FOR *IN VITRO* USE ONLY

foodproof® Beer Screening LyoKit

– 5'Nuclease –

Version 1, August 2017

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

Order No. R 602 02-1 / R 602 02-3

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

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

Component	Label	Contents / Function / Storage
foodproof® Beer Screening LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing a 8-tube strip mat • R 602 02-1 with white low profile tubes • R 602 02-3 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 °C 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.

Additional Equipment and Reagents Required

- R 602 02-1: Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and Cy5-labeled probes as well as for using low profile strip tubes. 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.
- R 602 02-3: Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and Atto490LS-labeled probes as well as for using low profile strip tubes.
- Sample Preparation Kit
  foodproof® StarPrep Two Kit (Order No. S 400 08)[1] or
  foodproof® StarPrep Three Kit (Order No. S 400 18)[1]
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex centrifuge Multispin MSC-3000/6000 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 **food**proof® Beer Screening LyoKit – 5'Nuclease – 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 addition to the detection of DNA from 31 known beer spoilage species in channel FAM (see table below)

and the specific identification of *Lactobacillus brevis* in channel HEX, 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 LyoKit**			
Lactobacillus	***Pediococcus***	***Pectinatus***	***Megasphaera***
L. acetotolerans	*Ped. damnosus*	*Pect. cerevisiiphilus*	*M. cerevisiae*
L. brevis	*Ped. inopinatus*	*Pect. frisingensis*	*M. paucivorans*
L. parabrevis	*Ped. parvulus*	*Pect. haikarae*	*M. sueciensis*
L. lindneri	*Ped. pentosaceus*	*Pect.* sp. DSM 20764	
L. casei	*Ped. acidilactici*		
L. paracasei	*Ped. claussenii*		
L. coryniformis			
L. buchneri			
L. parabuchneri			
L. collinoides			
L. paracollinoides			
L. pentosus			
L. plantarum			
L. paraplantarum			
L. perolens			
L. harbinensis			
L. rossiae			
L. backii			

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

In addition, the beer spoiling species *L. paucivorans* is detected in channel FAM.

*Lactobacillus brevis** is additionally detected in channel HEX to allow for the identification of this important beer spoiler.

The kit must not be used in diagnostic procedures.

The kit version R 602 02-1 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 R 602 02-3 has been developed for cyclers using Atto490LS.

* including *Lactobacillus* sp. DSM 6265 ("*L. brevisimilis*")

Note: A color compensation (Color Compensation Set 3; Order No. A 500 10) is necessary and will be supplied by BIOTECON Diagnostics for users of the LC 480 Systems I and II. Similarly, a dye calibration file for Atto490LS is necessary and will be supplied by BIOTECON Diagnostics. Please contact BIOTECON Diagnostics for further information.

2. How to Use this Product

2.1 Before You Begin

Precautions

Detection of DNA from beer spoilage bacteria using the **food**proof® Beer Screening 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.**

Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For preparation of genomic DNA from 1 ml of 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"*).

Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [**food**proof® Beer Screening Control Template (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 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

Program Setup

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 or Atto490LS (Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR-protocol for the **food**proof® Beer Screening LyoKit. For details on how to program the experimental protocol, see the Instrument Operator's Manual of your real-time PCR-cycler:

Program for R 602 02-1:		**Program for R 602 02-3:**	
Pre-incubation	**1** cycle	Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes	Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes	Step 2:	95 °C for 5 minutes
Amplification	**50** cycles	Amplification	**50** cycles
Step 1:	95 °C for 5 seconds	Step 1:	95 °C for 10 seconds
Step 2*:	60 °C for 60 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 **food**proof® Beer Screening LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For users of instruments with an Atto490LS channel: Click "Use Advanced Settings" (section "Run Settings") and enter the following values:

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

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. 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 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 **food**proof® 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: BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-3000/6000 (D 110 64) for PCR-strips or vortex centrifuge CVP-2 for PCR-plates (D 110 67). Dedicated protocols are available for this centrifuge.

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 at 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.

2.3 Data Interpretation

The amplification of the DNA of beer-spoilage bacteria is analyzed in the fluorescence channel suitable for FAM labeled probes detection. The amplification of the *Lactobacillus brevis*-specific sequence is analyzed 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 (R 602 02-1) or Atto490LS (R 602 02-3).
Compare the results from all four detection channels for each sample, and interpret the results as described in the table below.

FAM	HEX	ROX	Cy5 or Atto490LS	Result Interpretation
Positive	Positive	Positive	Positive or Negative	Positive for beer-spoilage bacteria, *L. brevis**, *hor*A and/or *hor*C
Positive	Negative	Positive	Positive or Negative	Positive for beer-spoilage bacteria, *hor*A and/or *hor*C
Positive	Positive	Negative	Positive or Negative	Positive for beer-spoilage bacteria, *L. brevis**
Positive	Negative	Negative	Positive or Negative	Positive for beer-spoilage bacteria
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

**including "L. brevisimilis"*

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.

Note that in a sample with multiple beer spoilage and/or hop-tolerant organisms, the detection of *Lactobacillus brevis* in channel HEX might be suppressed by the excess of other species that are positive in FAM and/or ROX.

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/Atto490LS.
	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 Atto490LS instruments.	• Use clear tube strips (kit version R 602 02-3).
	No data acquisition programmed.	• Check the cycle programs.
No signal increase in channel Cy5 or Atto490LS 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 **food**proof® Beer Screening lyophilized PCR Mix at 2 °C 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

How this Product Works

The **food**proof® Beer Screening 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 (R 602 02-1) or Atto490LS channel, respectively (R 602 02-3), whereas the bacterial DNA is detected in channels FAM (beer spoilage bacteria), HEX (*L. brevis*) 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 **food**proof® Beer Screening 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.

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.

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 **food**proof® Beer Screening LyoKit, decontamination can be achieved with the provided reagents.

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].

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 **food**proof® 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

Quality Control

The **food**proof® Beer Screening LyoKit is function tested using the LightCycler® 480 System (R 602 02-1) and the MyGo Pro instrument (R 602 02-3).

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

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First version of the package insert.

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