foodproof® Beer Screening Kit – Hybridization Probes -

Revision A, March 2024

PCR kit for the qualitative detection of beer spoilage bacteria DNA of the genera *Lactobacillus*, *Pediococcus*, *Pectinatus*, and *Megasphaera* and the identification of *Lactobacillus brevis*, *Lactobacillus lindneri*, *Pediococcus damnosus*, *Pediococcus inopinatus*, and *Megasphaera cerevisiae*.

Product No. KIT230066

Kit for 96 reactions for a maximum of 90 or 94 samples (depending on the instrument used)

Store the kit at -15 to -25 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

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1. What This Product Does

1.1 Number of Tests

The kit is designed for 96 reactions with a final reaction volume of 20 µL each. Up to 30 samples (single sample preparation) can be analyzed per LightCycler® Carousel-Based System run and up to 94 samples plus positive and negative control reactions per LC 480 Instrument II run (i.e., the complete kit allows analysis of a maximum of 90 samples or 94 samples). With the Dualo 32® Beverage, up to 30 samples plus positive and negative control can be analyzed per run.

1.2 Storage and Stability

- Store the kit at -15 to -25 °C through the expiration date printed on the label.
- Once the kit is opened, store the components as described in the following Contents table.

1.3 Kit Contents

Vial / Cap Color	Label	Contents / Function / Storage
1 yellow cap	foodproof Beer Screening Master Mix	• 3 x 420 µL • Ready-to-use primer and Hybridization Probe mix specific for DNA of beer spoilage bacteria and the beer spoilage bacteria-specific Internal Control (IC). • For amplification and detection of beer spoilage bacteria-specific sequences. • Store at -15 to -25 °C • **Avoid repeated freezing and thawing!** • **Protect from light!**
2 red cap	foodproof Beer Screening Enzyme Solution	• 3 x 32 µL • Contains FastStart Taq DNA Polymerase and Uracil-DNA Glycosylase for prevention of carry-over contamination. • Store at -15 to -25 °C
3 white cap	foodproof Beer Screening Internal Control - LC 1.x, 2.0	• 3 x 32 µL • Contains a stabilized solution of plasmid DNA. • For use as an internal amplification control using LightCycler 1.x, 2.0. • Store at -15 to -25 °C. • Store at 2 to 8 °C for up to 1 month after thawing or refreezing.
4 purple cap	foodproof Beer Screening Control Template	• 1 x 50 µL • Contains a stabilized solution of plasmid DNA. • For use as a PCR run positive control. • Store at -15 to -25 °C. • Store at 2 to 8°C for up to 1 month after thawing or refreezing.
5 colorless cap	H_2O PCR-grade	• 1 x 1 mL • Nuclease-free, PCR-grade H_2O. • For use as a PCR run negative control. • Store at -15 to -25 °C.
6 black cap	foodproof Beer Screening Internal Control - LC 480	• 3 × 32 µL • Contains a stabilized solution of plasmid DNA and a yellow dye for better visualization. • For use as an internal amplification control using LightCycler 480 II or the Dualo 32 Beverage. • Store at -15 to -25 °C. • Store at 2 to 8 °C for up to 1 month after thawing or refreezing. • Keep away from light!

1.4 Additional Equipment and Reagents Required

- Dualo 32® Beverage, LightCycler 1.x/2.0 or 480 real-time PCR instrument. For the LightCycler instruments, a Roche Color Compensation Set is necessary.

