July 2014

mericon® E. coli STEC O-Type Detection Handbook

For detection of *Escherichia coli* serotype O157:H7 and the *E. coli* non-O157:H7 serotypes O26, O45, O103, O111, O121, and O145, in food or animal feed samples using real-time PCR on the Rotor-Gene® Q

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- Nucleic acid and protein assays
- microRNA research and RNAi
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Contents

Kit Contents

***mericon* E. coli STEC O-Type Kit**		**(24)**	**(96)**
Catalog no.		**290233**	**290235**
Number of reactions		**24**	**96**
Yellow	*mericon* Assay*	2 x 12 reactions	1 x 96 reactions
	RNase-free water	1.9 ml	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl	1040 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).

Storage

The *mericon* Assays are shipped on dry ice. The Multiplex PCR Master Mix should be stored immediately at –15°C to –30°C upon receipt. All remaining kit components not reconstituted 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 *mericon* 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 at –15°C to –30°C for long-term storage.

Intended Use

The *mericon* E. coli STEC O-Type Kit is intended for molecular biology applications in food, animal feed, and pharmaceutical product testing. This product is 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 components.

Technical Assistance

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover). Technical Service in North America can be reached at 1-800-362-7737 and at QIAGEN GmbH at +49-2103-29-12400.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *mericon* E. coli STEC O-Type Kit is tested against predetermined specifications to ensure consistent product quality.

General Precautions for Real-Time PCR Assays

The *mericon* E. coli STEC O-Type assay uses PCR to detect DNA. Care must be taken to avoid contamination of the PCR reactions. It is important to include at least one negative control that lacks the template nucleic acid in every PCR setup, to detect possible contamination.

General physical and chemical precautions

- 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.
- Use a separate set of pipets for the PCR master mix and the DNA samples. Use of pipet tips with hydrophobic filters is strongly recommended.
- Use gloves and protective laboratory wear. Do not touch any PCR equipment and supplies (e.g., rotors, loading blocks, tubes, pipets) without wearing gloves.
- In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with a 1:10 dilution of a commercial

bleach solution. Afterwards, the benches and pipets should be rinsed with distilled water.

- All materials and media possibly containing the tested pathogen should be autoclaved for 20 min at 120°C, prior to disposal.

Assay-Specific Information

mericon E. coli STEC O-Type Kit

The *mericon* E. coli STEC O-Type Kit is designed for the qualitative detection of the *E. coli* serotype O157:H7 and *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145) in food and animal feed samples after enrichment.

Limit of detection

The *mericon* E. coli STEC O-Type Kit can detect as few as 10 copies of the respective *E.coli* target gene in a reaction.

Specificity

The *mericon* E. coli STEC O-Type Kit exhibits high specificity for *E. coli* strain O157:H7, O26, O45, O103, O111, O121, and O145. The kit was tested against an extensive inclusivity panel of *E. coli* O157:H7 and non-O157:H7 serotype strains, and an exclusivity panel of other *E. coli* and non-*E. coli* strains. All tested O157:H7 and non-O157:H7 serotype strains (O26, O45, O103, O111, O121, and O145)* were successfully detected and no cross-reactivity was observed with other pathogens using 2500 copies of tested DNA (Table 1). Information about full inclusivity/exclusivity test panels is available at www.qiagen.com/mericonPathogens.

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

Table 1. Results from cross-reactivity experiments

Pathogen	Strain no.	Result Orange Channel	Result Green Channel
E. coli O26:H-	DSM 8695	-	+
E. coli O45	SSI 81886	-	+
E. coli O103	NC09103	-	+
E. coli O103	SSI 82109	-	+
E. coli O111:H-	DSM 8698	-	+
E. coli O111:H-	DSM 10234	-	+
E. coli O121	NC09121	-	+
E. coli O145	SSI 82280	-	+
E. coli O157:H7	DSM 8579	+	-
E. coli O157:H7	DSM 13526	+	-
E. coli O157:H7	DSM 17076	+	-
E. coli O157:H7	DSM 19206	+	-
E. coli O1:H7	DSM 30083	-	-
E. coli O1:K1:H7	ATCC11775	-	-
E. coli O1:K1:H7	DSM 10750	-	-
E. coli O2:K1:H4	DSM 10777	-	-
E. coli O7:K1:H7	DSM 10858	-	-
E. coli O11:H10	DSM 11751	-	-
E. coli O12:H2	DSM 8703	-	-
E. coli O13:H11	DSM 1058	-	-
E. coli O18ac:K1:H7	DSM 10723	-	-
E. coli O19:H7	DSM 11752	-	-
E. coli O25:H4	DSM 22664	-	-
E. coli O29:H10	DSM 9026	-	-

