

April 2016

Product Insert for the AOAC-RI PTM-certified Automated and Manual *mericon*[®] E. coli O157 Screen Plus and *mericon*[®] E. coli STEC O-type Detection Workflow



mericon[®] E. coli O157 Screen Plus



mericon[®] E. coli STEC O-type



Sample & Assay Technologies

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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.

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Kit Contents

DNA extraction — automated workflow

QIAasymphony® mericon Bacteria Kit	(360)
Catalog no.	931156
Number of reactions	360
Reagent Cartridge*	2
Piercing Lid	2
TopElute Fluid	60 ml
Reuse Seal Set†	2
Product Insert	1
Quick-Start Protocol	1

* Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 8 for safety information.

† A Reuse Seal Set contains 8 Reuse Seal Strips.

DNA extraction — manual workflow

mericon® DNA Bacteria Kit	
Catalog no.	69525
Number of preps	100
Fast Lysis Buffer	2 x 25 ml
Product Insert	1
Quick-Start Protocol	1

Real-time PCR — automated and manual workflows

mericon® E. coli O157 Screen Plus Kit		(24)
Catalog no.		290403
Number of preps		24
Yellow	<i>mericon Assay</i> *	2 x 12 reactions
	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).

mericon® E. coli STEC O-Type Kit		(24)
Catalog no.		290233
Number of reactions		24
Yellow	<i>mericon Assay</i> *	2 x 12 reactions r
	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 and Shelf Life

The QIASymphony *mericon* Bacteria Kit should be stored at room temperature (15–25°C). Do not store the reagent cartridges at temperatures below 15°C. When stored properly, the kit is stable until the expiration date stated on the kit box. Partially used reagent cartridges can be stored for a maximum of 1 month. If a reagent cartridge is partially used, reseal all troughs with the provided Reuse Seal Strips. To avoid reagent evaporation, the reagent cartridge should be open for a maximum of 48 hours (including run times) at ambient temperature. Fast Lysis Buffer should be stored dry at room temperature (15–25°C). Under these conditions, the kit remains stable for 2 years.

The *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type 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 $2-8^{\circ}\text{C}$ 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 at -20°C for long-term storage.

Intended Use

Products for the automated and manual *mericon* Pathogen Detection workflows are intended for molecular biology applications in food, animal feed, water, and pharmaceutical product testing. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to regional guidelines that have been developed for working with pathogens and recombinant DNA.

Intended User

The automated and manual *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type detection workflows are designed to be used by qualified users in microbiology laboratories for the determination of the presence or absence of *E. coli* serotype O157 and the *E. coli* associated virulence genes *eae*, *stx1* and *stx2* (*mericon* E. coli O157 Screen Plus) and the detection of *E. coli* serotype O157:H7 and the *E. coli* non-O157:H7 serotypes O26, O45, O103, O111, O121 and O145 (*mericon* E. coli STEC O-type kit) in selected food products.

Applicability

The *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type detection workflows have been evaluated in an independent laboratory for use with the following food matrices: Ground beef, raw beef trim and spinach. The protocol includes preparation of an enrichment culture, followed by a manual or automated purification of *E. coli* DNA, and real-time PCR assay for presence or absence of pathogen using the *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type detection assays on the Rotor-Gene Q.

Environmental Factors

To allow for optimal real-time PCR detection quality using the Rotor-Gene Q, the instrument should be installed in a temperature-controlled, draft-free laboratory. Temperature should not be below 68°F (20°C) and should not fluctuate during the performance of the PCR assay. If the ambient temperature is below 68°F (20°C), it is recommended to preheat the Rotor-Gene Q at 95°C for 20 minutes before the run.

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 a 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.

Work with samples potentially contaminated with *E. coli* should be performed in laboratories meeting Biosafety Level 2 (BSL2) regulations.



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

The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. Reagents in this kit are highly flammable and are harmful by inhalation, skin contact and swallowing.

For safety information regarding the instruments, see the relevant instrument user manual.

Discard sample and assay waste according to your local safety regulations.

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

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) or on the web: <http://b2b.qiagen.com/about-us/contact/global-contacts/subsidiaries/> or <http://b2b.qiagen.com/about-us/contact/global-contacts/distributors-and-importers/>

Technical Service in North America can be reached at 1-800-362-7737 and at QIAGEN GmbH at +49-2103-29-12400.

Please also refer to the handbooks for the kits and user manuals for the instruments for comprehensive Troubleshooting Guides. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Principle of the assays

QIAGEN's *mericon* E. coli O157 Screen *Plus* and *mericon* E. coli STEC O-type Pathogen Detection assays are multiplex PCR assays that amplify specific DNA fragments from pathogenic *E. coli* in food and an internal control. The internal control provides data regarding the presence of inhibitors in the tested samples and the overall quality of the PCR run.

Each *mericon* Assay is an optimized mixture of PCR primer sets for target sequences specific for pathogenic *E. coli* and an internal control (IC), plus probes labeled with four distinct fluorescent dyes (see Table 1 for the *mericon* E. coli O157 Screen Plus Assay) or three fluorescent dyes (see Table 2 for the *mericon* E. Coli STEC O-type Assay). In addition, each kit includes internal control DNA and all reagents necessary to perform the analysis.

