August 2012

mericon® Quant Legionella Detection Handbook

For detection and quantification of *Legionella* spp. DNA in water samples using real-time PCR

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Contents

Kit Contents

***mericon* Quant Legionella spp Kit** ***mericon* Quant L. pneumophila Kit** See Table 1 (page 5) for more *mericon* kits		**(96)**
Number of reactions		**96**
Yellow	*mericon* Assay*	1 x 96 reactions
Red	Quant Control DNA	10 reactions
Green	Standard DNA	3 quantifications
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml
	RNase-Free Water	1.9 ml
Blue	Multiplex PCR Master Mix†	1040 µl
	50x ROX Dye Solution	210 µl
	Quick Start Protocol	1

* Contains target-specific primers and probes, as well as the internal control (IC)

† Contains HotStarTaq® *Plus* DNA Polymerase, dedicated multiplex real-time PCR buffer, and dNTP mix (dATP, dCTP, dGTP, dTTP).

Table 1. *mericon* Pathogen Detection Assays

Product name	Catalog no. (24)	Catalog no. (96)
mericon Quant Legionella spp Kit* For detection and quantification of *Legionella* subspecies	–	290085
mericon Quant L. pneumophila Kit* For detection and quantification of *Legionella pneumophila*	–	290095
mericon Salmonella spp Kit† For general detection of *Salmonella* subspecies	290013	290015
mericon Listeria spp Kit For general detection of *Listeria* subspecies	290123	290125
mericon L. monocytogenes Kit For specific detection of *Listeria monocytogenes*	290023	290025
mericon Campylobacter spp Kit For general detection of *Campylobacter* subspecies	290033	290035
mericon Campylobacter triple Kit For specific detection of *C. jejuni*, *C. lari*, and *C. coli*	290043	290045
mericon VTEC stx1/2 Kit For specific detection of verotoxin-producing *E. coli* (shigatoxin 1 and 2)	290053	290055
mericon Cronobacter spp Kit For general detection of *Cronobacter* subspecies (formerly *Enterobacter*)	290063	290065
mericon S. aureus Kit For specific detection of *Staphylococcus aureus*	290073	290075
mericon Vibrio triple Kit For specific detection of Vibrio vulnificus, Vibrio parahaemolyticus, and Vibrio cholerae	290133	290135
mericon Shigella spp Kit For general detection of *Shigella* subspecies	290103	290105
mericon Y. enterocolitica Kit For specific detection of *Yersinia enterocolitica*	290113	290115

* For more detailed information, see "Assay-specific information", page 10.

† AOAC-PTM-validated kit.

Storage

The *mericon* Quant Legionella spp. Kit and *mericon* Quant L. pneumophila kits are shipped on dry ice. 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. Stored under these conditions and handled correctly, assay performance remains unaffected until the date of expiration printed on the quality control label inside the kit box or envelope.

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.

Intended Use

mericon Quant Legionella Detection Assays are intended for molecular biology applications in food, water, animal feed, and pharmaceutical product testing. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

mericon Quant Legionella Detection Assays are a ready-to-use system for the detection of specific DNA fragments from *Legionella* spp. and/or *L. pneumophila* in water, food, animal feed, and pharmaceutical products using real-time polymerase chain reaction (PCR). These assays perform optimally on the Rotor-Gene® Q but have also been validated for block thermal cyclers. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology including HotStarTaq *Plus* DNA Polymerase, patented multiplex PCR technology such as Factor MP, and fast-cycling technology including Q-Bond®. Multiplex PCR Master Mix is also highly tolerant to PCR inhibitors. The analytical procedure of these protocols allows the user to perform analysis in accordance with local official requirements.

Each *mericon* Assay is an optimized mixture of PCR primer sets for a pathogen-specific target sequence and an internal control (IC), plus probes labeled with two distinct fluorescent dyes (the test sample is detected with FAM® reporter (495/520 nm), and the internal control is detected with MAX™ NHS Ester reporter (MAX; 524/557 nm). In addition, each kit includes positive control DNA and all reagents necessary to perform the analysis.

Principle

Pathogen detection by PCR is based on the amplification of a specific region of the relevant pathogen genome. In real-time PCR, the amplified product is detected via target-specific fluorescent probes that bind to the amplified product. Accumulation of PCR product results in increased fluorescent signal from the bound probes. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows the detection of the accumulating PCR product without having to re-open the reaction tubes after the PCR run.