Pathogen	Strain no.	Result Orange Channel	Result Green Channel
E. coli O104	NC09104	-	-
E. coli O107:H5	DSM 9033	-	-
E. coli O112ac:H-	DSM 9027	-	-
E. coli O113	SSI 82121	-	-
E. coli O124:H30	DSM 9031	-	-
E. coli O126:H2	DSM 8701	-	-
E. coli O143:H-	DSM 9028	-	-
E. coli RM-HYG118	VTEC Isolate	-	-
E. coli RM-HYG119	VTEC Isolate	-	-
E. coli RM-HYG120	VTEC Isolate	-	-
Bacillus cereus	ATCC 11778	-	-
Campylobacter jejuni	ATCC 33560	-	-
Clostridium perfringens	ATCC 13124	-	-
Enterobacter sakazakii	DSM 4485	-	-
E. coli	ATCC	-	-
E. coli	NCTC 9002	-	-
Legionella erythra	Sifin	-	-
Listeria monocytogenes	ATCC 7644	-	-
Shigella flexneri	ATCC 12022	-	-
Staphylococcus aureus	ATCC 25923	-	-
Vibrio vulnificus	ATCC 43478	-	-
Yersinia enterocolitica	ATCC 9610	-	-

Introduction

The *mericon* E. coli STEC O-Type Kit is a ready-to-use PCR assay for the detection of specific DNA fragments from pathogenic *Escherichia coli* in food, animal feed, and pharmaceutical products. This assay was developed for usage on the Rotor-Gene Q, but can be used with other real-time cyclers that can detect: FAM™ (495/520 nm), ROX™ NHS Ester (588/608 nm), and MAX™ NHS Ester (524/557 nm). The Multiplex PCR Master Mix contains QIAGEN proprietary technology, which includes HotStarTaq *Plus* DNA Polymerase; patented multiplex PCR technology, such as Factor MP; and fast cycling technology, including Q-Bond®. The Multiplex PCR Master Mix is also highly tolerant to PCR inhibitors. The analytical procedure of this protocol allows the user to perform analyses in accordance with local official requirements.

Each *mericon* Assay is an optimized mixture of PCR primer sets for a target sequence specific for pathogenic *E. coli* and an internal control (IC), as well as probes labeled with 3 distinct fluorescent dyes (Table 2). In addition, each kit includes internal control DNA and all reagents necessary to perform the analysis.

Table 2. Targets and channels of the *mericon* E. coli STEC O-Type Assay

	Green Channel FAM (495/520 nm)	**Orange Channel ROX NHS Ester (588/608 nm)**	**Yellow Channel MAX NHS Ester (524/557 nm)**
mericon E. coli STEC O-Type Assay	O26, O45, O103, O111, O121, O145	O157:H7	Internal Control (IC)

Principle and procedure

Pathogen detection by PCR is based on the amplification of a specific region of the relevant pathogen genome. 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 has been verified experimentally and using bioinformatics. Cross-reactivity has been investigated using bioinformatics and thoroughly tested with a panel of selected targets for each *mericon* PCR Assay. Each assay can detect as few as 10 copies of the respective *E.coli* target gene 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 to 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 mis-primed 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 to facilitate 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 specifically formulated to be highly tolerant to inhibitors commonly present in food.

Primer/probe mix with internal control

Each *mericon* PCR Assay includes rigorously designed primers and probes in a carefully balanced mix that amplifies 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 used as the reporter dye for the internal control with excitation/emission maxima of 524/557 nm and a non-fluorescent quencher, Iowa Black®.

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: www.qiagen.com/safety.

- Nucleic Acid Isolation Kit. We recommend the *mericon* DNA Bacteria Kit (cat. no. 69525) for Gram-negative bacteria or the QIAsymphony *mericon* Bacteria Kit (360) (cat. no. 931156).
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Rotor-Gene Q*
- Tube rack
- Microcentrifuge*
- Vortex mixer
- Autoclave*

PCR plastics for the Rotor-Gene Q

- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (250 or 2500) (cat. no. 981103 or 981106)
- Rotor-Disc® 72 (24 or 240) (cat. no. 981301 or 981303)
- Rotor-Disc Heat Sealing Film (60 or 600) (cat. no. 981601 or 981604)
- Loading Block 72 x 0.1 ml Tubes (cat. no. 9018901), Rotor-Disc 72 Loading Block (cat. no. 9018910)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Important Notes

General precautions

Please refer to handbook section "General Precautions for Real-Time PCR Assays", page 5. 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 quick vortexing), and centrifuge briefly.

