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Campylobacter Quantification Kit
Ready Reference Guide

Revision A, December 2023

Product No. KIT230041

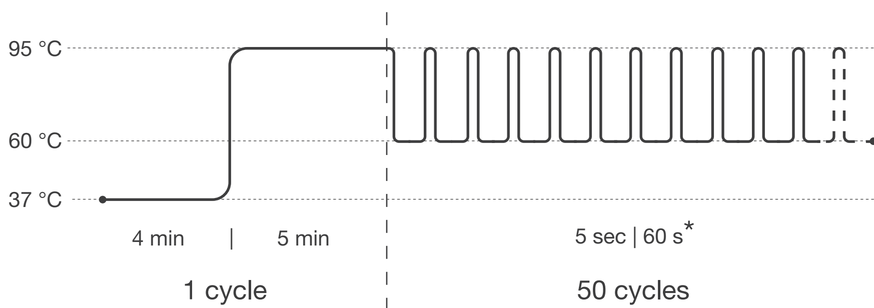
PCR kit for the quantitative detection of thermotolerant *Campylobacter* spp. and indentification of *C. jejuni* and *C. coli* using real-time PCR instruments.

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 (thermotolerant *Campylobacter* spp.), HEX (Internal Control), ROX (*C. jejuni*) and Cy5 (*C. coli*).



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 use of a passive reference dye must be specified. This kit contains probes with a non-fluorescent "dark" quencher and no passive reference dye.

PREPARATION OF STANDARD CURVE

Use the Quantification Standard (vial 4, purple cap) and Dilution Buffer (vial 6, blue cap) to prepare dilutions according to the table below.

For each dilution step, pipet 90 µL of Dilution Buffer into a new reaction tube. Transfer 10 µL from preceding step to the new dilution step. Mix well between pipetting steps.

A typical experiment consists of 13 wells needed for quantification standards (duplicates) and a negative control, plus n wells (n = number of samples).

Dilution Step	Dilution Factor	Concentration to Be Entered as Standard (CFUs/reaction)
1	Undiluted	1,000,000
2	1:10	100,000
3	1:100	10,000
4	1:1,000	1,000
5	1:10,000	100
6	1:100,000	10

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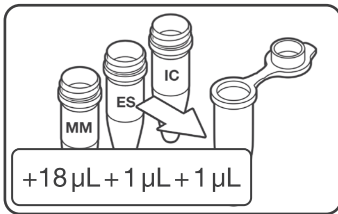
PREPARATION OF THE PCR MIX

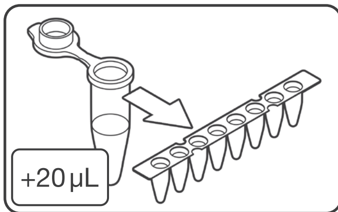
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.
Thaw reagents, mix (do not vortex!) and briefly spin vials before opening.



1. PREPARE PCR MIX

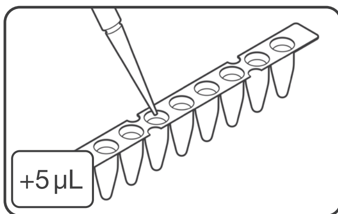
Add 18 µL of Master Mix (yellow cap), 1 µL of Enzyme Solution (red cap) and 1 µL of Internal Control (white cap) for each reaction to a suitable tube (n samples + 13 quantification standards + at least one additional reaction to cover pipetting loss).

Mix carefully but thoroughly by pipetting up and down.



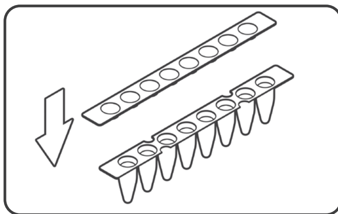
2. ADD PCR MIX

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



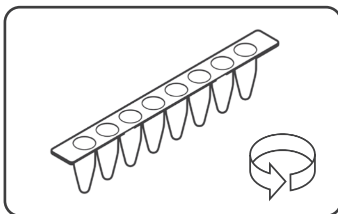
3. ADD SAMPLES AND CONTROLS

Pipet 5 µL of samples, standards and negative control (colorless cap) .



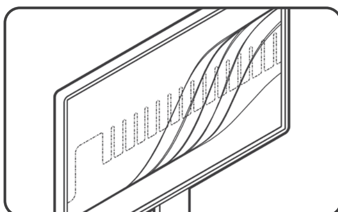
4. SEAL

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.