These assays were developed for use on the Rotor-Gene Q. The *mericon* E. coli O157 Screen Plus Kit and *mericon* E. coli STEC O-type are not compatible with other Real Time cyclers. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology, including HotStarTaq plus DNA Polymerase, patented multiplex PCR technology such as Factor MP, and fast cycling technology including Q-bond. [1]

Table 1. 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)
<i>mericon</i> E. coli O157 Screen Plus Assay	<i>stx1/stx2</i>	O157	<i>eae</i>	Internal control (IC)

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)
<i>mericon</i> E. coli STEC O-Type Assay	O26, O45, O103, O111, O121, O145	O157:H7	Internal Control (IC)

Data Analysis

The Rotor-Gene Q cycler produces raw data files that are further interpreted by the software using mathematical algorithms to characterize samples. The software guides the user through each step and provides simplicity for beginners as well as an experimental platform for advanced users. [2]

General Precautions for Real-Time PCR Assays

The *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type pathogen detection assays involve 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 potentially containing pathogens should be autoclaved for 20 min at 120°C prior to disposal.

Equipment and Reagents to Be Supplied by User

Automated Workflow

For the preparation of *E. coli* O157 Screen Plus and *E. coli* STEC O-type food enrichment cultures

- Modified Tryptic Soy Broth (mTSB) with casaminoacids (see MLG Appendix 1.08, p 21, revision 08).
- Lab paddle blender (e.g., Stomacher 3500,, Seward)*
- Filter homogenizer bags (e.g., VWR, cat. no. 129-9874)
- Balance*
- Incubators

- Vortex
- Microcentrifuge
- Precision Pipettors – For sampling and delivering of 10 – 1000 μL
- Dry bath incubator – Capable of maintaining $100 \pm 1^\circ\text{C}$
- Calibrated Thermometer – Capable of measuring $100 \pm 1^\circ\text{C}$

For sample preparation

- QIASymphony SP instrument (cat. no. 9001297)*
- QIASymphony *mericon* Bacteria Kit (cat. no. 931156)

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

Accessories and adapters for the QIASymphony SP

- Reagent Cartridge Holder (2) (cat. no. 997008)
- Insert, 2.0 ml v2, sample carrier. (24), Qsym (cat. no. 9242083)
- Cooling Adapter, EMT, v2, Qsym (cat. no. 9020730)

Consumables for the QIASymphony SP

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Microtubes 2 ml, PP, CB with screw-caps (cat no. 990382)
- Filter-Tips, 1500 μl (cat. no. 997024)
- Elution Microtubes CL with cap strips (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)

For assay set-up

- QIASymphony AS instrument (cat. no. 9001301)*
- *mericon* E. coli O157 Screen Plus (290403) and *mericon* E. coli STEC O-type Kit (Cat no 290233)

Accessories and adapters for the QIASymphony AS

- Cooling Adapter, Reagent Holder 1, Qsym (cat. no. 9018090)

For use with the Rotor-Gene Q 72 Rotor-Disc® (cat. no. 9018899)

- Adapter 2 x Rotor-Disc, Qsym (cat. no. 9242204)
- Rotor-Disc 72 Loading Block (cat. no. 9018910)
- Rotor-Disc 72 (cat. no. 981303 [240]/981301 [24])
- Rotor-Disc Heat Sealing Film (cat. no. 981604 [600]/981601 [60])
- Rotor-Disc Heat Sealer (cat. no. 9018898 [110 V]; cat. no. 9019725 [230 V])
- Rotor-Disc 72 Locking Ring (cat. no. 9018900)

Consumables for the QIA Symphony AS

- Filter-Tips, 200 μ l (cat. no. 990332)
- Filter-Tips, 50 μ l (cat. no. 997120)
- Micro tubes 2 ml, PP, CB with screw-caps (cat.no. 990382)
- Tip disposal bags (cat. no. 9013395)

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

Manual Workflow

For the preparation of *E. coli* O157 Screen Plus and *E. coli* STEC O-type food enrichment cultures (see Table 4 for validated matrix portions and culture volumes)

- Ground Beef, Raw Beef Trim, Spinach (modified Tryptic Soy Broth (mTSB) with Casaminoacids - see MLG Appendix 1.08, p 21, revision 08.)
- Lab paddle blender (e.g., Stomacher 3500, Seward)*
- Filter homogenizer bags (e.g., VWR, cat. no. 129-9874)
- Balance*
- Incubators
- Vortex
- Microcentrifuge
- Precision Pipettors – For sampling and delivering of 10 – 1000 μ L
- Dry bath incubator – Capable of maintaining $100 \pm 1^\circ\text{C}$
- Calibrated Thermometer – Capable of measuring $100 \pm 1^\circ\text{C}$

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

For sample preparation

- *mericon* DNA Bacteria Kit (cat. no. 69525)
- Vortexer
- SafeSeal Micro tubes 2 ml (Sarstedt, cat. no. 72.695) or microcentrifuge tubes with screw caps (2 ml)
- Microcentrifuge with rotor for 1.5 ml or 2 ml tubes
- Thermomixer* or heating block* suitable for 1.5 or 2 ml tubes and capable of attaining a temperature of 100°C. Alternatively, a water bath may be used.
- Pipets and pipet tips

For assay set-up

- Pipets and filter pipet tips

For use with the Rotor-Gene Q 72-Well Rotor (cat. no. 9018903)

- Loading Block, RG Strip Tubes 72, Qsym (cat. no. 9018092)
- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Locking Ring 72-Well Rotor (cat. no. 9018904)

Real-Time PCR

- Rotor-Gene Q 5-plex instrument (e.g., cat. no. 9001640)*
- Rotor-Gene Q software version 2.3 with assay package 3.0.4

Specifications of the AOAC-RI PTM-Certified *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type Detection Workflows

The automated and manual *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type workflows are being submitted for AOAC-RI PTM Certification. The specifications for these workflows can be found in Table 3, Table 4, and Table 5. For PCR assay setup, elution volumes of the automated workflow, and eluate dilutions of the manual workflow, are given in Table 6.

