BactoReal® Kit *Legionella pneumophila*

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *Legionella pneumophila*			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUB01153	50	FAM channel	Cy5 channel

Kit contents:

- Assay for detection of *Legionella pneumophila* 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 *Legionella pneumophila*
- Nuclease-free water

Pathogen information: *Legionellae* are gram-negative bacteria frequently found in aquatic systems and living within amoebae. The most common source of infection that have been found to transmit *Legionella* are domestic hot-water systems heated to less than 70°C, air conditioners and cooling towers. *Legionella* are transmitted to the lungs of human beings through aerosolized water. No person-to-person transmission has been yet observed. Many different species of *Legionella* (at least 40) have been discovered, and more than 66 serogroups of *Legionella* have been identified. Many thereof are considered to be potentially pathogenic for humans. Infection by *Legionella pneumophila* results mainly in Legionnaire's disease. However, some strains manifest a disorder called Pontiac Fever, which is a milder infection than the Legionnaire's disease and can lead to an atypical pneumonia after 2 – 10 days.

Intended purpose: BactoReal® Kit *Legionella pneumophila* is a non-automated CE-certified IVD real-time PCR test for the qualitative detection of DNA (23S-5S rDNA intergenic spacer region) of *L. pneumophila.* This test detects all known serogroups and subspecies of *L. pneumophila.* Other *Legionella* species occasionally causing pneumonia are not detected.
Proper specimens are DNA extracts isolated from samples of the human respiratory tract (BAL, sputum, throat swabs).
This test is suitable for patients of all ages with suspected infection with *L. pneumophila* (causative agent of legionellosis) 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. pneumophila* specific DNA. An internal DNA positive control (IPC) is detected in Cy5 channel 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 was also tested with a LightCycler® 480 II (Roche Diagnostics) and Mx3005P® (Agilent).

ingenetix GmbH
Haidingergasse 1, 1030 Vienna, Austria
T +43(0)1 36 198 01
office@ingenetix.com
www.ingenetix.com

The test 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 *Legionella pneumophila* detects at least 10 target copies/reaction. The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 15 copies/reaction. This kit is specific for *Legionella pneumophila*. Validation was performed with bacterial isolates and with 80 clinical samples.

Table 1 Results of clinical validation

	Value	95% CI
Sensitivity	100%	91.96% to 100%
Specificity	100%	90.26% to 100%
NPV	100%	90.26% to 100%
PPV	100%	91.96% to 100%
Prevalence	55 %	

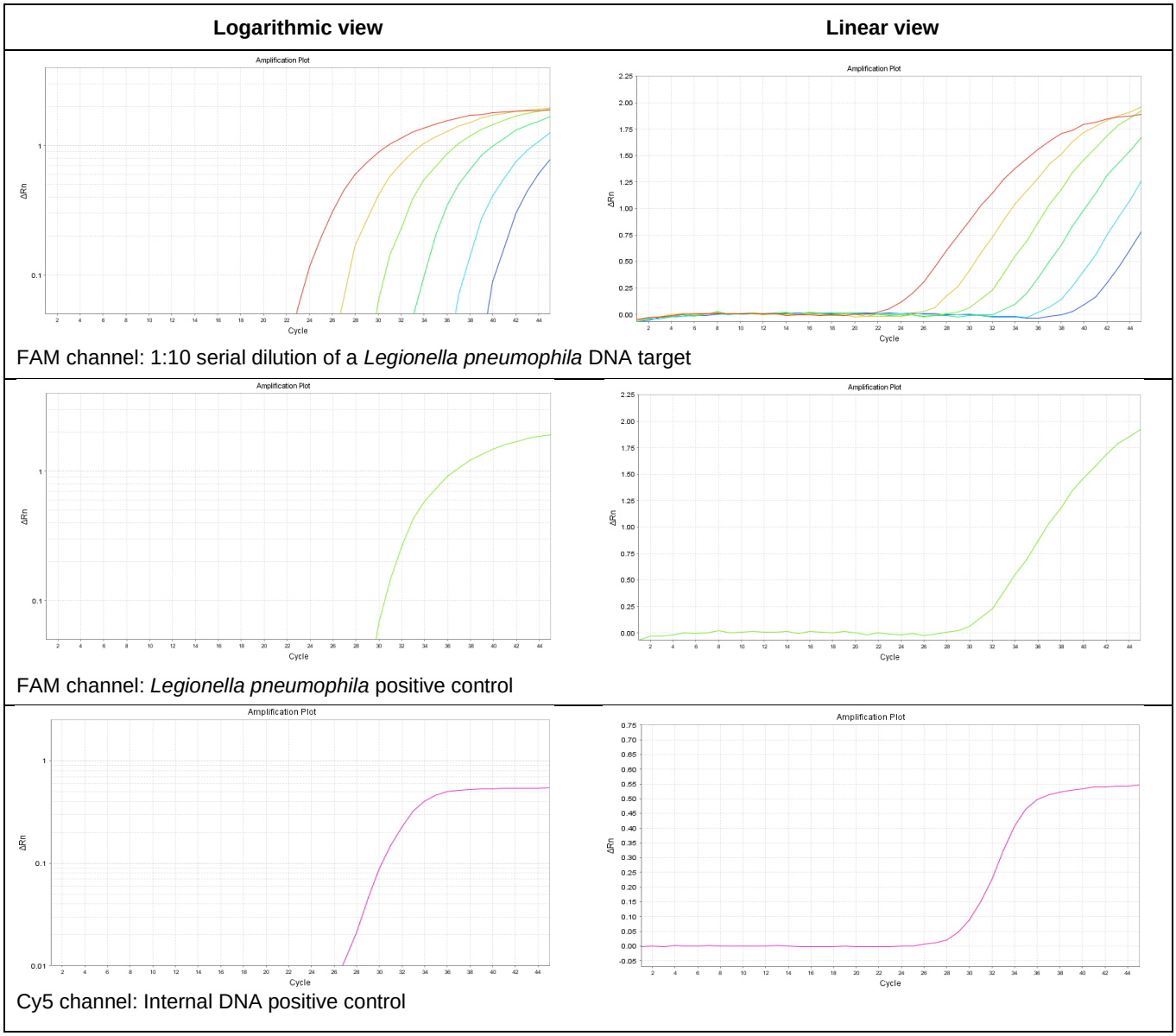

Figure 1 Performance of BactoReal® Kit *Legionella pneumophila*