foodproof®

STEC Identification LyoKit
Ready Reference Guide

Revision A, November 2023

Product No. KIT230079 (LP), KIT230080 (RP)

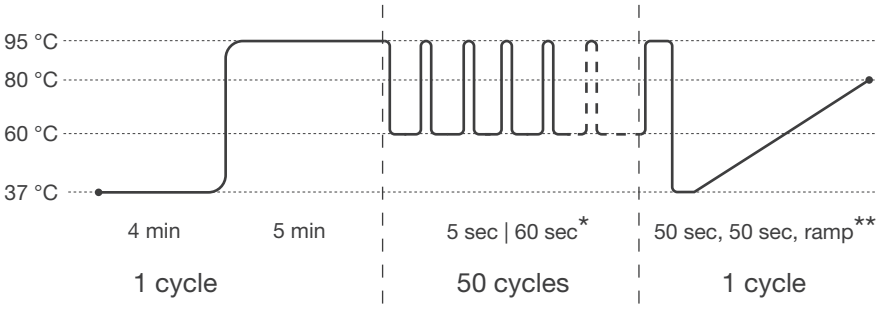
PCR kit for the qualitative detection of *Escherichia coli* (including STEC) of the following O-serotypes: O26, O45, O103, O104, O111, O121, O145 and O157.

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 (all 8 serotypes), HEX (O26, O103, O104), ROX (O111, O145, O157) and Cy5 (O45, O121, Internal Control).



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

Melting Curve: 1 cycle
Step 1 : 95 °C for 50 sec
Step 2 : 37 °C for 50 sec
Step 3**: ramp up to 80 °C

* Fluorescence detection ** Fluorescence detection during 37 - 80 °C ramp with 1 - 2 measurements/°C

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 a non-fluorescent "dark" quencher and no passive reference dye.
A Color Compensation is necessary for users of the LightCycler® 480 System: Color Compensation Set 3 (Product No. KIT230005).
For the CFX96™ system, the melting curve protocol is as follows: Step 1: 95 °C for 45 seconds; Step 2: Melt Curve, 37 °C to 80 °C, increments 0.5 °C, for 0.02 + Plate Read.

DATA INTERPRETATION

Verify results of positive (Control Template) and negative (H_2O) 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.

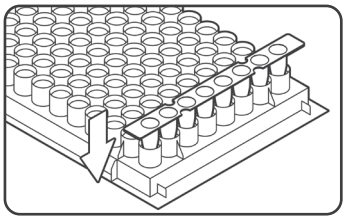
	FAM	Cy5	Result Interpretation
Amplification	+	+/-	Positive for one or more: O26, O45, O103, O104, O111, O121, O145, O157
	-	+	Negative for serotypes: O26, O45, O103, O104, O111,O121, O145, O157
	-	-	Invalid

	Channel	Melting Temperature (T_m) Range for LC480, LC96, AriaMx®, PikoReal24™		
Melting Curve	HEX	O26: 43 to 48 °C	O104: 48.5 to 54 °C	O103: 59 to 64 °C
	ROX	O157: 45 to 50 °C	O111: 51 to 56 °C	O145: 59 to 64 °C
	Cy5	O121: 50 to 55 °C	O45: 58 to 63 °C	IC: > 65 °C

	Channel	Melting Temperature (T_m) Range for iQ5, Mx3005P®, ABI 7500 Fast, CFX96		
Melting Curve	HEX	O26: 41 to 45.5 °C	O104: 46 to 51 °C	O103: 58 to 63 °C
	ROX	O157: 42 to 47 °C	O111: 48 to 53 °C	O145: 57 to 62 °C
	Cy5	O121: 48 to 53 °C	O45: 56 to 61 °C	IC: > 65 °C

PREPARATION OF THE PCR MIX

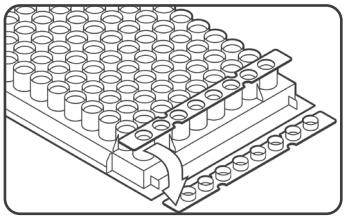
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.



1. PLACE STRIPS IN RACK

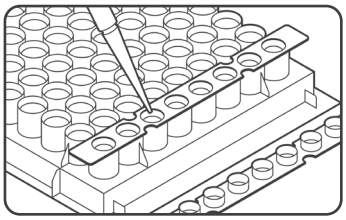
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

If needed, gently tap the tubes to move the lyophilized pellets to the bottom of all tubes.



2. DECAP

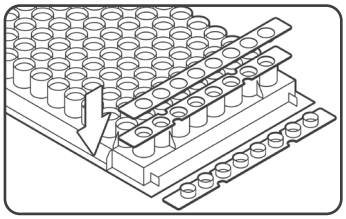
Immediately before filling, carefully open strips and discard caps.
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

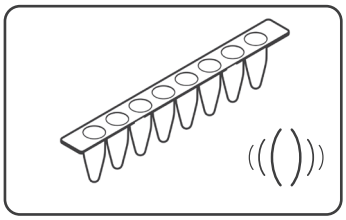
Pipette 25 µL of samples, Negative Control (colorless cap) or Control Template (purple cap) into respective wells.

If using less volume, add PCR-grade H_2O to reach 25 µL.



4. SEAL

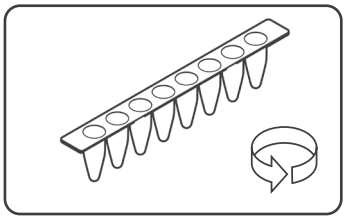
Carefully seal the tubes with the provided 8-cap strips.



5. MIX

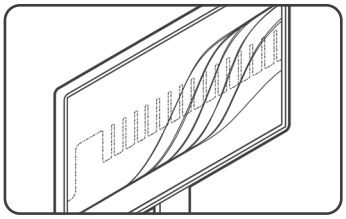
Resuspend pellet after sealing by mixing thoroughly.

Alternatively, resuspend pellet by pipetting up and down multiple times in Step 3.



6. CENTRIFUGE

Briefly spin strips, e.g., 5 seconds at 500 - 1,000 x g, in a suitable centrifuge.



7. START REAL-TIME PCR RUN

Cycle samples as described above.

Place tubes in a vertical, balanced order into the cycler, e.g., two strips can be placed in the first and last column.

foodproof®
STEC Identification LyoKit

KIT230079 /80
Kit for 48 reactions
Store kit at 2 to 8 °C

For food testing purposes
FOR IN VITRO USE ONLY
Made in Germany

Hygiena® | Camarillo, CA 93012 USA | diagnostics.support@hygiena.com | www.hygiena.com