Table 3. Overview of the specifications

AOAC-RI PTM-certified specification	Details
Target	<i>E. coli</i> strains containing virulence factors <i>stx1/stx2</i> , <i>eae</i> , and/or O157 (<i>E. coli</i> O157 Screen Plus assay) and <i>E. coli</i> with STEC O-type O157:H7 or the non-O157 STEC O-types O26, O45, O103, O111, O121, or O145 (<i>E. coli</i> STEC O-type assay).
DNA extraction Kit	Automated workflow: QIAsymphony <i>mericon</i> Bacteria Kit Manual workflow: <i>mericon</i> DNA Bacteria Kit
Real-time PCR assay	<i>mericon</i> E. coli O157 Screen Plus and <i>mericon</i> E. coli STEC O-type Kit
Enrichment broth	modified Tryptic Soy Broth (mTSB) with casaminoacids (prewarmed)
Enrichment temperature	42 ± 1°C
Enrichment time	10 ± 1 hours
Homogenizer bag	Filter bag
Sample matrices	Ground Beef Raw Beef Trim Spinach

Table 4. Validated matrix portions and culture volumes

Matrix	Sample amount	Culture volume
Ground Beef	325 g	975 ml
Raw Beef Trim	325 g	975 ml
Spinach	25 g	225 ml

Table 5. Limit of detection

Workflow segment	Limit of detection*
Overall automated and manual workflow	10 ³ cfu/ml
<i>mericon</i> E. coli O157 Screen Plus and <i>mericon</i> E. coli STEC O-type kit	10 copies/reaction

*Limit of Detection is derived from method development studies

Table 6. Sample volumes for *mericon* assay setup

Matrix	Manual workflow eluate dilution	Automated workflow elution volume
Ground Beef	1:10	400 μ l
Raw Beef Trim	1:10	400 μ l
Spinach	1:10	400 μ l

Confirmation of Positive Results:

All positive *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type results are presumptive positives and require confirmation by a reference method starting with the secondary selective enrichment. Appropriate standard methods for the determination of *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type include USDA/FSIS MLG 5.

Protocol: Preparation of *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type Enrichment Cultures – Food Matrices

Procedure

1. Add 25 g (325 g) of the potentially contaminated food sample to a filter homogenizer bag and add 225 ml (975 ml) pre-warmed mTSB plus casamino acids (see Table 4).
2. Homogenize the food sample using a lab paddle blender at 230 rpm for 1.5 min \pm 10 sec (spinach) or 2.0 min \pm 10 sec for raw ground beef or raw beef trim. Then, seal the homogenizer bag and incubate the homogenate at $42 \pm 1^\circ\text{C}$ for 10 ± 1 hour.
3. **Automated workflow:** After incubation of the enrichment culture, dispense 500 μl aliquots into 2 ml microtubes and start the automated QIASymphony DNA extraction protocol.
Manual workflow: After incubation of the enrichment culture, dispense 1 ml aliquots into 2 ml SafeSeal or screw cap tubes and start the manual DNA extraction protocol.

Automated Workflow

Protocol: Automated isolation of bacterial DNA on the QIASymphony SP

Procedure

1. Close all the drawers and hoods of the QIASymphony SP/AS instrument.
2. Switch on the instrument and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.
3. Log in to the instrument.
4. Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag, if necessary.
5. Load the required elution rack into the "Eluate" drawer and perform an inventory scan of the "Eluate" drawer.
6. Load the required reagent cartridge(s) and consumables into the "Reagents and Consumables" drawer.
7. Press the "R+C" button in the touchscreen to open the screen that shows the consumables status ("Consumables/8-RodCovers/Tubes/Filter-Tips/Reagent Cartridges"). Press the "Scan Bottle" button to scan the bar code of the TopElute bottle with the handheld bar code scanner. Press the "OK" button.
8. Perform an inventory scan of the "Reagents and Consumables" drawer.
9. Place the samples into the appropriate tube carrier and load them into the "Sample" drawer.
10. Using the touchscreen, enter the required information for each batch of samples to be processed.
11. Choose elution volumes according to Table 7.

Table 7. Elution volumes for *mericon* assay setup

Matrix	Elution volume
Ground Beef	400 μ l
Raw Beef Trim	400 μ l
Spinach	400 μ l

12. Press the “Run” button to start the purification procedure.
13. When sample processing is complete, perform a direct transfer of the elution rack to the QIASymphony AS via the transfer module (integrated operation). Press “Transfer” to transfer the elution rack from slot 1 of the QIASymphony SP to slot 2 of the QIASymphony AS.
14. If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips immediately after the end of the last protocol run to avoid evaporation.
15. Discard used sample tubes, plates, and waste according to your local safety regulations and replace the tip disposal bag.
16. Close the instrument drawers, and proceed with assay setup on the QIASymphony AS (page 19).
17. Clean the QIASymphony SP during the assay setup on the QIASymphony AS, or later.

Note: For daily maintenance, remove the waste bottle, tip park station, tip chute, tip guards, and magnetic-head guards and soak these in a glyoxal and quaternary ammonium salt-based disinfectant (e.g., gigasept[®] instru AF) for at least 15 minutes. Rinse with water and wipe dry with paper towels. Wipe the QIASymphony SP worktable and touch screen with an ethanol-based disinfectant (e.g., mikrozyd[®]) then wipe with a damp cloth and dry with a paper towel. For more information, please refer to the QIASymphony Instrument User Manuals.

