foodproof®

Yersinia enterocolitica plus *pseudotuberculosis* Detection Kit

Ready Reference Guide

Revision A, December 2023

Product No. KIT230018

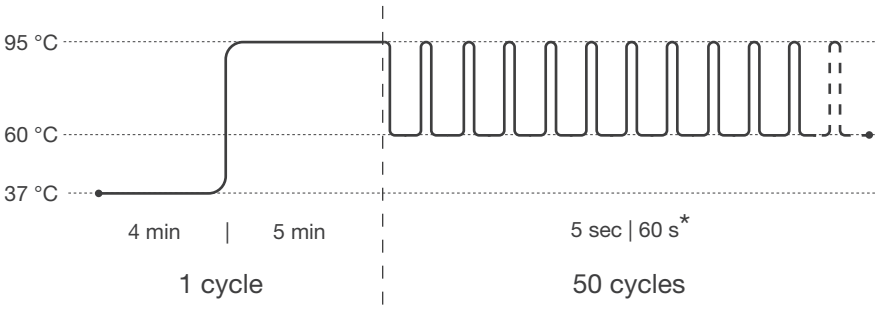
PCR kit for the qualitative detection of *Yersinia enterocolitica* plus *Yersinia pseudotuberculosis* DNA using real-time PCR instruments.

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 (*Yersinia enterocolitica*), HEX (*Yersinia pseudotuberculosis*) and ROX (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 s
Step 2*: 60 °C for 60 s

* 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).

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	HEX	ROX	Result Interpretation
+	+	+ or -	Positive for *Yersinia enterocolitica* plus *Yersinia pseudotuberculosis*
-	+	+ or -	Positive for *Yersinia pseudotuberculosis*
+	-	+ or -	Positive for *Yersinia enterocolitica*
-	-	+	Negative for *Yersinia enterocolitica* plus *Yersinia pseudotuberculosis*
-	-	-	Invalid

RR-KIT230018-RevA

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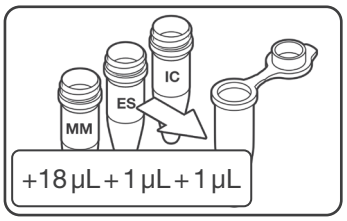
PREPARATION OF THE PCR MIX

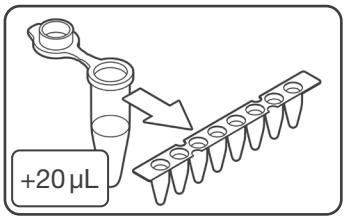
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.
Thaw reagents, mix (do not vortex!) and briefly spin vials before opening.



1. PREPARE PCR MIX

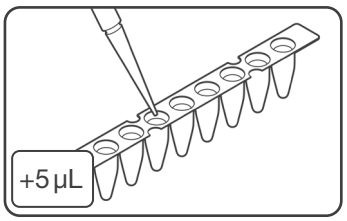
Add 18 µL of Master Mix (yellow cap), 1 µL of Enzyme Solution (red cap) and 1 µL of Internal Control (white cap) for each reaction to a suitable tube (n samples + 2 controls + at least one additional reaction to cover pipetting loss).

Mix carefully but thoroughly by pipetting up and down.



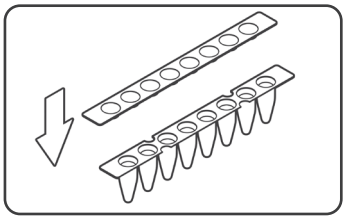
2. ADD PCR MIX

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



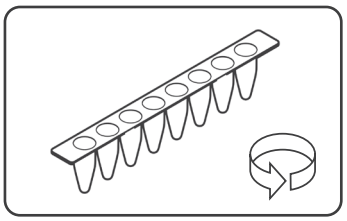
3. ADD SAMPLES AND CONTROLS

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



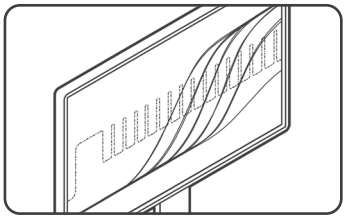
4. SEAL

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.