sensu lato sp." group on the principle of the amplification of the specific DNA sequence of a flagellin encoding gene by the „one tube nested“ Polymerase Chain Reaction (PCR) and the detection of the resulting amplification products by agarose electrophoresis. The detection kit takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. The reaction mix includes an internal standard controlling the possible inhibition of the PCR reaction and the uracil-DNA-glycosylase (UDG) controlling possible contamination of the PCR reaction by amplification products.

Kit composition

	Cat. No. BB/E/ISIN/025 25 reactions	Cat. No. BB/E/ISIN/050 50 reactions	Cat. No. BB/E/ISIN/100 100 reactions
MasterMix <i>Borrelia burgdorferi</i>	1 x 900 µl	2 x 900 µl	4 x 900 µl
Positive control <i>Borrelia burgdorferi</i> 102 copies/µl	1 x 50 µl	1 x 50 µl	2 x 50 µl

Storage and transportation conditions

Transport the kits at temperatures ranging from -20 °C to -80 °C. The kit remains stable for at least 6 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix and the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 36 µl directly to PCR tubes and hold in stock at -20°C. Positive control may be held in stock at 4°C.

User Manual

Sampling and sample storage

Skin biopsy sampling has to be performed from the location of the tick clinging or from the „exanthema migrans“ location. Samples should be placed into tubes „dry“ without any transportation media and conserved or transported at 4 °C within 24 hours. A sample of **incoagulable peripheral blood** should be sampled into the EDTA and transported into the laboratory at +4 °C within 24 hours. **Cerebrospinal and synovial fluids** from afflicted joints and **urine** samples should be sampled into tubes without transportation medium and preserved or transported at +4°C within 24 hours or long-term preserved at -20 to -80°C. If the examination of the removed tick is required, the removed arthropod has to be preserved in sterile environment at -20 to -80°C immediately after removing from the wound and transported into the laboratory as soon as possible. In case of longer storage all samples should be frozen at -20°C.

DNA isolation

Isolation recommended by means of commercial DNA isolation kits according to the particular protocols of the isolation kit manufacturers. The manufacturer recommends the following isolation kits: PathogenFree DNA isolation kit (GeneProof); QIAamp DNA Blood Mini Kit (QIAGEN); NucleoSpin Blood (Macherey-Nagel).

PCR amplification

1. Add 36 μl of the MasterMix and 4 μl of the DNA isolate or 4 μl of the Positive control into the PCR tube. The final reaction mix volume should be 40 μl .*
2. Insert the tubes into a thermocycler and amplify them according to the following recommended program:

Amplification program: **

UDG decontamination	37 °C/2 min.
initial denaturation	96 °C/10 min.
denaturation	96 °C/10 sec.
annealing	68 °C/10 sec.
extension	72 °C/40 sec.
number of cycles	30
denaturation	96 °C/10 sec.
annealing	54 °C/10 sec.
extension	72 °C/30 sec.
number of cycles	45
final extension	72 °C/2 min.

*Tubes should be maintained in cold environment when handled (at 0 – 15 °C)

**Hereby presented amplification conditions may differ for various types of thermocyclers. The above listed conditions apply for PTC 200 (MJ Research) thermocycler.

Agarose electrophoresis

The resulting amplification products should be separated on 2% agarose gel electrophoresis (5V/cm) containing Ethidium bromide (5 $\mu\text{g}/1\text{ ml}$) and visualized by means of UV illumination. Since the loading buffer is included in the reaction mix, there should be directly loaded at least 10 μl of the amplification product.

Detection evaluation

Positive sample - is the detected amplification product with the length of 276 bp, there may also be the detected amplification product of the internal standard with the length of 420 bp (Fig. 1).

Negative sample – only the amplification product of the internal standard with the length of 420 bp is detected.

Inhibiting sample - should inhibition of the reaction components occur or should the reaction fail to proceed, none of the above-listed amplification products would be detected and the examination should be repeated.

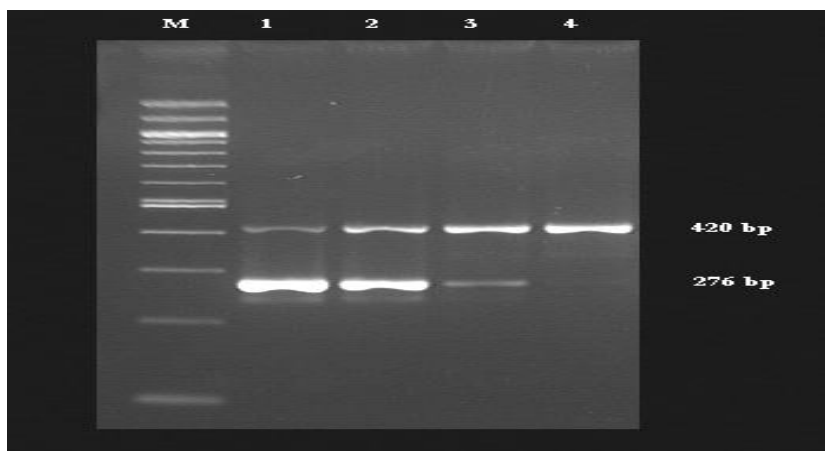


Fig. 1. Electrophoretical detection of amplification products on 2% agarose gel. 276 bp long fragment results from the amplification of the target sequence gene for flagellin of *Borrelia burgdorferi* sensu lato, 420 bp long fragment results from the amplification of the internal standard. **Track No. 1** – result of 320 spirochetes amplification per reaction, **Track No. 2** - 32 spirochetes, **Track No. 3** - 3 spirochetes, **Track No. 4** – 0.3 spirochetes per reaction, **M** - 100 bp weight marker.

Warning:

- Be very careful when handling the Positive control or the clinical material – incorrect handling could result in contamination and the consequent impairment of the kit components or the Master Mix! The manufacturer is not responsible for the kit impairment due to incorrect handling.
- The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations and the packaging materials are made of paper and polypropylene.
- Service fee assuring returnability and recycling of the waste packaging material has been paid under the identification number EK-F00041495.

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