Protocol: Assay setup on the QIASymphony AS

Things to do before starting

- **24 sample kit:** Add 130 μ l Multiplex PCR Master Mix (tube[s] with blue lid) to each vial of *mericon* Assay (yellow lid). Transfer the reconstituted *mericon* Assay to a labeled, fresh 2 ml microtube. Mix by pipetting up and down 5 times or by quick vortexing, and centrifuge briefly

Procedure

1. Insert the tip chute into its position on the right hand side in the front part of the QIASymphony AS module.
2. Install an empty tip disposal bag in the bag holder under the “Assays” drawer.
3. Switch user interface from sample preparation to assay setup.
4. Start the assay definition process.
5. For integrated operation (elution rack is automatically transferred from the QIASymphony SP into the AS module) the “Sample Rack(s)” screen will appear directly.

6. All stored sample information (sample status, sample ID, sample volume, and rack ID) is transferred to the QIASymphony AS module together with the elution rack and will automatically complete the required information in the "Sample Rack(s)" screen of the assay setup user interface.
7. If the assay setup is independent from a former QIASymphony SP run, select the rack file of the corresponding QIASymphony SP run or select the rack type of your elution rack for the highlighted "Sample" position (slot 2) and then either manually type in the "Rack ID" of the elution rack or choose "Automatic ID" for a new ID.
8. In the "Sample Rack Layout" screen of the assay setup user interface, the elution rack in slot 2 is pictured.

For integrated operation, or for independent operation in combination with a loaded rack file, sample IDs and sample volumes are automatically assigned to the corresponding positions.

For independent operation without a rack file, select the positions to be processed from the elution rack. Define the highlighted positions as "Sample" then reselect the defined samples and assign sample volumes.

9. In the "Assay Selection" screen, select the Assay Parameter Set(s) to use in the run.
10. In the "Assay Assignment" screen, assign the Assay Parameter Sets to samples.
11. In the "Assay Rack(s)" screen, define the assay rack ID. Either type in the assay rack ID manually or choose "Automatic ID" for a new ID.
12. The cooling of samples and reagents will start automatically. Check the temperature of the cooling positions.
13. The "Loading Information" screen displays the working table of the QIASymphony AS module with all previously defined sample and reagent rack types in the designated positions. The required position of the PCR reaction adapter is displayed as well as information on the required filter-tip types and number.
14. Place the reconstituted *mericon* Assay(s), the reconstituted Positive Control(s) and the Negative Control(s), without lids, into the appropriate positions of the precooled reagent adapters.
15. Open the "Eluate and Reagents" and "Assays" drawers.
16. Load the prepared reagent adapter into slot 3 of the "Eluate and Reagents" drawer according to the illustration in the "Loading Information" screen. Place the Rotor-Disc in the appropriate adapter and load the adapter into the designated slot of the "Assays" drawer.

17. Load disposable filter-tips into the "Eluate and Reagents" and "Assays" drawers, according to the required number of each tip type.
18. Close the "Eluate and Reagents" and "Assays" drawers.
19. Upon closing each drawer, press "Yes" to start the inventory scan for each drawer.
20. Press "Queue". Monitoring of the cooling starts.
21. Press "Run" to start the run.
22. After the run is finished, press "Remove" in the assay setup "Overview" screen. Open the "Assays" drawer and unload the PCR assay adapter.
23. Download the result and cycler files via the QIASymphony Management Console (QMC).
24. Proceed to "Protocol: PCR and data analysis on the Rotor-Gene Q", page 23.
25. Perform the regular maintenance/cleaning of the QIASymphony AS during the PCR run on the Rotor-Gene Q, or later.

For more information about regular cleaning procedures, please refer to the QIASymphony Instrument User Manuals.

Manual Workflow

Protocol: Manual isolation of DNA using the *mericon* DNA Bacteria Kit

Things to do before starting

- Prewarm a Thermomixer or heating block to 100°C for use in step 4.

Procedure

1. Pipet 1 ml enrichment culture into a 2 ml microcentrifuge SafeSeal or screw-cap tube (not supplied) and centrifuge at 13,000 x g for 5 minutes.
2. Discard the supernatant using a pipet, taking care to not disrupt the pellet.
3. Add 200 µl Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vortexing.
4. Place the microcentrifuge tube into a heating block or thermal shaker (800 rpm) set to 100 ± 1°C. Heat the sample for 10 min ± 10 sec.
5. Remove the sample and allow it to cool to room temperature (15–24°C) for 2 min ± 10 sec..
6. Centrifuge the tube at 13,000 x g for 5 minutes.

7. Transfer 100 μ l of the supernatant to a fresh 1.5 ml microcentrifuge tube. For the PCR reaction, use an aliquot of the collected supernatant diluted according to Table 8.

Table 8. Sample volumes for *mericon* manual assay setup

Matrix	DNA dilution
Ground Beef	1:10
Raw Beef Trim	1:10
Spinach	1:10

Protocol: Manual assay setup

Things to do before starting

- Please refer to “General Precautions for Real-Time PCR Assays”, page 11.
- PCR loading block should be stored refrigerated to ensure that PCR setup is performed under stable thermal conditions.

24 sample kit: Add 130 μ l Multiplex PCR Master Mix (tube[s] 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

Procedure

1. Place the desired number of PCR 72-well strip tubes into the adapters of the cooling block for the Rotor-Gene Q.
2. Set up the sample and control reactions according to Table 9.
3. Add reconstituted assay to the tubes first, then add the Sample DNA or controls.

