foodproof® **Beer Screening 2 LyoKit**

Revision A, January 2024

PCR kit for the qualitative detection of beer spoilage bacteria DNA of the genera *Lactobacillus, Pediococcus, Pectinatus* and *Megasphaera*, and detection of hop-tolerance genes *hor*A and *hor*C using real-time PCR instruments.

Product No. KIT230074 / KIT230075 / KIT230076

Kit for 96 reactions (lyophilized) for a maximum of 94 samples

Store the kit at 2 to 8 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

Table of Contents

1. Product Overview

1.1 Number of Tests

The kit is designed for 96 reactions with a final reaction volume of 25 µL each. Up to 94 samples (single sample preparation) plus positive and negative control reactions can be analyzed per run.

1.2 Storage and Stability of Kit/Components

- 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.

Component	Label	Contents / Function / Storage
foodproof® Beer Screening 2 LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230074 with white low profile tubes • KIT230075 with clear regular profile tubes • KIT230076 with clear low profile tubes	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for DNA of beer spoilage bacteria, hop-tolerance genes *hor*A and *hor*C, 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. • For amplification and detection of beer spoilage bacteria- and hop tolerance-specific sequences. • Store at 2 to 8 °C in the aluminum bag (sealed). • **Protect from light and moisture!**
Control Template	Vial 2 (purple cap)	• 1 x 250 µL • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • 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.

1.3 Additional Equipment and Reagents Required

- Dualo 32® Beverage or other real-time PCR instrument. For details, please refer to "*1.4 Applicability Statement*".
- DNA extraction kits:
  foodproof StarPrep Two Kit (Product No. KIT230177) or
  foodproof StarPrep Three Kit (Product No. KIT230187)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex centrifuge Multispin MSC-3000/6000 for PCR strips **with**
  - SR-32, Rotor for MSC-3000/6000
- Alternative: Vortex centrifuge CVP-2 for PCR plates

1.4 Applicability Statement

The foodproof Beer Screening 2 LyoKit is intended for the rapid screening for the presence of potentially beer-spoiling bacteria in preparations from potentially contaminated beer, enrichment broth or pitching yeast. In channel FAM, the beer-spoiling lactic acid bacteria, *Lactobacillus* and *Pediococcus* are detected; in channel HEX, the anaerobe spoilers *Pectinatus* and *Megasphaera* are detected (see table below). Channel ROX allows to screen for the genes *hor*A and *hor*C that are correlated with the ability of lactic acid bacteria to grow in beer.

Beer spoilage bacteria detected by the screening of the foodproof® Beer Screening 2 LyoKit			
FAM: *Lactobacillus and Pediococcus*		**HEX: *Pectinatus and Megasphaera***	
L. acetotolerans	*L. plantarum*	*Pect. cerevisiiphilus*	*M. cerevisiae*
*L. brevis**	*L. paraplantarum*	*Pect. frisingensis*	*M. paucivorans*
L. parabrevis	*L. perolens*	*Pect. haikarae*	*M. sueciensis*
L. lindneri	*L. harbinensis*	*Pect.* sp. DSM 20764	
L. casei	*L. rossiae*		
L. paracasei	*L. backii*		
L. coryniformis	*Ped. damnosus*		
L. buchneri	*Ped. inopinatus*		
L. parabuchneri	*Ped. parvulus*		
L. collinoides	*Ped. pentosaceus*		
L. paracollinoides	*Ped. acidilactici*		
L. pentosus	*Ped. claussenii*		
L. paucivorans			

Note: A few non-brewery relevant bacteria species like *Lactobacillus kefiri*, *L. vini* and *L. zymae*, may also be detected with the screening.

The kit must not be used in diagnostic procedures.

- KIT230074 / KIT230075: Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and Cy5-labeled probes as well as for using low profile tubes (LP; KIT230074) or regular profile tubes (RP; KIT230075). In cases the strip tubes don´t fit for the instrument the samples have to be transferred after resuspension of the lyophilized PCR mix to appropriate PCR vessels.
- KIT230076: Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and ATTO 490LS-labeled probes as well as for using low-profile strip tubes (DP).

The kit version KIT230074 (LP) and KIT230075 (RP) described in this instruction manual has been developed for real-time PCR instruments with a FAM, a HEX, a ROX and a Cy5 detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480, LightCycler® 96 (Roche Diagnostics), ABI 7500 FAST (Applied Biosystems™), AriaMx (Agilent Technologies), and PikoReal™ 24 (Thermo Scientific). The kit version KIT230076 (DP) has been developed for cyclers using ATTO 490LS, such as the Dualo 32 Beverage instrument.

