July 2015

mericon® E. coli O157 Screen Plus Kit Handbook

For detection of *Escherichia coli* serotype O157 and the *E. coli* associated virulence genes *eae*, *stx1*, and *stx2*, in food or animal feed samples using real-time PCR on the Rotor-Gene Q

Sample to Insight

QIAGEN

Contents

Kit Contents

***mericon* E. coli O157 Screen Plus Kit**		**(24)**
Catalog no.		**290403**
Number of preps		**24**
Yellow	*mericon* Assay*	2 x 12 reactions
Red	Positive Control	20 reactions
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml
	RNase-free water	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl
Quick-Start Protocol		1

* Contains target-specific primers and probes, as well as the internal control (IC).
† Contains HotStarTaq® Plus DNA Polymerase, dedicated multiplex real-time PCR buffer and dNTP mix (dATP, dCTP, dGTP, dTTP).

Storage

The *mericon* Assays are shipped on dry ice. Multiplex PCR Master Mix should be stored immediately at –30°C to –15°C upon receipt, in a constant-temperature freezer. Unreconstituted *mericon* Assays 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.

Once reconstituted, reagents 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 –30°C to –15°C for long-term storage.

Intended Use

The *mericon* E. coli O157 Screen Plus 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, or other applicable guidelines that have been developed for recombinant DNA experiments.

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.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *mericon* E. coli O157 Screen Plus Kit is tested against predetermined specifications to ensure consistent product quality.

General Precautions for Real-Time PCR Assays

The *mericon* E. coli O157 Screen Plus assay involves DNA detection by PCR. Care must be taken to avoid contamination of the PCR reactions.

It is extremely 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 O157 Screen Plus Assay

The *mericon* E. coli O157 Screen Plus Kit is designed for the qualitative detection of the *Escherichia coli* serotype O157 and *E. coli* virulence genes *intimin* (*eae*) and Shiga-toxin like proteins (*stx1* and *stx2*). The presence of *eae* and *stx1/stx2* suggests that further investigation into the *E. coli* O-serotype O157, or the non-O157 O-serotypes (O26, O45, O103, O111, O121, and O145) is required in food and animal feed samples after enrichment.

Limit of detection

The *mericon* E. coli O157 Screen Plus Kit can detect as few as 10 copies of the respective *Escherichia coli* target gene in a reaction.

Specificity

The *mericon* E. coli O157 Screen Plus Kit exhibits high specificity for *E. coli* strain O157 and the *E. coli* virulence genes *eae*, *stx1*, and *stx2*. The kit was tested against an extensive inclusivity panel of *E. coli* O157 and non-O157 serotype strains and an exclusivity panel of non-pathogenic *E. coli* strains and other non-*E. coli* strains. All tested O157 and non-O157 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 with *mericon* E. coli Screen Plus Kit

Pathogen/gene	Cross-reactivity
eae	+
E. coli O157:H7	+
VTEC* *stx1*	+
VTEC* *stx2*	+
Bacillus cereus	–
Campylobacter jejuni	–
Clostridium perfringens	–
Enterobacter sakazakii	–
Escherichia coli O6	–
Listeria monocytogenes	–
Shigella flexneri	–
Staphylococcus aureus	–
Yersinia enterocolitica	–

* VTEC: Verotoxigenic *E. coli*.

Introduction

The *mericon* E. coli O157 Screen Plus Kit is a ready-to-use system for the detection of specific DNA fragments from pathogenic *Escherichia coli* in food, animal feed, and pharmaceutical products using real-time polymerase chain reaction (PCR). This assay was developed for usage on the Rotor-Gene® Q. The *mericon* E. coli O157 Screen Plus Kit is not compatible with other Real Time cyclers. The Multiplex PCR Master Mix 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 this protocol allows the user to perform analysis in accordance with local official requirements.

Each *mericon* assay is an optimized mixture of PCR primer sets for pathogenic *Escherichia coli* and an internal control (IC), plus probes labeled with four distinct fluorescent dyes (see 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 O157 Screen Plus Assay

	Green channel: FAM™ (495/520 nm)	**Orange channel: ROX™ NHS Ester (588/608 nm)**	**Crimson channel: Cy®5.5 (685/707 nm)**	**Yellow channel: MAX™ NHS Ester (524/557 nm)**
***mericon* E. coli O157 Screen Plus Assay**	*stx1/stx2*	O157	*eae*	Internal control (IC)

Principle and Procedure

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 as few as 10 target copies of each target 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 –30°C to –15°C for up to 6 months. Repeated freezing and thawing should be avoided.