The probes of *mericon* PCR Assays are sequence-specific oligonucleotides with a fluorophore and a quencher moiety attached. The fluorophore is at the 5' end of the probe, and the quencher moiety is located at the 3' end. If the target DNA sequence is present, the probe is cleaved by the 5'→3' exonuclease activity of HotStarTaq *Plus* DNA Polymerase during the extension phase of PCR. This separates the fluorophore and the quencher moiety resulting in a detectable fluorescence that is proportional to the amount of accumulated PCR product.

Quantification of the target *Legionella* species is carried out based on a standard curve covering a concentration range of 25,000–25 copies per PCR reaction. The standard curve is prepared from purified *Legionella* Standard DNA, which is provided in both *mericon* Quant Legionella Detection kits at an accurately defined concentration.

The PCR primer set for each assay is highly specific and targets a unique and conserved DNA region of the tested pathogen genome that is verified bioinformatically and experimentally. Cross-reactivity has been bioinformatically investigated and thoroughly tested with a panel of selected targets for each *mericon* PCR Assay. Each assay can detect down to 10 target copies in a reaction.

Dedicated *mericon* sample preparation solutions are available from QIAGEN for a broad range of starting materials. These solutions were developed to complement *mericon* PCR Assays, and provide a complete and efficient workflow for food safety testing.

HotStarTaq *Plus* DNA Polymerase

HotStarTaq *Plus* DNA Polymerase is a modified form of QIAGEN® *Taq* DNA Polymerase. It is provided in an inactive state and has no enzymatic activity at ambient temperature, thereby preventing formation of misprimed products and primer–dimers during reaction setup and the first denaturation step. Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity and accurate quantification. The enzyme is activated first at the start of a reaction by a 5-minute, 95°C incubation step, which enables reactions to be set up rapidly and conveniently at room temperature. In addition, the concentration of the polymerase in the master mix is optimized to allow short extension times in the combined annealing/extension step of each PCR cycle.

Multiplex PCR Master Mix

The Multiplex PCR Master Mix is specifically developed for fast-cycling, multiplex, real-time PCR using sequence-specific probes. A novel additive in the buffer, Q-Bond, allows short cycling times on standard cyclers and on fast cyclers with rapid ramping rates. Q-Bond increases the affinity of HotStarTaq *Plus* DNA Polymerase for short single-stranded DNA, reducing the time required for primer/probe annealing to a few seconds. The buffer also contains Factor MP, which facilitates multiplex PCR. This synthetic factor increases the local concentration of primers and probes at the DNA template and stabilizes specifically bound primers and probes, allowing efficient annealing and extension. In addition, the Multiplex PCR Buffer is carefully formulated to be highly tolerant to inhibitors commonly present in food.

QuantiTect Nucleic Acid Dilution Buffer

QuantiTect Nucleic Acid Dilution Buffer is an optimized solution to dilute the nucleic acids used as positive controls for *mericon* PCR Assays. The buffer stabilizes DNA controls during dilution and reaction setup and prevents loss of nucleic acids on plastic surfaces, such as tubes or pipet tips. The buffer is ready to use and is free of DNases. Proper use of the buffer enables safe and accurate dilution of the small amounts of nucleic acids typically used as controls for analysis of nucleic acids. Aliquots of diluted positive control can be stored in QuantiTect Nucleic Acid Dilution Buffer at –15 to –30°C for up to 6 months. Repeated freezing and thawing should be avoided.

ROX Dye Solution, 50x

For certain real-time cyclers, the presence of ROX passive reference dye in real-time PCR compensates for non-PCR–related variations in fluorescence detection. The use of ROX dye is necessary for most instruments from Applied Biosystems® and is optional for Stratagene® cyclers from Agilent. ROX dye is not necessary for the Rotor-Gene Q, LightCycler® systems from Roche®, SmartCycler® instruments from Cepheid, and Bio-Rad instruments. ROX dye is provided in a 50x solution suitable for PCR instruments requiring a low ROX dye concentration, such as the Applied Biosystems models 7500 and 7500 Fast. Instructions for using the dye are provided in the protocol "Detection of Pathogen DNA by Real-time PCR with ROX" on page 20.

Primer/probe mix with internal control

Each *mericon* PCR Assay includes rigorously designed primers and probes in a carefully balanced mix that amplify a target sequence and an internal control (IC) with high specificity. This internal control provides information regarding the presence of inhibitors in tested samples and the overall success of the PCR. MAX NHS Ester is employed as the reporter dye for the internal control. With excitation/emission maxima of 524/557 nm and a non-fluorescent quencher (Iowa Black®), MAX dye has a spectral profile comparable to HEX®, JOE®, or VIC®, and can be used with most real-time cyclers.