Note: The Color Compensation Set 3 (Product No. KIT230005) is necessary for users of the LightCycler 480 System.

2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of DNA from beer spoilage bacteria using the foodproof Beer Screening 2 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 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 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 Beer Screening 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 enrichment broth, or enrichments of beer or pitching yeast, refer to the corresponding product package inserts of a suitable sample preparation kit (see *"Additional Equipment and Reagents Required"*).

2.1.3 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 Beer Screening Control Template (vial 2, purple cap)] or with a positive sample preparation control.

2.1.4 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H_2O 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

2.2.1 Program Setup for Dualo 32® Beverage (KIT230076)

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.

Note:

- For users of instruments with an ATTO 490LS channel: Click "Use Advanced Settings" (section "Run Settings") and enter the following values:

  Integration Time (s): 0.4
  Acquisitions per Cycle: 6

2.2.2 Program Setup for other cyclers (KIT230074/ KIT230075)

The following procedure is optimized for a real-time PCR instrument with a FAM (beer-spoilage bacteria), HEX (*Lactobacillus brevis*), ROX (hop-tolerance related genes) and Cy5 (Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR protocol for the foodproof Beer Screening 2 LyoKit. For details on how to program the experimental protocol, see the Instrument Operator's Manual of your real-time PCR cycler.

Program for KIT230074/ KIT230075**:**

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

Note:

- For some real-time PCR instruments the type of the probe quencher as well as the usage of a passive reference dye must be specified. The foodproof Beer Screening 2 LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.

2.3 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.

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 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. 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. 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 H_2O to achieve 25 µL).
   - For the negative control, add 25 µL PCR-grade H_2O (vial 3, colorless cap).
   - For the positive control, add 25 µL foodproof Beer Screening 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 recommends vortex centrifuges Multispin MSC-3000/6000 for PCR strips or vortex centrifuge CVP-2 for PCR plates. Dedicated protocols are available for this centrifuge.

Only KIT230074 and KIT230075**:** 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. Spin the PCR tube strips for 30 seconds at 150 – 200 g in a suitable centrifuge. If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1,000 g!

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

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

Note: 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.

For the Dualo 32 Beverage instrument, please make sure that the mount contains at least a strip in rows A and D or single tubes in wells A1, A8, D1 and D8. These positions can be filled with tubes containing reagents, or empty tubes. For more detailed information, please refer to the manual for the Dualo 32 Beverage.

2.4 Data Interpretation

The amplification of the DNA of *Lactobacillus* and *Pediococcus* is analyzed in the fluorescence channel suitable for FAM labeled probes detection. *Pectinatus* and *Megasphaera* DNA sequences are detected in the fluorescence channel suitable for the detection of HEX labeled probes, and the amplification of the hop-tolerance related sequences is analyzed in the fluorescence channel suitable for the detection of ROX labeled probes. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for Cy5 (KIT230074 / KIT230075) or ATTO 490LS (KIT230076).

Compare the results from all four detection channels for each sample, and interpret the results as described in the table below.

Compare the results from the FAM channel (*Lactobacillus* / *Pediococcus*), HEX channel (*Pectinatus* / *Megasphaera*), ROX channel (*hor*A / *hor*C) and Cy5 / ATTO 490LS channel (Process Control) for each sample, and interpret the results as described in the table below.

FAM	HEX	ROX	Cy5 or ATTO 490LS	Result Interpretation
Positive	Positive	Positive	Positive or Negative	Positive for beer-spoilage bacteria *Lactobacillus and/or Pediococcus*, and *Pectinatus* and/or *Megasphaera*, horA and/or *hor*C
Positive	Negative	Positive	Positive or Negative	Positive for *Lactobacillus* and/or *Pediococcus*, *hor*A and/or *hor*C
Positive	Positive	Negative	Positive or Negative	Positive for *Lactobacillus and/or Pediococcus*, and *Pectinatus* and/or *Megasphaera*
Negative	Positive	Negative	Positive or Negative	Positive for *Pectinatus* and/or *Megasphaera*
Negative	Negative	Positive	Positive or Negative	Positive for *hor*A and/or *hor*C
Negative	Negative	Negative	Positive	Negative for beer-spoilage bacteria, *hor*A and *hor*C
Negative	Negative	Negative	Negative	Invalid

Note: A prerequisite for the unambiguous discrimination of the target sequences in channels FAM, HEX and ROX as well as Internal Control DNA in this multi-color experiment is a suitable calibration of the PCR instrument for all used channels. Please refer to the operation manual of your real-time PCR cycler for further information.