- PCR reaction tubes or capillaries
  - **Dualo 32 Beverage**:
    - Reaction tube 8-strips in DP format (0.1 mL, clear plastic) with cap strips.
  - **LightCycler 480**:
    - White LightCycler 480 compatible PCR plate with optical sealing foil
  - **LightCycler 1.x/2.0**:
    - LightCycler 20 µL – Capillaries

- Centrifuge for reaction tubes or capillaries
  - **Dualo 32 Beverage**:
    - Small PCR strips centrifuge for 8-strips
  - **LightCycler 480**:
    - Standard swing bucket centrifuge containing a rotor for multiwell plates.
  - **LightCycler 1.x/2.0**:
    - Adapters that allow LightCycler Capillaries to be centrifuged in a standard microcentrifuge rotor ***or***
    - LC Carousel Centrifuge 2.0 for use with the LightCycler 2.0 Sample Carousel.
      Note: If you use a LightCycler Instrument version below 2.0, you will also need the LC Carousel Centrifuge 2.0 Bucket 2.1. To adapt the LightCycler 2.0 Sample Carousel to the former LC Carousel Centrifuge, you will need the LC Carousel Centrifuge 2.0 Rotor Set.

- DNA extraction kits:
  - foodproof StarPrep Two Kit (Product No. KIT230177) or
  - foodproof StarPrep Three Kit (Product No. KIT230187)

- Pipettors
- Nuclease-free, aerosol-resistant pipette tips
- Standard benchtop microcentrifuge containing a rotor for 2.0 mL reaction tubes.
- Sterile reaction tubes for preparing PCR mixes and dilutions

1.5 Applicability Statement

The foodproof Beer Screening Kit is intended for the rapid detection of fragments of beer spoilage bacteria-specific genes in preparations from potentially contaminated beer, enrichment broth or pitching yeast, listed in the following table:

Beer spoilage bacteria detected by the screening of the foodproof **Beer Screening 1 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			
L. paucivorans			

* including "*L. Brevisimilis*"

A few non-brewery-relevant bacteria species, such as *Lactobacillus kefiri*, *L. parakefiri* and *L. hilgardii*, may also be detected with the kit. The list contains species names that have changed, according to new research by Zheng et al. [1].

The kit must not be used in diagnostic procedures.

2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of beer spoilage bacterial DNA using the foodproof Beer Screening Kit requires DNA amplification by PCR. The kit provides all the 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:

- Prepare appropriate aliquots of the kit solutions and keep them 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.
- Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.
- To avoid cross-contamination, close all capillaries or tubes that contain sample DNA and negative controls before pipetting positive controls.

Keep the foodproof Beer Screening Master Mix (vial 1, yellow cap) away from light.

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 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”).

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 4, 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 5, 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 LightCycler Carousel-Based System Protocol

The following procedure is optimized for use with the LightCycler Carousel-Based System. Program the LightCycler Carousel-Based System before preparing the reaction mixes. A LightCycler Carousel-Based System protocol that uses the foodproof Beer Screening Kit contains the following programs: Pre-Incubation to prevent carry-over contamination (UNG), activate FastStart Taq DNA polymerase and DNA-denaturation, amplification of the target DNA, melting curve analysis of the DNA-probe-hybrids and cooling of the rotor and thermal chamber.

For details on how to program the experimental protocol, see the LightCycler Instrument Operator's Manual.

Pre-incubation		
Programs/Cycle Program Data	***Value***	
Cycles	1	
Analysis Mode	None	
Temperature Targets	***Segment 1***	***Segment 2***
Target/Target Temperature [°C]	37	95
Hold/Incubation Time [h:min:s]	00:02:00	00:15:00
Ramp Rate/Temperature Transition Rate [°C/s]	20	20
Sec Target/Secondary Target Temperature [°C]	0	0
Step Size [°C]	0.0	0.0
Step Delay [cycles]	0	0
Acquisition Mode	None	None

Amplification (of the target DNA)			
Programs/Cycle Program Data	***Value***		
Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target/Target Temperature [°C]	95	64	72
Hold/Incubation Time [h:min:s]	00:00:02	00:00:30	00:00:15
Ramp Rate/Temperature Transition Rate [°C/s]	20	20	20
Sec Target/Secondary Target Temperature [°C]	0	50	0
Step Size [°C]	0.0	0.5	0.0
Step Delay [cycles]	0	5	0
Acquisition Mode	None	Single	None

