BactoReal® Kit *Haemophilus influenzae*

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *Haemophilus influenzae*			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUB01053	50	FAM channel	Cy5 channel

Kit contents:

- Assay for detection of *Haemophilus influenzae* 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 *Haemophilus influenzae*
- Nuclease-free water

Pathogen information: The 16 species of *Haemophilus* are Gram-negative bacteria occasionally colonizing the mucosa of humans and animals. Some *Haemophilus* species are the causative agents of various infections. For example, *H. aegyptius* causes purulent conjunctivitis, *H. parainfluenzae* is a rare cause of a form of endocarditis. *Haemophilus influenzae* can be found exclusively in the mucosa of humans, mainly in the upper respiratory tract. Transmission occurs through close contact with contaminated respiratory droplets. In sum, six capsular serotypes (a-f) can be distinguished, out of which type b (Hib) is the most prevalent and virulent one causing bloodstream infections and meningitis mainly in children less than 5 years. In contrast, unencapsulated strains (also called nontypeable *H. influenzae*, NTHi) of *H. influenzae* are part of the normal bacterial flora of humans and are occasionally pathogen. These strains can cause otitis, sinusitis, epiglottitis, bronchitis, pneumonia and less frequently meningitis and bacteraemia. The Omp P6 gene of *H. influenzae* is present in both the encapsulated and unencapsulated strains of *H. influenzae.*

Intended purpose: BactoReal® Kit *Haemophilus influenzae* is a non-automated CE-certified IVD real-time PCR test for the qualitative detection of DNA (omp P6 gene) of encapsulated and unencapsulated (also called nontypeable *H. influenzae*, NTHi) *H. influenzae* strains. The test also recognizes some strains of *Haemophilus haemolyticus* and *Haemophilus parainfluenzae*, albeit with less sensitivity.
Proper specimens are DNA extracts isolated from of samples of the respiratory tract and cerebrospinal fluid.
This test is suitable for patients of all ages with suspected infection with *H. influenzae* 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 amplification of *H. influenzae* 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 Instrument II (Roche Diagnostics) and Mx3005P® QPCR System (Agilent). 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 *Haemophilus influenzae* detects at least 10 target copies/reaction. The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 36 copies/reaction. Diagnostic evaluation was performed with bacterial isolates and with clinical samples (sputum samples and spiked CSF extracts). All samples that were positive with BactoReal® Kit *Haemophilus influenza* also showed a positive signal with both reference methods. Cross reaction with some strains of *H. haemolyticus* and *H. parainfluenzae* was observed, albeit with lower sensitivity.

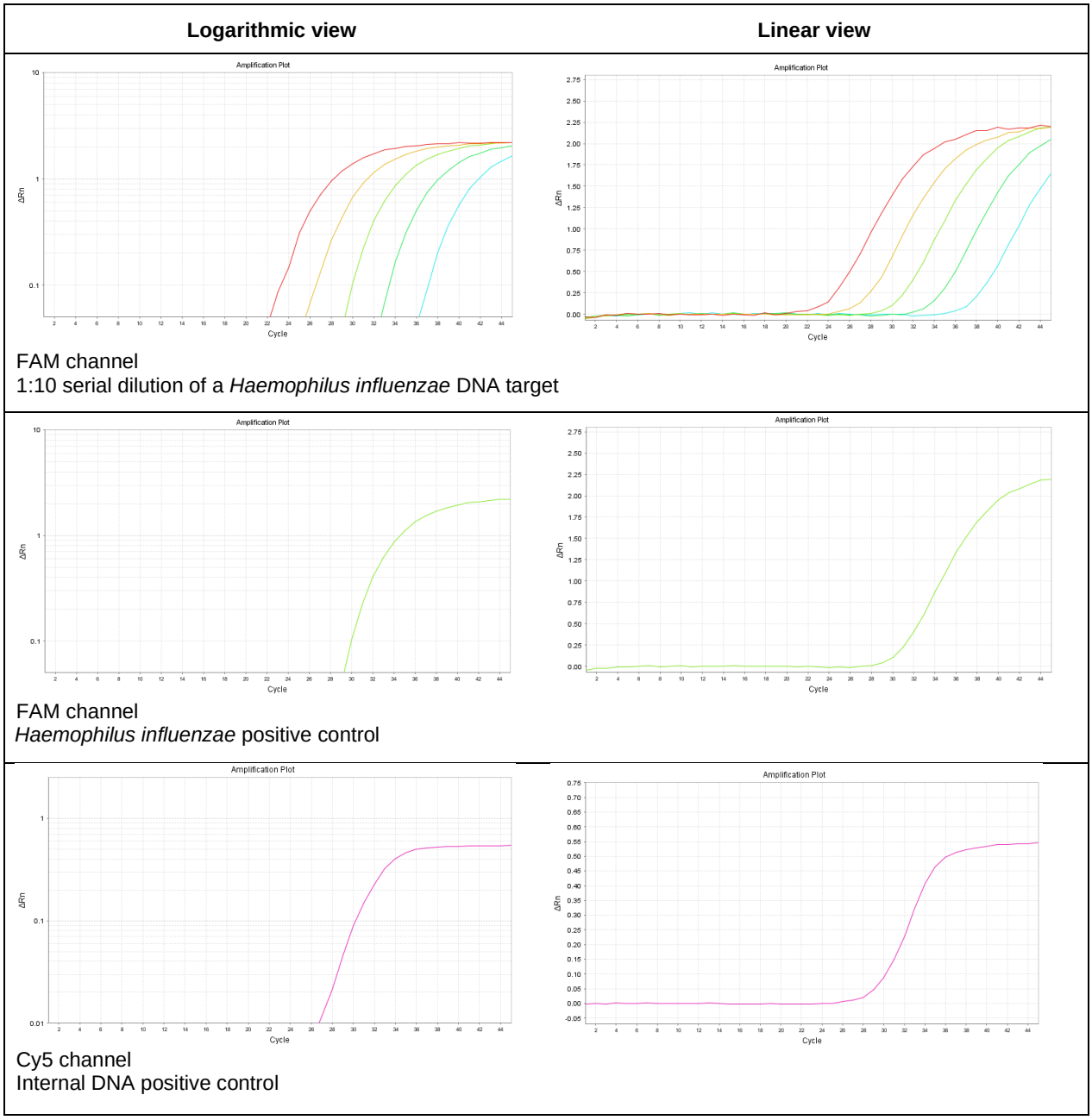

Figure 1 Performance of BactoReal® Kit *Haemophilus influenzae*

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