Relevant assay controls

Internal control

Each vial of *mericon* Assay contains an internal control to detect possible PCR inhibition from food or feed matrix enrichment cultures.

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 RNase-free water.

Positive PCR control

Positive controls are not provided with the product but may be included by the user 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.

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Protocol: Setup of Real-Time PCR on the Rotor-Gene Q

Important points before starting

- Before beginning the procedure, read "Important Notes", page 13.
- Take time to familiarize yourself with the Rotor-Gene Q. See the instrument user manual.
- Make sure that at least one negative control is included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube with yellow lid).
- **24-sample kit**: Add 130 µl 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 by quick vortexing, and centrifuge briefly.
- **96-sample kit**: 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 by quick vortexing, and centrifuge briefly.
- 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 –15 to –30°C for long-term storage.
- Before each use, all reagents should be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Procedure

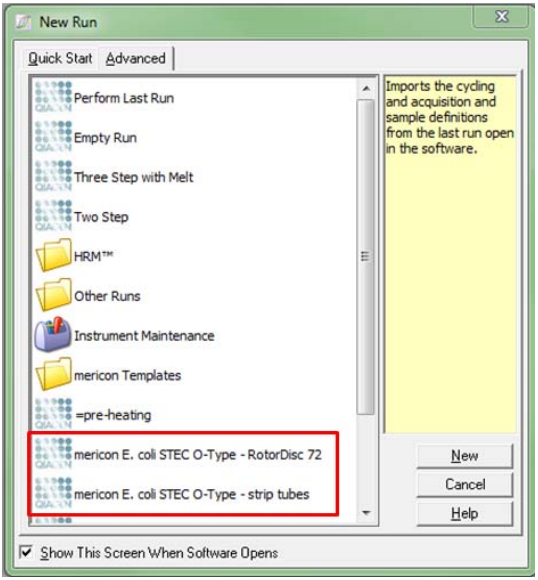
1. **Set up the sample and control reactions according to Table 3. Keep all samples and reaction tubes on ice or a cooled rack during setup.**

   Place the desired number of Strip Tubes or the Rotor-Disc into the appropriate cooled Loading Block for the Rotor-Gene Q.

Table 3. Setup of sample and control reactions

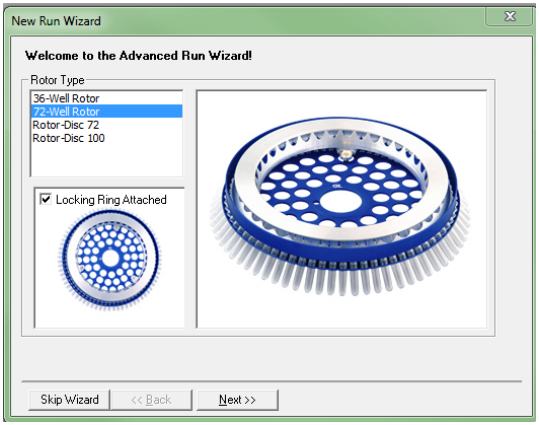
Component	Sample	Negative PCR control
Reconstituted *mericon* Assay	10 µl	10 µl
Sample DNA	10 µl	–
RNase-free water	–	10 µl
Total volume	20 µl	20 µl

2. **Close the Rotor-Disc or Strip Tubes and place them in the appropriate Rotor and attach the locking ring.**
   If you are using tubes, fill the empty positions in the rotor with empty Strip Tubes. Make sure that the locking ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.
3. **Open the Rotor-Gene Software. We recommend using the template file provided. In the Advanced Wizard, select "Open A Template In Another Folder…" and load the file "*mericon* E. coli STEC O-Type".**
   If you copy the template file "*mericon* E. coli STEC O-Type" in the Rotor-Gene Q Templates and in the Quick Start Templates folders, the template will appear directly in the Quick Start and Advanced Wizard windows.
4. **To setup cycling manually, select "Empty Run" and click "New".**
   We recommend using the provided template files to facilitate the reaction setup. When using template files, the settings may already be those described in the next step. In this case, click to the next screen.



