BactoReal® Kit *Listeria monocytogenes*

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *Listeria monocytogenes*			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUB00853	50	FAM channel	Cy5 channel

Kit contents:

- Assay for detection of *Listeria monocytogenes* 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 *Listeria monocytogenes*
- Nuclease-free water

Pathogen information: Listeriosis is a bacterial infection caused by the consumption of contaminated food (milk, cheeses, ice cream, vegetables, sausages, meats and fish). Although there are other types of Listeria (*L. ivanovii, L. innocua, L. welshimeri, L. seegligeri and L. grayi*), the culprit pathogen for most listeriosis cases is *L. monocytogenes*. Listeria are ubiquitously found in soil and water. Gastrointestinal symptoms may remain the only symptoms or precede more severe clinical manifestations such as sepsis and meningitis. People at risk include the elderly population, immunosuppressed patients, and foetuses of pregnant women. *L. monocytogenes* is a gram positive, facultative anaerobic, and facultative intracellular bacterium with an incubation time of at least seven days. It expresses a Beta hemolysin (hlyA gene) which causes destruction of red blood cells and can target other cells. The ability of *L. monocytogenes* to grow at temperatures as low as 0°C permits multiplication in refrigerated foods. *L. monocytogenes* is killed by pasteurization and cooking.

Intended purpose: BactoReal® Kit *Listeria monocytogenes* is a non-automated CE-certified IVD real-time PCR test for the qualitative detection and identification of DNA (hlyA gene) of *Listeria monocytogenes*.
Proper specimens are DNA extracts isolated from samples of CSF or aspirate samples.
This test is suitable for patients of all ages with suspected infection with *Listeria monocytogenes* and is intended as an aid in the diagnosis of infection with this pathogen 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 the amplification of *L. monocytogenes* specific DNA. The internal DNA positive control (IPC) is detected in the fluorescence channel Cy5 and serves as a control for DNA extraction and possible real-time PCR inhibition. The target for 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) and Mx3005P® (Agilent).

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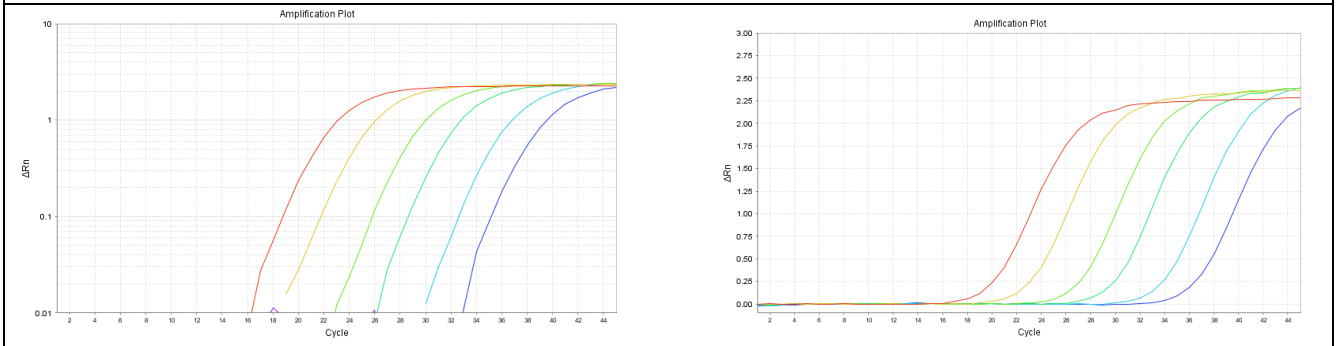
It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel (e.g., QuantStudio™ 5, QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), qTOWER^{3}G (Analytik Jena), Mic instrument (bio molecular systems), cobas z 480 Analyzer (Roche)).

Performance data: BactoReal® Kit *Listeria monocytogenes* is specific for *Listeria monocytogenes.* The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 6 copies/reaction. Diagnostic evaluation was performed with bacterial isolates and with 29 clinical samples.

