BactoReal® Kit *Borrelia burgdorferi* sensu lato

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *Borrelia burgdorferi* sensu lato			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUB05053	50	FAM channel	Cy5 channel

Kit contents:

- Assay for detection of *Borrelia* species belonging to the *B. burgdorferi* sensu lato complex and of internal DNA positive control (IPC)
- IPC-Target DNA (control of DNA extraction and of PCR amplification)
- DNA reaction mix (contains a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs with dUTP and Uracil-N glycosylase (UNG) to eliminate amplicon carryover, ROX™ dye (passive reference) and buffer components – additives optimized to handle PCR inhibitors)
- DNA positive control for *Borrelia burgdorferi* s.l.
- Nuclease-free water

Pathogen information: *Borrelia* are Gram-negative bacteria belonging to the *Spirochaetaceae*. The genus *Borrelia* consists of numerous species. BactoReal® Kit *Borrelia burgdorferi* sensu lato detects species belonging to the *B. burgdorferi* sensu lato-complex, which are the causative agents of Lyme borreliosis (LB). These bacteria are transmitted by ticks, mainly by *Ixodes ricinus*. Lyme disease is a multisystemic illness, which can affect various organs, especially the nervous system, skin, heart and joints.
In Europe, the main pathogenic and potentially pathogenic genospecies are *B. afzelii*, *B. garinii, B. spielmanii, B. bavariensis*, *B. valaisiana, B. bissettii and B. lusitaniae, while B. burgdorferi* sensu stricto is less common. Pathogenic species in North America are *Borrelia burgdorferi* senso stricto and *B. mayonii* so far found in the Upper Midwest of the USA. The *B. burgdorferi* sensu stricto pathogen probably migrated from Europe to North America some 60 000 years ago and dominates in this geographical area. Evidence is accumulating that different genospecies cause different clinical symptoms in humans.

Intended purpose: BactoReal® Kit *Borrelia burgdorferi* sensu lato is a non-automated CE-certified IVD real-time PCR test for the qualitative detection of DNA (ospA gene) of *Borrelia* species belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex (*Borrelia afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, *B. valaisiana*, *B. lusitaniae, B. bavariensis, B. bissettii,* and *B. spielmanii* (*B. spielmanii* is detected with lower sensitivity, *B. japonica* is not detected).
Proper specimens are DNA extracts isolated from samples of synovial fluid, cerebrospinal fluid, and skin biopsies.
This test is suitable for patients of all ages with suspected infection with *Borrelia* species belonging to the *Borrelia burgdorferi* s.l. complex (causative agent of Lyme borreliosis) and is intended as an aid in the diagnosis of infection with these pathogens in combination with patient history and additional clinical information.
The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.
A probe-specific amplification-curve in the FAM channel indicates amplification of *Borrelia burgdorferi* s.l. specific DNA. The internal DNA positive control (IPC) is detected in the Cy5 channel and serves as a control for DNA extraction and possible real-time PCR inhibition. The target of the DNA IPC (artificial target DNA) is added during sample extraction.

PCR-platforms: This test has been validated with the ABI® 7500 Fast Real-Time PCR System (fast cycle parameters are not supported, Thermo Fisher Scientific) and tested with a LightCycler® 480 II (Roche Diagnostics), Mx3005P® (Agilent), CFX Opus Real-time PCR System (Bio-Rad), CFX Dx (Bio-Rad) and T-COR 8 (Tetracore).
It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel (e.g., QuantStudio™ 5, QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), qTOWER^{3}G (Analytik Jena), Mic instrument (bio molecular systems), cobas z 480 Analyzer (Roche)).

Performance data: BactoReal® Kit *Borrelia burgdorferi* sensu lato is specific for *Borrelia* species belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex (*Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. lusitaniae, B. bavariensis, B. bissettii* and *B. spielmanii).* This kit detects at least 10 target copies/reaction. The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 42 copies/reaction. *Borrelia spielmanii* is detected with lower sensitivity, *B. japonica* is not detected. Validation was performed with bacterial isolates and with 80 clinical samples.

