FOR IN VITRO USE ONLY

foodproof® Spoilage Yeast Detection 1 LyoKit
For Detection and Quantification of Major Spoilage Yeast Genera

– 5´Nuclease –

Version 1, August 2018

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

Order No. R 602 47-1 / R 602 47-2
Kit for 48 reactions (lyophilized) for a maximum of 46 samples
Store the kit at 2 to 8 °C
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1. What this Product Does

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.

Storage and Stability

- Store the kit at 2 °C 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:

Kit Contents

<table>
<thead>
<tr>
<th>Component</th>
<th>Label</th>
<th>Contents / Function / Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>foodproof® Spoilage Yeast Detection 1 LyoKit Microplate</strong>, prefilled with 48 reactions (lyophilized)</td>
<td>Aluminum bag containing a 8-tube strip mat • R 602 47-1 with white low profile tubes • R 602 47-2 with clear regular profile tubes</td>
<td>• 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-DNA N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination. • Store at 2 °C to 8 °C in the aluminum bag (tightly sealed). • Protect from light and moisture!</td>
</tr>
<tr>
<td>Quantification Standard</td>
<td>Vial 2 (purple cap)</td>
<td>• 1 x 350 µl • Contains a stabilized solution of DNA. • For use as a PCR run positive control or Quantification Standard. • Store at 2 to 8 °C.</td>
</tr>
<tr>
<td>H₂O PCR-grade</td>
<td>Vial 3 (colorless cap)</td>
<td>• 2 x 1 ml • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control.</td>
</tr>
<tr>
<td>Cap strips</td>
<td>Plastic bag containing 8-cap strips</td>
<td>• 12 x 8-cap strip • For use in real-time PCR after addition of samples.</td>
</tr>
</tbody>
</table>

Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM, VIC/HEX, ROX and Cy5-labeled probes. In cases the strip tubes don’t fit the instrument, the samples have to be transferred after resuspension of the lyophilized PCR mix to appropriate PCR vessels.
- Sample Preparation Kit foodproof® StarPrep Two Kit (Order No. S 400 08)¹
- Reagent D (Order No. A 500 02)¹
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex centrifuge Multispin MSC-6000 for PCR-strips (Order No. D 110 66)¹ with
  • SR-32, Rotor for MSC-3000/6000 (Order No. D 110 65)¹ or
  • Vortex centrifuge CVP-2 for PCR-plates (Order No. D 110 67)¹

¹ Available from BIOTECON Diagnostics; see ordering Information for details
Applicability Statement

The foodproof® Spoilage Yeast Detection 1 LyoKit – 5’Nuclease – is intended for the rapid qualitative or quantitative detection of spoilage yeast DNA isolated from all kind 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 use of Reagent D.

The kit must not be used in diagnostic procedures.

The kit described in this Instruction Manual has been developed for real-time PCR instruments. Versions R 602 47-1 and -2 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, LightCycler® 96 (Roche Diagnostics), AriaMx® and Mx3005P® (Agilent Technologies), ABI 7500 FAST, PikoReal® 24 (Thermo Fisher Scientific) and CFX96 (BIO-RAD).

Note: A Color Compensation is necessary and will be supplied by BIOTECON Diagnostics for users of the LC 480 System I and LC 480 System II (Color Compensation Set 5; Order No. A 500 15).
2. How to Use this Product

2.1 Before You Begin

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

• Keep the kit components separate from other reagents in the laboratory.
• Use nuclease-free lab ware (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 carry-over 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 carry-over 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!

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”).

DNA Extraction
BIOTECON Diagnostics provides sample preparation kits suitable for all kind of food and environmental samples (see “Additional Equipment and Reagents Required”).
For more product information please refer to www.bc-diagnostics.com.

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 Quantification Standard (vial 2, purple cap)] or with a positive sample preparation control.

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 H₂O PCR-grade (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

Program Setup

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 (Internal Control) detection channels. Program the PCR instrument before preparing the PCR samples. For details on how to program the experimental protocol, see the Instrument Operator’s Manual of your real-time PCR cycler.