Assay-specific information

mericon Quant Legionella spp Kit

The *mericon* Quant Legionella spp Kit is designed for the detection and quantification of several *Legionella* subspecies in water after concentration, e.g., by filtration.

Limit of detection

The *mericon* Quant Legionella spp Kit can detect down to 10 copies of *Legionella* DNA in a reaction.

Specificity

The *mericon* Quant Legionella spp Kit exhibits high specificity for pathogens of the genus *Legionella*. No cross-reactivity was observed with other pathogens (Table 2, page 11) using 2500 copies of tested DNA.

mericon Quant L. pneumophila Kit

The *mericon* Quant L. pneumophila Kit is designed for the detection and quantification of *Legionella pneumophila* in water after concentration, e.g., by filtration.

Limit of detection

The *mericon* Quant L. pneumophila Kit can detect down to 10 copies of *Legionella pneumophila* DNA in a reaction.

Specificity

The *mericon* Quant L. pneumophila Kit exhibits high specificity for *Legionella pneumophila*. No cross-reactivity was observed with other pathogens (Table 3, page 12) using 2500 copies of tested DNA.

Table 2. Results from cross-reactivity experiments*

Pathogen	Result	Pathogen	Result
Legionella pneumophila	+	*Legionella erythra*	+
Legionella adelaidensis	+	*Legionella feeleii*	+
Legionella impletisoli	+	*Legionella jordanis*	+
Legionella israelensis	+	*Legionella tucsonensis*	+
Legionella worsleiensis	+	*Legionella beliardensis*	+
Legionella spiritensis	+	*Legionella lansingensis*	+
Legionella brunensis	+	*Legionella drozanskii*	+
Legionella geestiana	+	*Legionella nautarum*	+
Legionella santicruci	+	*Legionella shakespeare*	+
Salmonella abony	–	*Bacillus cereus*	–
Clostridium perfringens	–	*Campylobacter jejuni*	–
Escherichia coli	–	*Yersinia enterocolitica*	–
Staphylococcus aureus	–	*Listeria monocytogenes*	–
Shigella flexneri	–	*Cronobacter sakazakii*	–

* Cross-reactivity experiments are ongoing. For up-to-date information, visit http://www.qiagen.com/mericonPathogens.

Table 3. Results from cross-reactivity experiments*

Pathogen	Result	Pathogen	Result
Legionella pneumophila	+	*Legionella erythra*	–
Legionella adelaidensis	–	*Legionella feeleii*	–
Legionella impletisoli	–	*Legionella jordanis*	–
Legionella israelensis	–	*Legionella tucsonensis*	–
Legionella worsleiensis	–	*Legionella beliardensis*	–
Legionella spiritensis	–	*Legionella lansingensis*	–
Legionella brunensis	–	*Legionella drozanskii*	–
Legionella geestiana	–	*Legionella nautarum*	–
Legionella santicruci	–	*Legionella shakespeare*	–
Salmonella abony	–	*Bacillus cereus*	–
Clostridium perfringens	–	*Campylobacter jejuni*	–
Escherichia coli	–	*Yersinia enterocolitica*	–
Staphylococcus aureus	–	*Listeria monocytogenes*	–
Shigella flexneri	–	*Cronobacter sakazakii*	–

* Cross-reactivity experiments are ongoing. For up-to-date information, visit http://www.qiagen.com/mericonPathogens.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Nucleic acid isolation kit. For rapid, non-spin–column-based DNA extraction from concentrated pathogen solutions we recommend the *mericon* DNA Bacteria Kit for Gram-negative bacteria (cat. no. 69525) or the *mericon* DNA Bacteria Plus Kit (cat. no. 69534) for Gram-positive bacteria. For spin-column–based DNA extraction we recommend the QIAamp® UCP Pathogen Mini Kit
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Rotor-Gene Q or other real-time PCR instrument* with fluorescence detection for approximately 520 nm (FAM fluorescence) and approximately 560 nm (for yellow dyes). See Table 4 on page 15 for examples.
- PCR plastics for the thermal cycler to be used.

