

June 2015

***mericon*[®] Pathogen Detection Handbook**

For detection of pathogens in food or animal feed samples
using real-time PCR



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents	4
Storage	6
Intended Use	6
Safety Information	6
Introduction	7
Principle	7
Assay-specific information	10
Equipment and Reagents to Be Supplied by User	21
Important Notes	22
General precautions	22
Relevant assay controls	22
Internal control calibration for real-time cyclers	23
Protocols	
■ Detection of Pathogen DNA by Real-Time PCR without ROX	24
■ Detection of Pathogen DNA by Real-Time PCR with ROX	27
Analyzing the Results	30
Troubleshooting Guide	32
References	34
Ordering Information	35

Kit Contents

mericon Pathogen Detection Assays		(24)	(96)
See Table 1 (page 5) for specific kits			
Number of reactions		24	96
Yellow	<i>mericon Assay</i> *	2 x 12 reactions	1 x 96 reactions
Red	Positive Control DNA	20 reactions	20 reactions
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml	1.5 ml
	RNase-Free Water	1.9 ml	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl	1040 µl
	50x ROX Dye Solution	45 µl	210 µl
	Quick-Start Protocol	1	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)	(96)
<i>mericon</i> Salmonella spp Kit* For general detection of <i>Salmonella</i> subspecies [†]	290013	290015
<i>mericon</i> Listeria spp Kit For general detection of <i>Listeria</i> subspecies [†]	290123	290125
<i>mericon</i> L. monocytogenes Kit For specific detection of <i>Listeria monocytogenes</i>	290023	290025
<i>mericon</i> Campylobacter spp Kit For general detection of <i>Campylobacter</i> subspecies [†]	290033	290035
<i>mericon</i> Campylobacter triple Kit For specific detection of <i>C. jejuni</i> , <i>C. lari</i> , and <i>C. coli</i>	290043	290045
<i>mericon</i> VTEC stx1/2 Kit For specific detection of verotoxin-producing <i>E. coli</i> (shigatoxin 1 and 2)	290053	290055
<i>mericon</i> Cronobacter spp Kit For general detection of <i>Cronobacter</i> subspecies (formerly <i>Enterobacter</i>)	290063	290065
<i>mericon</i> S. aureus Kit For specific detection of <i>Staphylococcus aureus</i>	290073	290075
<i>mericon</i> Vibrio triple Kit For specific detection of <i>Vibrio vulnificus</i> , <i>Vibrio parahaemolyticus</i> , and <i>Vibrio cholerae</i>	290133	290135
<i>mericon</i> Quant Legionella spp Kit For detection and quantification of <i>Legionella</i> subspecies	–	290085
<i>mericon</i> Quant L. pneumophila Kit For detection and quantification of <i>Legionella pneumophila</i>	–	290095
<i>mericon</i> Shigella spp Kit For general detection of <i>Shigella</i> subspecies [†]	290103	290105
<i>mericon</i> Y. enterocolitica Kit For specific detection of <i>Yersinia enterocolitica</i>	290113	290115

* AOAC-PTM-validated kit.

[†] For more detailed information, see “Assay-specific information”, page 10.

Storage

mericon Pathogen Detection Assays 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 $2-8^{\circ}\text{C}$ 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* Pathogen Detection Assays should be dispensed into aliquots to avoid more than 5 freeze–thaw cycles, and stored at $2-8^{\circ}\text{C}$ for short-term storage (1 month) or -30 to -15°C for long-term storage.

Intended Use

mericon Pathogen Detection Assays are intended for molecular biology applications in food, 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.

Introduction

mericon Pathogen Detection Assays are a ready-to-use system for the detection of specific DNA fragments from pathogens in food, animal feed, and pharmaceutical products using real-time polymerase chain reaction (PCR). These assays perform optimally on the Rotor-Gene[®] Q but have been validated for block thermal cyclers as well. 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 the polymerase chain reaction (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.

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 –20°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 27.

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 Salmonella spp Kit (AOAC-PTM validated)

The *mericon* Salmonella spp Kit is an AOAC-PTM-validated PCR detection kit. It is designed for the qualitative detection of several *Salmonella* subspecies in food and animal feed samples after enrichment.