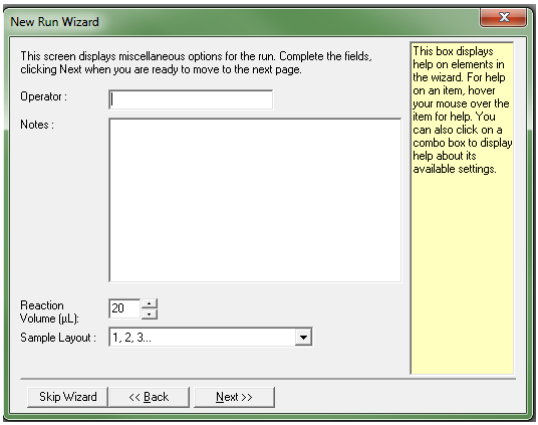
Select the *mericon* E. coli STEC O-Type Assay.

5. **Select the correct rotor and confirm the locking ring is attached by checking the check box. Click "Next" to continue.**



Select the correct rotor and confirm the locking ring is attached.

6. **Ensure that the reaction volume is set to 20 µl.**



Select 20 µl Reaction Volume.

7. **Click "Next" to continue.**
8. **Click "Edit Profile" and program the Rotor-Gene Q according to Table 4.**
   Data acquisition should be performed during the annealing step at 60°C.

Table 4. Cycling protocol for Rotor-Gene Q

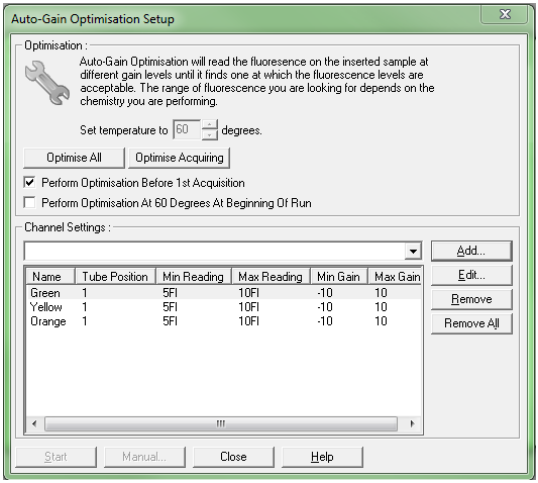
Step	Time	Temperature	Comment
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA Polymerase
3-step cycling			
Denaturation	15 s	95°C	Data collection at 60°C for Green, Yellow, and Orange Channels
Annealing	15 s	60°C	
Extension	10 s	72°C	
Number of cycles	40		
Gain optimization before first acquisition at 60°C for Green, Yellow, and Orange Channels			

9. **Click "OK" to close the window and return to the Wizard.**
10. **To set the gain optimization settings for the Green, Yellow, and Orange Channels click "Gain Optimisation".**

11. Select the 3 channels in the drop-down menu and click "Add".

12. In the dialog box that opens, confirm the standard settings. Click "Perform Optimisation Before 1st Acquisition". Then close the window.

Make sure that the tube at position 1 is not empty, since the gain optimization will be performed on this tube.



Perform Gain Optimization for the 3 channels before 1st acquisition.

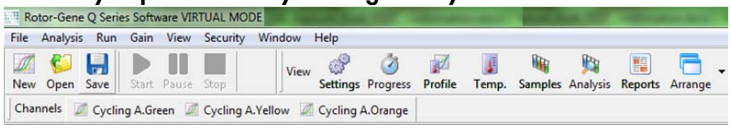
13. Start the PCR run.

Data Analysis on the Rotor-Gene Q

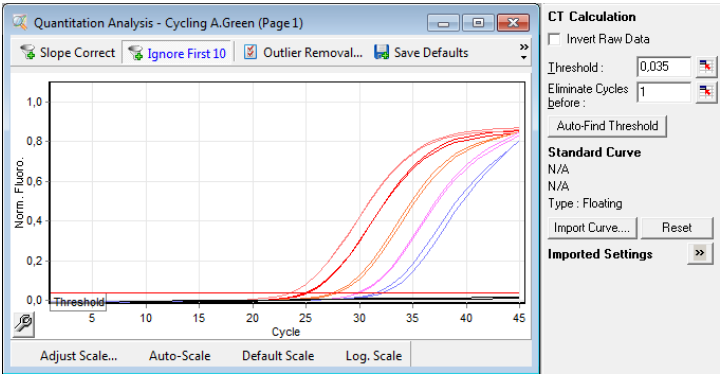
Note: Optimal analysis settings are a prerequisite for accurate real-time PCR data. Always use the following analysis settings.