Table 9. Setup of sample and control reactions

Component	Sample	Negative PCR control
Reconstituted <i>mericon</i> Assay	10 µl	10 µl
Sample DNA	10 µl	–
RNase-free water	–	10 µl
Total volume	20 µl	20 µl

Real-Time PCR

Protocol: PCR and data analysis on the Rotor-Gene Q using the open mode software

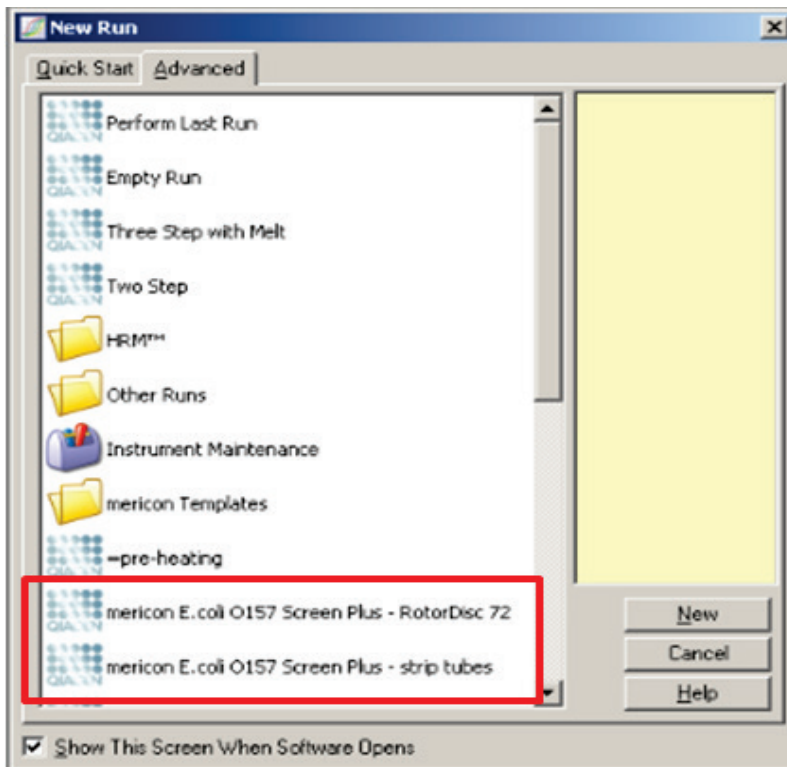
Procedure

- 1. Seal the Rotor-Disc after automated PCR setup, or close the strip tubes after manual PCR setup. Place Rotor-Disc or strip tubes in the respective rotor and make sure to apply the locking ring. If you are using tubes, fill the empty positions in the rotor with empty Strip Tubes. Place the rotor in the reaction chamber of the Rotor-Gene Q.**
- 2. 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 files “*mericon* E. coli O157 Screen Plus” and “*mericon* E. coli STEC O-Type”.**

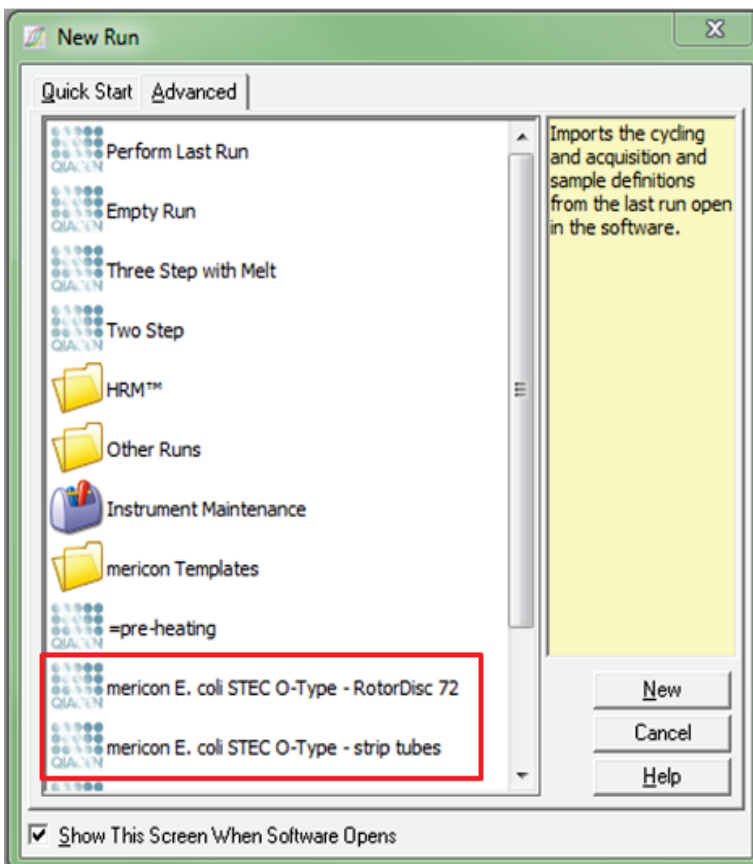
If you copy the template files “*mericon* E. coli O157 Screen Plus” and “*mericon* E. coli STEC O-Type” in the Rotor-Gene Q Templates and in the Quick Start Templates folders, the template will appear directly in the Quick Start and Advanced Wizard windows.

