foodproof® Spoilage Yeast Detection 1 LyoKit

For Detection and Quantification of Major Spoilage Yeast Genera

Revision A, January 2024

PCR kit for the qualitative or quantitative detection of *Dekkera*/*Brettanomyces* spp., *Zygosaccharomyces* spp. and *Saccharomyces* spp. using real-time PCR instruments.

Product No. KIT230121 (LP)

Product No. KIT230122 (RP)

Product No. KIT230123 (DP)

Kit for 48 reactions (lyophilized) for a maximum of 46 samples

Store at 2 to 8 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

Table of Contents

1. What this Product Does

1.1 Number of Tests

The kit is designed for 48 reactions with a final reaction volume of 25 µL each. Up to 46 samples (single sample preparation) plus positive control and negative control reactions can be analyzed per run.

1.2 Storage and Stability

- Store the kit at 2 to 8 °C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following Kit Contents table.

1.3 Kit Contents

Component	Description	Contents, Function, Storage
foodproof® Spoilage Yeast Detection 1 LyoKit Microplate, prefilled with 48 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230121 with white low-profile (LP) tubes* • KIT230122 with clear regular-profile (RP) tubes* • KIT230123 with clear deep-profile (DP) tubes*	• 48 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for DNA of the designated spoilage yeasts and the Internal Control (IC) as well as Taq DNA Polymerase and Uracil-N-Glycosylase (UNG, heat-labile) for prevention of carryover contamination. • Store at 2 to 8 °C in the aluminum bag with the silica pad (Keep tightly sealed). • **Protect from light and moisture!**
Control Template/ Quantification Standard	Vial 2 (purple cap)	• 1 x 350 µL • Contains a stabilized solution of DNA. • For use as a PCR positive control/Quantification Standard. • Store at 2 to 8 °C.
H_2O PCR-grade	Vial 3 (colorless cap)	• 2 x 1 mL • Nuclease-free, PCR-grade H_2O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	• 12 x 8-cap strip • For use in real-time PCR after addition of samples.

*Tube profile and instrument compatibility chart is available online: www.hygiena.com/documents

1.4 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, VIC-/HEX, ROX- and Cy5/ATTO 490LS-labeled probes. If the strip tubes do not fit into the instrument, the samples must be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix. Take precautions to avoid cross-contamination during this pipetting step.
- Sample preparation kit: foodproof StarPrep Two Kit (Product No. KIT230177)
- Reagent D (Product No. KIT230001)
- Pipettes
- Nuclease-free, aerosol-resistant pipette tips
- Vortex centrifuge Multispin MSC-6000 for PCR strips **with** SR-32, Rotor for MSC-3000/6000 **or** Vortex centrifuge CVP-2 for PCR plates

1.5 Applicability Statement

The foodproof Spoilage Yeast Detection 1 LyoKit is intended for the rapid qualitative or quantitative detection of spoilage yeast DNA isolated from a wide range of food and beverage samples that are potentially contaminated with *Dekkera*/*Brettanomyces* spp., *Zygosaccharomyces* spp. or *Saccharomyces* spp. DNA from dead yeast can be excluded from analysis by using Reagent D and a suitable protocol.

The kit must not be used in diagnostic procedures.

The kit described in these Product Instructions has been developed for real-time PCR instruments.

- Versions KIT230121 (LP) and KIT230122 (RP) are designed for instruments with FAM, VIC/HEX, ROX and Cy5 detection channels. The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480 and LightCycler 96 (Roche Diagnostics), Mx3005P® and AriaMx® (Agilent Technologies), ABI™ 7500 FAST (Thermo Scientific), CFX96 (Bio-Rad), PikoReal® 24 (Thermo Scientific) and CFX 96™ (Bio-Rad).
- Version KIT230123 is designed for instruments with FAM, VIC/HEX, ROX and ATTO 490LS detection channels. The performance of the kit was tested with the Dualo® 32 Beverage PCR Instrument (Hygiena Diagnostics).

