foodproof®

Enterobacteriaceae plus *Salmonella* Detection LyoKit

Ready Reference Guide

Revision A, November 2023

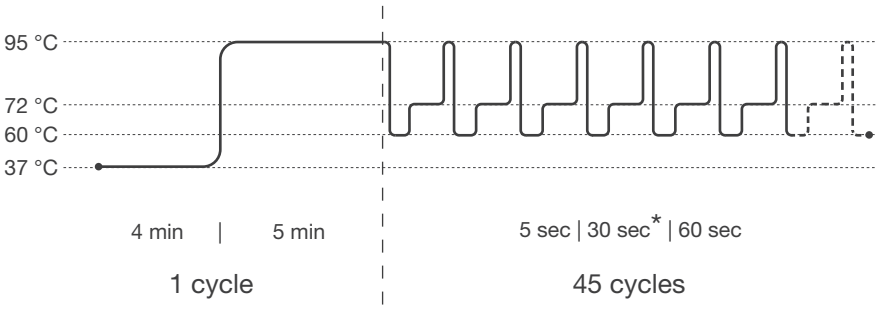
Product No. KIT230137 (LP), KIT230138 (RP), KIT230139 (DP)

PCR kit for the qualitative detection of *Enterobacteriaceae* plus simultaneous identification of *Salmonella* spp. 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 (*Salmonella*), VIC/HEX (*Enterobacteriaceae*) and ROX (Internal Control).



Pre-incubation: 1 cycle
Step 1: 37 °C for 4 min
Step 2: 95 °C for 5 min
Amplification: 45 cycles
Step 1 : 95 °C for 5 sec
Step 2*: 60 °C for 30 sec
Step 3 : 72 °C for 60 sec

* Fluorescence detection

For some real-time PCR instruments the probe quencher as well as the usage of a passive reference dye has to 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 5 (Product No. KIT230011).

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 controls (H_2O), before interpreting sample results. Always compare samples to positive and negative control. Review data from each channel and interpret results as described in the table.

FAM	VIC/HEX	ROX	Result Interpretation
+	+	+ or -	Positive for *Salmonella* and *Enterobacteriaceae*
-	+ (Cq < 40)	+ or -	Positive for *Enterobacteriaceae* (non-*Salmonella*)
-	- (Cq ≥ 40)	+	Negative for *Salmonella* and *Enterobacteriaceae*
-	- (Cq ≥ 40)	-	Invalid

If the Cq-Value in VIC/HEX is > 35, the result in FAM may be negative due to slight differences in the assay's limit of detection in this multiplex-PCR system. In this case, a second enrichment and a repetition of the analysis is recommended.

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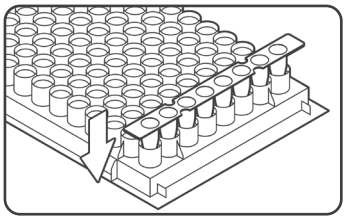
PREPARATION OF THE PCR MIX

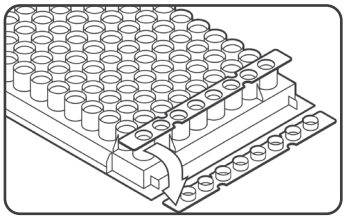
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.



1. PLACE STRIPS IN RACK

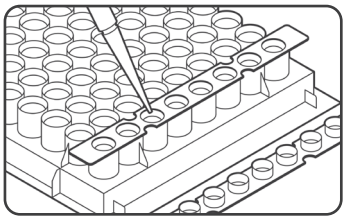
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

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



2. DECAP

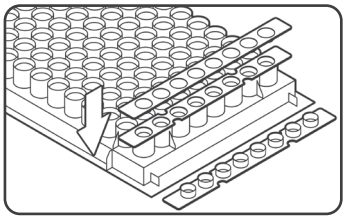
Immediately before filling, carefully open strips and discard caps.
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

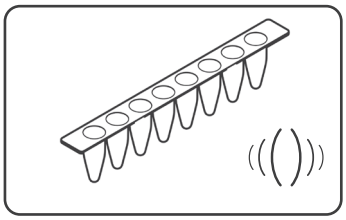
Pipette 25 µL of samples, Negative Control (colorless cap) or Control Template (purple cap) into respective wells.

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



4. SEAL

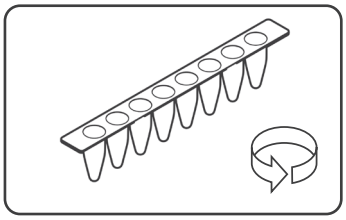
Carefully seal the tubes with the provided 8-cap strips.



5. MIX

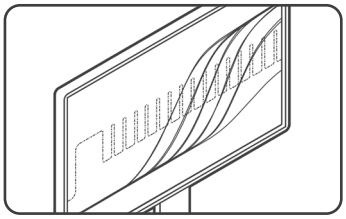
Resuspend pellet after sealing by mixing thoroughly.

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.