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®).

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 QIAsymphony *mericon* Bacteria Kit (360) (cat. no. 931156).
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Rotor-Gene Q*
- 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)
- Tube rack
- Microcentrifuge*
- Vortex mixer

* 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 6. 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 from food or feed matrix enrichment cultures.

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

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

A positive control might be optionally 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.

Note: The positive control is also suitable for the *mericon* E. coli STEC O-Type Assay (cat. no. 290233)

Protocol: Screening for *E. coli* O157 and Virulence Factors *eae* and *stx1/stx2* by Real-Time PCR on the Rotor-Gene Q

Important points before starting

- Before beginning the procedure, read "Important Notes", page 15.
- 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. A positive control might be included optionally.
- Things to do before starting
- Prepare the *mericon* Assay (tube with yellow lid).
- 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 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 at –30°C to –15°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. 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 should be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

- Download the Rotor-Gene Q template files for starting and analyzing the PCR run. To download the *mericon* E. coli O157 Screen Plus files, go to the Product Resources tab at **www.qiagen.com/mericonEcoliScreenPlus**.

Procedure

1. Set up the sample and control reactions according to Table 3, page 18. 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	Positive PCR control
Reconstituted *mericon* Assay	10 µl	10 µl	10 µl
Sample DNA	10 µl	–	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	10 µl	–
Dissolved Positive Control DNA	–	–	10 µl
Total volume	**20 µl**	**20 µl**	**20 µl**

2. Close the Rotor-Disc or Strip Tubes and place them in the appropriate Rotor, and attach the locking ring.

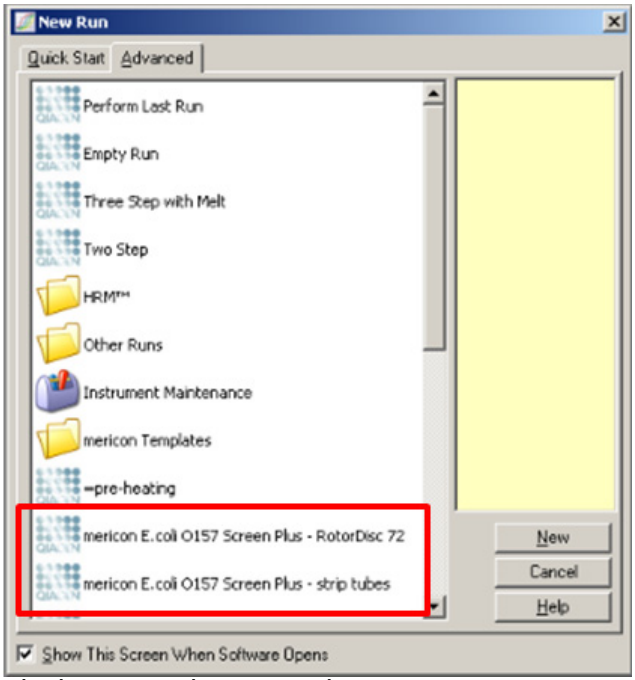
   If you are using tubes, empty positions in the rotor should be filled with empty Strip Tubes. Make sure that the locking ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.

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 O157 Screen Plus".

If you copy the template file "*mericon* E. coli O157 Screen Plus" in the Rotor-Gene Q Templates and in the Quick Start Templates folders, the template will appear directly in the Quick Start and in the Advanced Wizard windows.

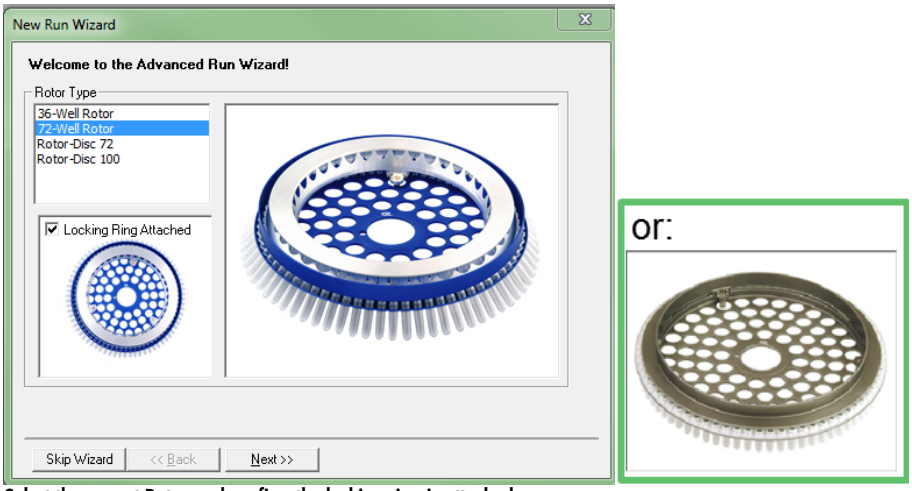
4. To setup cycling manually, select "Empty Run", and click "New".