  For Rotor-Gene Q: Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106) or PCR Tubes, 0.2 ml, for use with 36-well rotor (cat. no. 981005 or 981008)†
- For Rotor-Gene Q: Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901, or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905
- Tube rack
- Microcentrifuge*
- Vortexer*
- Ice bucket with ice or cooled Loading Block

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. Use of yellow dye to detect the internal control of the *mericon* PCR Assays requires calibration on some instruments. See Table 4, page 15 for details.

Important Notes

General precautions

The user should always pay attention to the following:

- Use gloves as well as sterile pipet tips with filters.
- All materials and media possibly containing the tested pathogen should be autoclaved for 20 min at 120°C after use.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly.

Relevant assay controls

Internal control

Each vial of *mericon* Assay contains an internal control to detect possible PCR inhibition.

Standard dilutions

A Standard DNA is provided with both *mericon* Quant Legionella Detection kits to prepare a standard curve. It is provided to allow for 3 independent quantification experiments. The Standard DNA is diluted in defined steps and the resulting 4 Standard dilutions are integrated into the PCR reaction setup in duplicates. The applied Standard dilutions cover a copy number range of 25,000–25 copies in the final PCR reaction.

Quantification Control

The Quantification Control contains purified *Legionella* spp. DNA and serves as a positive control for the quantification experiment. In both *mericon* Quant Legionella Detection kits the copy number of the Quantification Control is adjusted to yield 250 ± 100 copies in the final PCR reaction and is integrated into the PCR reaction setup in duplicates. During a quantification experiment the verification of the Quantification Control copy number ensures preparation of an accurate standard curve and a correct quantification calculation.

Negative PCR control

Negative controls should be included in each analysis run to check for possible contamination of the *mericon* Assay during reaction setup. Instead of adding sample DNA to a reaction vial containing Multiplex PCR Master Mix, add the same volume of QuantiTect Nucleic Acid Dilution Buffer or RNase-free water.

Internal control calibration for real-time cyclers

MAX NHS Ester (MAX) dye is used to detect the internal control of *mericon* PCR Assays. Table 4 lists common thermal cyclers with their calibration requirement and the detection channel or filter set for this dye. Refer to the manufacturer's manual of the thermal cycler to be used for detailed calibration instructions.

Table 4. Calibration requirements and detection channel for MAX NHS Ester (MAX) dye

Thermal cycler	Dye calibration*	Filter suitable for MAX NHS Ester detection
Rotor-Gene Q	Not required	Yellow
Applied Biosystems models 7000, 7300, 7500, 7500 Fast, 7700, 7900HT, StepOne™, StepOnePlus™	Required for new instruments†	VIC
Stratagene (Agilent) models Mx3005P®, Mx3000P®	Not required	Filter set 535/550 nm (HEX, JOE, VIC)

* For information on detection channel settings for instruments not listed in Table 4, contact QIAGEN Technical Services.

† If the instrument is new, a dye calibration for the individual channels (e.g., VIC) of the real-time cycler must be performed. See the manufacturer's manual for details on calibration.

Protocol: Detection of Pathogen DNA by Real-time PCR without ROX

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 14.
- Take time to familiarize yourself with the Rotor-Gene Q or other real-time PCR instrument to be used before starting the protocol. See the instrument user manual.
- Make sure that the Quantification Control and at least one negative control are included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube with yellow lid).

  Add 1040 µl Multiplex PCR Master Mix (tube with blue lid) to the vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

  Note: If the reconstituted *mericon* Assay will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

- Dissolve the dried Quantification Control DNA (tube with red lid). Add 100 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly.

  Note: If the reconstituted Quantification Control will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

- Dissolve the dried Standard DNA (tube with green lid). Add 100 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial (Standard 1) and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly.
  Use this solution for the preparation of defined Standard DNA dilutions. Assign the copy numbers given in Table 5, page 17.

  Note: The reconstituted Standard DNA allows for 3 independent quantification experiments. If not used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

Table 5. Preparation of the Standard DNA dilution series

Standard	Dilutions	Copy number per PCR reaction
Standard 1	Standard DNA dissolved in 100 µl QT NA Dilution Buffer	25,000
Standard 2	5 µl Standard 1 + 45 µl QT NA Dilution Buffer	2500
Standard 3	5 µl Standard 2 + 45 µl QT NA Dilution Buffer	250
Standard 4	5 µl Standard 3 + 45 µl QT NA Dilution Buffer	25

- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.
- Standard dilutions as well as Quantification Control and negative template controls (NTC) are assayed in duplicates.