Melting Curve Analysis (of the DNA probe hybrids)			
Programs/Cycle Program Data	***Value***		
Cycles	1		
Analysis Mode	Melting Curves		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target/Target Temperature [°C]	95	38	80
Hold/Incubation Time [h:min:s]	00:00:00	00:01:00	00:00:00
Ramp Rate/Temperature Transition Rate [°C/s]	20	20	0.1
Sec Target/Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [cycles]	0	0	0
Acquisition Mode	None	None	Cont
Cooling (the rotor and thermal chamber)			
Programs/Cycle Program Data	***Value***		
Cycles	1		
Analysis Mode	None		
Temperature Targets	***Segment 1***		
Target/Target Temperature [°C]	40		
Hold/Incubation Time [h:min:s]	00:00:30		
Ramp Rate/Temperature Transition Rate [°C/s]	20		
Sec Target/Secondary Target Temperature [°C]	0		
Step Size [°C]	0.0		
Step Delay [cycles]	0		
Acquisition Mode	None		

2.2.2 Fluorescence and Run Setup Parameter

Parameter	Setting	
All LightCycler Software Versions		
Seek Temperature	30 °C	
LightCycler Software prior to Version 3.5		
Display Mode	Fluorescence channel F2 or F3	
Fluorescence Gains	**Fluorimeter**	**Gain Value**
	Channel 1 (F1)	1
	Channel 2 (F2)	15
	Channel 3 (F3)	30

LightCycler Software Version 3.5	
Display Mode • during run • for analysis	 • Fluorescence channel F2 or F3 • F2/Back-F1 or F3/Back-F1
Fluorescence Gains	Not required In data created with LightCycler® Software Version 3.5, all fluorescence values are normalized to a fluorescence gain of “1”. This produces a different scale on the Y-axis than that obtained with previous LightCycler® Software Versions. This difference does not affect the crossing points nor any calculated concentrations obtained.
LightCycler Software Version 4.x	
Default channel • during run • for analysis	 • Fluorescence channel 640 or 705 • 640/Back 530 or 705/Back 530
Fluorescence Gains	Not required
“Max. Seek Pos”	Enter the number of samples, including controls.
“Instrument Type”	‘6 Ch.’: for LightCycler® 2.0 Instrument (selected by default). ‘3 Ch.’: for LightCycler® 1.5 Instrument and instrument versions below.
“Capillary Size”	Select ‘20 µL’ as the capillary size for the experiment (For the ‘6 Ch.’ instrument type only).

2.2.3 LightCycler 480 Instrument II Protocol

The following procedure is optimized for use with the LightCycler 480 Instrument II. Program the LightCycler 480 Instrument II before preparing the reaction mixes. A LightCycler 480 Instrument II protocol that uses the foodproof Beer Screening Kit contains the following programs:

- Pre-incubation to prevent carry-over contamination (UNG), to activate FastStart Taq DNA polymerase and for DNA-denaturation
- Amplification of the target DNA
- Melting Curve Analysis of the DNA-probe-hybrids
- Cooling of the LightCycler 480 Instrument II.

For details on how to program the experimental protocol, see the LightCycler 480 Instrument Operator's Manual.

Set-Up		
Detection Format	**Block Type**	**Reaction Volume**
Multi Color HybProbe	96	20 µL
Filtering Setting	dynamic mode: Fluos (465-510, Red 640 (498-640) and Cy 5/ Cy 5.5 (498-660)	
Programs		
Program Name	**Cycles**	**Analysis Mode**
Pre-Incubation	1	None
Amplification	45	Quantification
Melting Curve	1	Melting Curves
Cooling	1	None

Step	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target Temperature [°C]	Step Size [°C]	Step Delay [cycles]
Pre-Incubation								
Segment 1	37	None	00:04:00	4.4		0	0.0	0
Segment 2	95	None	00:15:00	4.4		0	0.0	0
Amplification								
Segment 1	95	None	00:00:10	4.4		0	0.0	0
Segment 2	64	Single	00:00:40	2.2		50	0.5	5
Segment 3	72	None	00:00:25	4.4		0	0.0	0
Melting Curve								
Segment 1	95	None	00:00:10	4.4				
Segment 2	38	None	00:01:00	2.2				
Segment 3	76	Continuous		0.08	2.5			
Cooling								
	40	None	00:00:30	2.2		0	0.0	0

2.2.4 Dualo® 32 Beverage Protocol

The Dualo 32 Beverage cycler (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.