Note: Color Compensation is necessary and will be supplied by Hygiena Diagnostics for users of the LightCycler 480 System I and LightCycler 480 System II (Color Compensation Set 5; Product No. KIT230011).

2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of spoilage yeast DNA using the foodproof Spoilage Yeast Detection 1 LyoKit requires DNA amplification by PCR. The kit provides all the reagents required for the PCR. To achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carryover- or cross-contamination:

- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- To avoid carryover contamination, transfer the required solutions for one experiment into a fresh tube rather than directly pipetting from stock solutions.
- Physically separate the workplaces for DNA preparation, PCR setup and PCR to minimize the risk of carryover contamination. Use a PCR hood for all pipetting steps.

Keep the foodproof Spoilage Yeast Detection 1 LyoKit lyophilized PCR Mix away from light and moisture.

2.1.2 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration and absence of inhibitors. For preparation of genomic DNA from various samples, refer to the corresponding product package inserts of a suitable sample preparation kit (see "*Additional Equipment and Reagents Required*").

2.1.3 DNA Extraction

Hygiena Diagnostics provides sample preparation kits suitable for all kinds of foods and environmental samples (see "*Additional Equipment and Reagents Required*").

For more product information, please refer to www.hygiena.com.

2.1.4 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [foodproof Spoilage Yeast Detection 1 Control Template (Quantification Standard, vial 2, purple cap)] or with a positive sample preparation control.

2.1.5 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with foodproof Spoilage Yeast Detection 1 PCR-grade water (vial 3, colorless cap). Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.2 Procedure

2.2.1 Program Setup for the Dualo® 32 Beverage Instrument (KIT230123)

The Dualo 32® Beverage (Product No. MCH230008) can be started from a pre-installed run template: Click on 'New', select the appropriate template, and press 'Select'. After loading the samples, the instrument can be started by clicking on 'Start Run'.

For detailed instructions on how to program and start the PCR run on the Dualo 32® Beverage, please refer to the manual for this instrument.

2.2.2 Program Setup for other cyclers (KIT230121 / KIT230122)

The following procedure is optimized for a real-time PCR instrument with FAM (*Dekkera*/*Brettanomyces* spp.), VIC/HEX (*Zygosaccharomyces* spp.), ROX (*Saccharomyces* spp.) and Cy5/ATTO 490LS (Internal Control) detection channels. Program the PCR instrument before preparing the reaction mixes. The amplification is carried out according to the following temperature-time program. For details on how to program the experimental protocol, see the operation manual for the real-time PCR cycler used.

Use the following real-time PCR protocol for the foodproof Spoilage Yeast Detection 1 LyoKit, Product Nos. KIT230121-22-23:

Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes
Amplification	**50** cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds

* Fluorescence detection in step 2

Notes:

- For some real-time PCR instruments, the type of probe quencher as well as the use of a passive reference dye must be specified. The foodproof Spoilage Yeast Detection 1 LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For users of the Agilent Mx3005P instrument: Click 'Instrument → Filter Set Gain Settings' to open the Filter Set Gain Settings dialog box. For FAM and VIC/HEX, modify the Filter Set Gain Setting to 'x4'.

2.2.3 Preparation of the PCR Mix

Proceed as described below to prepare a 25 µL standard reaction. Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors.

Always wear gloves when handling tube strips or caps.

Note: The PCR strips must be stored in the provided aluminum bag with the silica gel pads to avoid liquid absorption.

2.2.4 Procedure A: Qualitative Detection

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or a scalpel to cut the strips apart.

   Note: Tightly seal the bag afterward and store under the recommended conditions.

2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. If the reagent pellets are not at the bottom of the tubes, briefly centrifuge or flick the pellets to the bottom before proceeding.

3. Carefully uncap the tube strips and discard the cap strips.

   Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, only open strips shortly before filling.

