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E. coli and *Shigella* Detection LyoKit
Ready Reference Guide

Revision A, November 2023

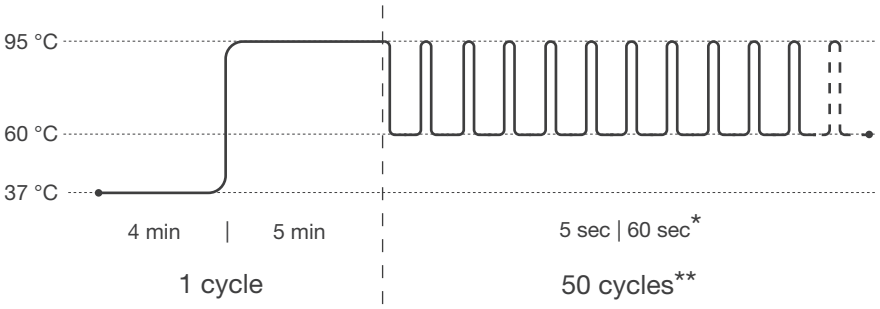
Product No. KIT230020 (LP), KIT230021 (RP), KIT230329 (DP)

PCR kit for the qualitative detection of *Escherichia coli* and *Shigella* spp. DNA using real-time PCR instruments.

PROGRAM SETUP

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

- FAM (*Escherichia coli* and *Shigella* spp.) and HEX (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

* Fluorescence detection
** 35 cycles for confirmation of single colonies

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.

For the Dualo 32® R2 real-time PCR instrument, please open the software, click on 'New', and select the respective template file. Template files can be added by clicking on 'Add' in the 'Select template file' window.

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.

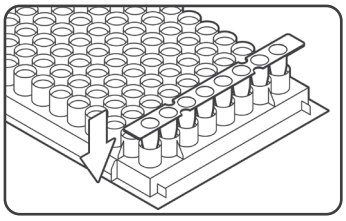
FAM	HEX	Result Interpretation
+	+ or -	Positive for *Escherichia coli* and/or *Shigella* spp.
-	+	Negative for *Escherichia coli* and *Shigella* spp.
-	-	Invalid

Note: The table is valid for protocols with 35 cycles only. If 50 cycles are used, Cq values > 35 should be considered as negative results.

RR-KIT230020-021-329-RevA

PREPARATION OF THE PCR MIX

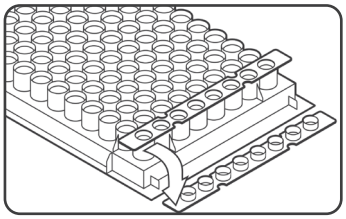
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.



1. PLACE STRIPS IN RACK

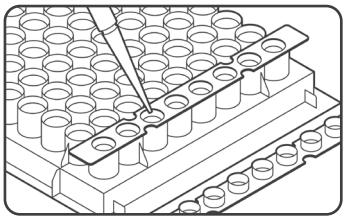
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

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



2. DECAP

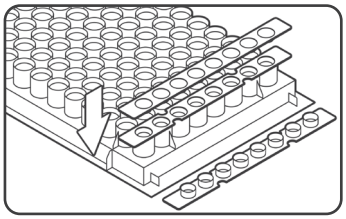
Immediately before filling, carefully open strips and discard caps.
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

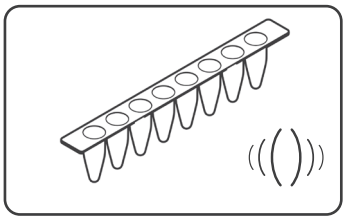
Pipette 25 µL of samples, Negative Control (colorless cap) or Control Template (purple cap) into respective wells.

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



4. SEAL

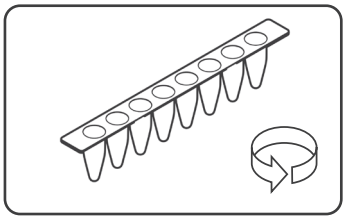
Carefully seal the tubes with the provided 8-cap strips.



5. MIX

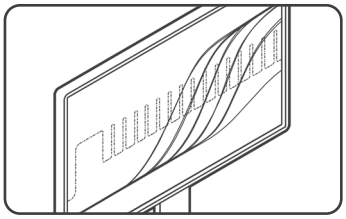
Resuspend pellet after sealing by mixing thoroughly.

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.

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Detection LyoKit

KIT230020 /021 /329
Kit for 96 reactions
Store kit at 2 to 8 °C

For food testing purposes
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