4everyone Detection Kit B
LP Identification

USER GUIDE

Identification of the Real Beer Spoiling Bacteria Species of Lactobacillus and Pediococcus

For research *in vitro* use only

SKU# 2301-37 4everyone Detection Kit B 200ul LP Identification
includes skirted 200 µl PCR tubes, frosted

Kits include all buffers and reagents necessary for DNA extraction and PCR together with PCR tubes in strips of 8 and strips of 5, containing specific primers and probes aliquoted in a dried format.

Therefore, 4everyone Detection Kits are extremely temperature stable and ship at room temperature. Storage at 2-8 °C upon arrival is recommended.

The 4everyone Detection Kit B LP Identification contains sufficient reagents for 6 samples / 78 reactions.

Table of Contents

Section 1

4everyone Detection Kit B LP Identification

This test allows the quantitative identification of the real beer spoiling species of lactic acid bacteria.

The species which are proven to cause beer spoilage are the *Lactobacillus* species *L. acetotolerans*, *L. backii*, *L. brevis*, *L. casei*, *L. collinoides*, *L. coryniformis*, *L. lindneri*, *L. parabuchneri* ("*frigidus*"), *L. perolens*, *L. plantarum*, *L. rossiae*, and the *Pediococcus* species *P. damnosus* and *P. inopinatus*.

It is common knowledge that a multiplex PCR test doesn't allow the safe parallel identification of a target present at a very low concentration whenever another target is present at a high concentration at the same time. The low concentration target usually will be outcompeted by the high concentration target in a multiplex PCR reaction, and therefore wouldn't show a positive result.

For a safe identification of all spoiler species within a sample, regardless of their concentrations, 4everyone Detection Kits are strictly designed using single species assays. All beer spoiling species from a sample are tested side by side in single tube assays, each species is detected and quantified in a separate PCR.

Section 2

Introduction to the 4everyone Detection Kit Technology

Today, the use of PCR is accepted as the standard method for detecting nucleic acids from numerous microorganisms in a diversity of food and beverages, both functional species as well as spoilers. Real time PCR is one of the most powerful, specific and reliable methods for the quantitative detection and identification of microorganisms at an early process stage to prevent spoilage and to maintain overall product quality.

The 4everyone Detection Kit system is based on DNA amplification and detection of microorganisms by real time PCR. The specific PCR reagents, primers and probes, come in a ready-made dried format in the PCR tubes for unrivalled ease of use and temperature stability.

All PCR tests use the FAM channel (495/520 nm) for detection of the target microorganisms and the VIC/HEX channel (530/550 nm) for an internal control reaction. This allows 4everyone Detection Kits to prevent false negatives due to sample inhibition, allowing you to be truly confident about negative results.

A typical workflow includes the following four steps:

In order to achieve lowest detection limits, we recommend sample enrichment in our PCR certified FastOrange® enrichment media (https://pika-weihenstephan.com).

Section 3

Kit Components

The 4everyone Detection Kit B LP Identification kit contains sufficient reagents for 6 samples.

Kit material for DNA isolation and analysis	Amount	Storage
Washing buffer A (yellow cap)	2 x 10.0 mL	dark at 2-8 °C
Lysis buffer B (blue cap)	1 x 10.0 mL	
Rehydration buffer B (white cap)	1 x 5.0 mL	
Positive control DNA (red cap)	1 x 40 µL	
PCR tubes (strips of 8 + 5) with mix of primers and probes	6 + 6	
2 x Master mix (green cap)	1 x 1,300 µL	
Cap strips (strips of 8) for covering the PCR reaction tubes	12	2-8 °C or ambient

Table 1: Material supplied

Section 4

Shelf Life and Storage

Once received, the kit must be stored at 2-8 °C in the dark. Unless storage and/or time in transit at conditions above 8 °C wasn't exceeding 14 days in total, all reagents can be used until the best before date as indicated on the package label.