Use the following real-time PCR-protocol for the foodproof® Spoilage Yeast Detection 1 LyoKit:

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 the probe quencher as well as the usage of a passive reference dye has to be specified. The foodproof® Spoilage Yeast Detection 1 LyoKit contains probes with a non-fluorescent (“dark”) quencher and no passive reference dye.

Preparation of the PCR Mix

Proceed as described below to prepare a 25 µl standard reaction. Always wear gloves when handling strips or caps. Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors.

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

Procedure A: Qualitative Detection

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or scalpel to cut the strips apart. Tightly seal the bag afterwards and store away at the recommended conditions.

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

3. Decap the tube strips cautiously and discard the cap strips.

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

4. Pipet 25 µl sample into each PCR-vessel:
   • For the samples of interest, add 25 µl sample DNA (if using less volume, add PCR-grade H₂O to achieve 25 µl).
   • For the negative control, add 25 µl PCR-grade H₂O (vial 3, colorless cap).
   • For the positive control, add 25 µl 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 vessels accurately and tightly with the colorless cap strips.
6. Mix thoroughly using a vortex centrifuge.

**Note:** BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-6000 (D 110 66) for PCR-strips or vortex centrifuge CVP-2 for PCR-plates (D 110 67). Dedicated protocols are available for these centrifuges.

**Note:** Alternatively resuspend the pellet by manual mixing. This may be achieved by cautiously 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 g in a suitable centrifuge.

**Note:** If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds. Avoid centrifugation forces exceeding 1,000 g!

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

**Note:** For using any LightCycler 480 instrument, a special adapter (Order No. Z 100 24) 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 column 1 and 12.

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 scalpel to cut the strips apart. Tightly seal the bag afterwards and store away at the recommended conditions.

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

3. Decap the tube strips cautiously and discard the cap strips.

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

4. Pipet 25 µl sample into each PCR-vessel:
   - For the samples of interest, add 25 µl sample DNA (if using less volume, add PCR-grade H₂O to achieve 25 µl).
   - For the negative control, add 25 µl PCR-grade H₂O (vial 3, colorless cap).
   - For the positive control, add 25 µl each of four dilutions (in duplicate) of the foodproof® Spoilage Yeast Detection 1 Quantification Standard (vial 2, purple cap) to generate a standard curve (see table below).

**Note:** Therefore, 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 samples of interest. Since 48 reactions can be run with the kit, up to 39 samples may be analyzed quantitatively during one PCR run.

**Note:** Once a standard curve has been established with a specific lot of reagents it is possible to apply this as an external standard curve to quantify samples in successive PCR runs. Only a single dilution of the foodproof® Spoilage Yeast Detection 1 Quantitative Standard then needs to be added to the run to serve as a calibrator. Please inquire at BIOTECON Diagnostics for more information on how to generate and use an external standard curve.
Dilution of Quantification Standard

Quantification of spoilage yeasts via the standard curve procedure requires the stepwise dilution of the foodproof® Spoilage Yeast Detection 1 Quantification Standard (vial 2, purple cap) with the foodproof® Spoilage Yeast Detection 1 H₂O PCR-grade (vial 3, colorless cap) as shown below. Prepare each dilution step with a final volume of 100 μl by using 10 μl of the previous dilution step.

<table>
<thead>
<tr>
<th>Dilution step</th>
<th>Dilution</th>
<th>Concentration to be entered as standard [GE/reaction*]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FAM channel</td>
</tr>
<tr>
<td>1</td>
<td>Undiluted</td>
<td>30,000</td>
</tr>
<tr>
<td>2</td>
<td>1:10</td>
<td>3,000</td>
</tr>
<tr>
<td>3</td>
<td>1:100</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>1:1,000</td>
<td>30</td>
</tr>
</tbody>
</table>

* 1 GE (genome equivalent) ideally corresponds to a diploid genome (~1 yeast cell) present in the sample.

