foodproof®

Yeast and Mold Quantification LyoKit
Ready Reference Guide

Revision A, November 2023

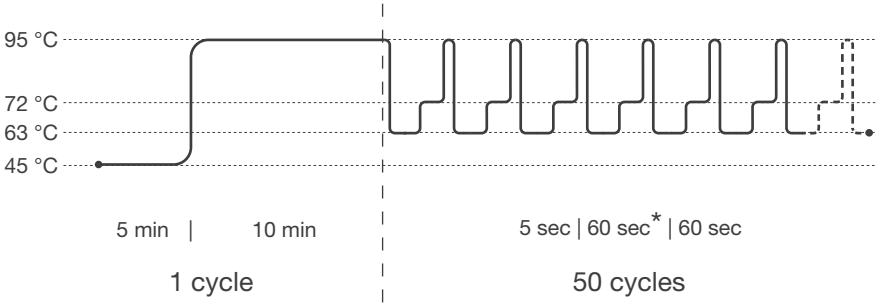
Product No. KIT230112 (LP), KIT230113 (RP), KIT230114 (DP)

PCR kit for the quantitative detection of yeast and mold 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 (yeast and mold) and HEX (Internal Control).



Pre-incubation: 1 cycle
Step 1: 37 °C for 5 min
Step 2: 95 °C for 10 min
Amplification 50: cycles
Step 1 : 95 °C for 5 sec
Step 2*: 63 °C for 60 sec
Step 3 : 72 °C for 60 sec

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

For each dilution step, pipet 90 µL of Negative Control into a new reaction tube. Transfer 10 µL from 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 (n = number of food samples).

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

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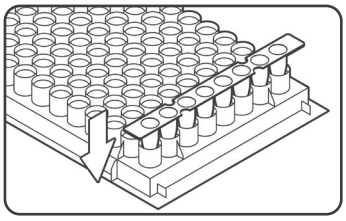
PREPARATION OF THE PCR MIX

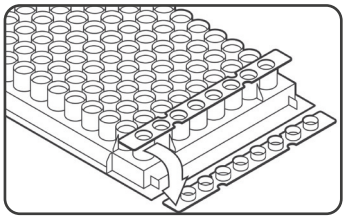
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.
For data interpretation and calculation, refer to the complete product manual.



1. PLACE STRIPS IN RACK

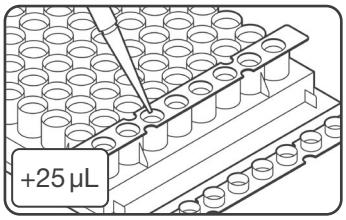
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

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



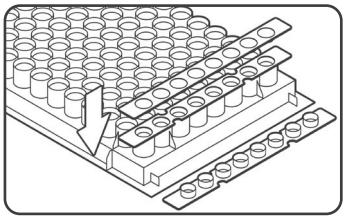
2. DECAP

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



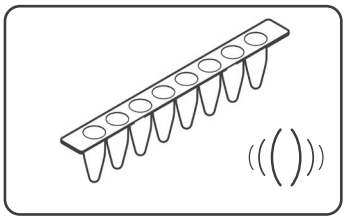
3. ADD SAMPLES AND CONTROLS

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.



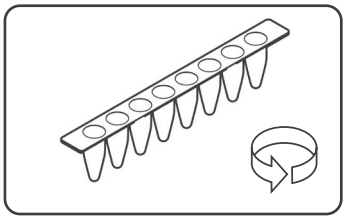
4. SEAL

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



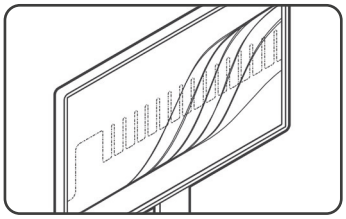
5. MIX

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.

foodproof®
Yeast and Mold
Quantification LyoKit

KIT230112 / 13 / 14
Kit for 96 reactions
Store kit at 2 to 8 °C

For food testing purposes
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