Section 5

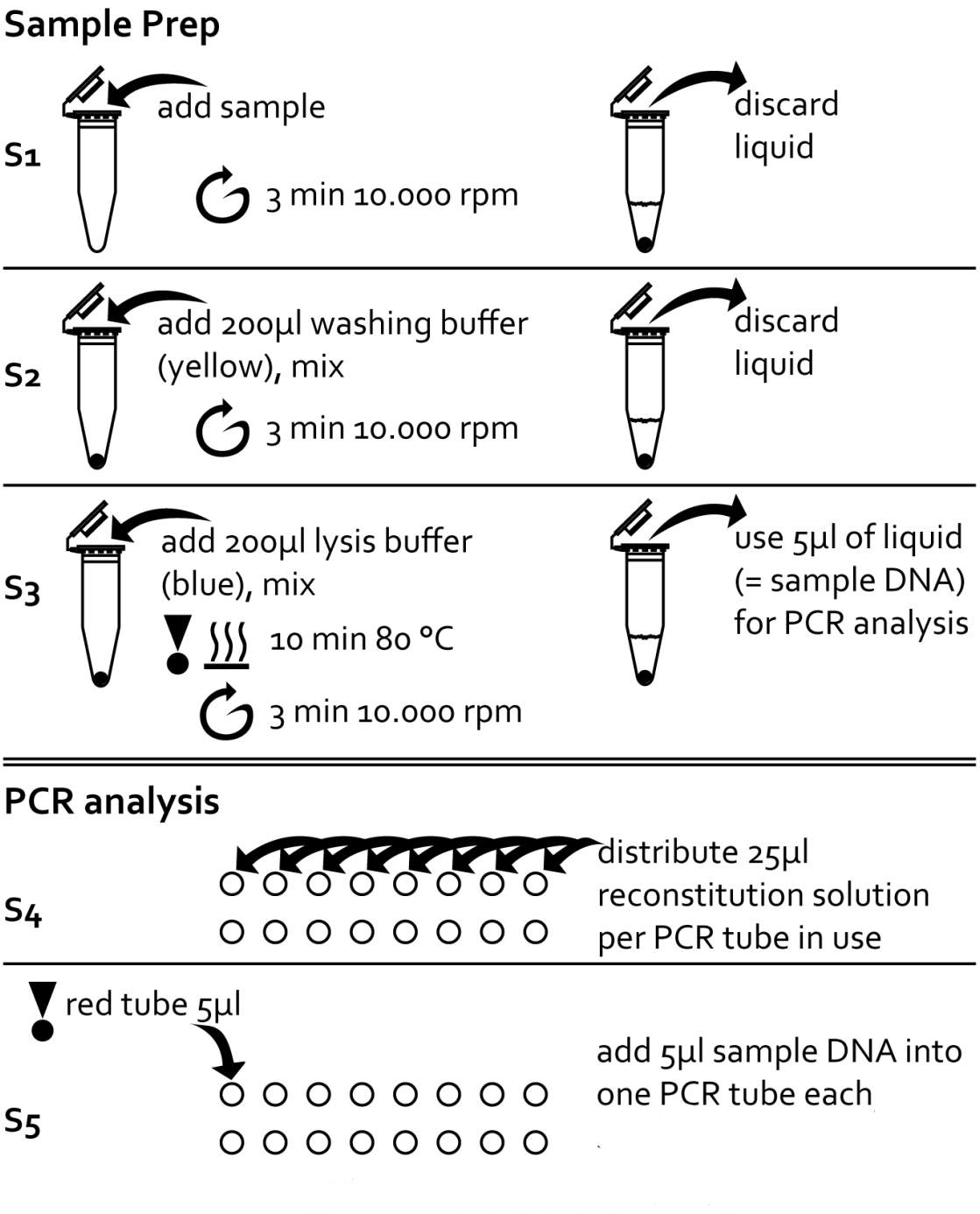
Material Required but Not Supplied

Equipment	Supplies
Real time PCR thermocycler for 0.1 or 0.2 mL tubes with detection channels for FAM (520 nm emission) and VIC/HEX (550 nm emission)	1.5 mL reaction tubes, 2 per sample plus 1 per run
Benchtop microcentrifuge for 1.5 mL reaction tubes, 10,000 rpm (RCF: 10,000 x g) minimum	Pipette tips with filters
Centrifuge for 8-tube strips 0.1 or 0.2 mL or adaptor for benchtop microcentrifuge	Gloves, powder free
Reaction tube mixer (Vortexer) (optional)	
Thermoincubator, dry bath or water bath set to 80 °C ± 5 °C	
Microliter pipettes for DNA extraction 100-1,000 µL variable volume 200 µL fixed volume (optional)	Use different pipettes for DNA extraction and PCR set-up
Microliter pipettes for PCR set-up 100-1,000 µL variable volume 25 µL fixed volume 5 µL fixed volume, or variable equivalent	

Table 2: Additional material required

Section 6

Overview



Refer to Section 9 'PCR Mix Calculation Guide' for preparation of reconstitution solution.

Section 7

Detailed Instructions

Warning! Read the manual and the Safety Data Sheets before starting the analysis. Safety Data Sheets are available in the download area from our website https://pika-weihenstephan.com. All handling steps should be performed under sterile conditions. Wear appropriate protective clothing and powder free gloves. The use of filter tips is recommended.

Sample Preparation

Before you start: Heat water bath, dry bath or thermoincubator to 80 °C

1. Transfer the samples into a 1.5 mL reaction tube:
   a) *Liquid samples*:
   - Clear samples (rinse water, filtered beer, enrichment without visible growth, etc):
     - Standard volume, sample after enrichment:
       - Use 1.0 - 1.5 mL. Proceed to step 2.
     - Larger volume, sample without enrichment: use larger volume, up to 50 mL:
       - Centrifuge sample 5 min at 4,000 - 5,000 rpm (RCF: 2,700-3,000 x g)
       - Slowly decant the liquid, leave about 1 mL in the cone of the tube
       - Mix liquid in the cone with a pipet tip and transfer it completely into a 1.5 mL reaction tube
       - Proceed to step 2.
   - Turbid samples, if turbidity caused by bacterial growth (previously enriched sample, yellow color in FastOrange® B, or contaminated product):
     - Use 50 µL. Proceed to step 2.
   - Turbid sample, if a high yeast concentration is known in the sample (fermenter, pitching yeast, unfiltered beer):
     - Use 50 - 200 µL to reach a pellet size of app. 2 mm in diameter after centrifugation (see fig. 1). Proceed to step 2.

   b) *Colonies:* Single colonies as well as a couple of different colonies together can be processed as one sample
     - First transfer 200 µL Washing buffer A into a 1.5 mL reaction tube
     - Add cell material from all colonies to be analyzed into the liquid. Proceed to step 2.
2. Centrifuge in a mini centrifuge for 3 min at >10,000 rpm (RCF: 8,500 x g) or alternatively in a larger centrifuge for 10 min at 4,000 – 5,000 rpm (RCF: 2,700 – 3,000 x g)
3. Control the pellet sizes, which is the cell material, of your samples. The pellet size should not exceed 2 mm in diameter (see fig. 1).
   - If necessary, remove part of the pellet together with the liquid phase in step 4.
4. Remove the liquid phase carefully and discard

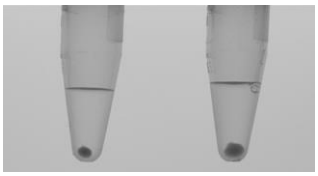


Fig. 1: recommended pellet sizes
Left: maximum pellet size for turbidity from bacteria
Right: maximum pellet size for yeast containing samples