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 or Cy5 / ATTO 490LS.
	Pipetting errors.	• Check for correct reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	White tube strips used for the Dualo 32 Beverage instrument.	• Use clear tube strips (Product No. KIT230076).
	No data acquisition programmed.	• Check the cycle programs.
No signal increase in channel Cy5 or ATTO 490LS is observed.	Inhibitory effects of the sample material (*e.g.,* caused by insufficient purification).	• Use the recommended DNA sample preparation kit to generate template DNA. • Dilute samples or pipet a lower amount of sample DNA (e.g., 5 µL instead of 25 µL).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof Beer Screening 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. Resuspended PCR mix is still in the upper part of the vessel.	• Always centrifuge PCR strips.
	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 Beer Screening 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 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 (KIT230074 / KIT230075) or ATTO 490LS channel (KIT230076), respectively, whereas the bacterial DNA is detected in channels FAM (*Lactobacillus* and/or *Pediococcus*), HEX (*Pectinatus and/or Megasphaera*) and ROX (*hor*A and *hor*C). 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 DNA of beer spoilage bacteria and hop-tolerance related genes in the sample. The foodproof Beer Screening 2 LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of DNA of the target organisms. Primers and probes provide specific detection in beer 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 beer spoilage bacteria and hop-tolerance related sequences.
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.

4.3 Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCR's. 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 bacterial 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 Beer Screening 2 LyoKit, decontamination can be achieved with the provided reagents.

4.4 Background Information

A spoiled beer may be recognized in different ways. In less severe cases, unwanted turbidity may be observed, either due to the high number of contaminating microorganisms or as a result of pH changes and protein flocculation. In other cases, microorganisms cause an undesired change of flavor. Beer is a difficult culture medium for microorganisms to grow in, due to the presence of alcohol, carbon dioxide, hop bitter compounds, low amount of oxygen, etc. However, some microorganisms have adapted to these conditions – among them, *Lactobacillus*,

Pediococcus, *Pectinatus* and *Megasphaera* are the most troublesome [1]. The ability of some *Lactobacillus* and *Pediococcus* species to tolerate hop acids is a multi-factorial trait, but especially the presence of the plasmid-borne genes *hor*A and *hor*C has been shown to correlate with the ability of isolates to grow in beer [2]. Different stages of beer production are monitored for the presence of spoilage microorganisms to guarantee product consistency. Since conventional microbiological methods for the detection and identification of beer spoilage bacteria are very time-consuming, PCR as a highly sensitive and specific detection method has been introduced into the beverage/beer producing industry [3, 4].

5. References

1. Jespersen, L. and Jakobsen, M. 1996. Specific spoilage organisms in breweries and laboratory media for their detection. *Int. J. Food Microbiol*. 33, 139-155.
2. Suzuki, K. 2011. 125th Anniversary Review: Microbiological Instability of Beer Caused by Spoilage Bacteria. *Journal of the Institute of Brewing*, 117(2), 131–155.
3. Berghof K, Fandke M, Pardigol A, Tauschmann A, Kiehne M. 2003. Fast Detection of Beer Spoilage Microorganisms by Consensus Polymerase Chain Reaction with foodproof® Beer Screening. In Brewing Yeast Fermentation Performance (2nd Edition). Blackwell Publishing. 13-21.
4. Methner, F.-J., Schuster, E. and Schackmann, A. 2004. Screening of Beer- Spoilage Bacteria Using the LightCycler® PCR Workflow System. *Biochemica* 2004 (1), 9-11.

6. Supplementary Information

6.1 Quality Control

The foodproof Beer Screening 2 LyoKit is function tested using the LightCycler 480 System and the Dualo 32 Beverage instrument.

6.2 Ordering Information

Hygiena offers a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

6.3 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.

6.4 Trademarks

foodproof® is a registered trademark of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

6.5 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.

6.6 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers:
R 602 06-1 (KIT230074), R 602 06-2 (KIT230075), R 602 06-3 (KIT230076)

7. Change Index

Revision A, January 2024:
First version.

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

Manufactured by
Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam
Germany
www.hygiena.com