

## ***mericon*<sup>®</sup> E. coli STEC O-Type Kit for Rotor-Gene<sup>®</sup> Q**

Multiplex PCR Master Mix should be stored immediately upon receipt at  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$ , in a constant-temperature freezer. All other components should be stored at  $2-8^{\circ}\text{C}$  and protected from light. Reconstituted PCR Assay 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  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  for long-term storage.

### **Further information**

- *mericon* E. coli *STEC O-Type Kit Handbook*: [www.qiagen.com/handbooks](http://www.qiagen.com/handbooks)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: USA 800-362-7737, Germany +49 2103-29-12400, or <http://www.qiagen.com/support/technical-support/contact-technical-service/>

### **Notes before starting**

- Use gloves and sterile pipet tips with filters.
- Ensure that at least one negative control is included per PCR run.
- PCR tubes should be kept on ice until they are placed in the thermal cycler.
- Prepare the *mericon* Assay (tubes(s) with yellow lid). See the *mericon* E. coli *STEC O-Type Kit Handbook* for more information.
- Before each use, all reagents must be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

### **Procedure**

1. Set up the sample and control reactions according to Table 1. Keep all samples and reaction tubes on ice during setup. Place the desired number of PCR tubes or strips into the cooled Loading Block for the Rotor-Gene Q.

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**Table 1. Setup for 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

2. Close the Rotor-Disc® or Strip Tubes, 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.
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*.
4. To set up cycling manually, program the Rotor-Gene Q according to Table 2.

**Table 2. Cycling protocol for Rotor-Gene Q**

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq® Plus DNA Polymerase
3-step cycling			
Denaturation	15 s	95°C	Data collection at 60°C 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			

5. Start the PCR run.

## Data analysis

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 for all 3 channels and adjust the take-off point by clicking **Take Off Point Adjustment** and **OK**. Do not activate **Slope Correct**. Set the threshold for Green and Yellow Channels to 0.035, and for the Orange Channel to 0.08.
4. To export the results to Excel®, go to **File**, followed by **Save As**, and then **Excel Analysis Sheet**. To create a printable report go to **Reports** and create a **Quantitation (Full Report)** for each channel.

## Interpretation of 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 Rotor-Gene Q Software. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold. Possible outcomes are shown in Table 3.

**Table 3. Possible outcomes**

Amplification of samples	Amplification of internal controls	Results
$C_T$ 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 $C_T$	$C_T \geq 28.01$ or No $C_T$	IC invalid, PCR inhibited; dilute sample and repeat test

## Test scheme for presence of *E. coli* O157:H7 serotype and non-O157:H7 serotype (O26, O45, O103, O111, O121, and O145) 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 4 indicates the further actions required with each possible combination of test results.

**Table 4. Test results and next actions**

No.	O157:H7	Non-O157:H7		Next action
	Orange Channel	serotypes Green Channel	IC Yellow Channel	
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

1. The sample is negative for *E. coli* O157:H7 and the non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145).
2. The test result is positive for the *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145). Additional testing is required.
3. The test result is positive for *E. coli* O157:H7. Additional testing is required.
4. The test result is positive for *E. coli* O157:H7 and the non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145). Additional testing is required.
5. PCR inhibitors may be present. Repeat with diluted sample.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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