

# GeneProof®

## *Borrelia burgdorferi*

### PCR Kit



### *in vitro* Diagnostics

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

#### Kit composition

Cat. No.	Internal Standard is included in the MasterMix for inhibition control			Contains a separate tube of Internal Standard for inhibition and isolation process control		
	BB/ISIN/025 25 reactions	BB/ISIN/050 50 reactions	BB/ISIN/100 100 reactions	BB/ISEX/025 25 reactions	BB/ISEX/050 50 reactions	BB/ISEX/100 100 reactions
<b>MASTERMIX</b> <i>Borrelia burgdorferi</i>	1 x 750 µl	2 x 750 µl	4 x 750 µl	1 x 750 µl	2 x 750 µl	4 x 750 µl
<b>POSITIVE CONTROL</b> <i>Borrelia burgdorferi</i> 10 <sup>2</sup> copies/µl	1 x 200 µl	1 x 200 µl	2 x 200 µl	1 x 200 µl	1 x 200 µl	2 x 200 µl
<b>INTERNAL STANDARD</b> <i>Borrelia burgdorferi</i>	-	-	-	1 x 1000 µl	1 x 1000 µl	2 x 1000 µl

#### Storage and transportation conditions

Transport the kits at temperatures ranging from -20°C to -80°C. The kit remains stable for 9 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix, Internal Standard or the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 30 µl directly to PCR tubes and hold in stock at -20°C. Positive control and the Internal Standard may be held in stock at 4°C.

## Pathogen information

*Borrelia burgdorferi* sensu lato is an agent of a multi-organ disease – Lyme borreliosis. This disease is transmitted by infected ticks and proceeds in three stages. The first stage is typical with the occurrence of erythema chronicum migrans; the second stage is characteristic by hematogenous dispersion with skin symptoms possibly resulting in CNS infection (aseptic meningitis, radiculoneuropathy, lymphocytic meningitis, eye infection, etc.) and the third stage features arthritis, acrodermatitis chronica atrophicans and limb paresis. Laboratory diagnostics of Lyme borreliosis predominantly use indirect diagnostic methods based on the detection of specific serum antibodies IgM and IgG or intrathecal antibodies by the ELISA method or by the immunoblotting confirmation method (western blot). Due to the long-term persistence of serum antibodies, comparatively high seroprevalence of the anti-borreliosis antibodies in people living in endemic regions, existence of seronegative forms of Lyme borreliosis, possibly crossed reactions or the impossibility to examine clinical materials such as biopsies (skin), synovial fluid, etc. it is expedient to complement the serological methods with direct *Borrelia* detection for the purpose of the diagnosis establishment. PCR diagnostics is the only clinically available method for direct borreliosis detection and it is perfectly suited to complement the current range of serological tests. This sensitive method provides for borrelia detection in actually any type of clinical material. In the primary, acute stage of the disease, the borrelias may be detected in the skin biopsy samples and later, in the stage of the hematogenous dispersion and even in later borreliosis recurrences they may be detected in the samples of peripheral blood and urine. In the later stages of the disease the PCR method may be used for borrelia detection in the cerebrospinal fluid or in synovial fluid and also in the vitreous fluid in the late stage of eye borreliosis. Clinical samples for the *B. burgdorferi* detection by the PCR method are to be sampled in those stages of the disease when the pathogen presence in the sampled materials can be expected. It is also possible to preventively test the removed tick.

## Method principles

This kit is designed for the detection of the species from the "*Borrelia burgdorferi* sensu lato" group (*B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. lusitanae*, *B. andersonii*, *B. bissettii*, *B. japonica*, *B. tanukii*, *B. turdi*, *B. sinica*). It is based on the principle of the sequence-specific detection of a chromosomal gene encoding bacterial 16S RNA, specific for *B. burgdorferi* sensu lato group using real-time polymerase chain reaction (real-time PCR) method. This method has been tested on a large set of European borrelia and spirochete strains. Borrelia DNA presence in the sample is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction and the efficiency of the DNA isolation process. IS positive amplification is detected in the fluorescence channel for the JOE fluorophore. The detection kit takes an advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. It contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. Sensitivity of the PCR detection kit runs in single copies of the borrelia genome in a reaction, providing for maximum sensitivity of the laboratory detection of body fluids (synovial fluid, cerebrospinal fluid, urine) or blood. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

GeneProof PCR kits are designed for use with real-time devices from various manufacturers.

*Borrelia burgdorferi* PCR Kit has been validated with the following devices:

Rotor-Gene™ 3000 (Corbett Life Science)  
Rotor-Gene™ 6000 (Corbett Life Science)  
7500 Real-Time PCR System (Applied Biosystems)  
LightCycler® 2.0 (Roche)  
LightCycler® 480 System (Roche)  
SLAN Real-time Quantitative PCR Fluorescent Detection System (Shanghai Odin Scienc & Technology Co.)

