

foodproof®

E. coli O157 Detection Kit

Ready Reference Guide

Revision A, December 2023

Product No. KIT230042

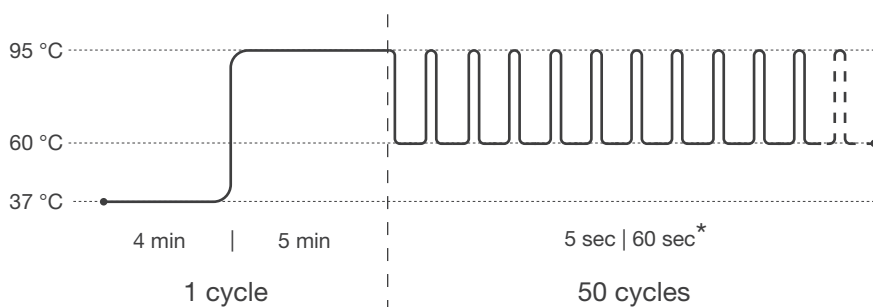
PCR kit for the qualitative detection of *Escherichia coli* O157 DNA using real-time PCR instruments. Before starting, it is strongly recommended to read the entire product manual available on our website.

PROGRAM SETUP

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

- ▶ FAM (*E. coli* O157) and VIC (Internal Control).

As an alternative to VIC, HEX can be used. For the PikoReal® 24, Yakima Yellow must be selected.



Pre-incubation: 1 cycle

Step 1: 37 °C for 4 min

Step 2: 95 °C for 5 min

Amplification: 50 cycles

Step 1 : 95 °C for 5 sec

Step 2*: 60 °C for 60 sec

* Fluorescence detection

For some real-time PCR instruments the probe quencher as well as the use of a passive reference dye must be specified. This kit contains probes with TAMRA as quencher and no passive reference dye.

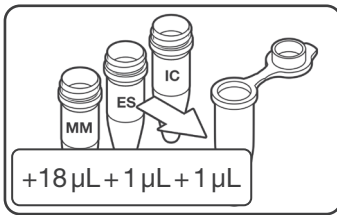
DATA INTERPRETATION

Verify results of positive (Control Template) and negative (H₂O) controls, before interpreting the sample results. Always compare samples to positive and negative controls. Review data from each channel and interpret results as described in the table.

FAM	VIC	Result Interpretation
+	+ or -	Positive for <i>E. coli</i> O157
-	+	Negative for <i>E. coli</i> O157
-	-	Invalid

PREPARATION OF THE PCR MIX

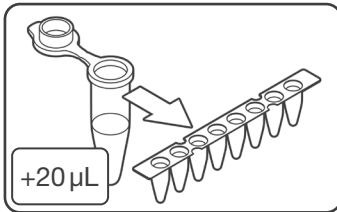
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves. Thaw reagents, mix (do not vortex!) and briefly spin vials before opening.



1. PREPARE PCR MIX

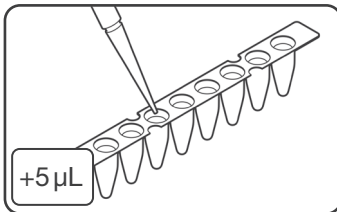
Add 18 µL of Master Mix (yellow cap), 1 µL of Enzyme Solution (red cap) and 1 µL of Internal Control (white cap) for each reaction to a suitable tube (n samples + 2 controls + at least one additional reaction to cover pipetting loss).

Mix carefully but thoroughly by pipetting up and down.



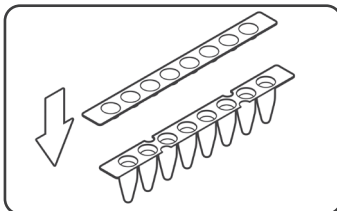
2. ADD PCR MIX

Pipet 20 µL of prepared PCR mix into each strip or plate well.



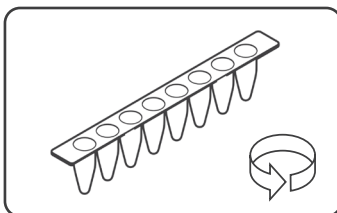
3. ADD SAMPLES AND CONTROLS

Pipet 5 µL of samples, Negative Control (colorless cap) or Control Template (purple cap) into respective wells.



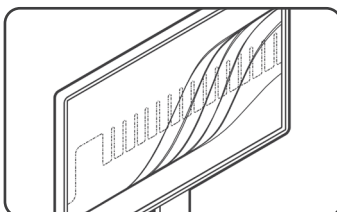
4. SEAL

Seal strips/plate accurately.



5. CENTRIFUGE

Briefly spin strips/plate in a suitable centrifuge.



6. START REAL-TIME PCR RUN

Cycle samples as described above.