2.2.5 Preparation of the PCR Mix

Proceed as described below to prepare a 20 µL standard reaction.

The kit contains **two different internal amplification controls**, depending on the instrument used:

- **Vial 3, white cap,** "foodproof Beer Screening Internal Control (LC 1.x, 2.0)"**:** Use in combination with the LightCycler 1.x or 2.0
- **Vial 6, black cap,** "foodproof Beer Screening Internal Control (LC 480)"**:** Use in combination with the Dualo 32 Beverage cycler or the LightCycler 480 Instrument II.

LightCycler 1.x or 2.0: Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries. Depending on the total number of reactions, place the required number of LightCycler Capillaries in centrifuge adapters or in a LightCycler Sample Carousel in a LC Carousel Centrifuge Bucket.

LightCycler 480 Instrument II: Do not touch the upper surface of the PCR multiwell plate.

Preparation:

1. Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully but thoroughly by pipetting up and down.
2. In a 1.5 mL reaction tube, prepare the PCR Mix by adding the following components in the order mentioned below, then mix gently but thoroughly by pipetting up and down:

The volumes indicated below are based on a single 20 µL standard reaction. Prepare the PCR mix by multiplying the amount in the "Volume" column by the number of reactions to be cycled plus one or two additional reactions to cover pipetting losses.

Component	Volume
foodproof Beer Screening Master Mix (vial 1, yellow cap)	13 µL
foodproof Beer Screening Enzyme Solution (vial 2, red cap)	1 µL
foodproof Beer Screening Internal Control - LC 1.x, 2.0 (vial 3, white cap) → for LightCycler 1.x or 2.0 **or** foodproof Beer Screening Internal Control - LC 480 (vial 6, black cap) → for Dualo 32 Beverage and LightCycler 480 II	1 µL
Total volume	15 µL

3. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
    - Pipet 15 µL PCR mix into each LightCycler capillary or into each well of the PCR plate or PCR 8-strips.
    - For the samples of interest, add 5 µL sample DNA to a capillary (seal with a stopper) or to a well.
    - For the negative control, add 5 µL H_2O, PCR-grade (vial 5, colorless cap) to a capillary (seal with a stopper) or to a well.
    - For the positive control, add 5 µL foodproof Beer Screening Control Template (vial 4, purple cap) to a capillary (seal with a stopper) or to a well.
    - Seal the plate accurately with optical sealing foil or seal the 8-strip tubes with caps.
4. Centrifugation
    - LightCycler 1.x and 2.0 Carousel-Based System:
        i. Place the adapters (containing the capillaries) in a standard benchtop microcentrifuge. (Place the centrifuge adapters in a balanced arrangement within the centrifuge.)
        ii. Centrifuge at 700 x *g* for 5 s (3,000 rpm in a standard benchtop microcentrifuge).
        iii. Alternatively, use the LC Carousel Centrifuge for spinning the capillaries.
    - LightCycler 480 Instrument II:
        i. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s.
    - Dualo 32 Beverage:
        i. Briefly spin strips, e.g., at 500 - 1,000 x g for 5 s, in a suitable centrifuge.
5. Load the PCR instrument and cycle the samples as described above.

2.3 Analysis

2.3.1 Color Compensation (LightCycler)

The use of the previously generated color compensation file or color compensation object is a prerequisite for the unambiguous discrimination of beer spoilage bacteria DNA and Internal Control (IC) DNA amplification in this dual-color experiment. For additional information on the generation and use of a color compensation file or object, refer to the LightCycler Instrument Operator's Manual or the LightCycler 480 Instrument Operator's Manual and the package insert for the LightCycler Color Compensation Set. The LightCycler Color Compensation Set is intended for the LightCycler Carousel-Based System but can also be used for this kit in combination with the LightCycler 480 Instrument II.

