

foodproof®

Clostridium Quantification LyoKit

Ready Reference Guide

Revision A, February 2024

Product No. KIT230230 (LP), KIT230231(RP)

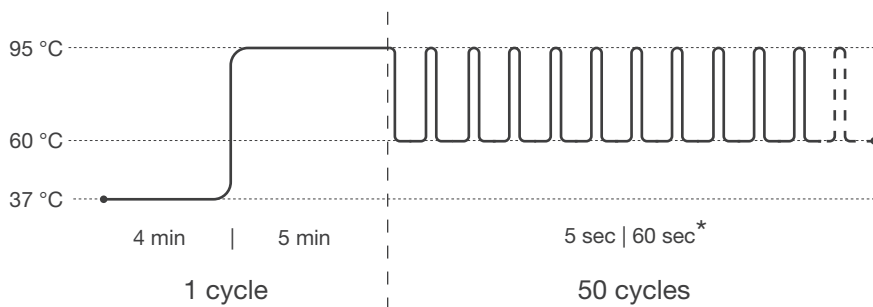
PCR kit for the quantitative detection of *Clostridium* spp. using real-time PCR instruments.

Before starting, it is strongly recommended to read the entire product instructions available on our website.

PROGRAM SETUP

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

- ▶ FAM (*Clostridium* 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

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.

PREPARATION OF A STANDARD CURVE

Use the Quantification Standard (vial 2, purple cap) and Negative Control (vial 3, colorless cap) to prepare serial dilutions according to table below.

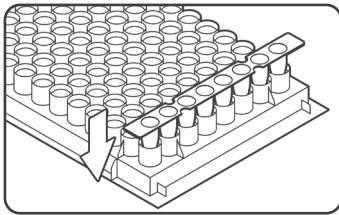
For each dilution step, pipet 90 µL Negative Control into a new reaction tube. Transfer 10 µL from the preceding step to new dilution step. Mix well between pipetting steps.

A typical experiment consists of 9 wells needed for standards (duplicates) and negative control, plus n wells for samples (n = number of food samples).

Dilution Step	Dilution Factor	Concentration to be entered as Standard (GU/reaction)
1	Undiluted	100,000
2	1:10	10,000
3	1:100	1,000
4	1:1,000	100

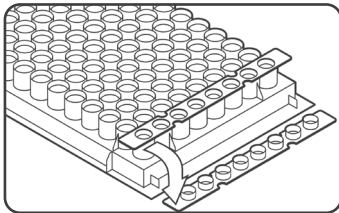
PREPARATION OF THE PCR MIX

Take appropriate precautions to prevent contamination, e.g., by using aerosol-barrier tips and wearing gloves. For data interpretation and calculation, refer to the complete product instructions.



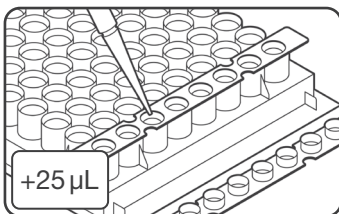
1. PLACE STRIPS IN RACK

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 each tube.



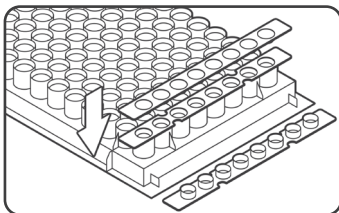
2. UNCAP

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



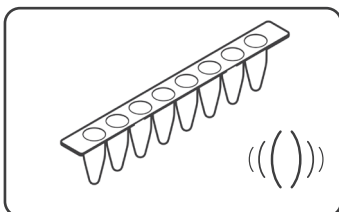
3. ADD SAMPLES AND CONTROLS

Pipet 25 µL of samples, standards and Negative Control (colorless cap) into respective wells. If using less volume for samples, add Negative Control to reach 25 µL.



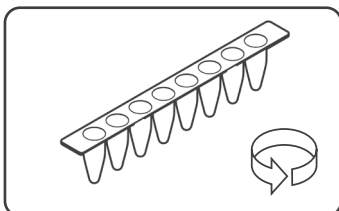
4. SEAL

Seal the tubes with the provided 8-cap strips properly.



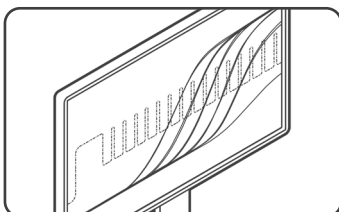
5. MIX

Resuspend pellet by mixing thoroughly.



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 columns.