4. Pipette sample into each PCR vessel:
   - For the samples of interest, add 25 µL of sample DNA (if using less volume, add PCR-grade H_2O up to 25 µL).
   - For the negative control, add 25 µL of foodproof Spoilage Yeast Detection 1 PCR-grade H_2O (vial 3, colorless cap).
   - For the positive control, add 25 µL of foodproof Spoilage Yeast Detection 1 Quantification Standard (vial 2, purple cap).

   Note: To reduce the risk of cross-contamination, it is recommended to prepare only one PCR tube strip at a time.

5. Seal the PCR vessels tightly with the colorless cap strips.

6. Mix thoroughly using a vortex centrifuge.

   Note: Hygiena Diagnostics recommends vortex centrifuges Multispin MSC-6000 for PCR strips or vortex centrifuge CVP-2 for PCR plates. Dedicated protocols are available for these centrifuges.

   Alternatively, resuspend the pellet by manual mixing. This may be achieved by carefully pipetting the sample up and down multiple times during step 4 or flipping the tube strips after sealing while holding down the cap strip.

7. Spin the PCR tube strips for 30 seconds at 150 – 200 x g in a suitable centrifuge.

   Note: If your centrifuge exceeds 200 x g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1000 x g!

8. Place the samples in your PCR cycler and run the program as described above.

   Note: When using the LightCycler 480 instrument, a special adapter (Product No. MIS230005) is necessary.

   For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example, two strips can be placed in columns 1 and 12.

2.2.5 Procedure B: Quantitative Detection Using a Standard Curve

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or a scalpel to cut the strips apart.

   Note: Tightly seal the bag afterward and store under the recommended conditions.

2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. If the reagent pellets are not at the bottom of the tubes, briefly centrifuge or flick the pellets to the bottom before proceeding.

3. Carefully uncap the tube strips and discard the cap strips.

   Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, only open strips shortly before filling.

4. Pipette sample into each PCR vessel:
   - For the samples of interest, add 25 µL of sample DNA (if using less volume, add PCR-grade H_2O up to 25 µL).
   - For the negative control, add 25 µL of foodproof Spoilage Yeast Detection 1 PCR-grade H_2O (vial 3, colorless cap).
   - For the standard curve, add 25 µL of each dilution (in duplicate) of foodproof Spoilage Yeast Detection 1 Quantification Standard (vial 2, purple cap) to generate the standard curve (see table below).

   Note: A typical experiment consists of 9 reactions needed for controls, plus n x reactions needed for the samples of interest, where (n) indicates the number of food samples of interest. Since 48 reactions can be made with the kit, up to 39 samples can be analyzed quantitatively during one PCR run.

   Dilution of Quantification Standard

   Quantification of spoilage yeast via the standard curve procedure requires a serial dilution of the Control Template/Quantification Standard, as shown below. Prepare a dilution of the standard at a final volume of 100 µL by adding 10 µL of the previous dilution step to 90 µL PCR-grade H2O. Then, close the tube, vortex for 10 seconds, and briefly spin for 10 seconds in a centrifuge. Repeat for every dilution step.

Dilution Step	Dilution	Concentration to Enter as Standard [GE/Reaction]		
		FAM Channel	HEX Channel	ROX Channel
1	undiluted	30,000	10,000	5,000
2	1:10	3,000	1,000	500
3	1:100	300	100	50
4	1:1,000	30	10	5

5. Seal the PCR vessels tightly with the colorless cap strips.

6. Mix thoroughly using a vortex centrifuge.

   Note: Hygiena Diagnostics recommends vortex centrifuges Multispin MSC-6000 for PCR strips or vortex centrifuge CVP-2 for PCR plates. Dedicated protocols are available for these centrifuges.

   Alternatively, resuspend the pellet by manual mixing. This may be achieved by carefully pipetting the sample up and down multiple times during step 4 or flipping the tube strips after sealing while pressing down the cap strip.