   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.



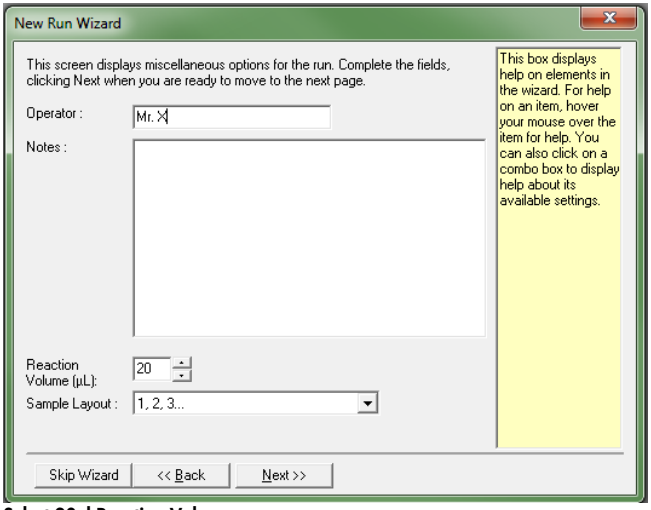
Select the *mericon* E. coli O157 Screen Plus assay.

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 20 µl.



Select 20µl Reaction Volume.

7. Click “Next” to continue.
8. Click “Edit Profile” and program the Rotor-Gene Q according to Table 4.

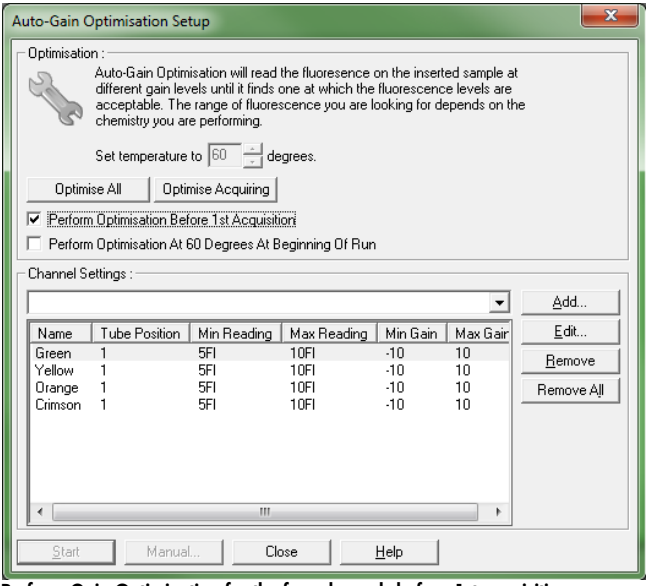
   Data acquisition should be performed during the annealing step at 60°C.

Table 4. Cycling protocol for Rotor-Gene Q

Component	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 for channels green, yellow, orange and crimson
Annealing*	15 s	60°C*	
Extension	10 s	72°C	
Number of cycles	40		
Gain optimization before first acquisition at 60°C for channels green, yellow, orange, and crimson			

9. Click “OK” to close the window and return to the Wizard.
10. To set the gain optimization settings for the green, yellow, orange, and crimson channels, click “Gain Optimisation”.
11. Select the four 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 four channels before 1st acquisition.

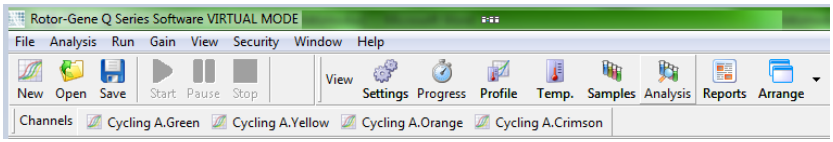
13. Start the PCR run.

Data Analysis

Note: Optimal analysis settings are a prerequisite for accurate real-time PCR data. Always use the following analysis settings. Alternatively, these analysis settings are available at **www.qiagen.com/mericonEcoliScreenPlus** and can be imported into the file.