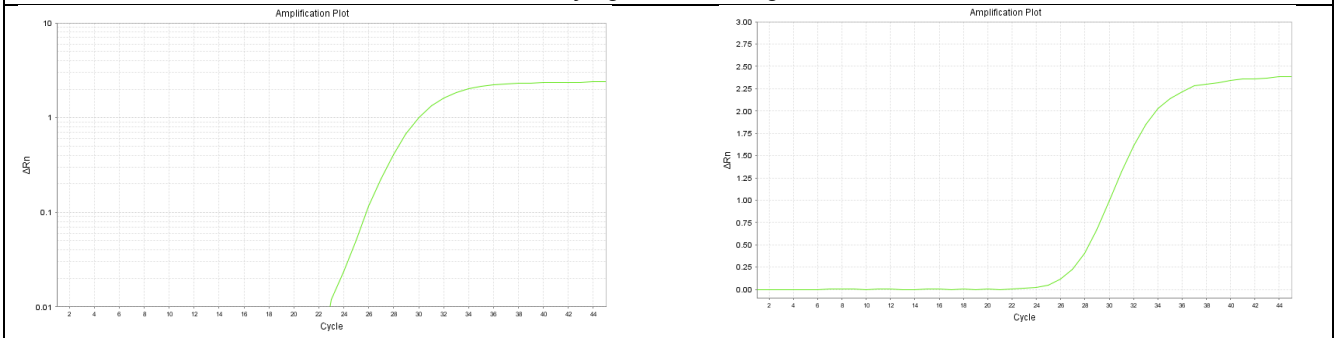
Results of clinical validation:

	Value	95% CI
Sensitivity	100.00 %	76.84 % to 100.00 %
Specificity	100.00 %	78.20 % to 100.00 %
NPV	100.00 %	78.20 % to 100.00 %
PPV	100.00 %	76.84 % to 100.00 %
Prevalence	48.3 %	

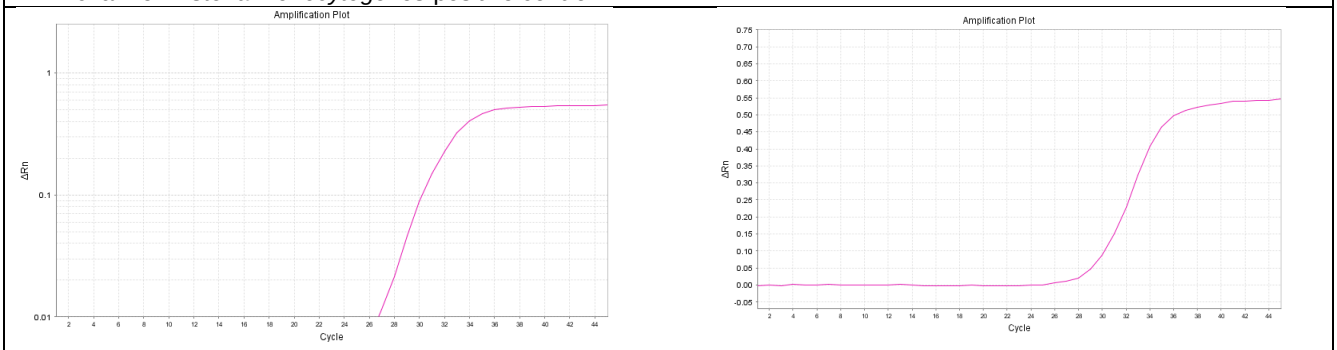
Logarithmic view | **Linear view**



FAM channel: 1:10 serial dilution of a *Listeria monocytogenes* DNA target



FAM channel: *Listeria monocytogenes* positive control



Cy5 channel**:** Internal DNA positive control

Figure 1 Performance of BactoReal® Kit *Listeria monocytogenes*