Limit of detection

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

Specificity

The *mericon* Salmonella spp Kit exhibits high specificity for pathogens of the genus *Salmonella*. It was tested against an extensive inclusivity panel of salmonella strains and exclusivity panel of non-salmonella strains. All tested salmonella strains were successfully detected and no cross-reactivity was observed with other pathogens. <http://www.qiagen.com/mericonPathogens>.

Table 2 gives an overview of selected non-salmonella targets using 2500 copies of tested DNA. Information about full inclusivity/exclusivity test panels is available at <http://www.qiagen.com/mericonPathogens>.

Table 2. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Salmonella</i> spp.	+	<i>Cronobacter sakazakii</i>	–
<i>Staphylococcus aureus</i>	–	<i>Bacillus cereus</i>	–
<i>Clostridium perfringens</i>	–	<i>Legionella erythra</i>	–
<i>Listeria monocytogenes</i>	–	<i>Campylobacter jejuni</i>	–
<i>Yersinia enterocolitica</i>	–	<i>Legionella pneumophilia</i>	–
<i>Shigella flexneri</i>	–	VTEC stx1/stx2	–
<i>Escherichia coli</i>	–		

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

mericon *Listeria* spp Kit

The *mericon* *Listeria* spp Kit is designed for the qualitative detection of several *Listeria* subspecies in food and animal feed samples after enrichment.

Limit of detection

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

Specificity

The *mericon* *Listeria* spp Kit exhibits high specificity for pathogens of the genus *Listeria*. No cross-reactivity was observed with other pathogens (Table 3) using 2500 copies of the tested DNA.

Table 3. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Listeria monocytogenes</i>	+	<i>Listeria innocua</i>	+
<i>Listeria welshimeri</i>	+	<i>Listeria marthii</i>	+
<i>Listeria seeligeri</i>	+	<i>Listeria grayi</i>	+
<i>Listeria ivanovii</i>	+	<i>Bacillus cereus</i>	–
<i>Listeria rocourtiae</i>	–	<i>Yersinia enterocolitica</i>	–
<i>Campylobacter jejuni</i>	–	<i>Clostridium perfringens</i>	–
<i>Escherichia coli</i>	–	<i>Legionella erythra</i>	–
<i>Cronobacter sakazakii</i>	–	<i>Shigella flexneri</i>	–
<i>Legionella pneumophila</i>	–	VTEC stx1/stx2	–
<i>Staphylococcus aureus</i>	–	<i>Salmonella abony</i>	–

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

mericon L. monocytogenes Kit

The mericon L. monocytogenes Kit is designed for the qualitative detection of *Listeria monocytogenes* in food and animal feed after enrichment.

Limit of detection

The mericon L. monocytogenes Kit can detect down to 10 copies of *Listeria monocytogenes* DNA in a reaction.

Specificity

The mericon L. monocytogenes Kit exhibits high specificity for *Listeria monocytogenes*. No cross-reactivity was observed with other pathogens (Table 4) using 2500 copies of tested DNA.

Table 4. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Listeria monocytogenes</i>	+	<i>Cronobacter sakazakii</i>	–
<i>Staphylococcus aureus</i>	–	<i>Bacillus cereus</i>	–
<i>Clostridium perfringens</i>	–	<i>Legionella erythra</i>	–
<i>Salmonella abony</i>	–	<i>Campylobacter jejuni</i>	–
<i>Yersinia enterocolitica</i>	–	<i>Legionella pneumophila</i>	–
<i>Shigella flexneri</i>	–	VTEC stx1/stx2	–
<i>Escherichia coli</i>		<i>Shigella flexneri</i>	–
<i>Listeria innocua</i>	–	<i>Listeria welshimeri</i>	–
<i>Listeria ivanovii</i>	–	<i>Listeria grayi</i>	–
<i>Listeria seeligeri</i>	–		

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

mericon Campylobacter spp Kit

The *mericon* Campylobacter spp Kit is designed for the detection of several *Campylobacter* subspecies in food and animal feed samples after enrichment.

Limit of detection

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

Specificity

The *mericon* Campylobacter spp Kit exhibits high specificity for pathogens of the genus *Campylobacter*. No cross-reactivity was observed with other pathogens (Table 5) using 2500 copies of tested DNA.