5. Wash the pellet as follows:
    - Add 200 µL Washing buffer A to the pellet
    - Resuspend pellet by vortexing or pipetting up and down, and repeat steps 2. - 4.
    - Extended washing might be necessary for samples known to likely be inhibited:
        - Repeat the whole washing procedure as above, and/or
        - Use a higher volume of washing buffer - up to 1,000 µl per wash
6. Add 200 µL of Lysis buffer B and again resuspend the cell pellet thoroughly
7. Incubate samples at 80 °C ± 5 °C for 10 min in a thermoincubator, dry bath or water bath
8. Centrifuge again as in step 2.
    - Attention! Do **NOT** discard supernatant now as it contains your sample DNA!
    - The pellet contains cell debris and other waste particles, which were separated from the DNA
9. Use the liquid phase for PCR, take care **NOT** to touch the pellet in the bottom of the tube when pipetting
10. For overnight or long term storage, transfer 100 µL of the liquid phase from 8. into a new 1.5 mL reaction tube
11. Store at 2-8 °C for same day PCR analysis; for long-term storage, freeze at -18 to -20 °C

DNA Analysis by real time PCR

All reaction components for PCR except the 2-fold concentrated Master mix are provided in a dried form in the PCR tubes. Each tube contains a mix which delivers primers and probes for the detection of one target species. Tube 5/Strip of 5 contains the reagents for the internal positive control (IPC) additionally.

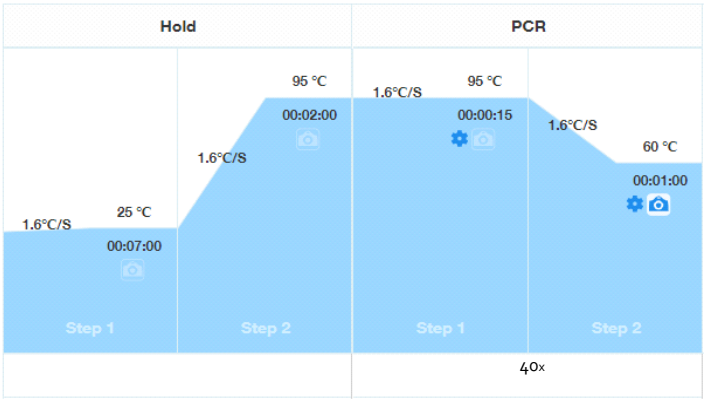
Preparation and Distribution of the Reconstitution Solution

1. Prepare one strip of 8 plus one strip of 5 PCR reaction tubes for a sample.
    - ✓ Take care **NOT** to touch caps or tubes when not wearing gloves!
    - ✓ Be careful about the orientation of the PCR tubes, as each tube contains a different primer/probe mix which is specific for the detection of one species. For easy orientation, there are numbers shown on the tube strips from 1 to 8.
    - ✓ In the strip of 8, tubes 1 is intended for the positive control. In the following tubes 2 to 8, different spoiler species are identified.
    - ✓ In the strip of 5, each tube detects a different *Lactobacillus* species, except for tube 5 which detects *Pediococcus damnosus* and *P. inopinatus* together, as well as the internal positive control (IPC).
2. Prepare the reconstitution solution by pipetting the required amounts of Rehydration buffer and 2 x Master mix into a fresh 1.5 mL reaction tube.
    - ✓ Refer to table 5 for the volumes needed for one sample
    - ✓ A 10% overage to account for pipetting losses is also included in the total calculation
3. After adding both solutions, close the reaction tube, which now contains the reconstitution solution. Mix briefly by vortexing or inverting the tube a couple times. Follow up with a quick spin down to collect the liquid at the bottom of the tube
4. Pipet 25 µL of the reconstitution solution into each PCR tube needed (one strip of 8 plus one strip of 5 PCR tubes)

5. Prepare the control reaction:
    - ✓ Positive control: Pipet 5.0 µL of the provided DNA into the first PCR tube of the strip of 8 tubes. Do not add any sample.
6. Prepare the samples: Pipet 5.0 µL of the extracted sample DNA (from part: Sample Preparation) into one PCR tube each, starting with tube 2 of the strip of 8 tubes, and also in each of the all tubes of the strip of 5 tubes
7. Close all PCR tubes with the provided cap strips
8. Spin down the PCR tubes shortly (10-15 seconds) to collect the liquid at the bottom (max. 2,000 rpm), and check for trapped air bubbles
9. If trapped bubbles are present, repeat step 8.
10. Transfer all PCR tubes into the thermocycler and follow instructions according to the software