Procedure

1. **Set up the sample and control reactions according to Table 6, page 18. Keep all samples and reaction tubes on ice during setup.**

   If using the Rotor-Gene Q, place the desired number of PCR tubes or strips into the cooled Loading Block for the Rotor-Gene Q.

Table 6. 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 thermal cycler, securing them according to the instrument manual.**

   If using the Rotor-Gene Q, make sure that the locking ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.

3. **Program the thermal cycler. If using the Rotor-Gene Q, use the cycling protocol in Table 7. For all other real-time cyclers, use the cycling protocol in Table 8.**

   Note: For information on instrument detection settings for the MAX NHS Ester dye (MAX) used to detect the internal control of *mericon* Assays, see Table 4 on page 15.

4. **Start the PCR run.**
5. **Proceed to the protocol "Analyzing the Results" on page 24.**

Table 7. Cycling protocol for Rotor-Gene Q

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

Table 8. Cycling protocol for real-time cyclers other than Rotor-Gene Q

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	23 s*	60°C	
Extension	10 s	72°C	
Number of cycles	40		

Detection	Reporter	Excitation/emission	Channel
Target	FAM	495/520 nm	FAM
Internal control	MAX	524/557 nm	VIC†

* For some instruments, the shortest annealing time possible is longer than 23 s (in the range of 32 s). Use the shortest annealing time permitted by the instrument.

† See Table 4, page 15, for information on instrument-specific detection channel or filter set.

Protocol: Detection of Pathogen DNA by Real-time PCR with ROX

For certain real-time thermal cyclers, the use of a ROX passive reference dye during the PCR is necessary to compensate for variations in the fluorescence signal that are not related to the PCR.

Important points before starting

- Before beginning the procedure, read "Important Notes", page 14.
- Take time to familiarize yourself with the real-time PCR instrument to be used before starting the protocol. See the instrument user manual.
- Make sure that the Quantification Control and at least one negative control are included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube with yellow lid) by adding Multiplex PCR Master Mix (tube with blue lid) and ROX dye to the tube of *mericon* Assay according to Table 9. Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

  Note: The provided 50x ROX Dye solution is suitable for PCR instruments, requiring a low ROX Dye concentration (see Table 9). For PCR instruments requiring a high ROX Dye concentration please contact QIAGEN Technical Service.

Table 9. Components to add to the *mericon* Assay

Thermal cycler	ROX dye	Multiplex PCR Master Mix
Applied Biosystems model 7500, 7500 Fast	43.3 µl	1040 µl
Rotor-Gene models, Stratagene Mx models, LightCycler 480, SmartCycler models, Bio-Rad instruments	ROX reference dye not necessary	

Note: If the reconstituted *mericon* Assay will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

- Dissolve the dried Quantification Control DNA (tube with red lid). Add 100 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix by pipetting up and down 5 times or vortexing.

  Note: If the reconstituted Quantification Control will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

- Dissolve the dried Standard DNA (tube with green lid). Add 100 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial (Standard 1) and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly.
  Use this solution for the preparation of defined Standard DNA dilutions. Assign the copy numbers given in Table 10.

  Note: The reconstituted Standard DNA allows for 3 independent quantification experiments. If not used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

Table 10. Preparation of the Standard DNA dilution series

Standard	Dilutions	Copy number per PCR reaction
Standard 1	Standard DNA dissolved in 100 µl QT NA Dilution Buffer	25,000
Standard 2	5 µl Standard 1 + 45 µl QT NA Dilution Buffer	2500
Standard 3	5 µl Standard 2 + 45 µl QT NA Dilution Buffer	250
Standard 4	5 µl Standard 3 + 45 µl QT NA Dilution Buffer	25

- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.
- Standard dilutions as well as Quantification Control and negative template controls (NTC) are assayed in duplicates.

