



foodproof® Spoilage Yeast Detection 2 LyoKit®

Revision A1, April 2026

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

Product No. KIT230124 (LP)

Product No. KIT230125 (RP)

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 volume of 25 µL per reaction. Up to 46 samples (single sample preparations) 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 the expiration date printed on label.
- Once the 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 2 LyoKit® Microplate, prefilled with 48 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat <ul style="list-style-type: none"> • KIT230124 with white low-profile (LP) tubes • KIT230125 with clear regular-profile (RP) tubes 	<ul style="list-style-type: none"> • 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 to 8 °C in the aluminum bag with the silica pad (Keep tightly sealed). • Protect from light and moisture!
Control Template	Vial 2 (purple cap)	<ul style="list-style-type: none"> • 1 x 350 µL • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.
H ₂ O PCR-grade	Vial 3 (colorless cap)	<ul style="list-style-type: none"> • 2 x 1 mL • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	<ul style="list-style-type: none"> • 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. If the PCR strip tubes do not fit the instrument, the samples must be transferred to appropriate PCR vessels after resuspending the lyophilized PCR mix.
- 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** the SR-32, Rotor for MSC-3000/6000 **or** Vortex centrifuge CVP-2 for PCR plates



1.5 Applicability Statement

The foodproof Spoilage Yeast Detection 2 LyoKit is intended for the rapid qualitative detection of spoilage yeast DNA isolated from all kinds of food and beverage samples that are potentially contaminated with *Saccharomyces cerevisiae* var. *diastaticus*, *Wickerhamomyces anomalus*, *Kazachstania exigua* or *Schizosaccharomyces pombe*. DNA from dead yeast can be excluded from analysis by using Reagent D.

The kit must not be used in diagnostic procedures. The kit described in these Product Instructions is intended for use with real-time PCR instruments.

Both KIT230124 (LP) and KIT230125 (RP) are designed for instruments with FAM, VIC/HEX, ROX and Cy5/ATTO 490LS 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 available from Hygiena® Diagnostics for users of the LC 480 System I and LC 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 2 LyoKit requires DNA amplification by PCR. The kit provides all the reagents required for the PCR. However, 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 labware (e.g., pipettors, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-barrier 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 cycler runs to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.

Keep the foodproof Spoilage Yeast Detection 2 LyoKit lyophilized PCR Mix away from light and moisture!

2.1.2 Sample Material

Use any sample material suitable for PCR, with regard to purity, concentration and the 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, 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 (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 PCR-grade H₂O (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

The following procedure is optimized for a real-time PCR instrument with FAM (*Saccharomyces cerevisiae* var. *diastaticus*), VIC/HEX (*Wickerhamomyces anomalus*), ROX (*Kazachstania exigua* and *Schizosaccharomyces pombe*) and Cy5/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 your real-time PCR cycler.

Use the following real-time PCR protocol for the foodproof Spoilage Yeast Detection 2 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.

<u>Melting Curve</u>	1 cycle
Step 1:	95 °C for 50 seconds
Step 2:	50 °C for 50 seconds
Step 3*:	Ramp up to 85 °C

* Fluorescence detection during 50 – 85 °C ramp with 1 measurement/°C.

Note: For some real-time PCR instruments, the type of probe quencher and the use of a passive reference dye must be specified. The foodproof Spoilage Yeast Detection 2 LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.

2.2.2 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.



