foodproof®

Spoilage Yeast Detection 2 LyoKit
Ready Reference Guide

Revision A, November 2023

Product No. KIT230126 (DP)

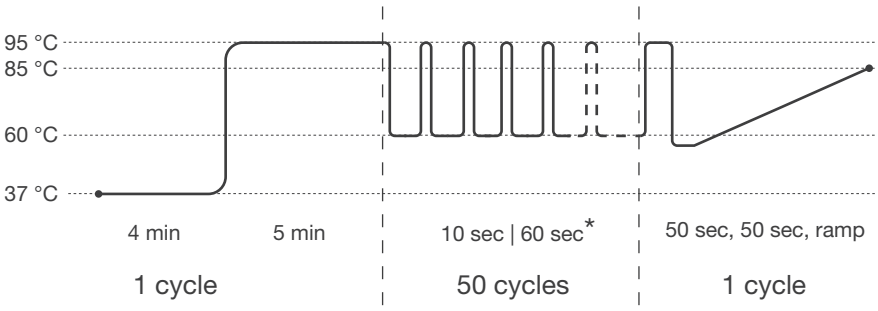
PCR kit for the qualitative detection of *Saccharomyces cerevisiae* var. *diastaticus*, *Wickerhamomyces anomalus, Kazachstania exigua* and *Schizosaccharomyces pombe* using real-time PCR instruments.

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 (*Saccharomyces cerevisiae* var. *diastaticus*), HEX (*Wickerhamomyces anomalus*), ROX (*Kazachstania exigua* and *Schizosaccharomyces pombe*) and ATTO 490LS (Internal Control).



* Fluorescence detection

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 10 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 85 °C

For the Dualo 32® (for beverage testing) real-time PCR instrument, please open the software, click on 'New', and select the respective template file. Template files can be added by clicking on 'Add' in the 'Select template file' window.

DATA INTERPRETATION

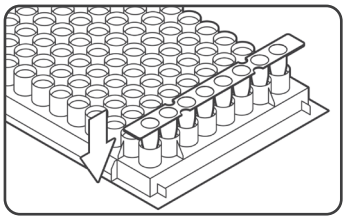
Verify results of positive (Control Template) and negative controls (H_2O), before interpreting sample results. Always compare samples to positive and negative controls. Review data from each channel and interpret results as described in the table.

FAM	HEX	ROX	ATTO 490LS	Result Interpretation
+	+ or -	+ or -	+ or -	Positive for *Saccharomyces cerevisiae* var. *diastaticus*
+ or -	+	+ or -	+ or -	Positive for *Wickerhamomyces anomalus*
+ or -	+ or -	+	+ or -	Positive for *K. exigua* and *Schizosaccharomyces pombe*
-	-	-	+	Negative for targeted spoilage yeasts
-	-	-	-	Invalid

Melting Curve	Channel	Organism	Tm-Range
	ROX	*Kazachstania exigua*	68 ± 2°C
		Schizosaccharomyces pombe	78 ± 2°C

PREPARATION OF THE PCR MIX

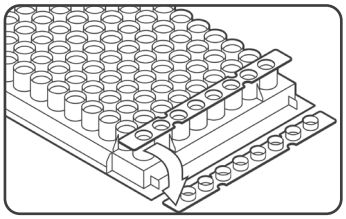
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.



1. PLACE STRIPS IN RACK

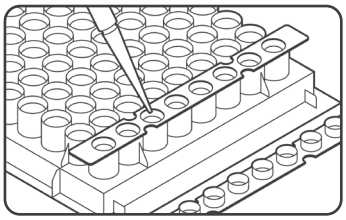
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

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



2. DECAP

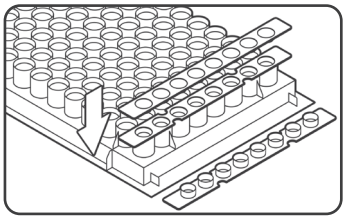
Immediately before filling, carefully open strips and discard caps.
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

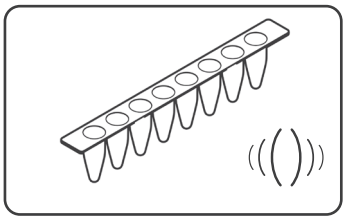
Pipette 25 µL of samples, Negative Control (colorless cap) or Control Template (purple cap) into respective wells.

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



4. SEAL

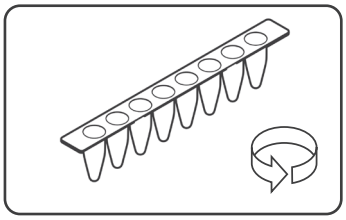
Carefully seal the tubes with the provided 8-cap strips.



5. MIX

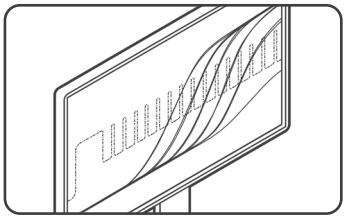
Resuspend pellet after sealing by mixing thoroughly.

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® Spoilage Yeast Detection 2 LyoKit

KIT230126
Kit for 48 reactions
Store kit at 2 to 8 °C

For food testing purposes
FOR IN VITRO USE ONLY
Made in Germany

Hygiena® | Camarillo, CA 93012 USA | diagnostics.support@hygiena.com | www.hygiena.com