5. Seal the vessels accurately and tightly with the colorless cap strips.

6. Mix thoroughly using a vortex centrifuge.

**Note:** BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-6000 (D 110 66) for PCR-strips or vortex centrifuge CVP-2 for PCR-plates (D 110 67). Dedicated protocols are available for these centrifuges.

**Note:** Alternatively resuspend the pellet by manual mixing. This may be achieved by cautiously 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 g in a suitable centrifuge.

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

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

**Note:** For using any LightCycler 480 instrument, a special adapter (Order No. Z 100 24) 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 column 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 probes detection. The amplification of DNA specific for yeasts belonging to the genus *Zygosaccharomyces* is analyzed in the fluorescence channel suitable for VIC/HEX. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for Cy5.

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

**Procedure A – Qualitative Detection**

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

<table>
<thead>
<tr>
<th>Channel FAM</th>
<th>Channel HEX</th>
<th>Channel ROX</th>
<th>Channel Cy5</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive or Negative</td>
<td>Positive or Negative</td>
<td>Positive or Negative</td>
<td>Positive for <em>Dekkera/Brettanomyces</em> spp.</td>
</tr>
<tr>
<td>Positive or Negative</td>
<td>Positive</td>
<td>Positive or Negative</td>
<td>Positive or Negative</td>
<td>Positive for <em>Zygosaccharomyces</em> spp.</td>
</tr>
<tr>
<td>Positive or Negative</td>
<td>Positive or Negative</td>
<td>Positive</td>
<td>Positive or Negative</td>
<td>Positive for <em>Saccharomyces</em> spp.</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative for targeted spoilage yeasts</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

**Procedure B – Quantification of spoilage yeast in GE/ml**

In the real-time PCR cycler software define the 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. It is recommended to use the “Spoilage Yeast Detection 1 Quantification Template” provided by BIOTECON Diagnostics for analysis.

\[
\text{result [GE/ml]} = \frac{\text{result [GE/reaction]} \times \text{elution volume} [\mu l] \times \text{recovery factor}}{\text{PCR reaction volume} [\mu l] \times \text{sample volume} [ml]}
\]

- PCR reaction volume = volume used per PCR reaction
- elution volume = final volume after sample preparation
- recovery factor = inverse fraction of Rinse Buffer recovered after washing the filter
- sample volume = initial volume used for filtration
When requiring a GE count for larger volumes (e.g. \( Y = 500 \) ml), use this general formula:

\[
\text{result \left[ \frac{\text{GU}}{\text{Y ml}} \right]} = \frac{\text{result \left[ \frac{\text{GU}_{\text{reaction}}}{\mu l} \right] \times \text{elution volume} [\mu l] \times \text{recovery factor} \times Y}{\text{PCR reaction volume} [\mu l] \times \text{sample volume} [\text{Y ml}]}
\]

**Example:**
The following calculation is suitable for samples prepared with the foodproof\textsuperscript{®} StarPrep Two Kit (S 400 08), assuming filtration of 500ml of a beverage sample:
- PCR reaction volume = 25 \( \mu l \)
- Elution volume = 250 \( \mu l \)
- Recovery factor = 1000 \( \mu l / 700 \mu l \) Rinse Buffer = 1.43
- Sample volume = 500 ml
- \( Y = 500 \) ml

\[
\text{result \left[ \frac{\text{GU}}{500 \text{ ml}} \right]} = \frac{\text{result \left[ \frac{\text{GU}_{\text{reaction}}}{25 [\mu l]} \times 250 [\mu l] \times 1.43 \times 500 [500 \text{ ml}] \right]}{25 [\mu l] \times 500 [500 \text{ ml}]}} = \text{result} \times 14.4 \left[ \frac{\text{GU}}{500 \text{ ml}} \right]
\]

**Note:** Elution volume and recovery factor depend on the respective sample preparation protocol. Use the “Spoilage Yeast Detection 1 Quantification Template” tailored to the sample preparation protocol in use.