2.2.3 Qualitative Detection

1. Remove the needed number of PCR tube strips from the aluminum bag. Use scissors or a scalpel to cut the strips apart. Tightly seal the bag afterward and store it 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. Uncap the tube strips cautiously and discard the cap strips.
Note: Do not leave strips open for extended periods. To avoid unwanted liquid absorption, open strips 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 2 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.
Note: 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 1,000 x g!
8. Place the samples in your PCR cycler and run the program as described above.
Note: For use with any LightCycler 480 instrument, a special adapter (Product No. MIS230005) is required. 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. The amplification of DNA specific for *Wickerhamomyces anomalus* is analyzed in the fluorescence channel suitable for VIC/HEX. The amplification of DNA specific for *Kazachstania exigua* and *Schizosaccharomyces pombe* is analyzed in the fluorescence channel suitable for ROX. The 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	Melt Curve ROX	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>Wickerhamomyces anomalus</i>
Positive or Negative	Positive or Negative	Positive	Positive or Negative	67 ± 2 °C	Positive for <i>Kazachstania exigua</i>
Positive or Negative	Positive or Negative	Positive	Positive or Negative	77 ± 2 °C	Positive for <i>Schizosaccharomyces pombe</i>
Negative	Negative	Negative	Positive	---	Negative for targeted spoilage yeasts
Negative	Negative	Negative	Negative	---	Invalid

Note: The Control Template contains a mixture of all target sequences and, therefore, usually generates significantly higher fluorescent values than samples that are positive for only one or two of the targets. This can affect positive/negative calls in automatic analysis of amplification curves by the respective instrument software. Always check results visually for plausibility.



3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> Check for correct reaction setup. Repeat the PCR run. Always run a positive control along with your samples.
	No data acquisition programmed.	<ul style="list-style-type: none"> Check the cycle programs.
No signal increase in channel Cy5/ATTO 490LS is observed, while other channels are also negative.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> Store the foodproof Spoilage Yeast Detection 2 LyoKit lyophilized PCR Mix at 2 to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	<ul style="list-style-type: none"> Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline	Resuspension of the lyophilized PCR mix is not complete.	<ul style="list-style-type: none"> Always resuspend the lyophilized PCR mix thoroughly.
Negative control samples are positive.	Carry-over contamination.	<ul style="list-style-type: none"> 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 the sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Resuspended PCR mix is still in the upper part of the vessel.	<ul style="list-style-type: none"> Always centrifuge PCR strips. Check that no air bubbles are formed or remain in tubes/strips after centrifugation.
	The 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.	<ul style="list-style-type: none"> Always store the lyophilized PCR mix in the aluminum bag with the silica gel pad. Open strips shortly before filling.



4. Additional Information on this Product

4.1 How this Product Works

The foodproof Spoilage Yeast Detection 2 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 amplification inhibition, an Internal Control (IC) is included. A hydrolysis probe was designed to bind specifically to the IC, allowing detection in the Cy5/ATTO 490LS channel, whereas DNA from spoilage yeasts is detected in the FAM, HEX and ROX channels. In case of a negative result due to amplification inhibition 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 2 LyoKit minimizes contamination risk and includes all reagents (except template DNA) needed for detecting 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

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*, *Wickerhamomyces anomalus*, *Kazachstania exigua* and *Schizosaccharomyces pombe*.

The PCR instrument detects these amplified fragments in real time by measuring fluorescence generated from cleavage of the hybridized probe by the 5'-nuclease activity of Taq DNA polymerase. The probe is labeled at the 5'-end with a reporter fluorophore and at the 3'-end with a quencher.

During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal amplicon sequence and is cleaved by Taq DNA polymerase's 5'-nuclease activity. This cleavage of the probe separates the reporter and quencher dyes, increasing the reporter dye signal.

The PCR instrument measures the fluorescence emitted by 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 subsequent 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 2 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 "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 beer spoilers, as it ferments dextrins, giving rise to super-attenuated beers. This yeast is particularly notable for its ability to secrete extracellular glucoamylase, 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 [6].

Wickerhamomyces anomalus (i.e., *Pichia anomala*) is frequently found in the environment and known as a spoiler of beer, wine, fruit, dough and meat. This species can produce ethyl acetate from glucose [4]. It is estimated that *Wickerhamomyces anomalus* is found in 4 – 5% of all spoiled beverages [2]. *Wickerhamomyces anomalus* is a frequent constituent of biofilms, particularly those detected in bottling sections of breweries [5]. In addition, *Wickerhamomyces anomalus* is one of the yeasts that can grow abundantly in wine, with fully aerobic or weakly fermentative metabolism, leading to film formation on the surface of bulk wine in unfilled containers with low sulfite levels. Due to the production of high levels of ethyl acetate and acetic acid before and during initial fermentation steps, *Wickerhamomyces anomalus* can lead to serious wine deterioration [7].