Procedure

1. **Open the run file using the Rotor-Gene Q Software. Go to "File", followed by "Open", and then "Browse" to locate the saved file.**
2. **Edit analysis parameters by clicking "Analysis".**



3. **Click "Ignore First" to ignore the first 10 cycles when calculating the threshold for all 3 channels. Adjust the take-off point by clicking "Take Off Point Adjustment" and "OK". Do not activate "Slope Correct". Set the threshold for the Green and Yellow Channels to 0.035, and for the Orange Channel to 0.08.**



4. **To export the results to Excel®, go to the "File" menu, followed by "Save As" and "Excel Analysis Sheet". To create a printable report go to "Reports" and create a "Quantitation (Full Report)" for each channel.**

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 5.

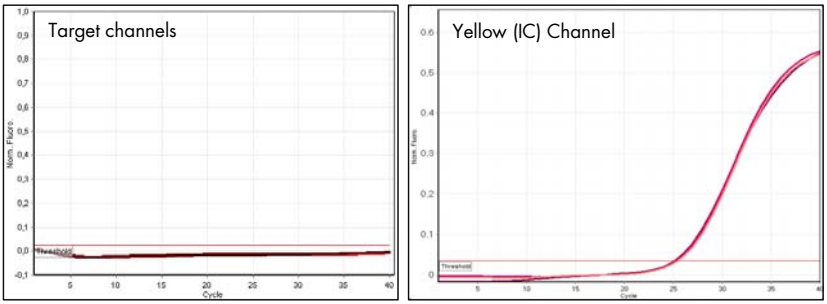


Figure 1. The sample is negative for tested pathogen. The 3 sample curves in the target channels (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the Yellow Channel (right) are above the threshold and have a C_T value of 24–28, indicating that the PCR was successful and not inhibited.

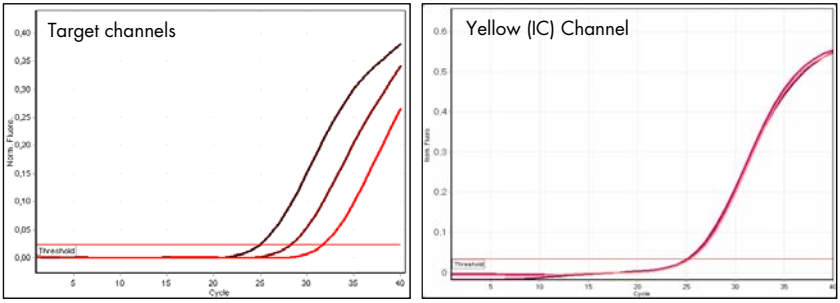


Figure 2. The sample is positive for tested pathogen. The 3 sample curves in the target channel (left) are above a preset threshold indicating the presence of pathogen DNA. The corresponding curves of the internal control in the Yellow Channel (right) are above the threshold and have a C_T value of 24–28, indicating that the PCR was successful and not inhibited.

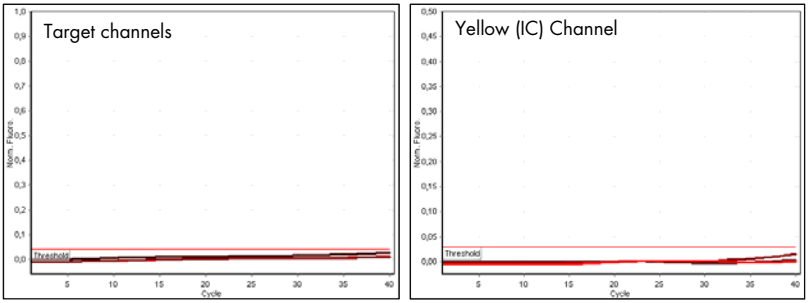


Figure 3. The PCR is inhibited. No amplification of the 3 samples in the target channel (left) or the internal control in the Yellow Channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 5. Summary of possible outcomes

Amplification of sample	Amplification of internal control	Result
C_T 10–38	C_T 24–28	Sample is positive
C_T 38,01–40	C_T 24–28	Sample is indeterminate; repeat test
No C_T	C_T 24–28	Sample is negative
No C_T	C_T ≥28,01 or No C_T	IC invalid, PCR inhibited; dilute sample and repeat test

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 between 24 and 28. A cycle threshold value above 28 indicates inhibition. In the event of PCR inhibition, dilute the extracted samples 1:10 with RNase-free water and repeat the test. If DNA template concentration is very high, a shift of the internal control to lower cycle values might occur. This does not influence its sensitivity toward PCR inhibitors or amplification of the target DNA.