- 3. 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

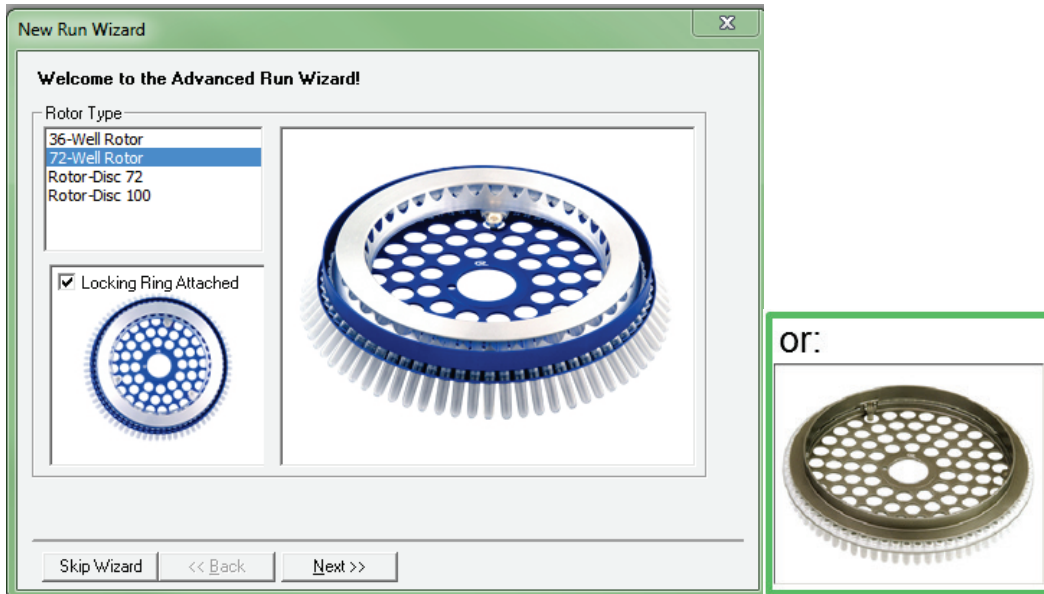


Select the *mericon E. coli* O157 Screen Plus assay.

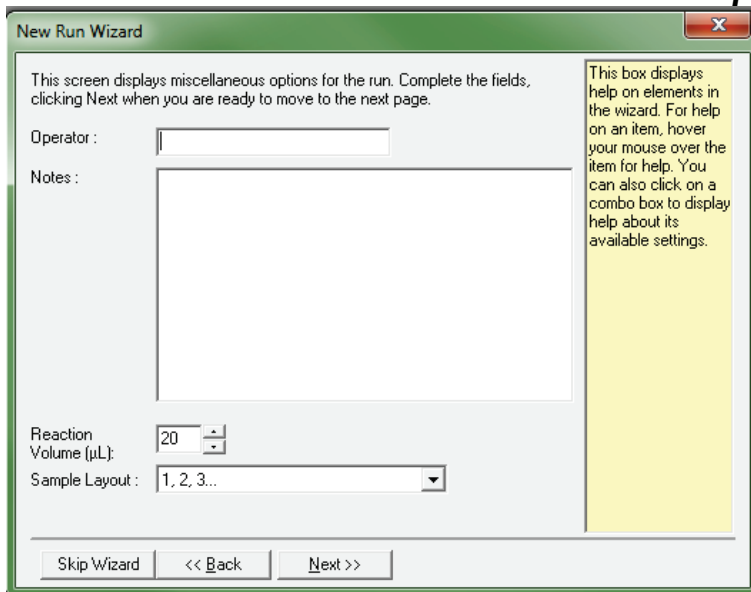


Select the *mericon E. coli* STEC O-type assay.

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



5. Ensure that the reaction volume is set to 20 μ l.



6. Click "Next" to continue.
7. Click "Edit Profile" and program the Rotor-Gene Q according to Table 10.

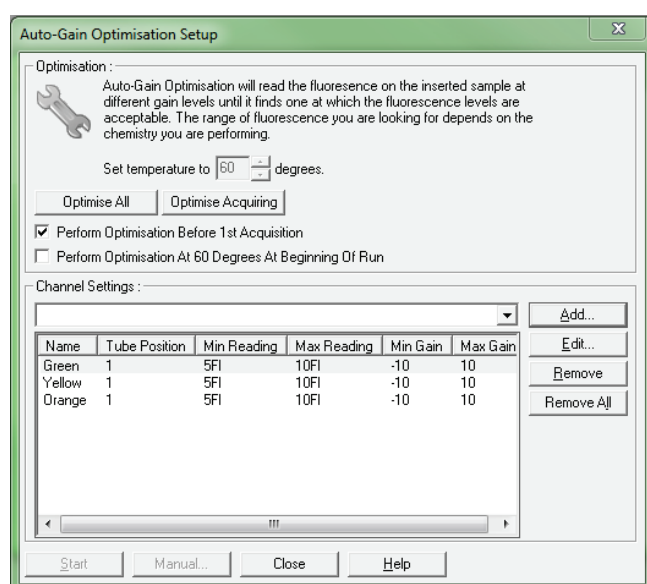
Data acquisition should be performed during the annealing step at 60°C.

Table 10. Cycling protocol for Rotor-Gene Q

Step	Time	Temperature	Comment
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 for green, crimson, orange and yellow 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, crimson, orange and yellow channels			

8. Click "OK" to close the window and return to the Wizard.
9. To set the gain optimization settings for the Green, Yellow, and Orange Channels click "Gain Optimisation".
10. Select the 3 channels in the drop-down menu and click "Add".
11. 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.



12. Start the PCR run.

Data Analysis on the Rotor-Gene Q

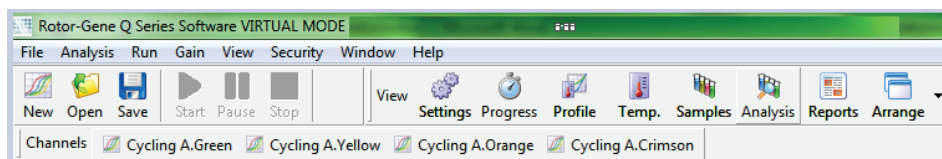
Note: Optimal analysis settings are a prerequisite for accurate real-time PCR data. Always use the following analysis settings.

Procedure

Open the run file using the Rotor-Gene Q Software. Go to “File”, followed by “Open”, and then “Browse” to locate the saved file.

