# Xpert qDetect *L.pneumophila*

#GDK18.0100 (100 rxns)
(FOR RESEARCH ONLY)

**Product:** Nearly half of all known *Legionella* species are associated with human disease, such as Pontiac Fever and Legionnaires´ disease (LD). LD is the most severe form of infection, with clinical symptoms including pneumonia and a fatality rate of around 10%. The vast majority of the isolates associated with LD in previously healthy individuals are *L. pneumophila*[1][2].

*L.pneumophila* is very common to the aqueous environment with amoebae being among their natural hosts. *L.pneumophila* infection occurs via inhalation of water droplets from a contaminated source that has allowed the organism to grow and spread. *L.pneumophila* can often been found, in high concentration, in a wide variety of environmental water sources such as ponds, hot-water tanks, hot tubs, showerheads, whirlpools, spas, air-conditioning, cooling towers, and public fountains, if those water systems are not managed correctly (e.g. in case of stagnation and warm temperatures).

Traditional microbiological detection and confirmation methods are long established and well developed but are laborious and typically require many days in order to obtain results, as *Legionella* are slow growing bacteria. This qPCR Detection Kit provides a fast, sensitive and reliable method for the detection of *L.pneumophila* based on qPCR using precisely designed specific primers and FAM-labeled Taqman® probe, requiring only a few hours. This immense time reduction allows taking appropriate action, if needed, much sooner.

This kit is compatible with instruments equipped with FAM and ROX channels. The detection limit is approximately 5pg of *L.pneumophila* DNA allowing the detection of as little $10^3$ cells/L of water, with a specificity of 100%.

**Applications:** Detection of *L.pneumophila* in water (following concentration by membrane filtration and subsequent DNA extraction (see: "prior to use")).

**Contents:** The qPCR Detection Kit (#GDK18.0100) for *L.pneumophila* contains sufficient reagents for 100 qPCR reactions.

| Component | GDK18.0100 |
|---|---|
| Lp Mix A | 2x 840 µl |
| Lp Mix B | 210 µl |
| Positive Control (Lp+) | 70 µl |
| Negative Control (Lp-) | 70 µl |

**Note:** This product does not include reagents and other materials required for DNA extraction.

**Properties:**
Fast, Easy and Reliable
Low limit of Detection
Validated according to ISO 22118:2011
100% Specificity
Compatible with instruments equipped with **FAM** and **ROX** channels

**Storage:** -20ºC and protected from light for at least 1 year. Minimize repeated freeze/thawing, consider preparation of aliquots.

## Prior to use:

**Safety Considerations**
*Legionella* species are pathogenic to humans; hence isolation and identification must be carried out by trained laboratory personnel, in a properly equipped laboratory. Most activities can be carried out in a Containment Level 2 laboratory, however, in some cases the nature of the work with *L.pneumophila* may dictate full Containment Level 3 conditions. Care must be taken in the sterilization and disposal of all test materials. All procedures must be performed in the designated area of the laboratory. Disposable gloves must be worn during all procedures. All relevant National and Local Regulations must be met.

**Laboratory Set-Up**
In order to prevent erroneous results due to contamination and/or degradation of DNA, it is strongly recommended to have dedicated materials, equipment and area for the DNA extraction, separated from the preparation of PCR procedures. Workflow in the laboratory should be unidirectional, starting with DNA isolation and moving to preparation of PCR amplification and finally to (q)PCR and Detection. Moreover, all molecular biology procedures, including DNA extraction, require qualified personnel to prevent risk of erroneous results and/or inadequate interpretation of results.

**Bacterial Enrichment**
This kit is for the detection of DNA from *L.pneumophila* present in total DNA previously purified from a concentrate obtained by membrane filtration. Representative water samples (optionally including sediment or biofilms) should be prepared conform standard procedures and under GLP conditions [3]. Water samples should be concentrated according to ISO 11731:2017 [4].