Table 5. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Campylobacter jejuni</i>	+	<i>Campylobacter coli</i>	+
<i>Campylobacter lari</i>	+	<i>Campylobacter upsaliensis</i>	+
<i>Salmonella abony</i>	–	<i>Staphylococcus aureus</i>	–
<i>Clostridium perfringens</i>	–	<i>Escherichia coli</i>	–
<i>Legionella erythra</i>	–	<i>Yersinia enterocolitica</i>	–
<i>Legionella pneumophila</i>	–	<i>Listeria monocytogenes</i>	–
<i>Bacillus cereus</i>	–	<i>Cronobacter sakazakii</i>	–
<i>Shigella flexneri</i>	–		

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

mericon Campylobacter triple Kit

The mericon Campylobacter triple Kit is designed for the detection of *Campylobacter jejuni*, *C. coli*, and *C. lari* in food and animal feed samples after enrichment.

Limit of detection

The mericon Campylobacter triple Kit can detect down to 10 copies of *C. jejuni*, *C. coli* or *C. lari* DNA in a reaction.

Specificity

The mericon Campylobacter triple Kit exhibits high specificity for *C. jejuni*, *C. coli*, and *C. lari*. No cross-reactivity was observed with other pathogens (Table 6) using 2500 copies of tested DNA.

Table 6. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Campylobacter jejuni</i>	+	<i>Campylobacter coli</i>	+
<i>Campylobacter lari</i>	+	<i>Campylobacter upsaliensis</i>	–
<i>Salmonella abony</i>	–	<i>Cronobacter sakazakii</i>	–
<i>Clostridium perfringens</i>	–	<i>Shigella flexneri</i>	–
<i>Legionella pneumophila</i>	–	<i>Yersinia enterocolitica</i>	–
<i>Legionella erythra</i>	–	<i>Listeria monocytogenes</i>	–
<i>Staphylococcus aureus</i>	–	<i>Bacillus cereus</i>	–
<i>Escherichia coli</i>	–		

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

mericon VTEC stx1/2 Kit

The mericon VTEC stx1/2 Kit is designed for the detection of shigatoxin-producing *Escherichia coli* (VTEC stx1 and VTEC stx2) in food and animal feed samples after enrichment. VTEC is an abbreviation for verotoxin-producing *Escherichia coli* and is synonymous with STEC, or shigatoxin-producing *Escherichia coli*. This nomenclature results from the interchangeable names for the two toxin groups: shigatoxins 1 and 2 (stx 1 and stx 2) and verotoxins 1 and 2.

Limit of detection

The mericon VTEC stx1/2 Kit can detect down to 10 copies of shigatoxin-producing *Escherichia coli* DNA in a reaction.

Specificity

The mericon VTEC stx1/2 Kit exhibits high specificity for VTEC stx 1 and VTEC stx2. No cross-reactivity was observed with other pathogens (Table 7) using 2500 copies of tested DNA.

Table 7. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
VTEC stx1	+	VTEC stx2	+
<i>Campylobacter jejuni</i>	–	<i>Escherichia coli</i>	–
<i>Campylobacter coli</i>	–	<i>Clostridium perfringens</i>	–
<i>Campylobacter lari</i>	–	<i>Bacillus cereus</i>	–
<i>Campylobacter upsaliensis</i>	–	<i>Yersinia enterocolitica</i>	–
<i>Legionella pneumophila</i>	–	<i>Listeria monocytogenes</i>	–
<i>Legionella erythra</i>	–	<i>Shigella flexneri</i>	–
<i>Staphylococcus aureus</i>	–	<i>Cronobacter sakazakii</i>	–
<i>Salmonella abony</i>	–		

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

mericon Cronobacter spp Kit

The *mericon* Cronobacter spp Kit is designed for the detection of several *Cronobacter* subspecies (formerly *Enterobacter*) in food and animal feed after enrichment. Due to a recent change in taxonomy and according to regulation of the EU Commission (365/2010) the name *Cronobacter* replaces the former *Enterobacter*.

Limit of detection

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

Specificity

The *mericon* Cronobacter spp Kit exhibits high specificity for pathogens of the genus *Cronobacter*. No cross-reactivity was observed with other pathogens (Table 8) using 2500 copies of tested DNA.

