

Legionella pneumophila Quantitative qPCR Standard

Catalogue number: MD07251

General Description

The Legionella pneumophila Quantitative qPCR Standard is a highly specific product designed for quantitative real-time PCR (qPCR) applications, and it is explicitly tailored for use in conjunction with the Legionella pneumophila qPCR Kit (MD05281). The essence of this product resides in its provision of a DNA fragment from *Legionella pneumophila*, meticulously measured to be at to 2×10^6 copies/ μL . When a quantitative analysis of *L. pneumophila* samples is required, a standard curve should be generated using Legionella pneumophila Quantitative qPCR Standard and analysed in a qPCR run. Serial dilutions of the Quantitative Standard must be meticulously prepared with a defined genome copies per microliter (copies/ μL). The data obtained from these serial dilutions is then plotted to form a standard curve, which serves as a reference for determining the concentration of *L. pneumophila* in the test sample. qPCR method serves as a gold standard in molecular diagnostics due to its exceptional accuracy, specificity, and sensitivity. Please be reminded that this kit is designed for use by trained laboratory personnel and should be used strictly in accordance with the accompanying Instructions for Use (IFU) to ensure accurate and reliable results. Execution of the outlined procedures is crucial in minimizing potential errors and maintaining the integrity of the testing process.

Kit composition

The kit provides a comprehensive set of reagents, allowing for the generation of multiple quantitative Standard Curves of significance:

Component	Tubes	Volume	Cap color
L. pneumophila Quantitative Standard (2×10^6 copies/ μL)	1	100 μL	Red
Positive Control Dilution Buffer	2	1500 μL	Green

Storage Conditions and Kit Stability

This Molecular Diagnostic Quantitative qPCR Standard is shipped at room temperature (RT). Although kit components are stable at room temperature, they should be immediately stored at -85°C to -15°C upon arrival. Also proceed with the following recommendations:

- Minimise the number of freeze-thaw cycles by storing kit components in working aliquots.
- If the package that protects the kit arrived damaged, please contact NZYTech.
- Beware of the expiry date indicated on the packaging. NZYTech does not recommend using the kit after the expiry date. On this date, the Quantitative qPCR Standard must be discarded.

Required Reagents and Equipment

- This Quantitative qPCR Standard is to be used solely with NZYTech's Legionella pneumophila qPCR Kit (MD05281).
- Commercially available Real-time PCR Instruments that detect FAM™ and HEX™/JOE™/VIC™ fluorescent dyes (emission wavelengths of 520 and 556/555/554 nm, respectively).
- RNase/DNase-free qPCR plasticware: PCR tubes, strips, caps, 96-well plates, and adhesive films (also available at NZYTech).
- Pipettes and filter tips (RNase/DNase-free).
- Disposable gloves.
- Vortex and centrifuge.

Standard Curve Preparation and Real-time PCR Detection Protocol

1. Sample Processing and Nucleic Acids Extraction

Initiate sample preparation as delineated in the IFU associated with the Legionella pneumophila qPCR Kit (MD05281). Please note that for quantitative applications, it is advised against the pooling of samples, as this could introduce variability and affect the accuracy of quantification. All dilutions conducted with the sample need careful consideration, particularly if the end goal is to extrapolate the initial copy number quantification based on the starting volume or mass of the sample. This diligence ensures a reliable and accurate representation of Acanthamoeba spp. concentration in the original specimen, crucial for the validity of the subsequent qPCR analyses.

2. Reaction Setup, Quantitative Standard dilution and Amplification

Follow the IFU associated with the Legionella pneumophila qPCR Kit (MD05281) while ensuring to incorporate the reactions for additional seven serial dilutions of the L. pneumophila Quantitative Standard into the assay. To prepare the nucleic acids for the Quantitative Standard curve, proceed as follows:

- 2.1 Pipette 90 μL of Positive Control Dilution Buffer into 6 tubes labelled 2 to 7.

