foodproof®

Vibrio Detection LyoKit
Ready Reference Guide

Revision A, November 2023

Product No. KIT230117 (LP), KIT230118 (RP)

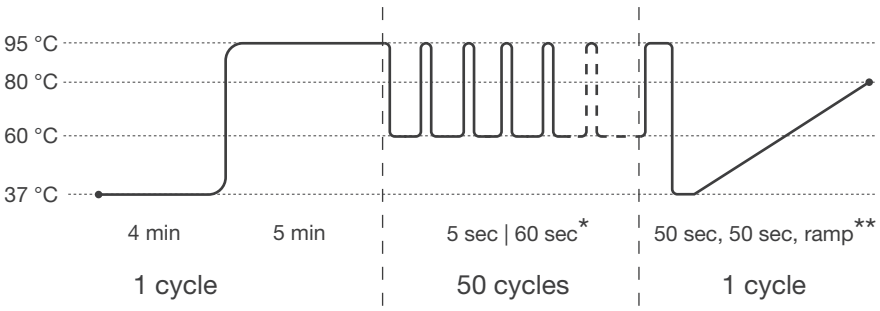
PCR kit for the qualitative detection of *Vibrio parahaemolyticus, V. vulnificus* and *V. cholerae* DNA and their respective toxin genes using real-time PCR instruments.

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 (*V. parahaemolyticus, tdh*), HEX (*V. vulnificus, trh1* and *trh2*), ROX (*V. cholerae, ctx*) and Cy5 (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

Melting Curve: 1 cycle
Step 1 : 95 °C for 50 sec
Step 2 : 37 °C for 50 sec
Step 3**: ramp up to 65 °C

* Fluorescence detection ** Fluorescence detection during 37 - 65 °C ramp with 2 - 4 measurements/°C

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.
Color Compensation is necessary for users of the LightCycler® 480 System: Color Compensation Set 3 (Product No. KIT230005) has to be used for LC480 I instrument; Color Compenation Set 5 (Product No. KIT230011) has to be used for LC480 II instrument.

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. For melting curve interpretation of toxin genes (*tdh*, *trh1*, *trh2* and *ctx*), refer to the product manual.

FAM	HEX	ROX	Cy5	Result Interpretation
+	+	+	+ or -	Positive for *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*
-	+	+	+ or -	Positive for *V. vulnificus* and *V. cholerae*
+	-	+	+ or -	Positive for *V. parahaemolyticus* and *V. cholerae*
+	+	-	+ or -	Positive for *V. parahaemolyticus* and *V. vulnificus*
-	+	-	+ or -	Positive for *V. vulnificus*
+	-	-	+ or -	Positive for *V. parahaemolyticus*
-	-	+	+ or -	Positive for *V. cholerae*
-	-	-	+	Negative for *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*
-	-	-	-	Invalid

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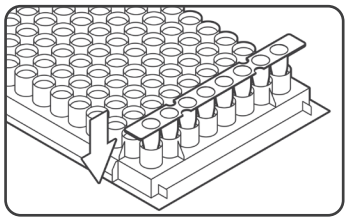
PREPARATION OF THE PCR MIX

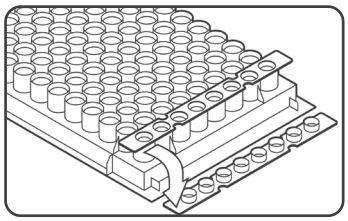
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.



1. PLACE STRIPS IN RACK

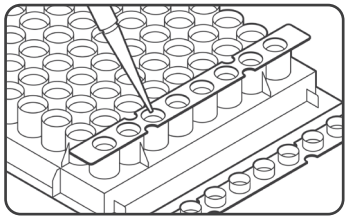
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

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



2. DECAP

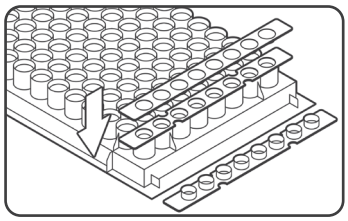
Immediately before filling, carefully open strips and discard caps.
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

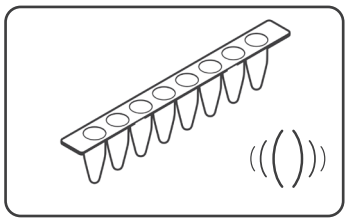
Pipette 25 µL of samples, Negative Control (colorless cap) or Control Template (purple cap) into respective wells.

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



4. SEAL

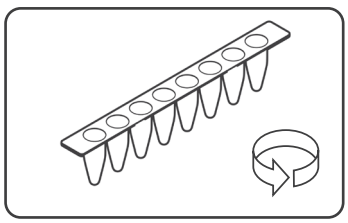
Carefully seal the tubes with the provided 8-cap strips.



5. MIX

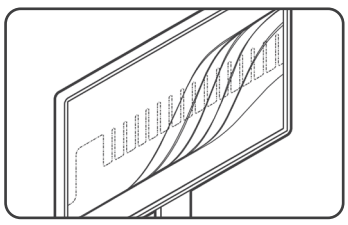
Resuspend pellet after sealing by mixing thoroughly.

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.

foodproof®
Vibrio Detection LyoKit

KIT230117 /18
Kit for 96 reactions
Store kit at 2 to 8 °C

For food testing purposes
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Made in Germany

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