Table 8. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Cronobacter sakazakii</i>	+	<i>Cronobacter dubliensis</i>	+
<i>Cronobacter turingensis</i>	+	<i>Cronobacter malonatus</i>	+
<i>Cronobacter muytjensii</i>	+	<i>Legionella pneumophila</i>	–
<i>Campylobacter jejuni</i>	–	<i>Listeria monocytogenes</i>	–
<i>Campylobacter coli</i>	–	<i>Salmonella abony</i>	–
<i>Campylobacter lari</i>	–	<i>Shigella flexneri</i>	–
<i>Campylobacter upsaliensis</i>	–	<i>Yersinia enterocolitica</i>	–
<i>Staphylococcus aureus</i>	–	<i>Clostridium perfringens</i>	–
<i>Bacillus cereus</i>	–	<i>Escherichia coli</i>	–
<i>Legionella erythra</i>	–	VTEC stx1/stx2	–

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

mericon *S. aureus* Kit

The mericon *S. aureus* Kit is designed for the detection of *Staphylococcus aureus* in food and animal feed after enrichment.

Limit of detection

The mericon *S. aureus* Kit can detect down to 10 copies of *Staphylococcus aureus* DNA in a reaction.

Specificity

The mericon *S. aureus* Kit exhibits high specificity for *Staphylococcus aureus*. No cross-reactivity was observed with other pathogens (Table 9) using 2500 copies of tested DNA.

Table 9. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Staphylococcus aureus</i>	+	<i>Staphylococcus saprophyticus</i>	–
<i>Staphylococcus epidermis</i>	–	<i>Staphylococcus sciuri</i>	–
<i>Staphylococcus haemolyticus</i>	–	<i>Staphylococcus warneri</i>	–
<i>Staphylococcus lugdunensis</i>	–	<i>Staphylococcus xylosus</i>	–
<i>Salmonella abony</i>	–	<i>Campylobacter upsaliensis</i>	–
<i>Cronobacter sakazakii</i>	–	<i>Clostridium perfringens</i>	–
<i>Legionella pneumophila</i>	–	<i>Listeria monocytogenes</i>	–
<i>Bacillus cereus</i>	–	<i>Legionella erythra</i>	–
<i>Yersinia enterocolitica</i>	–	VTEC stx1/stx2	–
<i>Campylobacter jejuni</i>	–	<i>Escherichia coli</i>	–
<i>Campylobacter coli</i>	–	<i>Shigella flexneri</i>	–
<i>Campylobacter lari</i>	–		

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

mericon Vibrio triple Kit

The *mericon* Vibrio triple Kit is designed for the detection of *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae* in food and animal feed after enrichment.

Limit of detection

The *mericon* Vibrio triple Kit can detect down to 10 copies of *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae* DNA in a reaction.

Specificity

The *mericon* Vibrio triple Kit exhibits high specificity for *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae*. No cross-reactivity was observed with other pathogens (Table 10) using 2500 copies of tested DNA.

Table 10. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Vibrio vulnificus</i>	+	<i>Staphylococcus aureus</i>	–
<i>Vibrio parahaemolyticus</i>	+	<i>Bacillus cereus</i>	–
<i>Vibrio cholerae</i>	+	<i>Salmonella abony</i>	–
<i>Vibrio alginolyticus</i>	–	<i>Clostridium perfringens</i>	–
<i>Vibrio furnissii</i>	–	<i>Shigella flexneri</i>	–
<i>Legionella pneumophila</i>	–	<i>Escherichia coli</i>	–
<i>Campylobacter jejuni</i>	–	<i>Cronobacter sakazakii</i>	–
<i>Listeria monocytogenes</i>	–	<i>Yersinia enterocolitica</i>	–

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

mericon Shigella spp Kit

The *mericon* Shigella spp Kit is designed for the detection of several *Shigella* subspecies in food and animal feed after enrichment.