Users of **LightCycler Software 3.5** proceed as described below to use a stored color compensation file after the PCR run on the LightCycler Carousel-Based System:

1. Select the data file in the LightCycler Data Analysis module of the LightCycler Software.
2. Click on the 'Select a Program' button and select the program to be analyzed.
3. Under the Color Compensation menu, select 'Load Calibration Data', then highlight the stored 'CCC' color compensation file. Alternatively, click the 'Select CCC Data' button and choose 'Import CCC File'.
4. To display the color-compensated data, click the 'Color Compensation' button. Alternatively, select 'Enable' under the 'Color Compensation' pull-down menu.
5. To return to the raw data, click the 'Color Compensation' button again. Alternatively, select 'Disable' under the 'Color Compensation' pull-down menu.

Users of **LightCycler Software 4.x** proceed as described below to use a stored color compensation object after the PCR run on the LightCycler Carousel-Based System:

1. Add the analysis module, click 'Color Compensation' in the analysis window, then select 'Select Color Compensation'.
2. Select the color compensation object you want to apply, then click 'OK'.
3. A small dialog box opens so you can select the channels to compensate. The number of channels displayed depends on the number of channels used in the color compensation experiment. By default, all channels are selected.
4. Deselect any channels you do not want to compensate (*i.e.,* for this kit, select channels 530, 640, and 705 only), then click 'OK'.
5. The analysis charts are redrawn using the compensated data. Notice that the Color Compensation menu label now says "(On)".

Users of **LightCycler 480 Software 1.5** proceed as described below to use a stored color compensation object after the PCR run on the LightCycler 480 Instrument II:

1. Add the analysis module, click 'Color Comp' in the analysis window, then select between the options '*In Use'* or '*In Data-base'*.
2. Select the color compensation object you want to apply, then click 'OK'.
3. A small dialog box opens so you can select the channels to compensate. The number of channels displayed depends on the number of channels used in the color compensation experiment. By default, all channels are selected.

4. Deselect any channels you do not want to compensate (*i.e.,* for this kit, select channels Fluos (465-510), Red 640 (498-640), Cy 5 / Cy 5.5 (498-660) only), then click 'OK'.
5. The analysis charts are redrawn using the compensated data. Notice that the 'Color Comp' menu label now says "(On)".

2.3.2 Data Interpretation of DNA Amplification

LightCycler Software 3.5: Analyze real-time PCR results in channels F2/Back-F1 and F3/Back-F1 using the Quantification module.
LightCycler Software 4.x: Analyze results in channels 640/Back 530 and 705/Back 530 using the Qualitative Detection module.
LightCycler 480 Software 1.5: Analyze results in channels Red 640 (498-640) and Cy 5/ Cy 5.5 (498-660), using Abs Quant/2nd Derivative Max analysis type.
Dualo 32 Software 3.5: Analyze results in channels 640 Tm (A) and 705 Tm (A).

Check for a positive result of the **Internal Control** [signal in *Internal Control Channels*, see table] for each sample that is negative for beer spoilage bacteria DNA [no signal in *Beer Spoilage Bacteria Channels*, see table]. Compare the results from both channels (Beer Spoilage Bacteria and Internal Control) for each sample, and interpret the results as described in the table below:

Beer Spoilage Bacteria Channels **Channel F2/Back-F1** **Channel 640/Back 530** **Channel Red 640 (498-640)** **Dualo 32: 640 Tm (A)**	**Internal Control Channels** **Channel F3/Back-F1** **Channel 705/Back 530** **Channel Cy 5 / Cy 5.5 (498-660)** **Dualo 32: 705 Tm (A)**	**Result Interpretation**
Positive	Positive	Positive
Negative	Positive	Negative
Positive	Negative	Positive
Negative	Negative	Invalid

Note:

- Use the "High Sensitivity" setting of the LightCycler 480 Software 1.5 to calculate results.
- Always check the software results for plausibility by inspecting the amplification curves.