Results of clinical validation:

	Value	95% CI
Sensitivity	95.12%	83.47% to 99.40%
Specificity	100.00%	90.97% to 100.00%
NPV	95.12%	83.46% to 98.69%
PPV	100.00%	
Prevalence	51.25%	39.81% to 62.59%
Accuracy	97.50%	91.26% to 99.70%

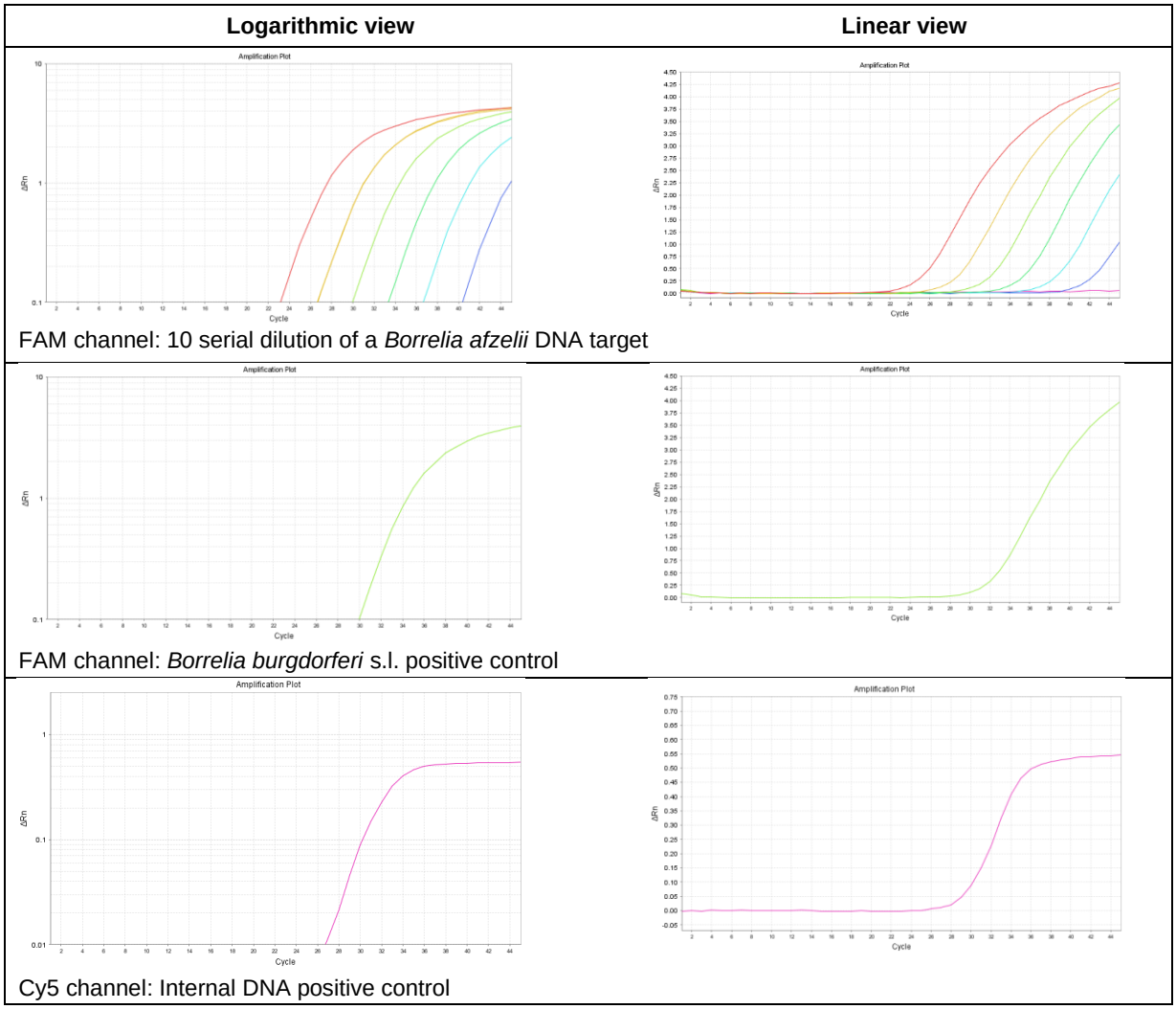

Figure 1 Performance of BactoReal® Kit *Borrelia burgdorferi* sensu lato