Procedure

1. **Set up the sample and control reactions according to Table 11. Keep all samples and reaction tubes on ice during setup.**

Table 11. Setup of Standard, Quantification Control, and sample reactions for PCR instruments requiring ROX Dye at a low concentration

Component	Standard dilutions	Quantification control	Samples	Negative control
Reconstituted *mericon* Assay	10.4 µl	10.4 µl	10.4 µl	10.4 µl
Respective Standard dilution	9.6 µl	–	–	–
Quantification Control DNA	–	9.6 µl	–	–
Sample DNA	–	–	9.6 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	–	9.6 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 thermal cycler, securing them according to the instrument manual.**
3. **Program the real-time cycler according to Table 12 (page 23).**

   Note: For information on instrument detection settings for the MAX NHS Ester dye (MAX) used to detect the internal control of *mericon* Assays, see Table 4 on page 15.
4. **Start the PCR run.**
5. **Proceed to "Analyzing the Results" on page 24.**

Table 12. 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	23 s*	60°C	
Extension	10 s	72°C	
Number of cycles	40		

Detection	Reporter	Emission maximum	Channel
Target	FAM	520 nm	Green (FAM)
Internal control	MAX	560 nm	Yellow (HEX)

* For some instruments, the shortest annealing time possible is longer than 23 s (in the range of 32 s). Use the shortest annealing time permitted by the instrument.

Analyzing the 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. Figures 1–3 are examples of possible outcomes, which are summarized in Table 13 (page 25).

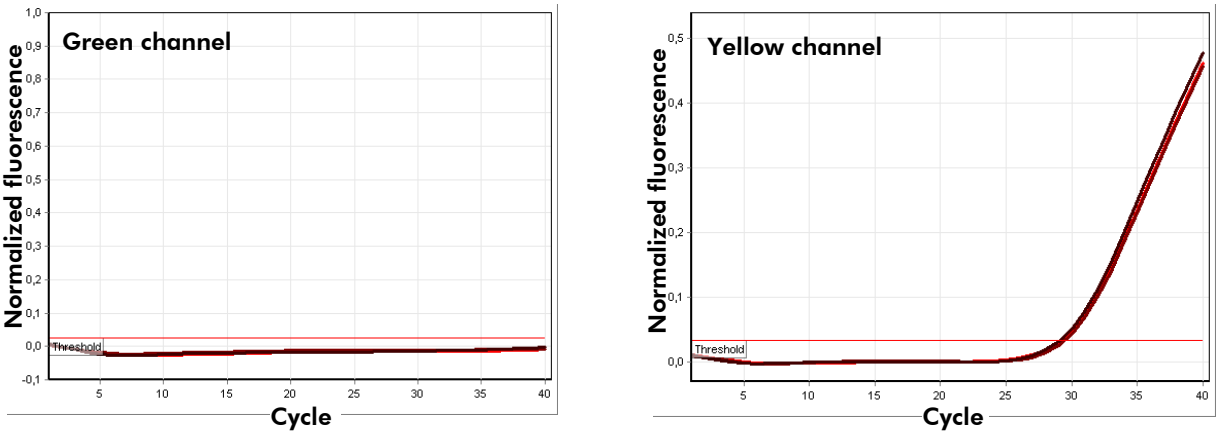

Figure 1. The sample is negative for tested pathogen. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

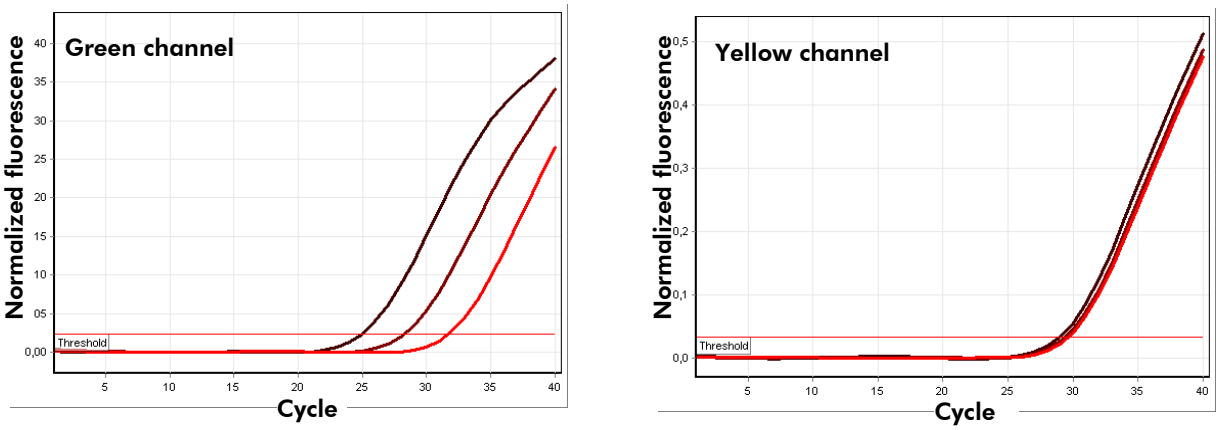

Figure 2. The sample is positive for tested pathogen. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of pathogen DNA in the sample and a successful PCR.

