

# *mericon*<sup>®</sup> *E. coli* O157 Screen Plus Kit for the Rotor-Gene<sup>®</sup> Q

Multiplex PCR Master Mix should be stored immediately at  $-20^{\circ}\text{C}$  upon receipt, 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  $-20^{\circ}\text{C}$  for long-term storage.

## Further information

- *mericon E. coli O157 Screen Plus 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: toll-free 00800-22-44-6000, or [www.qiagen.com/contact](http://www.qiagen.com/contact)

## Notes before starting

- Use gloves as well as sterile pipet tips with filters.
- Ensure that at least one negative control is included per PCR run. A positive control can be included, optionally.
- PCR tubes should be kept on ice until they are placed in the thermal cycler.
- Prepare the *mericon* Assay (tube[s] with yellow lid) and dried Positive Control DNA (tube with red lid). See the *mericon E. coli O157 Screen Plus 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.

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.

**Table 1. Setup of sample and control reactions**

Component	Sample	Negative PCR control	Positive PCR control
Reconstituted <i>mericon</i> 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
<b>Total volume</b>	<b>20 µl</b>	<b>20 µl</b>	<b>20 µl</b>

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.
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".
4. To setup cycling manually, program the Rotor-Gene Q according to Table 2.

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

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq® Plus DNA Polymerase
<b>3-step cycling</b>			
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			

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** and ignore the first 10 cycles for all four channels. For all channels (Green, Crimson, Yellow and Orange).
4. 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**.
5. Set the threshold for channels Green, Crimson and Yellow to **0.035** and for channel Orange to **0.08**.
6. To export the results to Excel®, go to File, followed by "Save As" and then "Excel Analysis Sheet". The results will be saved in \*.csv format

## 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 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 $C_T$	$C_T > 30$ or No $C_T$	IC invalid, PCR inhibited; dilute sample and repeat test

## 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 serotype, *eae*, *stx1*, and *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 4 indicates the further actions required with each possible combination of test results.

**Table 4. 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

1. The samples are negative for *E. coli* O157, *eae*, and *stx1/stx2*.
2. The test result is negative for *E. coli* virulence factor DNA.
3. The test result is negative for *E. coli* virulence factor DNA.
4. *E. coli* O157 DNA is present. Additional testing is required.
5. *E. coli* virulence factor DNA is present. Additional testing is required.
6. *E. coli* virulence factor DNA is present. Additional testing is required.
7. PCR inhibitors may be present. Repeat with diluted sample.