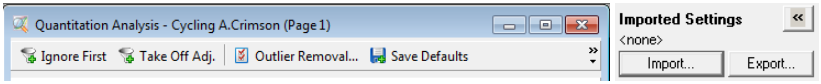
Procedure

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. Click "Analysis" to edit analysis parameters.



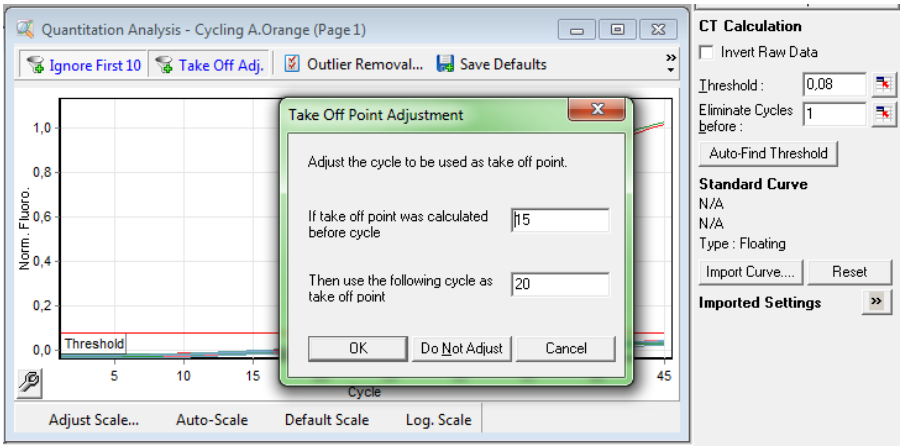
Open the Quantitation Analysis windows under "Analysis".

3. To import the analysis settings to each channel, activate the "Quantitation Analysis" window for the channel and select the respective template file from your directory with the "Import" function.



4. To set up the analysis parameters manually, continue with step 5.
5. Click "Ignore First" and ignore the first 10 cycles for all four channels.
6. For all channels (Green, Crimson, Yellow, and Orange), click "Take Off Point Adjustment".

7. Adjust the settings so that if the take-off point was calculated before cycle 15, then cycle 20 is used as the take-off point. Click "OK".



8. Set the threshold for channels Green, Crimson, and Yellow to 0,035 (or 0.035 depending on language settings).
9. Set the threshold for channel Orange to 0,08 (or 0.08).
10. To export the results to Excel®, go to the "File" menu, followed by "Save As" and then "Excel Analysis Sheet". The results will be saved in *.csv format.

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. Figure 1–3 are examples of possible outcomes, which are summarized in Table 5 (page 26).

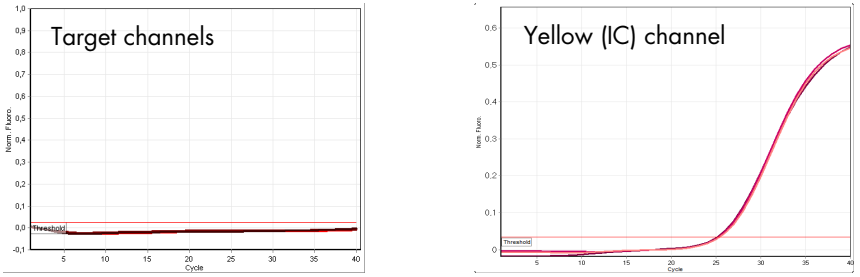


Figure 1. The sample is negative for tested pathogen. The 3 sample curves in the target channels (left) are at the baseline and below a present threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold and have a CT value of 24–28, indicating that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

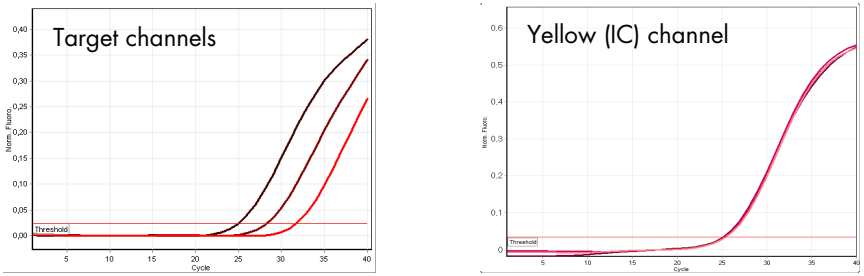


Figure 2. The sample is positive for tested pathogen. The 3 sample curves in the target channel (left) are above a preset threshold indicating the presence of pathogen DNA. The corresponding curves of the internal control in the yellow channel (right) are above the threshold and have a CT value of 24–28,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