Limit of detection

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

Specificity

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

Table 11. Results from cross-reactivity experiment*

Pathogen	Result	Pathogen	Result
<i>Shigella flexneri</i>	+	<i>Shigella dysenteriae</i>	+
<i>Shigella boydii</i>	+	<i>Shigella sonnei</i>	+
<i>Campylobacter jejuni</i>	–	<i>Escherichia coli</i>	–
<i>Legionella pneumophila</i>	–	<i>Yersinia enterocolitica</i>	–
<i>Listeria monocytogenes</i>	–	<i>Clostridium perfringens</i>	–
<i>Bacillus cereus</i>	–	<i>Cronobacter sakazakii</i>	–
<i>Salmonella abony</i>	–	<i>Staphylococcus aureus</i>	–

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

mericon *Y. enterocolitica* Kit

The *mericon Y. enterocolitica* Kit is designed for the detection of *Yersinia enterocolitica* in food and animal feed after enrichment. The kit specifically detects human pathogen serotypes 0:3, 0:5, 0:6, 0:8, 0:9, and 0:5:27.

Limit of detection

The *mericon Y. enterocolitica* Kit can detect down to 10 copies of *Yersinia enterocolitica* DNA in a reaction.

Specificity

The *mericon Y. enterocolitica* Kit exhibits high specificity for *Yersinia enterocolitica*. No cross-reactivity was observed with other pathogens (Table 12) using 2500 copies of tested DNA.

Table 12. Results from cross-reactivity experiments*

Pathogen	Result	Pathogen	Result
<i>Yersinia enterocolitica</i>	+	<i>Listeria monocytogenes</i>	–
Non-pathogenic <i>Yersinia enterocolitica</i>	–	<i>Bacillus cereus</i>	–
<i>Clostridium perfringens</i>	–	<i>Campylobacter jejuni</i>	–
<i>Salmonella abony</i>	–	<i>Legionella pneumophila</i>	–
<i>Staphylococcus aureus</i>	–	VTEC stx1/stx 2	–
<i>Shigella flexneri</i>	–	<i>Cronobacter sakazakii</i>	–
<i>Escherichia coli</i>	–		

* 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. We recommend the *mericon* DNA Bacteria Kit (cat. no. 69525) for Gram-negative bacteria or the *mericon* DNA Bacteria Plus Kit (cat. no. 69534) for Gram-positive bacteria
- 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 13 on page 23 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 13, page 23 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.

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.

Positive PCR control

Positive controls should be included in each analysis run to check the functionality of the Multiplex PCR Master Mix. Instead of adding sample DNA to a reaction vial containing Multiplex PCR Master Mix, add the same volume of the positive control included in the kit.

Internal control calibration for real-time cyclers

MAX NHS Ester (MAX) dye is used to detect the internal control of *mericon* PCR Assays. Table 13 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 13. 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 13, 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 22.
- 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 at least one positive and one negative control are included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube with yellow lid).
24-sample kit: Add 130 μ l Multiplex PCR Master Mix (tube with blue lid) to each vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.
96-sample kit: Add 1040 μ l 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 Positive Control DNA (tube with red lid). Add 200 μ l 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 dissolved positive 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.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Procedure

- 1. Set up the sample and control reactions according to Table 14, page 25. 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 14. Setup of sample and control reactions

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted <i>mericon</i> Assay	10 μ l	10 μ l	10 μ l
Sample DNA	10 μ l	–	–
Dissolved Positive Control DNA	–	10 μ l	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	10 μ l
Total volume	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 15. For all other real-time cyclers, use the cycling protocol in Table 16.**

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 13 on page 23.

- 4. Start the PCR run.**
- 5. Proceed to “Analyzing the Results”, page 30.**

Table 15. 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 16. 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 13, page 23, for information about instrument-specific detection channels 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 22.
- 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 at least one positive and one negative control are included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube(s) with yellow lid) by adding Multiplex PCR Master Mix (tube(s) with blue lid) and ROX dye to the tube(s) of *mericon* Assay according to Table 17. Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

Note: The 50x ROX Dye solution provided is suitable for PCR instruments, requiring a low ROX Dye concentration (Table 17). For PCR instruments requiring a high ROX Dye concentration please contact QIAGEN Technical Services.

Table 17. Components to add to the *mericon* Assay

Thermal cycler	Kit size	ROX dye	Multiplex PCR Master Mix
Applied Biosystems model 7500 and 7500 Fast	24 96	5.4 μ l per vial 43.3 μ l	130 μ l per vial 1040 μ l
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 Positive Control DNA (tube with red lid). Add 200 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix by pipetting up and down 5 times or vortexing.

Note: If the dissolved positive 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.