For detailed information about PCR kit use with specific devices see the Manufacturer's web site ([www.geneproof.com](http://www.geneproof.com)) or request the information from your kit supplier.

If you want to use the kit with other real-time devices, contact the manufacturer, please: [support@geneproof.com](mailto:support@geneproof.com)

### Warning:

- The kit has been manufactured according to the EC Directive 98/79/EC as an *in vitro* medical diagnostic device.
- 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 MasterMix! 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.

# 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

DNA isolation should be performed by isolation kits available at the market according to specific protocols for the particular microorganism isolation. The manufacturer recommends the following isolation kits: PathogenFree DNA Isolation Kit (GeneProof).

All GeneProof PCR kits include an Internal Standard (IS) providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by genetic engineering methods GeneProof develops and sells two basic variants of PCR kits which differ in the Internal Standard composition.

### PCR Kit ISIN (Cat. No. BB/ISIN...)

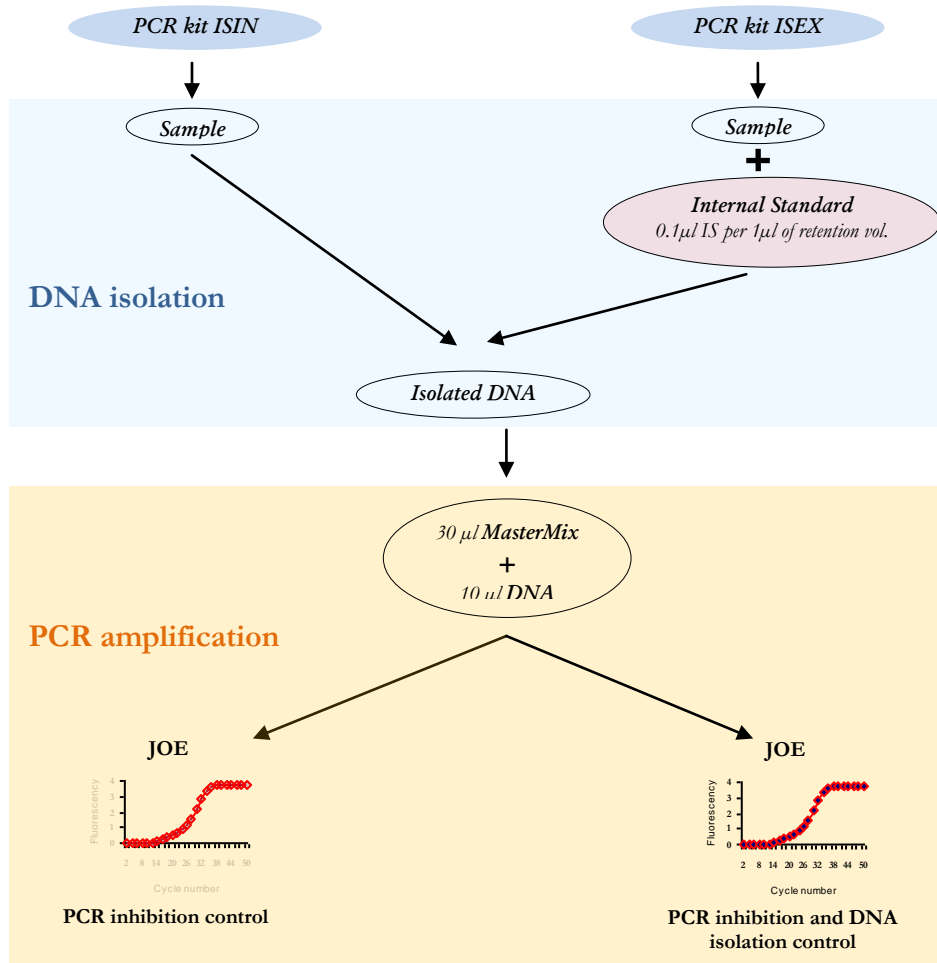
In this version of the PCR kit the Internal Standard (IS) is included directly in the MasterMix tube. This version of the kit provides **efficient control of the PCR reaction inhibition.**

### PCR Kit ISEX (Cat. No. BB/ISEX...)

In this PCR kit version the Internal Standard (IS) is included in a separate tube within the package. This version of the PCR kit can be used for both **PCR reaction inhibition control and DNA isolation efficiency control.**

**When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:**

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Standard	2.5 µl	5 µl	10 µl	20 µl



## PCR amplification

1. Add **30 µl of the MasterMix** and **10 µl of the DNA isolate** or **10 µl of the Positive Control** into a PCR tube. The final reaction mix volume should be 40 µl.
2. Close the tubes, centrifuge shortly, insert into the device and program according to the following table:

### Amplification program:

UDG decontamination	37°C/2 min.
initial denaturation	95°C/10 min.
denaturation	95 °C/5 sec.
annealing	60°C/40 sec. - reading of the fluorescence signal
extension	72°C/20 sec.
number of cycles	45

## Qualitative evaluation of detection