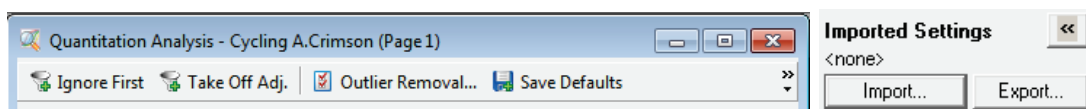
mericon E. Coli O157 Screen Plus:

1. Click “Analysis” to edit analysis parameters.



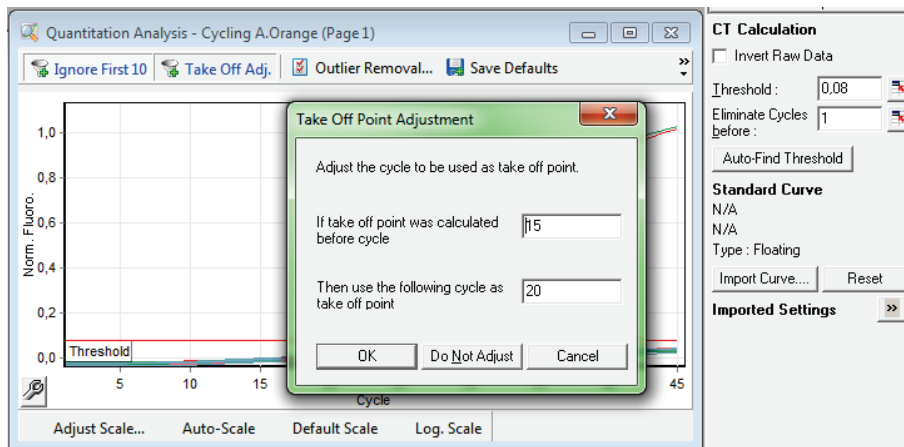
Open the Quantitation Analysis windows under “Analysis”.

2. 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.



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

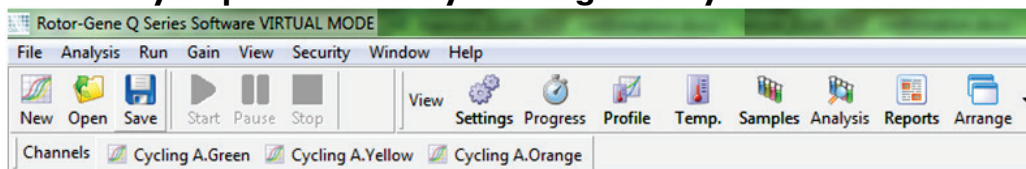
- Adjust the settings so that if the take off point was calculated before cycle 15, then cycle 20 is used as the take off point. Click "OK".



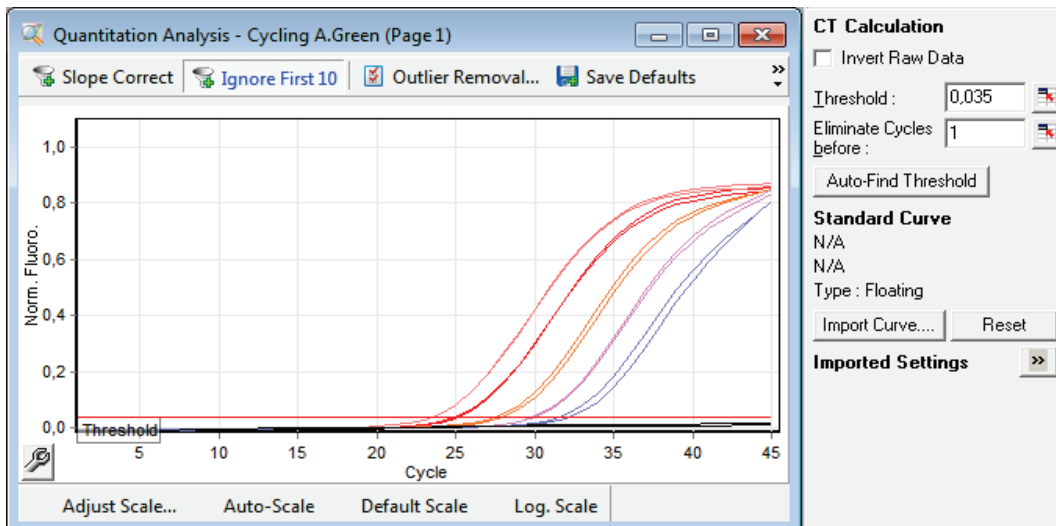
- Set the threshold for channels Green, Crimson, and Yellow to 0.035.
- Set the threshold for channel Orange to 0.08.
- To export the results to Excel®, go to the "File" menu, followed by "Save As" and then "Excel Analysis Sheet". The results will be saved in *.csv format.

For *mericon* E. coli STEC O-type:

- Edit analysis parameters by clicking "Analysis".



- 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



3. 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 11, (page 31).

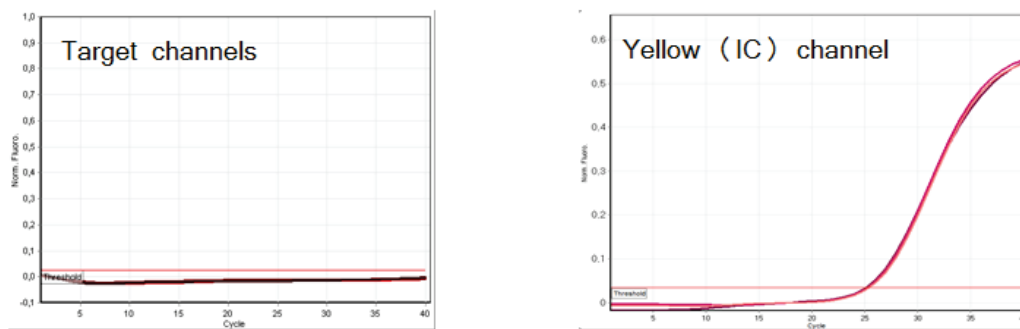


Figure 1. The sample is negative for tested pathogen. The 3 sample curves in the target channels (left) are at the baseline and below a present threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold and have a CT value of 24–30, indicating that the PCR was successful and not inhibited.