- Before each use, all reagents must be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Procedure

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

Table 18. Setup of sample and control reactions for PCR instruments requiring ROX dye at a low concentration

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted <i>mericon</i> Assay	10.4 µl	10.4 µl	10.4 µl
Sample DNA	9.6 µl	–	–
Dissolved Positive Control DNA	–	9.6 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	9.6 µl
Total volume	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 19.

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 13 on page 23.

4. Start the PCR run.

5. Proceed to “Analyzing the Results”, page 30.

Table 19. Cycling protocol

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq <i>Plus</i> 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 20 (page 31).

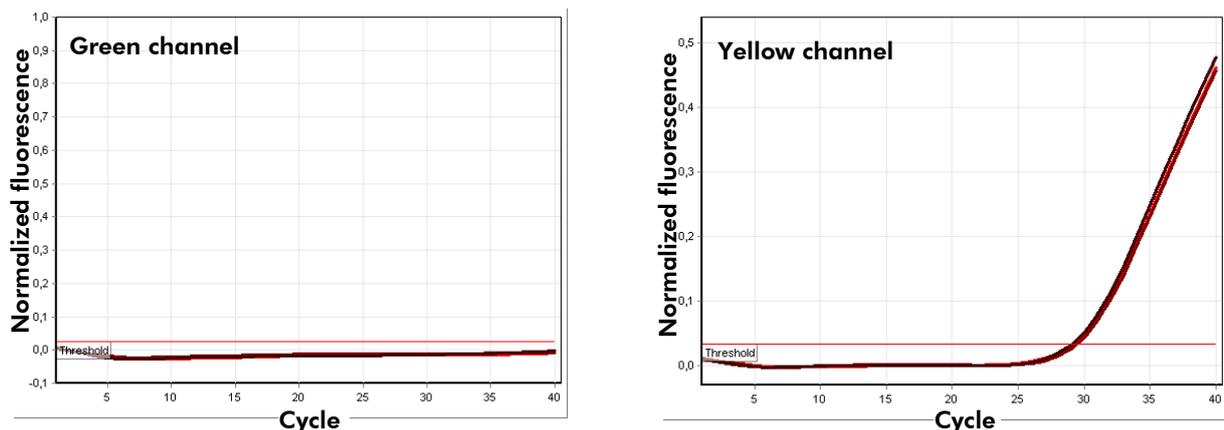


Figure 1. The sample is negative for tested pathogen. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

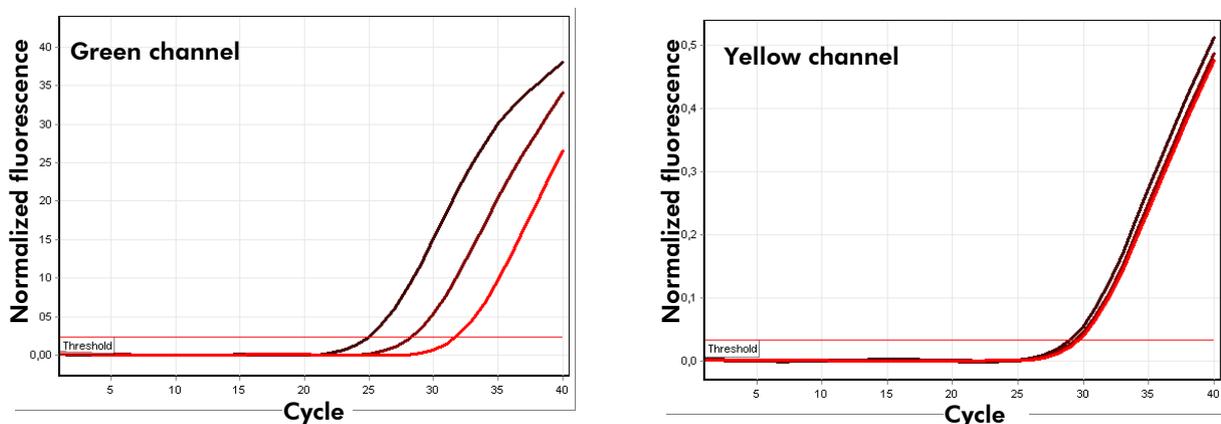


Figure 2. The sample is positive for tested pathogen. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of pathogen DNA in the sample and a successful PCR.

