



foodproof® Spoilage Yeast Detection 3 LyoKit

Revision B, July 2024

PCR kit for the qualitative detection of *Saccharomyces cerevisiae* var. *diastaticus* and *Brettanomyces/Dekkera* spp. with specific identification of *Brettanomyces bruxellensis* and *Brettanomyces anomalus* using real-time PCR instruments.

Product No. KIT230140 (LP)

Product No. KIT230141 (RP)

Product No. KIT230142 (DP)

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

Store the kit at 2 to 8 °C

FOR *IN VITRO* USE ONLY



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1. What this Product Does

1.1 Number of Tests

The kit is designed for 48 reactions with a final reaction volume of 25 μ L 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 kit at 2 to 8 °C through expiration date printed on label.
- Once kit is opened, store components as described in the following Kit Contents table:

1.3 Kit Contents

Component	Description	Contents / Function / Storage
foodproof [®] Spoilage Yeast Detection 3 LyoKit Microplate, prefilled with 48 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230140 with white low-profile (LP) tubes • KIT230141 with clear regular-profile (RP) tubes • KIT230142 with clear deep-profile (DP) tubes	• 48 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for DNA of designated spoilage yeasts and Internal Control (IC), as well as Taq DNA Polymerase and Uracil-DNA N- Glycosylase (UNG, heat labile) which is for prevention of carry-over contamination. • Store at 2 to 8 °C in supplied aluminum bag with the silica pad (Keep tightly sealed). • Protect from light and moisture!
Control Template	Vial 2 (purple cap)	• 1 x 350 μL • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.
H2O PCR-grade	Vial 3 (colorless cap)	• 1 x 1 mL • Nuclease-free, PCR-grade H2O. • 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.

1.4 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM, HEX, ROX and Cy5/ATTO 490LS-labeled probes. For situations where PCR strip tubes don't fit the cycler, samples can 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)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- 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 3 LyoKit is intended for rapid qualitative detection of spoilage yeast DNA isolated from a wide range of beverage samples that are potentially contaminated with *Saccharomyces cerevisiae* var. *diastaticus* and/or *Brettanomyces/Dekkera* spp. DNA from dead yeast can be excluded from analysis by use of 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 KIT230140 and KIT230141 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 (Thermo Scientific), QuantStudio™ 5 (Thermo Scientific) and CFX96™ (Bio-Rad).
- Version KIT230142 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 3 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 3 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 food 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 3 Control Template (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 3, 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 (KIT230142)

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 (KIT230140 / KIT230141)

The following procedure is optimized for real-time PCR instruments with FAM (*Saccharomyces cerevisiae* var. *diastaticus*), VIC/HEX (*Brettanomyces/Dekkera* spp.), ROX (*B. bruxellensis* and *B. anomalus*) and Cy5 or ATTO 490LS (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 for the real-time PCR cycler used.

Use the following real-time PCR protocol for the foodproof Spoilage Yeast Detection 3 LyoKit:

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

* Minimum denaturation time may be longer on some instruments. Set to the shortest denaturation time possible in this case.

** Fluorescence detection in Step 2

Notes:

- For some real-time PCR instruments, the type of the probe quencher as well as the use of a passive reference dye has to be specified. The foodproof Spoilage Yeast Detection 3 LyoKit contains probes with a non-fluorescent (“dark”) quencher and no passive reference dye.



2.2.3 Preparation of the PCR Mix

Proceed as described below to prepare a 25 μ L 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.

2.2.4 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. Tightly seal the bag afterwards and store under 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. Uncap 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. Pipette 25 μ L sample into each PCR vessel:
 - For the samples of interest, add 25 μ L sample DNA (if using less volume, add PCR-grade H₂O to achieve 25 μ L).
 - For the negative control, add 25 μ L PCR-grade H₂O (vial 3, colorless cap).
 - For the positive control, add 25 μ L foodproof Spoilage Yeast Detection 3 Control Template (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: Hygiena Diagnostics recommends vortex centrifuge Multispin MSC-6000 for PCR strips or vortex centrifuge CVP-2 for PCR plates. Dedicated protocols are available for these centrifuges upon request.

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 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 any 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.



2.3 Data Interpretation

Amplification of DNA specific for *Saccharomyces cerevisiae* var. *diastaticus* is analyzed in the fluorescence channel suitable for FAM-labeled probes detection. DNA-specific amplification for *Brettanomyces/Dekkera* spp. is analyzed in the fluorescence channel suitable for VIC/HEX. Amplification of DNA specific for *Brettanomyces bruxellensis* and *Brettanomyces anomalus* is analyzed in the fluorescence channel suitable for ROX. Specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for Cy5/ATTO 490LS.