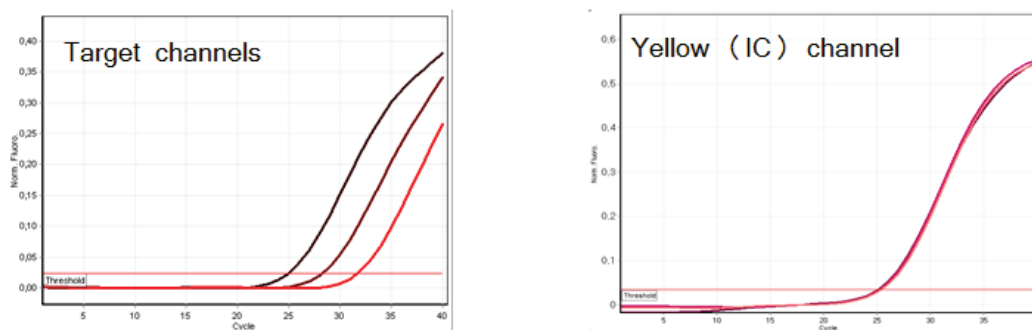


Figure 2. The sample is positive for tested pathogen. The 3 sample curves in the target channel (left) are above a preset threshold indicating the presence of pathogen DNA. The corresponding curves of the internal control in the yellow channel (right) are above the threshold and have a CT value of 24–30, indicating that the PCR was successful and not inhibited.

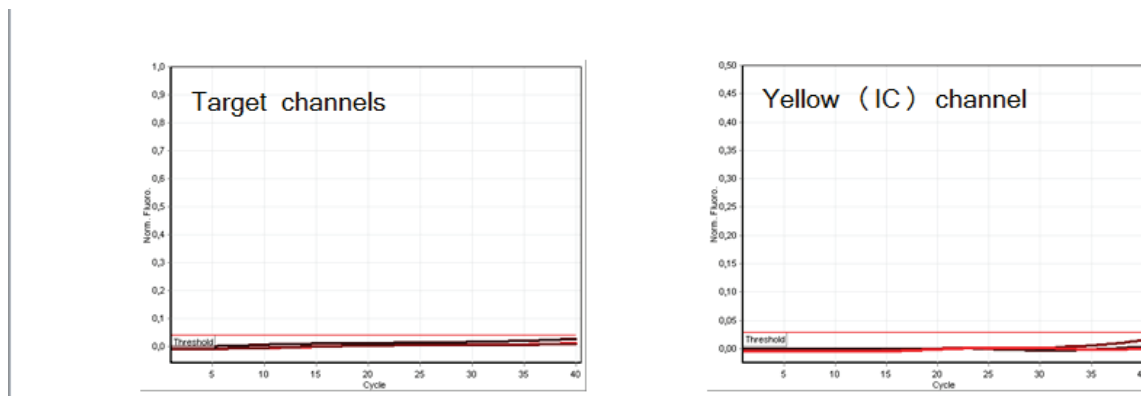


Figure 3. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 11. Summary of possible outcomes

Sample	Internal Control (IC)	Result
$C_T < 38$	$C_T 24-30$	Sample is positive (+)
$C_T < 38$	$C_T \geq 30.01$ Or No C_T	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	$C_T \geq 30.01$ 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 C_T values. As a guideline, the uninhibited internal control should give a C_T value ranging between 24 and 30. A cycle threshold above 30 indicates potential inhibition.

In the event of a PCR inhibited Internal Control ($C_T \geq 30.01$ or No C_T) and a positive sample result, repeating the test is not necessary. In the event of a PCR inhibited Internal Control ($C_T \geq 30.01$ or No C_T) and a negative or indeterminate sample result, dilute the extracted sample 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

The *mericon* *E. coli* O157 Screen Plus 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 12 indicates the further actions required with each possible combination of test results.

Table 12. Test results and next actions

No.	Orange O157	Green <i>stx1/stx2</i>	Crimson <i>eae</i>	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* 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 or the non-O157 serotypes, O26, O45, O103, O111, O121, and O145. 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.

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

The *mericon* *E. coli* STEC O-type 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 13 indicates the further actions required with each possible combination of test results.

Table 13. 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 <http://b2b.qiagen.com/knowledge-and-support/troubleshooting-and-support/>. 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, orange, and crimson channel for the samples and the yellow channel for the internal control. See the cycling protocols in Table 8, page 25. |
| b) Incorrect programming of the real-time PCR instrument | Compare the temperature profile with the protocol. See the cycling protocols in Table 8, page 25. Refer to the Rotor-Gene Q manual. |
| c) Incorrect configuration of the PCR | Ensure that reactions were set up according to Table 8, page 25. 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 7) | 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. |

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 8, page 25. Repeat PCR with corrected settings, if necessary. |
| b) The PCR was inhibited | Use the recommended DNA isolation method in this workflow. If there is inhibition, dilute DNA sample and repeat PCR |

Comments and suggestions

- | | |
|---|---|
| c) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 7) | 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 the 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 the work space and instruments are decontaminated at regular intervals. |

References

- [1] QIAGEN GmbH: *mericon*[®] Automated Pathogen Detection Workflow Handbook, March 2012. <http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=c2df0606-cae7-4041-b618-541989a03de3&lang=en>
- [2] QIAGEN GmbH . *QIASymphony*[®] SP/AS Handbook User Manual May 2013. <http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=363d44fa-0560-4b0c-8aab-96c25b30cf39&lang=en>

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