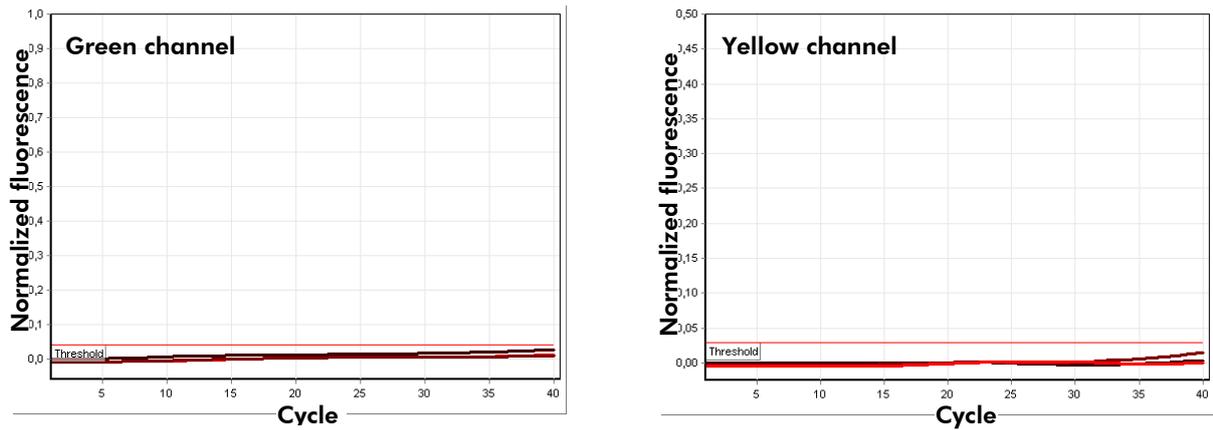


Figure 3. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 20. 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 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.

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 positive 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 15 or Table 16 on page 26, or Table 19 on page 29. 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 15 or Table 16 on page 26, or Table 19 on page 29. 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 13 (page 23) or Table 18 (page 28). 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 <i>mericon</i> 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

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 15 or Table 16 on page 26, or Table 19 on page 29. 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 <i>mericon</i> 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. |
| d) The <i>mericon</i> 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.
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
Related products		
mericon GMO detection assays		
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
mericon Quant GMO detection assays		
<i>mericon</i> 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
<i>mericon</i> 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		
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> sample preparation kits		
DNeasy® <i>mericon</i> Food Kit (50)	50 QIAquick® Spin Columns, Proteinase K, buffers	69514
<i>mericon</i> DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
<i>mericon</i> DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534
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

* Larger kit sizes available; please inquire.

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.

Notes

Notes

Trademarks: QIAGEN®, QIAquick®, DNeasy®, HotStarTaq®, mericon®, Q-Bond®, QuantiTect®, Rotor-Gene® (QIAGEN Group); Mx3005P®, Mx3000P®, Stratagene® (Agilent Technologies); SmartCycler® (Cepheid); Roche® (Hoffman-La Roche, Inc.); Iowa Black®, MAX™ (Integrated DNA Technologies, Inc.); Applied Biosystems®, FAM®, HEX®, JOE®, StepOne™, StepOnePlus™, VIC® (Applied Biosystems Corporation or its subsidiaries); LightCycler® (Roche Group). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

Developed by: ifp Institut für Produktqualität, Teltowkanalstr. 2, 12247 Berlin, Germany. www.produktqualitaet.com

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,804,375, 5,538,848, 5,723,591, 5,876,930, 6,030,787 and 6,258,569. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product solely in Food Testing Applications and Industrial Microbiology Applications, including reporting results of purchaser's activities for a fee or other commercial consideration, and also for the purchaser's own internal research. No right under any other patent claim is conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

This multiplex PCR product is licensed under US5,582,989 and corresponding patents.

The purchase of this product includes a limited, non-transferable right to use the purchased amount of the product to perform Applied Biosystems' patented Passive Reference Method for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

License Probe manufactured by Integrated DNA Technologies, Inc. under license from Applied Biosystems. License Probe incorporates IDT's Iowa Black quencher technology and is accompanied by a limited research use only license from IDT.

Limited License Agreement for the mericon Pathogen Detection Handbook

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

© 2012–2015 QIAGEN, all rights reserved.

www.qiagen.com

Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com