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

2.3.1 Qualitative Detection

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

Channel FAM	Channel HEX	Channel ROX	Channel Cy5/ATTO 490LS	Result Interpretation
Positive	Positive or Negative	Positive or Negative	Positive or Negative	Positive for <i>Saccharomyces cerevisiae</i> var. <i>diastaticus</i>
Positive or Negative	Positive	Positive or Negative	Positive or Negative	Positive for <i>Brettanomyces/Dekkera</i> spp.
Positive or Negative	Positive	Positive	Positive or Negative	Positive for <i>B. bruxellensis</i> and/or <i>B. anomalus</i>
Negative	Negative	Negative	Positive	Negative for targeted spoilage yeasts
Negative	Negative	Negative	Negative	Invalid



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 or ATTO 490LS. • 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 pipette 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 3 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 not complete	• Always resuspend lyophilized PCR mix thoroughly.
Negative control samples are positive.	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.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Resuspend 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 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

4.1 How this Product Works

The foodproof Spoilage Yeast Detection 3 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. A hydrolysis probe was designed to bind specifically the IC, allowing detection in the Cy5 channel or ATTO 490LS channel, whereas the DNA from spoilage yeasts is detected in channels FAM, HEX and ROX. In case of a negative result due to inhibition of the amplification by the sample DNA extract of interest, the amplification of the IC is suppressed as well, whereas a negative result for the sample DNA extract of interest and amplification of the IC clearly indicates the absence of spoilage yeast DNA in the sample. The foodproof Spoilage Yeast Detection 3 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.

4.2 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 species *Saccharomyces cerevisiae* var. *diastaticus* and *Brettanomyces/Dekkera* spp.
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 amplicon sequence 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.

4.3 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 3 LyoKit, decontamination can be achieved with the provided reagents.

4.4 Background Information

Spoilage yeasts are usually defined as species or strains of yeast unintentionally introduced into a fermentation process or final product, which can compromise the quality of food and beverages. Extreme examples of yeast spoilage include exploding packages, "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].

Saccharomyces cerevisiae var. *diastaticus* is considered one of the most dangerous spoilers of beer, as it ferments dextrins, leading to super-attenuation. This yeast is particularly noted for its ability to extracellularly secrete glucoamylase, which is encoded by the STA1 gene, and to ferment starch [2,3].



Saccharomyces cerevisiae var. *diastaticus* causes extremely high bottle internal pressures, leading even to explosions and sensory changes such as dry mouthfeel and phenolic off-flavor [4].

The genus *Dekkera/Brettanomyces* is comprised of five recognized species: *B. anomalus*, *B. bruxellensis*, *B. custersianus*, *B. naardenensis* and *B. nanus*. *Dekkera/Brettanomyces* yeast are spoilers of many 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 [5]. 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, the polymerase chain reaction (PCR) method [5]. *Dekkera/Brettanomyces* spp. present markedly different ability in their potential to spoil different beverages. *B. anomalus* and *B. bruxellensis* present the largest potential, being able to spoil even low pH, high alcohol beverages such as beer and wine. Also, *B. custersianus* exhibits beer spoilage properties [5]. In contrast, the growth of *B. nanus* and *B. naardenensis* appears to be limited in beer but these yeasts have been reported as important spoilers of beer mixed beverages and non-alcoholic beverages [1,2,5,6].

4.5 References

1. Bartram, J., Stradford, M. (2006). Food and beverage spoilage yeasts. In: Querol, A.; Fleet, G.H. (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.
3. Yamashita I., Suzuku K., Fukui S. (1985). Nucleotide Sequence of the Extracellular Glucoamylase Gene STAI in the Yeast *Saccharomyces diastaticus*. *J. Bacteriol. Microbiol.*, vol. 161, no. 2, p.567–573.
4. Hutzler, M., Wellhoener, U., Tenge, Chr., Geiger, E. (2008). Beer mixed beverages: dangerous spoilage yeasts, susceptible beverages? *Brauwelt International IV* p.206-211.
5. Shimotsu S, Asano S, Iijima K, Suzuki K, Yamagishi H, Aizawa M (2015) Investigation of beer-spoilage ability of *Dekkera/Brettanomyces* yeasts and development of multiplex PCR method for beer-spoilage yeasts. *Journal of The Institute of Brewing*. 121, 177-180.
6. Hutzler, M., Riedl, R., Koob J., Jacob, FA. (2012). Fermentation and Spoilage Yeasts and their Relevance for the Beverage Industry – A Review. *Brewing Science*. 65, 33-52.

4.6 Product characteristics

The foodproof Spoilage Yeast Detection 3 LyoKit has been designed to detect all strains belonging to the species *Saccharomyces cerevisiae* var. *diastaticus* and the genus *Dekkera/Brettanomyces* by qualitative PCR. Performance has been tested with representative beverage matrices, e.g. beer, wine, non-alcoholic beverages and juices.

Specificity: The foodproof Spoilage Yeast Detection 3 LyoKit inclusivity has been tested with 45 strains including 23 strains of *Saccharomyces cerevisiae* var. *diastaticus* and 22 strains of *Dekkera/Brettanomyces*. The exclusivity was determined using 42 unrelated wild yeast species. No false positives or false negatives were determined.

Sensitivity: At least 10² cfu/ml can be directly detected from beverages and enrichment cultures with a sensitive protocol using the foodproof StarPrep Two Kit (Product No. KIT230177). A sensitivity of 1 cell per sampled volume can be achieved with suitable pre-enrichment procedures.



4.7 Quality Control

The foodproof Spoilage Yeast Detection 3 LyoKit is function tested using the LightCycler 480 System and the Dualo 32 Beverage.

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, please visit our website at www.hygiena.com.

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 GmbH, please contact our Technical Support staff (www.hygiena.com/support). 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.

5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers:
R 602 69-1, R 602 69-2, R 602 69-3

6. Change Index

Version 1, July 2021

First version of the package insert.

Revision A, January 2024:

Rebranding and new layout.

R 602 69 20 -> INS-KIT230140-41-42-RevA

Revision B, July 2024:

Minor edits – 2.2.2 Step 2, Pre-incubation time



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