Kazachstania exigua (i.e., *Saccharomyces exiguus*), albeit detected at a lower frequency of about 1.1% in spoiled beverages, is considered a more potent spoilage yeast than *Wickerhamomyces anomalus* [2,6]. *Kazachstania exigua* has been isolated from a number of foods and beverages, including beer, non-alcoholic beverages, olives, yogurt, sourdough, sauerkraut and meat [5]. *Kazachstania exigua* has a spoilage potential similar to *Saccharomyces sensu stricto* yeasts, as it is fast-growing and has a high fermentation activity [4].

Schizosaccharomyces pombe is an osmotolerant, heat- and preservative-resistant spoilage yeast that can grow at 37 °C [4]. This yeast has a high fermentation activity and has historically been used in the production of African beers, rum and other indigenous beverages. It has mostly been isolated as a spoiler from juices, syrups and even beer [2,4,5].

4.5 References

1. Bartram J., 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*.
3. Yamashita I, Suzuku K, Fukui S. (1985). Nucleotide Sequence of the Extracellular Glucoamylase Gene *STA1* in the Yeast *Saccharomyces diastaticus*, *J. Bacteriol. Microbiol.*, 161(2): 567 – 573.
4. Pitt JI and Hocking AD. (2009). *Fungi and Food Spoilage*. Heidelberg: Springer.
5. Deak T. (2008). *Handbook of Food Spoilage Yeasts*, 2nd ed. Boca Raton: CRC Press.
6. Hutzler M, Wellhoener U, Tenge Chr, Geiger E. (2008). Beer Mixed Beverages: Dangerous Spoilage Yeasts, Susceptible Beverages. *Brauwelt International* 26:206 – 211.
7. Loureiro V, Malfeito-Ferreira M. (2003). Spoilage yeasts in the wine industry. *Int J Food Microbiol.* 86(1 – 2): 23 – 50.

4.6 Product characteristics

The foodproof Spoilage Yeast Detection 2 LyoKit has been designed to detect all strains belonging to the species *Saccharomyces cerevisiae* var. *diastaticus*, *Wickerhamomyces anomalus*, *Kazachstania exigua* and *Schizosaccharomyces pombe* by real-time PCR. Performance has been tested with representative beverage matrices, e.g., beer, wine, non-alcoholic beverages and juices.



Specificity: The foodproof Spoilage Yeast Detection 2 LyoKit inclusivity has been tested with 49 strains, including 25 strains of *Saccharomyces cerevisiae* var. *diastolicus*, 14 strains of *Wickerhamomyces anomalus*, 5 strains of *Kazachstania exigua* and 5 strains of *Schizosaccharomyces pombe*. Besides *Kazachstania exigua*, the closely related species *Kazachstania turicensis*, *Kazachstania humaticus* and *Kazachstania bulderi* may also be detected in the ROX channel. The exclusivity was determined using 65 unrelated wild yeast species. No false positives nor false negatives were determined.

Sensitivity: At least 10^2 CFU/mL can be detected in enrichment cultures using a sensitive protocol with the foodproof StarPrep Two Kit (Product No. KIT230177).

4.7 Quality Control

The foodproof Spoilage Yeast Detection 2 LyoKit is functionally 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 our website at www.hygiena.com.

5.2 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 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 are committed to providing rapid and effective assistance. 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 48-1 and R 602 48-2



6. Change Index

Version 1, August 2018

First version of the package insert.

Revision A, February 2024:

Rebranding and new layout.

R 602 48 20 -> INS-KIT230124-25-26-RevA

Revision A1, April 2026:

Removed a discontinued product.

INS-KIT230124-25-26-RevA -> INS-KIT230124-25-RevA1



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