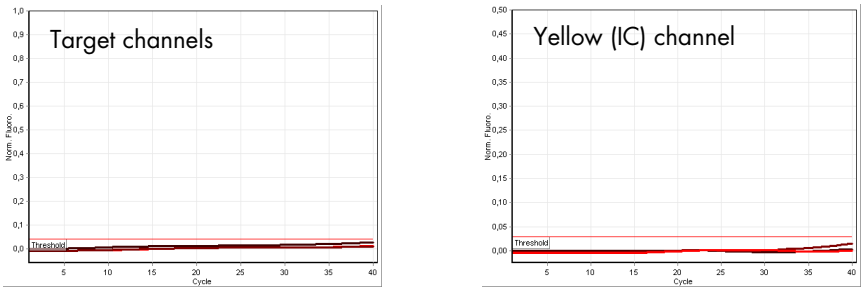


Figure 3. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 5. Summary of possible outcomes

Amplification of sample	Amplification of internal control	Result
C_T <38	C_T 24–30	Sample is positive
C_T 38,01–40	C_T 24–30	Sample is indeterminate; repeat test
No C_T	C_T 24–30	Sample is negative
No C_T	C_T ≥30,01 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 thresholds (CT) values. As a guideline, the uninhibited internal control should give a cycle threshold value ranging between 24 and 30. A cycle threshold value above 30 indicates inhibition.

In the event of a PCR inhibited internal control channel and a positive target results, repeating the test is not necessary.

In the event of a PCR inhibited internal control channel and a negative target result, 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, which does not influence its sensitivity toward PCR inhibitors or amplification of the target DNA.

Test scheme for presence of *E. coli* O157 and virulence factors *eae* and/or *stx1/stx2* DNA

This assay screens for the *E. coli* O157, *eae*, and *stx1/stx2* genes. This serves to identify situations in which virulence factors are present and in which further studies to confirm the presence of the *E. coli* O157:H7 serotype, and the non-O157 serotypes O26, O45, O103, O111, O121, and O145 are required. Table 6 indicates the further actions required with each possible combination of test results.

Table 6. Test results and next actions

No.	Orange O157	Green *stx1/stx2*	Crimson *eae*	Yellow IC	Next action
1	–	–	–	Valid	No further action
2	–	+	–	Valid	No further action
3	–	–	+	Valid	No further action
4	+	–	–	Valid	Additional testing required
5	–	+	+	Valid	Additional testing required
6	+	+	+	Valid	Additional testing required
7	–	–	–	Invalid	Dilute sample and repeat test

Several different test results are possible for the screening assay:

1. If the sample curves in the target channels (green, orange, and crimson) are at the baseline and below a preset threshold, the samples are negative for *E. coli* O157, *eae* and *stx1/stx2*. No further action is required.

2. If the sample curve for the *stx1/stx2* targets is above a preset threshold and the sample curves for *eae* and O157 are at the baseline, the test result is negative for *E. coli* virulence factor DNA.
3. If the sample curve for the *eae* target is above a preset threshold and the sample curves for *stx1/stx2* and O157 are at the baseline, the test result is negative for *E. coli* virulence factor DNA.
4. If the sample curve in the orange channel (O157) is above a preset threshold, this indicates the presence of *E. coli* O157 DNA. As a consequence, additional testing is required.
5. If the sample curves in the green and crimson channels (*stx1/stx2* and *eae*) are above a preset threshold, this indicates the presence of the *E. coli* virulence factor DNA and the likelihood that the samples will be positive for the non-O157 serotypes, O26, O45, O103, O111, O121, and O145. As a consequence, additional testing is required.
6. If the sample curves in the green, orange and crimson channels (*stx1/stx2, O157* and *eae*) are above a preset threshold, this indicates the presence of the *E. coli* virulence factor DNA and the likelihood that the samples will be positive for O157:H7. A co-infection with the non-O157 serotypes, O26, O45, O103, O111, O121 and O145 might be indicated, as well. As a consequence, additional testing is required.
7. 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/or protocols in this handbook or sample and assay technologies (for contact information, 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, orange, and crimson channel for the samples and the yellow channel for the internal control. See the cycling protocols in Table 4 on page 21.
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 manual.
c)	Incorrect configuration of the PCR	Ensure that reactions were set up according to Table 4 on page 21. 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 5)	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 5)	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 O157 Screen Plus Kit (24)	For 24 reactions: PCR Assay E. coli O157 Screen Plus, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water	290403
Related products		
***mericon* Pathogen detection assays (duplex real-time PCR assays)**		
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 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

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.

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

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