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Legionella Quantification LyoKit
Ready Reference Guide

Revision A, November 2023

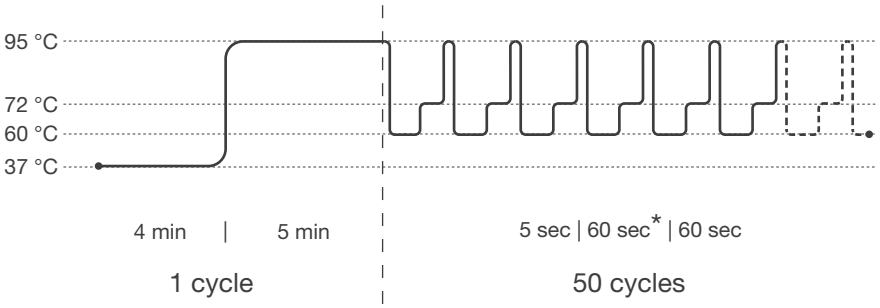
Product No. KIT230119 (LP), KIT230120 (RP)

PCR kit for the quantitative detection of *Legionella* DNA using real-time PCR instruments. Before starting, it is strongly recommended to read the entire product manual available on our website.

PROGRAM SETUP

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

- FAM (*Legionella pneumophila*), HEX (*Legionella* spp.), ROX (*Legionella pneumophila* serogroup 1) and Cy5 (Internal Control).



Pre-incubation: 1 cycle
Step 1: 37 °C for 4 min
Step 2: 95 °C for 5 min

Amplification 50: cycles
Step 1 : 95 °C for 5 sec
Step 2*: 60 °C for 60 sec
Step 3 : 72 °C for 60 sec

* Fluorescence detection

For some real-time PCR instruments the probe quencher as well as the use of a passive reference dye must be specified. This kit contains probes with a non-fluorescent "dark" quencher and no passive reference dyes.

For colony confirmation, a shortened PCR protocol is available. Please refer to the manual.

PREPARATION OF STANDARD CURVE

Use Quantification Standard A, B, C and D to prepare a standard curve (see table below).

Briefly vortex and centrifuge Quantification Standards before use.

For each Quantification Standard, pipet 25 µL into the designated wells in duplicates.

A typical experiment consists of 9 wells needed for standards (duplicates) and a negative control, plus n wells (n = number of samples).

Quantification Standard	Cap Color	Concentration to Be Entered as Standard (GU/reactions)		
		FAM Channel	HEX Channel	ROX Channel
A	purple	25,000	25,000	25,000
B	red	2,500	2,500	2,500
C	yellow	250	250	250
D	white	25	25	25

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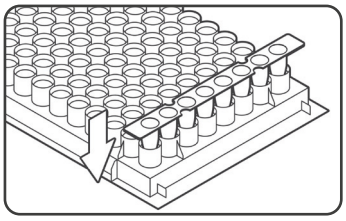
PREPARATION OF THE PCR MIX

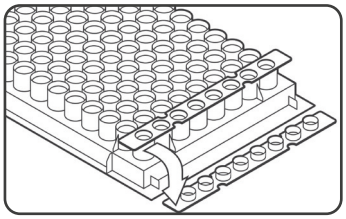
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.
For data interpretation and calculation, refer to the complete product manual.



1. PLACE STRIPS IN RACK

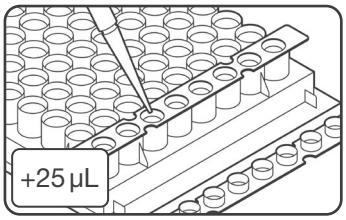
Take needed number of PCR tube strips out of aluminum bag.
Important: Seal bag tightly afterwards. Place strips in a suitable PCR tube rack.

If needed, gently tap the tubes to move the lyophilized pellets to the bottom of each tube.



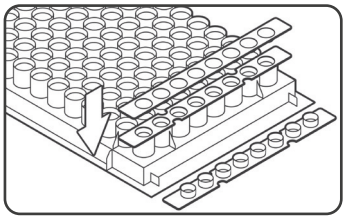
2. DECAP

Immediately before filling, carefully open strips and discard caps.
Do not leave open longer than necessary.



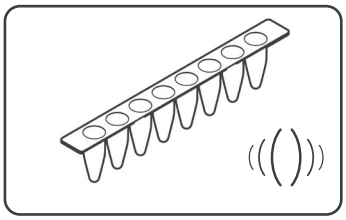
3. ADD SAMPLES AND CONTROLS

Pipet 25 µL of samples, standards and Negative Control (colorless cap) into respective wells. If using less volume, add PCR-grade H_2O to reach 25 µL.



4. SEAL

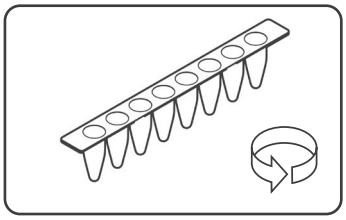
Tightly seal the tubes with the provided 8-cap strips.



5. MIX

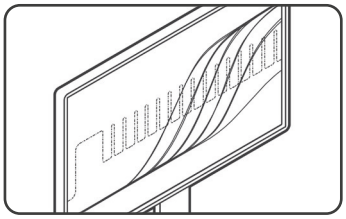
Resuspend pellet by mixing thoroughly.

Alternatively resuspend pellet by pipetting up and down multiple times in step 3.



6. CENTRIFUGE

Briefly spin strips, e.g., 5 seconds at 500 - 1,000 x g, in a suitable centrifuge.



7. START REAL-TIME PCR RUN

Cycle samples as described above.

Place tubes in a vertical, balanced order into the cycler, e.g., two strips can be placed in the first and last columns.

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Legionella
Quantification LyoKit

KIT230119 / 20
Kit for 96 reactions
Store kit at 2 to 8 °C

FOR IN VITRO USE ONLY
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