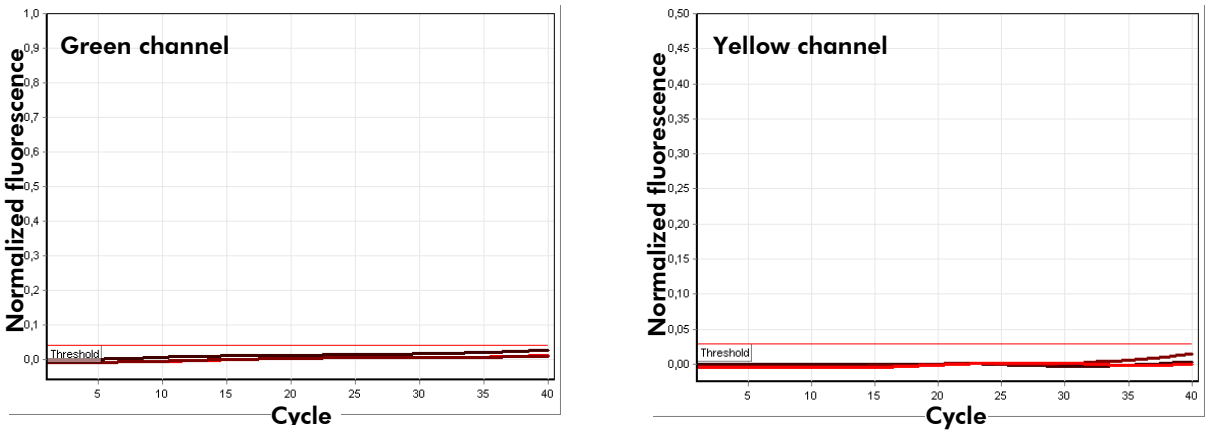

Figure 3. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 13. Summary of 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 threshold (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.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No signal with Quantification Control	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis, select the green channel (FAM) for the samples and the yellow channel (VIC or corresponding filter set) for the internal control. See the cycling protocols in Table 7 on page 19, Table 8 on page 19, or Table 12 on page 23. For Applied Biosystems software, the channel for the internal control is specified during calibration with MAX NHS Ester dye. Refer to the manufacturer's manual of the cycler to be used.
b) Incorrect programming of the real-time PCR instrument	Compare the temperature profile with the protocol. See the cycling protocols in Table 7 on page 19, Table 8 on page 19, or Table 12 on page 23. Refer to the manufacturer's manual of the cycler to be used.
c) Incorrect configuration of the PCR	Ensure that reactions were set up according to Table 4 (page 15), Table 6 (page 18), or Table 11 (page 22). Repeat the PCR, if necessary.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 6)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *mericon* PCR Assay has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Comments and suggestions	
Determination of a copy no of the Quantification Control, which is not in the 250 ± 100 copy range	
a) Standard curve is nor prepared correctly	Ensure that the Standard DNA dilutions were strictly prepared according to Table 5 (page 17) or Table 10 (page 21). Provided copy numbers refer to final copy numbers per PCR reaction.
b) Standard curve is not plotted or read-off properly	For preparation of the standard curves C_T values (y-axis) are plotted against the logarithm of the copy numbers (x-axis) provided in Table 5 (page 17) or Table 10 (page 21). The data correlation needs to follow a linear regression. Copy numbers correlating with C_T values of the Quantification Control should be read off the standard curves by the real-time cycler software or alternatively calculated via the linear equation corresponding to the data fit of the standard curve.
c) Quality of the standard curve is low	Accuracy of the standard curve is verified via the Quantification Control, which contains 250 ± 100 copies. The standard curve is correctly prepared if the Quantification Control is determined within a 150–350 copy number range.
Weak or no signal in the internal amplification control	
a) The PCR conditions do not comply with the protocol	Check that PCR conditions match the cycling protocols in Table 7 on page 19, Table 8 on page 19, or Table 12 on page 23. Repeat the PCR with corrected settings, if necessary.
b) The PCR was inhibited	Use the recommended DNA isolation method and closely follow the manufacturer's instructions. QIAGEN offers dedicated sample preparation kits developed to complement *mericon* PCR Assays, and provide a complete and efficient workflow for food safety testing.
c) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 6)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