2.3.3 Data Interpretation of Melting Curve Analysis

In case of a positive screening result, the detected beer spoilage bacteria can be identified by Melting Curve/Tm Calling analysis in detection channel F3/Back-F1, 705/Back 530, Cy 5 / Cy 5.5 (498-660), or 705 Tm (A), respectively. Melting curve analysis should be performed manually because automated Tm Calling cannot cope with the complex melting peak patterns in many cases. The following table shows the specific melting temperatures, Tm, for the Internal Control and the most relevant beer spoilage bacteria and the corresponding detection channel of the melting peaks.

Detection Channel	Tm of melting peak	Result Interpretation
F3/Back-F1 705/Back 530 Cy 5 / Cy 5.5 (498-660) 705 Tm (A)	Peak at 73.0 °C (± 1 °C) and/or Peak at 69.0 °C (± 1 °C)	Internal Control (IC)
	Peak at 60.0 °C (± 1 °C)	Control Template
F3/Back-F1 705/Back 530 Cy 5 / Cy 5.5 (498-660) 705 Tm (A) with or without the melting peak of the Internal Control	Peak at 43.0 °C (± 1 °C)	*Megasphaera cerevisiae*
	Peak at 47.0 °C (± 1 °C)	*Pediococcus inopinatus*
	Peak at 52.5 °C (± 1 °C)	*Pediococcus damnosus*
	Peak at 60.0 °C (± 1 °C)	*Lactobacillus brevis (*and *"L. brevisimilis ")*
	Peak at 65.5 °C (± 1 °C) and in cases of high amount of initial DNA Peak at 61.0 °C (± 1 °C)	*Lactobacillus lindneri*

Note: For melting curve analysis, always compare the sample curve with the curve of the Internal Control.

The melting peak of *M. cerevisiae* is only slightly higher than the curve of the Internal Control. For confirmation, the melting curve in channel Red 640 (498-640) or 640 Tm (A) must be analyzed. The curve of *M. cerevisiae* has one melting peak at 59 – 60 °C.

The quality of the melting curves depends on the initial amount of DNA in the sample.

- For the LightCycler, the best results for identification by Melting Curve analysis will be obtained at crossing points between approx. 20 and 28. For samples with a very low amount of beer spoilage DNA, e.g., a crossing point higher than approx. 28 in channel F2/Back-F1, 640/Back 530, or Red 640 (498-640), melting curve analysis in channel F3/Back-F1, 705/Back 530 or Cy 5 / Cy 5.5 (498-660) might not be possible.

Following data interpretation by Melting Curve/Tm Calling analysis in channel F3/Back-F1, 705/Back 530, Cy 5 / Cy 5.5 (498-660), or 705 Tm (A), Melting Curve/Tm Calling analysis in channel F2/Back-F1, 640/Back 530, Red 640 (498-640), or 640 Tm (A) can be applied optionally to:

- Confirm the result for identification of *M. cerevisiae, P. damnosus/inopinatus* and *L. brevis/lindneri*
- Identify the *Pectinatus* group
- Identify the less relevant beer spoilage bacteria species or groups of species.

Note: The results and interpretations of Melting Curve/Tm Calling analysis in channel F2/Back-F1, 640/Back 530, Red 640 (498-640), or 640 Tm (A) is strongly dependent in many cases on the amount of input genome equivalents. Please contact Hygiena Diagnostics for more information on analyzing the melting curves in this channel.