7. Spin the PCR tube strips for 30 seconds at 150 – 200 x g in a suitable centrifuge.

Note: If your centrifuge exceeds 200 x g, do not centrifuge for more than 5 seconds. Avoid centrifugation forces exceeding 1000 x g!

8. Place the samples in your PCR cycler and run the program as described above.

   Note: When using the LightCycler 480 instrument, a special adapter is necessary.

   For some PCR instruments, the PCR strips should be placed into the cycler block in a balanced order. For example, two strips can be placed in columns 1 and 12.

2.3 Data Interpretation

The amplification of DNA specific for yeasts belonging to the genus *Dekkera/Brettanomyces* is analyzed in the fluorescence channel suitable for FAM-labeled probe detection. The amplification of DNA specific for yeasts belonging to the genus *Zygosaccharomyces* is analyzed in the fluorescence channel suitable for VIC-/HEX-labeled probe detection. The amplification of DNA specific for yeasts belonging to the genus *Saccharomyces* is analyzed in the fluorescence channel suitable for ROX-labeled probe detection. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for Cy5/ATTO 490LS-labeled probe detection.

Compare the results from FAM, VIC/HEX, ROX and Cy5/ATTO 490LS (Internal Control) channels for each sample, and interpret the results as described in the table below.

2.3.1 Procedure A – Qualitative Detection

For qualitative detection, compare the results from FAM, VIC/HEX, ROX and Cy5/ATTO 490LS (Internal Control) channels for each sample and interpret the results as described in the table below:

Channel FAM	Channel VIC/HEX	Channel ROX	Channel Cy5/ATTO 490LS	Result Interpretation
Positive	Positive or Negative	Positive or Negative	Positive or Negative	Positive for *Dekkera/Brettanomyces* spp.
Positive or Negative	Positive	Positive or Negative	Positive or Negative	Positive for *Zygosaccharomyces* spp.
Positive or Negative	Positive or Negative	Positive	Positive or Negative	Positive for *Saccharomyces* spp.
Negative	Negative	Negative	Positive	Negative for targeted spoilage yeasts
Negative	Negative	Negative	Negative	Invalid

2.3.2 Procedure B – Quantification of Spoilage Yeast in GE/mL

In the real-time PCR cycler software, define positions of the dilutions of the foodproof Spoilage Yeast Detection 1 Quantification Standard as "Standard" with the respective concentrations given in the table above to generate a standard curve. Alternatively, a given standard curve from a previous PCR run can be imported if the real-time PCR instrument provides this functionality.

The foodproof Spoilage Yeast Detection 1 Quantification Standard is defined as GE/reaction (GE = genomic equivalent, amount of DNA equivalent to a single bacterial cell). The use of the calibration curve results in such a value for every sample analyzed. GE/reaction may be converted to GE/mL in a sample according to the following equation.

$$\textbf{result}\left[\frac{\textbf{GE}}{\textbf{mL}}\right] = \frac{\textbf{result}\left[\frac{\textbf{GE}}{\textbf{reaction}}\right] \times \textbf{elution volume}\ [\mu\textbf{L}] \times \textbf{recovery factor}}{\textbf{PCR reaction volume}\ [\mu\textbf{L}] \times\ \textbf{sample volume}\ [\textbf{mL}]}$$

- Elution volume = final volume after sample preparation
- Recovery factor = inverse fraction of the Rinse Buffer recovered after washing the filter
- PCR reaction volume = volume used per PCR reaction
- Sample volume = initial volume used for filtration

When requiring a GE count for larger volumes (e.g., *Y* = 500 mL), use this general formula:

$$\textbf{result}\left[\frac{\textbf{GU}}{\boldsymbol{Y}\ \textbf{mL}}\right] = \frac{\textbf{result}\left[\frac{\textbf{GU}}{\textbf{reaction}}\right] \times \textbf{elution volume}\ [\mu\textbf{L}] \times \textbf{recovery factor} \times \boldsymbol{Y}}{\textbf{PCR reaction volume}\ [\mu\textbf{L}] \times\ \textbf{sample volume}\ [\boldsymbol{Y}\ \textbf{mL}]}$$

