mericon® Quant Legionella Detection on the Rotor-Gene® Q

Multiplex PCR Master Mix and ROX dye should be stored immediately at –20°C upon receipt, in a constant-temperature freezer. All remaining not reconstituted kit components should be stored at 2–8°C and protected from light. Reconstituted reagents of *mericon* Quant Legionella Detection Assays should be dispensed into aliquots to avoid more than 5 freeze–thaw cycles, and stored at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

Further information

- *mericon* Quant Legionella Detection Handbook: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- The protocol below is for use with the Rotor-Gene Q. For other cyclers, please see the mericon *Quant Legionella Detection Handbook*.
- Use gloves as well as sterile pipet tips with filters.
- Ensure that the Quantification Control and at least one negative control are included per PCR run.
- PCR tubes should be kept on ice until they are placed in the thermal cycler.
- Prepare the *mericon* Quant Legionella Assay (tube with yellow lid), dried Quantification Control DNA (tube with red lid), and Standard DNA (tube with green lid). See the mericon *Quant Legionella Detection Handbook* for more information.
- Before each use, all reagents must be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

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1. Set up the sample and control reactions according to Table 1. Keep all samples and reaction tubes on ice during setup. Place the desired number of PCR tubes or strips into the cooled Loading Block for the Rotor-Gene Q.

Table 1. Setup of Standard, Quantification Control, and sample reactions

Component	Standard dilutions	Quantification control	Samples	Negative control
Reconstituted *mericon* Assay	10 µl	10 µl	10 µl	10 µl
Respective Standard Dilution	10 µl	–	–	–
Quantification Control DNA	–	10 µl	–	–
Sample DNA	–	–	10 µl	–
QuantiTect® Nucleic Acid Dilution Buffer or RNase-free water	–	–	–	10 µl
Total volume	**20 µl**	**20 µl**	**20 µl**	**20 µl**

2. Close the PCR tubes or strips and place them in the reaction chamber of the Rotor-Gene Q. Ensure that the locking ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.
3. Program the Rotor-Gene Q according to Table 2.

Table 2. Cycling protocol

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA Polymerase
3 step cycling			
Denaturation	15 s	95°C	Data collection at 60°C
Annealing	15 s	60°C	
Extension	10 s	72°C	
Number of cycles	40		
Detection	**Reporter**	**Excitation/emission**	**Channel**
Target	FAM™	495/520 nm	Green
Internal control	MAX™	524/557 nm	Yellow

4. Start the PCR run.
5. Analyze the results; see next page.

Interpretation of results

Determining the presence or absence of pathogen DNA is carried out based on the amplification of the target sequence and is visualized in real time on the amplification plot generated by the application software of the real-time PCR instrument used. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold.

Table 3. Possible outcomes

Amplification of internal control	Amplification of sample	Result
+	+	Sample is positive
+	–	Sample is negative
–	–	PCR failed

Partial inhibition of the PCR due to the presence of detectable but tolerable concentrations of inhibitors in the samples is typically indicated by a shift of the internal control to higher cycle thresholds (C_T) values. As a guideline, the uninhibited internal control should give a cycle threshold value ranging between 28 and 32. A cycle threshold value above 33 indicates inhibition.

In the event of PCR inhibition, dilute the extracted samples 1:10 with RNase-free water and repeat the test.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.