3. Troubleshooting

Observation	Possible cause	Recommendation
No signal increase in amplification is observed, even with positive controls.	No correct detection channel has been chosen.	Set channel settings to F2/Back-F1, 640/Back 530, Red 640 (498-640), or 640 Tm (A), and F3/Back-F1, 705/Back 530, Cy 5 / Cy 5.5 (498-660), or 705 Tm (A). Note: Fluorescence data is acquired for all channels during the run, regardless of the channel settings (LightCycler). If the incorrect channel is selected, there is NO need to abort and redo a run.
	Pipetting errors or omitted reagents.	• Check for correct pipetting scheme and reaction set-up. • Repeat the PCR run. • Always run a positive control along with your samples.
	Inhomogeneity of reagent.	• Thaw the foodproof Beer Screening Master Mix and Internal Control thoroughly before pipetting. • Mix these reagents and the PCR mix well. • Repeat the PCR run.
	No data acquisition programmed.	• Check the cycle programs. • Select acquisition mode ‘single’ at the end of each annealing segment of the PCR program. • Repeat the PCR run.
No signal increase in channel F3/Back-F1 705/Back 530 Cy 5 / Cy 5.5 (498-660) 705 Tm (A) is observed.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Use the recommended DNA sample preparation kit to purify template DNA. • Dilute samples or pipet a lower amount of sample DNA (e.g., 2.5 µL instead of 5 µL, substitute with H_2O, PCR-grade).
Fluorescence intensity is too high.	Incorrect gain settings.	• Gain settings cannot be changed during or after the run. • Before repeating the run, check the gain settings in the cycle program (only applicable when using LightCycler 1.x with software version below 3.5).

Observation	Possible cause	Recommendation
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof Beer Screening Master Mix (vial 1) at -15 to -25 °C, protected from light. • Avoid repeated freezing and thawing.
	Low initial amount of target DNA.	Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
	foodproof Beer Screening Master Mix (vial 1) is not homogenously mixed.	Mix the foodproof Beer Screening Master Mix (vial 1) thoroughly before pipetting.
Strong and continuous increase of fluorescence signal in negative sample.	Auto-fluorescence of sample material.	See "inhibitory effects“.
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 capillaries and negative control capillaries have been sealed with stoppers.
Fluorescence intensity varies.	Insufficient centrifugation of the capillaries, PCR tube strips or plate.	Always centrifuge capillaries or PCR plates (loaded with the PCR mix) as described.
	Outer surface of the capillary tip, tube caps or the sealing foil is dirty (e.g., by direct skin contact).	Always wear gloves when handling the capillaries, tube caps or sealing foil.
Melting peaks cannot be differentiated.	Initial amount of target DNA is too low or too high.	Increase or decrease, respectively, the amount of sample DNA to obtain a crossing point in channel F2/Back-F1 [640/Back 530, Red 640 (498-640)] between approx. 20 and 28. For the Dualo 32, the Cq value should be between 15 and 23.
	C° to Average setting is too high.	Lower the number of C° to Average (8.0 for F2/Back-F1 and 6.0 for F3/Back-F1 is recommended). (Applicable for LightCycler Software Version 3.5 only)

4. Additional Information on this Product

4.1 How this Product Works

The foodproof Beer Screening Kit provides PCR primers and Hybridization Probes, ready-to-use amplification and detection reagents and a control template to ensure accurate amplification of beer spoilage bacterial DNA. Sample DNA is added to the final reaction mixes, thus minimizing contamination risks. Sequence-specific primers and Hybridization Probes provide specific detection of DNA of obligatory beer spoilage bacteria in beer samples. The foodproof Beer Screening Kit is a rapid detection method for the testing of enrichment cultures inoculated with beer samples that are potentially contaminated. The kit allows testing for the presence and absence of all relevant beer spoilage bacteria in the brewery. It covers 31 different species of the genera *Lactobacillus*, *Pediococcus*, *Pectinatus*, and *Megasphaera*. Beyond supplying a rapid result, the assay provides superior detection sensitivity and specificity to breweries and eliminates the need for time-consuming traditional detection methods. It also minimizes the risk of sample contamination and false-positive and false-negative 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 supplied with the kit (vial 3, white cap for the LightCycler Carousel-Based System and vial 6, black cap for the LightCycler 480 Instrument II and the Dualo 32 Beverage). The IC must be added to each reaction. Hybridization Probes were designed to bind specifically the IC, allowing detection in channel F3 (LightCycler Software 3.5 and versions below), 705 (LightCycler Software 4.x, Dualo 32 Beverage) or Cy 5 / Cy 5.5 (498-660) (LightCycler 480 Software 1.5), whereas the beer spoilage bacteria DNA is detected in channel F2 (LightCycler Software 3.5 and versions below), 640 (LightCycler Software 4.x, Dualo 32 Beverage) or Red 640 (498-640) (LightCycler 480 Software 1.5). In case of a negative result due to inhibition of amplification by the sample DNA of interest, the amplification of the IC is suppressed as well. However, a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of beer spoilage bacteria DNA in the sample. The foodproof Beer Screening Detection Kit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of beer spoilage bacteria DNA.