Example:

The following calculation is suitable for samples prepared with the foodproof StarPrep Two Kit, assuming filtration of 500mL of a beverage sample:

- PCR reaction volume = 25 µL
- Elution volume = 250 µL
- Recovery factor = 1000 µL / 700 µL Rinse Buffer = 1.43
- Sample volume = 500 mL
- Y = 500 mL

$$\textbf{result}\left[\frac{\textbf{GU}}{\textbf{500 mL}}\right] = \frac{\textbf{result}\left[\frac{\textbf{GU}}{\textbf{reaction}}\right] \times \textbf{250}\ [\mu\textbf{L}] \times \textbf{1.43} \times \textbf{500}}{\textbf{25}\ [\mu\textbf{L}] \times\ \textbf{500}\ [\textbf{500 mL}]} = \textbf{result} \times \textbf{14.4}\left[\frac{\textbf{GU}}{\textbf{500 mL}}\right]$$

Note: The elution volume and recovery factor depend on the respective sample preparation protocol.

3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	• Set Channel settings to FAM, HEX, ROX and Cy5/ATTO 490LS. • If your instrument does not have a HEX channel, use VIC instead.
	Pipetting errors or omitted reagents.	• Check for correct pipetting scheme and reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	• Check the cycle programs.
No signal increase in the Cy5/ATTO 490LS channel is observed with negative results in all other channels.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Use a recommended DNA sample preparation kit to purify template DNA. • Dilute samples or pipette a lower amount of sample DNA (e.g., 20 µL of PCR-grade H_2O and 5 µL of sample instead of 25 µL of sample).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof Spoilage Yeast Detection 1 LyoKit lyophilized PCR Mix at 2 to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	• Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline.	Resuspension of lyophilized PCR mix is not complete.	• Always resuspend lyophilized PCR mix thoroughly.
Negative control samples are positive.	Carryover contamination is present.	• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carryover contamination. • Add positive controls after sample and negative control reaction vessels have been sealed.

Observation	Possible Reason	Recommendation
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Prepared PCR mix is still in the upper part of the vessel.	• Always centrifuge PCR strips. • Check that no air bubbles are formed or remain in tube after centrifugation.
	Outer surface of the vessel or seal is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the vessel and seal.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	• Always store the lyophilized PCR mix in the aluminum bag with the silica gel pad. • Open the PCR strip shortly before filling.

4. Additional Information on this Product

4.1 How this Product Works

The foodproof Spoilage Yeast Detection 1 LyoKit provides all necessary reagents and a control template for reliable interpretations of results. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included. The hydrolysis probe was designed to bind specifically to the IC, allowing detection in the Cy5/ATTO 490LS channel, whereas the spoilage yeast DNA is detected in the FAM, VIC/HEX and ROX channels.

In cases of a negative result due to inhibition of amplification by the sample DNA of interest, the amplification of the IC is suppressed as well. Therefore, a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of spoilage yeast DNA in the sample.

The foodproof Spoilage Yeast Detection 1 LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for detection of spoilage yeast DNA. Primers and probes provide specific detection of spoilage yeast DNA in food and beverage samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only.

4.2 Test Principle

1. Using the supplied sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and its associated reagents amplify and simultaneously detect fragments of genomic DNA originating from spoilage yeasts belonging to the genera *Dekkera*/*Brettanomyces*, *Zygosaccharomyces* and *Saccharomyces*.
2. The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probe due to the 5´-nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5´-end with a reporter fluorophore and at the 3´-end with a quencher.
3. During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal sequence of the amplicon downstream from one of the primer sites and is cleaved by the 5' nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.
4. The real-time PCR instrument measures the emitted fluorescence of the reporter dye.