- 2.2 Pipette 10 μL of *L. pneumophila* Quantitative Standard (2×10^6 copies/ μL) into tube 2.
- 2.3 Vortex thoroughly and spin.
- 2.4 Change tip and pipette 10 μL from tube 2 into tube 3.
- 2.5 Vortex thoroughly and spin.
- 2.6 Repeat steps 2.2 (from sequential tubes) to 2.5. to complete the dilution series. The final Standard Curves have the following copy number per reaction:

Standard Curve	Copy number (per μL)	Copy number per reaction (per 5 μL)
Tube 1 - <i>L. pneumophila</i> Quantitative Standard	2×10^6	10^7
Tube 2	2×10^5	10^6
Tube 3	2×10^4	10^5
Tube 4	2×10^3	10^4
Tube 5	2×10^2	10^3
Tube 6	20	10^2
Tube 7	2	10

- 2.7 Pipette 5 μL of each dilution into each well containing 15 μL of reaction mix, according to your experimental plate set-up (prepare reaction mix according with *Legionella pneumophila* qPCR Kit (MD05281) IFU). The final volume in each well should be 20 μL . To minimize the risk of potential cross-contaminations, Quantitative Standards should be added to the corresponding qPCR well plate after all previous samples and control reactions are set up.
- 2.8 Proceed with qPCR as recommended in the IFU of the respective kit.

3. Data Analysis - Validity and Quantitative Result

Prior to proceeding with the analysis of sample results, we strongly advise validating the integrity of the real-time PCR test. This can be achieved by ensuring that both Positive and Negative controls, along with the Internal Extraction Controls (IEC), have performed as anticipated, as outlined in the kit's Instructions for Use (IFU).

The processing of the amplification data can be conducted automatically, utilizing the dedicated software tool that accompanies your Real-Time PCR thermal cycler. However, should you opt for a more manual approach, the threshold should intersect with the FAM curve in its linear phase of increase (using the log scaling of the y-axis). This setting of the threshold thereby dictates the Crossing Point (Cp) or Cycle threshold (Ct), which is inversely correlated with the initial concentration of target gene copies' present in the Real-Time PCR reaction.

It is important to note that only curves demonstrating a typical exponential amplification should be considered positive. These are characterized by an initial flat baseline, followed by a clear exponential increase in fluorescence, potentially culminating in a plateau phase when viewed with a logarithmic y-axis scaling.

In the subsequent figure, you can find a typical Standard Curve that has been generated using this kit:

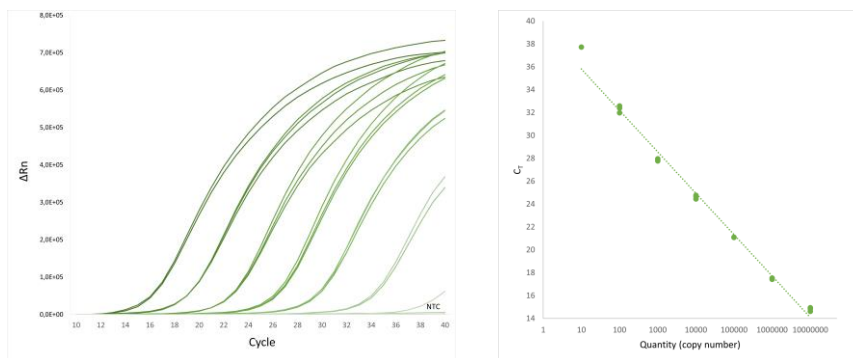


Figure 1: Amplification plot (cycle number versus fluorescence - ΔRn) (A) and the respective standard curve (copy number versus Ct) (B) of 1:10 serial dilutions of *L. pneumophila* Quantitative Standard, from 1×10^7 copies to 10 copies per reaction detected through the FAM channel using *Legionella pneumophila* qPCR Kit (MD05281). NTC, No Template Control (negative control).

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