4.2 Test Principle

1. Using the kit's supplied sequence-specific primers in a polymerase chain reaction (PCR), the LightCycler System and its associated reagents amplify and detect fragments of beer spoilage species of the genera *Lactobacillus*, *Pediococcus*, *Pectinatus*, and *Megasphaera* simultaneously.
2. The LightCycler System and the Dualo 32 Beverage PCR instruments detect these amplified fragments in real time through fluorescence generated by their corresponding pair of sequence-specific Hybridization Probes. For each amplicon, one probe is labeled at the 5´-end with an acceptor fluorophore and, to avoid extension, is modified at the 3´-end by phosphorylation. The other oligonucleotide probe is labeled at the 3´-end with a donor fluorophore.
3. During the annealing phase of each PCR cycle, these probes hybridize to an internal amplicon sequence. Only if hybridized in close proximity to each other can fluorescence resonance energy transfer (FRET) between the two fluorophores occur. During FRET, the light of the PCR instrument excites the donor fluorophore and part of the excitation energy is transferred to the acceptor fluorophore.
4. The PCR instrument measures the emitted fluorescence of the acceptor fluorophore.

4.3 Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA 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 pre-treatment 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 of 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 Detection Kit, 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. This is either due to the high number of contaminating microorganisms (more than 10^7 CFU/mL) or is the result of pH changes and protein flocculation. In more complicated 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, low amount of oxygen, etc. However, some microorganisms have adapted to these conditions – among them, *Lactobacillus, Pediococcus, Pectinatus* and *Megasphaera* are the most troublesome [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].

4.5 Product Characteristics

4.5.1 Specificity

The foodproof Beer Screening Kit is sequence-specific for the beer spoilage members of the genera *Lactobacillus, Pediococcus, Pectinatus,* and *Megasphaera*.

4.5.2 Sensitivity

The foodproof Beer Screening Kit detects, in combination with the foodproof ShortPrep III Kit, approximately down to 10^3 cells/mL of enrichment cultures (sensitivity may vary slightly depending on sample type).

4.6 References

1. Jinshui Zheng et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae. International Journal of Systematic and Evolutionary Microbiology*, Vol. 70, Issue 4.
2. Jespersen, L. and Jakobsen, M. 1996. Specific spoilage organisms in breweries and laboratory media for their detection. *Int. J. Food Microbiol*. 33, 139-155.
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 Beerscreening. 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.

4.7 Quality Control

The foodproof Beer Screening Kit is function-tested using the LightCycler Carousel-Based, the LightCycler 480 System and the Dualo 32 Beverage.

5. Supplementary Information

5.1 Ordering Information

Hygiena Diagnostics is offering 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.

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5.3 Trademarks

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5.4 Contact and Support

If you have questions or experience problems with this or any other Hygiena Diagnostics GmbH product, 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 number: R 310 02

6. Change Index

Version 1, August 2016
Primers and probes for *Lactobacillus acetotolerans* were integrated into the foodproof Beer Screening Kit.

Version 2, March 2017
License Notice changed.

Version 3, September 2017
License Notice changed.

Revision A, March 2024
Rebranding and new layout.
Dualo 32 Beverage added.
R 310 02 20 -> INS-KIT230066-RevA

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