Test Scheme for Presence or Absence of Suspected *E. coli* DNA

This assay detects the presence of *E. coli* O157:H7 and non-O157:H7 serotype (O26, O45, O103, O111, O121, and O145) DNA. This serves to confirm a positive result from the *mericon* E. coli O157 Screen Plus Assay. A confirmed positive food sample cannot be cleared. It might be harmful for the customer and additional testing by authorized laboratories is required. Table 6 indicates the further actions required with each possible combination of test results.

Table 6. Test results and next actions

No.	O157:H7 Orange Channel	non-O157:H7 serotypes Green Channel	IC Yellow Channel	Next action
1	–	–	Valid	No further action
2	–	+	Valid	Additional testing required
3	+	–	Valid	Additional testing required
4	+	+	Valid	Additional testing required
5	–	–	Invalid	Dilute sample and repeat test

Several different test results are possible for the *mericon* E. coli STEC O-Type assay:

1. If the sample curves in the target channels (Green and Orange) are at the baseline and below a preset threshold, the samples are negative for *E. coli* O157:H7 and the non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145). No further action is required.
2. If the sample curve for the *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145) is above a preset threshold and the sample curve for O157:H7 is at the baseline, the test result is positive and additional testing is required.

3. If the sample curve for *E. coli* O157:H7 is above a preset threshold and the sample curve for *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145) is at the baseline, the test result is positive and additional testing is required.
4. If the sample curves for *E. coli* O157:H7 and *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145) are above a preset threshold, the test result is positive and additional testing is required.
5. If IC is invalid, the sample might contain PCR inhibitors. In this case the test needs to be repeated with diluted sample.

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 protocols 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 and Orange Channels for the samples and the Yellow Channel for the internal control. See the cycling protocols in Table 4.
b) Incorrect programming of the real-time PCR instrument	Compare the temperature profile with the protocol. See the cycling protocols in Table 4. Refer to the Rotor-Gene Q user manual.
c) Incorrect configuration of the PCR	Ensure that reactions were set up according to Table 4. 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 4)	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.
Weak or no signal in the amplification internal control	
a) The PCR conditions do not comply with the protocol	Check that PCR conditions match the cycling protocols in Table 4. Repeat the PCR with corrected settings, if necessary.

	Comments and suggestions
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. If there is still inhibition, dilute the DNA 1:10.
c) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 4)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
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 your 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.

Ordering Information

Product	Contents	Cat. no.
mericon E. coli STEC O-Type Kit (24)	For 24 reactions: PCR Assay E. coli STEC O-Type, Multiplex PCR Master Mix, RNase-Free Water	290233
mericon E. coli STEC O-Type Kit (96)	For 96 reactions: PCR Assay E. coli STEC O-Type, Multiplex PCR Master Mix, RNase-Free Water	290235
Related products		
***mericon* pathogen detection assays (duplex real-time PCR Assays)**		
mericon E. coli O157 Screen Plus Kit (24)	For 24 reactions: PCR Assay E. coli O157 Screen Plus, Multiplex PCR Master Mix, RNase-Free Water	290403
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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
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
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

* Larger kit sizes available; please inquire

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
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* sample preparation kits**		
QIAsymphony *mericon* Bacteria Kit (360)	For 360 preparations: 2 Reagent Cartridges, Piercing Lid, TopElute Fluid (60 ml), Reuse Seal Set	931156
mericon DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
mericon DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534

* Larger kit sizes available; please inquire

Product	Contents	Cat. no.
Instruments		
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	9001640

* The *mericon* E. coli O157 Screen Plus Kit is also compatible with Rotor-Gene Q 5plex HRM, and Rotor-Gene Q 6plex.

Notes

Limited License Agreement for *mericon* E. coli STEC O-Type Detection

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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

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