4.3 Prevention of Carryover Contamination

The heat-labile Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions and the pretreatment of all successive PCR mixtures with the heat-labile UNG. The UNG cleaves DNA at any site where a dUTP residue has been incorporated. The resulting abasic sites are hydrolyzed due to the high temperatures during the initial denaturation step and can no longer serve as PCR templates. The heat-labile UNG is inactivated during the initial denaturation step. Native DNA (e.g., the isolated yeast genomic DNA) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in the foodproof Spoilage Yeast Detection 1 LyoKit, decontamination can be achieved with the provided reagents.

4.4 Background Information

Spoilage yeasts are usually defined as species or strains of yeast that are unintentionally introduced into a fermentation process or final product and that are capable of compromising the quality of food and beverages. Extreme examples of yeast spoilage include 'blown cans' of soft drinks, cloudy re-fermented wine, pink or red slime dripping from refrigerated meat, white yeast colonies on food and tainted fruit juices [1]. Species belonging to one of the three genera *Dekkera*/*Brettanomyces*, *Zygosaccharomyces* or *Saccharomyces* are considered as obligatory spoilage yeasts in both alcoholic and non-alcoholic beverages and constitute the most significant group of spoilage yeasts [1,2].

The genus *Dekkera*/*Brettanomyces* is presently made up of five different species: *D. anomala*, *D. bruxellensis*, *B. custersianus*, *B. naardenensis* and *B. nanus*. *Dekkera*/*Brettanomyces* yeasts are known as spoilers of various beverages, such as beer, juice and wine, and have harmful effects on flavor and/or visual appearance. These yeasts produce acetic acid, 4-ethylphenol and 4-ethylguaiacol, causing off-flavors in wine. *Dekkera*/*Brettanomyces* species also cause haze and turbidity in bottled wine and lambic beer [3]. It is possible that resistance to citric acid, together with the ability to utilize nitrate, may enhance the ability of *Dekkera*/*Brettanomyces* spp. to spoil low-nutrient soft drinks [1]. Therefore, for beverage manufacturers, control of these species is very important to prevent spoilage incidents in their products. Generally, *Dekkera*/*Brettanomyces* yeasts grow slowly on culture media, and the detection of spoilage *Dekkera*/*Brettanomyces* strains typically takes 3 – 7 days, depending on the medium used for detection. Therefore, rapid detection and identification methods for *Dekkera*/*Brettanomyces* yeasts have been previously reported, e.g., the polymerase chain reaction (PCR) method [3].

Zygosaccharomyces is osmophilic and resistant to ethanol, SO_2, sorbate, and other commonly used preservatives. Some species can even grow at temperatures as low as 2.5 °C (36.5 °F) [4]. *Zygosaccharomyces* causes spoilage by forming gas, sediment and/or cloudiness in bottled wines. Synthesis of other compounds, namely succinic, acetic and lactic acids, as well as acetaldehyde and glycerol, have also been reported [4]. *Zygosaccharomyces bailii*, *Z. bisporus* and *Z. lentus*, which are highly fermentative and very highly resistant to preservatives, give rise to safety concerns due to explosions of plastic and glass bottles. Such spoiled preserved foods usually result in expensive (and damaging) public recalls of products and 'deep-cleaning' of the factories involved. The presence of osmophilic *Z. rouxii* and *Z. bailii* in foods preserved by high sugar concentrations (e.g., candied fruits or confectionary) would require cheaper, silent recalls of products from the supply chain as well as assessments of the manufacturing process to identify errors that had allowed contamination by viable cells. Rapid identification of these yeasts from contaminated batches before the product leaves the factory is thus paramount [5].