**Note:** When performing quantification with an external standard curve from a previous run, a “calibration factor” has to be applied to account for run-to-run variance in Cp values. This factor is calculated from the difference between the Cp of the foodproof\textsuperscript{®} Spoilage Yeast Detection 1 Quantification Standard calibrator and matching Cp values of the pre-recorded standard curve.
### 3. Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Reason</th>
<th>Recommendation</th>
</tr>
</thead>
</table>
| No signal increase is observed, even with positive controls. | Incorrect detection channel has been chosen.                                      | • Set Channel settings to FAM, HEX, ROX and Cy5.  
• If your instrument does not have a HEX Channel, use VIC instead. |
| Pipetting errors.                                 |                                                                                  | • Check for correct 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 channel Cy5 is observed, with other channels also negative. | Inhibitory effects of the sample material (e.g., caused by insufficient purification). | • Use the recommended DNA sample preparation kit to purify template DNA.  
• Dilute samples or pipet a lower amount of sample DNA (e.g., 20 µl PCR-grade H₂O and 5 µl sample DNA instead of 25 µl sample DNA). |
| Fluorescence intensity is too low.                | Inappropriate storage of kit components.                                        | • Store the foodproof Spoilage Yeast Detection 1 LyoKit lyophilized PCR Mix at 2 °C to 8 °C, protected from light and moisture. |
| Strong decrease of fluorescence baseline          | Low initial amount of target DNA.                                               | • Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur. |
| Negative control samples are positive.            | Resuspension of lyophilized PCR mix not complete                                | • Always resuspend lyophilized PCR mix thoroughly.                                                   |
| Fluorescence intensity varies.                    | Carry-over contamination.                                                       | • 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 carry-over contamination.  
• Add positive controls after sample and negative control reaction vessels have been sealed. |
| Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact). |                                                                                  | Always wear gloves when handling the vessels and seal.                                             |
| Pellets are difficult to dissolve.                | The lyophilized PCR mix started to rehydrate.                                  | • Store the lyophilized PCR mix always in the aluminum bag with the silica gel pad  
• Open Strip shortly before filling.                                                                  |
4. Additional Information on this Product

How this Product Works

The foodproof® Spoilage Yeast Detection 1 LyoKit – 5’Nuclease – 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. A hydrolysis probe was designed to bind specifically the IC, allowing detection in the Cy5 channel, whereas the DNA from spoilage yeasts is detected in channels FAM, HEX/VIC and ROX. In case of a negative result due to inhibition of the amplification by the sample DNA of interest, the amplification of the IC is suppressed as well, whereas 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 the 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.

Test Principle

1. Using the kit’s sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify 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 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 PCR instrument measures the emitted fluorescence of the reporter dye.

Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over 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 deoxyuridine 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.

Background Information

Spoilage yeasts are usually defined as species or strains of yeast unintentionally introduced into a fermentation process or final product, which 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 to 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. For example, polymerase chain reaction (PCR) method [3].

Zygosaccharomyces is osmophilic, resistant to ethanol, SO₂, 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 has 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 in plastic and glass bottles and such spoiled preserved foods would normally result in an expensive (and damaging) public recall 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, for example candied fruits or confectionary, would require a cheaper silent recall of products from the supply chain, and assessment of the manufacturing process to identify errors that had allowed access 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 which 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 as the most prominent and dangerous foreign yeast in breweries due to its potential to over-ferment beer through its unique enzyme glucoamylase [2]. Also other species such as S. pastorianus, S. bayanus or S. paradoxus lead to product spoilage such as haze, sensory changes and pressure increases [6].

References
Product characteristics

The foodproof® Spoilage Yeast Detection 1 LyoKit has been 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 (Order No. S 400 08).

Quality Control

The foodproof® Spoilage Yeast Detection 1 LyoKit – 5’Nuclease – is function tested using the LightCycler® 480 System (R 602 47-1 and -2).

5. Supplementary Information

5.1 Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

5.2 License

License Notice

The purchase price of this product includes limited, nontransferable rights under U.S. 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 about this or any other product of BIOTECON Diagnostics, please contact our Technical Support staff (for details see www.bc-diagnostics.com). Our scientists commit themselves to providing rapid and effective help. We also want you to 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.

6. Change Index

Version 1, July 2018

First version of the package insert.