	Comments and suggestions
d) The *mericon* PCR Assay has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Signals present for the negative controls	
a) Contamination occurred during PCR setup	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure to pipet the positive controls last. Make sure that work space and instruments are decontaminated at regular intervals.
b) Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
mericon Quant Legionella spp Kit	For 96 reactions: PCR Assay Legionella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290085
mericon Quant L. pneumophila Kit	For 96 reactions: PCR Assay L. pneumophila, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290095
Related products		
mericon Salmonella spp Kit (24)*	For 24 reactions: PCR Assay Salmonella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290013
mericon Listeria spp Kit (24)*	For 24 reactions: PCR Assay Listeria spp, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290123
mericon L. monocytogenes Kit (24)*	For 24 reactions: PCR Assay L. monocytogenes, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290023
mericon Campylobacter spp Kit (24)*	For 24 reactions: PCR Assay Campylobacter spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290033
mericon Campylobacter triple Kit (24)*	For 24 reactions: PCR Assay Campylobacter triple, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290043
mericon VTEC stx1/2 Kit (24)*	For 24 reactions: PCR Assay VTEC stx1/2, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290053

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
mericon Cronobacter spp Kit (24)*	For 24 reactions: PCR Assay Cronobacter spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290063
mericon S. aureus Kit (24)*	For 24 reactions: PCR Assay S. aureus, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290073
mericon Vibrio triple Kit (24)*	For 24 reactions: PCR Assay Vibrio triple, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290133
mericon Shigella spp Kit (24)*	For 24 reactions: PCR Assay Shigella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290103
mericon Y. enterocolitica Kit (24)*	For 24 reactions: PCR Assay Y. enterocolitica, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290113
***mericon* GMO detection assays**		
mericon Screen 35S Kit (24)*	For 24 reactions: PCR Assay Screen 35S, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291013
mericon Screen Nos Kit (24)*	For 24 reactions: PCR Assay Screen Nos, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291043
mericon RR Soy (24)*	For 24 reactions: PCR Assay RR Soy, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291113

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Product	Contents	Cat. no.
mericon GMO Screen bar Kit (24)*	For 24 reactions: PCR Assay Screen bar, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291063
mericon GMO Screen CTP2-CP4EPSPS Kit (24)*	For 24 reactions: PCR Assay Screen CTP2-CP4EPSPS, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291053
mericon MON 810 Corn Kit (24)*	For 24 reactions: PCR Assay MON 810 Corn, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291073
mericon GMO Screen 35S-pat Kit (24)*	For 24 reactions: PCR Assay Screen 35S-pat, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291023
***mericon* Quant GMO detection assays**		
mericon Quant RR Soy (48)	For 48 reactions: PCR Assay RR Soy, PCR Assay Reference System, Quantification Control DNA, Standard DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291514
mericon MON 810 (48)	For 48 reactions: PCR Assay MON 810, PCR Assay Reference System, Quantification Control DNA, Standard DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291524
***mericon* ingredient authentication assays**		
mericon Pig Kit (24)*	For 24 reactions: PCR Assay Pig, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292013

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
mericon Soy Kit (24)*	For 24 reactions: PCR Assay Soy, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293013
mericon Apricot Kernels Kit (24)*	For 24 reactions: PCR Assay Apricot Kernels, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293033
mericon Cattle Kit (24)*	For 24 reactions: PCR Assay Cattle, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292023
mericon Chicken Kit (24)*	For 24 reactions: PCR Assay Chicken, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292033
mericon Corn Kit (24)*	For 24 reactions: PCR Assay Corn, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293023
mericon Turkey Kit (24)*	For 24 reactions: PCR Assay Turkey, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292043
mericon Sheep Kit (24)*	For 24 reactions: PCR Assay Sheep, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292063
mericon Goat Kit (24)*	For 24 reactions: PCR Assay Goat, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292053
mericon Ruminant Kit (24)*	For 24 reactions: PCR Assay Ruminant, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	202073

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Product	Contents	Cat. no.
***mericon* sample preparation kits**		
DNeasy® *mericon* Food Kit (50)	50 QIAquick® Spin Columns, Proteinase K, buffers	69514
mericon DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
mericon DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534
QIAamp Pathogen Kit		
QIAamp UCP Pathogen Mini Kit	50 QIAamp UCP Mini Columns, collection tubes (2 ml), tube extenders (20 ml), elution tubes, VacConnectors, buffers, and Proteinase K	50214
Instrumentation		
Rotor-Gene Q 5plex	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire

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