**DNA extraction**
Resuspend the filtrate in 1 ml of water and centrifuge at 10,000g-12,000g for 5 minutes. Discard the supernatant and add 200µl of Lysis Buffer (e.g. 10mM Tris-HCl pH 8.0 containing 1mM EDTA and 0.1% Tween®20) to the pellet. Resuspend by vortexing or pipetting up and down in the tube. Incubate at 95°C-100°C for 10-15 minutes using a water bath or heating block. Centrifuge 10,000g-12,000g for 2 minutes and transfer the cleared supernatant to a new microtube. Use 2µl of the supernatant as template DNA sample in the qDetect protocol.

Alternatively, DNA is extracted from the filtrate using an appropriate kit for the extraction of DNA from Gram-negative bacteria (not included) using the manufacturer´s instructions. The obtained highly-purified DNA, free of possible PCR inhibitors, can be used immediately or stored at -20°C for up to several years for future analysis. Use 2µl of the eluate as template DNA sample in the qDetect protocol.

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**References**


1. Public Health England (2015) Identification of *Legionella* species. UK standards for Microbiology Investigations. ID 18 Issue 3

2. Benitez and Winchell (2013) Clinical application of a multiplex real-time PCR assay for simultaneous detection of *Legionella* species, *Legionella pneumophila*, and *Legionella pneumophila* serogroup 1. J. Clin. Microb. **51**:348-351

3. Public Health England (2016) Preparation of samples and dilutions, plating and sub-culture. Microbiology Services. Food, Water & Environmental Microbiology Standard Method FNES26 (F2); Version 2.

4. Water quality – Enumeration of *Legionella* (ISO 11731:2017)

## qDetect - Basic Protocol

Unambiguous detection of *L.pneumophila.* (and of the internal control) requires a suitable calibration of both FAM and ROX channel. Please refer to the manufacturer´s instruction of the real time PCR cycler.

1. **Mix for each qPCR reaction**:

| Component | Volume |
|---|---|
| Lp Mix A | 16 µl |
| Lp Mix B | 2 µl |

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control, and a positive control, by mixing all components (N+2), except template DNA (nor control DNA), dividing the mixture equally into each PCR tube (18 µl each), briefly spin tubes (or tap down) and then add 2 µl template DNA or control DNA directly in the mixture.

2. **Set-up qPCR cycling**:

| N° cycles | Temp | Time | Acquisition |
|---|---|---|---|
| 1x | 50°C | 2 min | No |
| 1x | 95°C | 5 min | No |
| 40x | 95°C | 30 sec | No |
| | 56°C | 30 sec | Yes |
| | 72°C | 30 sec | No |

After an initial cycle of 2 min at 50°C and 5 min at 95°C (Enzyme activation and denaturation of template), cycle 40 times for 30 seconds at 95°C, 30 seconds at 56°C and 30 seconds at 72°C. Acquire data for the detection of *L.pneumophila* on the **FAM** channel. Probe to detect specific amplification of the internal control, which is included in Lp Mix A and which is amplified simultaneously with the target DNA, should be detected in the **ROX** channel.

## Results

**Controls**
In order to validate the assay, controls must have the following results. If the signal of one of the controls does not match, the whole experiment, including all samples, must be repeated.

| Control | FAM channel | ROX channel |
|---|---|---|
| Negative Control | Ct=N/A | positive |
| Positive Control | positive | unimportant* |

N/A = Not applicable (signal below threshold).
*unimportant: is expected to be positive, however, if negative but other controls match expected results, this makes no difference

**Samples**
For each sample, there are 4 possible outcomes, as summarized in the table below.

| FAM channel | ROX channel | Result |
|---|---|---|
| positive | positive | positive |
| positive | Ct=N/A | positive |
| Ct=N/A | positive | negative |
| Ct=N/A | Ct=N/A | inhibition* |

*) in case both *L.pneumophila* (FAM channel) and Internal Control (ROX channel) have signals below threshold, but all the controls resulted in signals as expected, the sample must be retested, as the qPCR reaction was inhibited. Inhibition often is the result of a too high DNA concentration and therefore it is recommended that retesting should be carried out with a 10-fold dilution of the original DNA sample.