Saccharomyces spp. (also called *Saccharomyces sensu stricto*) foreign yeasts pose a threat to many products due to their very high spoilage potential. This genus comprises many obligate spoilage microorganisms that cause high internal pressures or even explosions in bottled products, such as beverages. In many cases, sensory changes arise from spoilage by *Saccharomyces cerevisiae*, in particular its variant *Saccharomyces cerevisiae* var. *diastaticus* [6]. The latter is considered the most prominent and dangerous foreign yeast in breweries due to its potential to over-

ferment beer through its unique enzyme glucoamylase [2]. Other species, such as *S. pastorianus*, *S. bayanus* or *S. paradoxus,* lead to product spoilage, such as haze, sensory changes and pressure increases [6].

4.5 References

1. Bartram J and Stradford M. (2006). Food and beverage spoilage yeasts. *In*: Querol A; Fleet GH (eds.). *The Yeast Handbook Volume 2: Yeasts in Food and Beverages.* Springer-Verlag, Berlin, Germany, p.336 – 379.
2. Hutzler M. (2009). Dissertation: Entwicklung und Optimierung von Methoden zur Identifizierung und Differenzierung von getränkerelevanten Hefen. [in German]
3. Shimotsu S, Asano S, Iijima K, Suzuki K, Yamagishi H and Aizawa M (2015). Investigation of beer-spoilage ability of *Dekkera*/*Brettanomyces* yeasts and development of multiplex PCR method for beer-spoilage yeasts. *J Inst Brewing*. 121:177 – 180.
4. Fugelsang KC and Edwards CG. (2007). Wine Microbiology: Practical Applications and Procedures, 2nd ed.; Springer: New York, NY, USA.
5. Harrison E, Muir A, Stratford M and Wheals A. (2011). Species-specific PCR primers for the rapid identification of yeasts of the genus *Zygosaccharomyces*. FEMS Yeast Res. 11:356 – 365.
6. Hutzler M, Wellhoener U, Tenge C and Geiger E. (2008). Beer mixed beverages: dangerous spoilage yeasts, susceptible beverages? *Brauwelt Int*. 26:206 – 211.

4.6 Product Characteristics

The foodproof Spoilage Yeast Detection 1 LyoKit is designed to detect all species belonging to the genera *Dekkera*/*Brettanomyces*, *Zygosaccharomyces* and *Saccharomyces* by quantitative PCR. Performance has been tested with representative food and beverage matrices, e.g., beer and non-alcoholic beverages.

Specificity: The foodproof Spoilage Yeast Detection 1 LyoKit inclusivity has been tested with 108 strains, including all 5 species of *Dekkera*/*Brettanomyces*, all 14 species of *Zygosaccharomyces* and all 12 species of *Saccharomyces*.

The exclusivity was determined using 37 unrelated, wild yeast species. No false positives nor false negatives were determined.

Sensitivity: At least 10^2 CFU/mL can be detected from enrichment cultures with a sensitive protocol using the foodproof StarPrep Two Kit.

4.7 Quality Control

The foodproof Spoilage Yeast Detection 1 LyoKit is function-tested using the LightCycler 480 System.

5. Supplementary Information

5.1 Ordering Information

Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview and for more information, visit us at www.hygiena.com and contact us via email or phone.

5.2 License Notice

The purchase price of this product includes limited, nontransferable rights under US Patent No. 7,687,247 owned by Life Technologies Corporation to use only this amount of the product to practice the claims in said patent solely for activities of the purchaser for bioburden testing, environmental testing, food testing, or testing for genetically modified organisms (GMO) in accordance with the instructions for use accompanying this product. No other rights are conveyed, including no right to use this product for *in vitro* diagnostic, therapeutic, or prophylactic purposes. Further information on purchasing licenses under the above patent may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008.

Email: outlicensing@lifetech.com.

5.3 Trademarks

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5.4 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics, contact our Technical Support staff (for details see www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. Contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers:

R 602 47-1, R 602 47-2, R 602 47-3

6. Change Index

Version 1, July 2018
First version of the package insert.

Revision A, January 2024
Rebranding and new layout.
R 602 47 20 -> INS-KIT230121-22-23-REVA.

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