Instrument and Software Setup

Set up a PCR test as follows:



Detectors:	FAM (520 nm emission)
	VIC/HEX (550 nm emission)
Quencher:	TAMRA or BHQ
Data collection	
Sample voume	30 µL

All times given are in hours:minutes:seconds

Fig. 2: Temperature scheme of thermocycler

Data Analysis

1. Follow user manual of thermocycler instrument
2. Evaluate the thermocycler results as follows:
    i. Verify the curves
    ii. Evaluation of the measured Cq/Ct values:
        - FAM channel detects target organisms
        - VIC/HEX channel detects internal positive control reaction

Detection of target (FAM channel)	**Internal control reaction ONLY INCLUDED in tube 5 (Strip of 5)** (VIC/HEX channel)	**Result from analysis**
+	**+**	Positive: DNA of target (ref. Table 4) is present
+	**-**	Positive: DNA of target (ref. Table 4) is present
-	**+**	Negative: DNA of target (ref. Table 4) is not detected
-	**-**	Result is not evaluable: Either: Dilute extracted DNA with rehydration buffer 1:1,000 and repeat PCR Or: Repeat the DNA extraction with a smaller amount of sample, applying more extensive washing – refer to Section 8

Table 3: Manually evaluating PCR results

tube 1: Positive control

tubes 2-8 and 1-5: samples

tube 5/ strip of 5: Including internal control VIC reaction

Position	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8
Strip of 8	Positive control	Lactobacillus acetotolerans	Lactobacillus backii	Lactobacillus brevis	Lactobacillus casei	Lactobacillus collinoides	Lactobacillus coryniformis	Lactobacillus lindneri

Position	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
Strip of 5	Lactobacillus parabuchneri ("frigidus")	Lactobacillus perolens	Lactobacillus plantarum	Lactobacillus rossiae	Pediococcus damnosus & Pediococcus inopinatus

Table 4: Result per tube from 4everyone Detection Kit LP Identification

Section 8

Precautions and Recommendations for Best Results

- ✓ This test must be performed by trained persons
- ✓ All potentially infectious material should be autoclaved before disposal
- ✓ The quality of results depends on strict compliance with Good Laboratory Practices (for example, the EN ISO 7218 standard), especially concerning PCR:
  - The laboratory equipment (pipets, tubes, etc.) must not circulate from one workstation to another
  - It is essential to use positive and negative controls for each series of amplification reactions
  - Do not use reagents after their expiration date
  - Periodically verify the accuracy and precision of pipets and the correct functioning of the instruments
- ✓ Change gloves often, especially if you suspect they are contaminated
  - Clean work spaces periodically with at least 5% bleach or other DNA decontaminating agents such as DNA AWAY
  - Use powder-free gloves and avoid fingerprints and writing on tube caps as this can interfere with data acquisition
- ✓ It is strongly advised to follow the general requirements described in the standard EN ISO 22174 (Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection and quantification of microorganisms - General requirements and definitions)

Section 9 Appendix

PCR Mix Calculation Guide

You find the corresponding volumes of Rehydration buffer and 2 x Master mix for one sample in the table (13 reactions per sample). A 10% overage to account for pipetting losses is also included in the total calculation.

	volume to pipet in µL		total
Number of samples	Rehydration buffer	2x Master mix	Volume in µL
1	143,0	214,5	357,5
2	286,0	429,0	715,0
3	429,0	643,5	1.072,5
4	572,0	858,0	1.430,0
5	715,0	1.072,5	1.887,5
6	858,0	1.287,0	2.145,0

Table 5: Pipetting volumes depending on sample numbers

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Use of product:
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