4everyone Detection Kit BY
Beer Spoiling Bacteria + Yeasts Typing

USER GUIDE

Detection and Identification of all Beer Spoilers in One Test

- *The Real Beer Spoiling Bacteria Species of Lactobacillus and Pediococcus*
- *The anaerobic beer spoiling bacteria Megasphaera and Pectinatus species*
- *The Superattenuating Yeasts Dekkera (Brettanomyces) and Saccharomyces cerevisiae var. diastaticus*

4everyone Detection Kit BY Typing 200ul

All kits come with breakable 96 well PCR plates 200 µl, clear, using a strip of 8 PCR tubes per test.

SKU # 2305-12-61 for 12 samples, 8 tubes each
SKU # 2305-48-61 Multipack 48 - for 48 samples, 8 tubes each
SKU # 2305-96-61 Multipack 96 - for 96 samples, 8 tubes each

For research *in vitro* use only

Depending on package size, one 4everyone Detection Kit BY Typing contains sufficient reagents for 12, 48, or 96 samples with 8 reactions each.

Kits include all buffers and reagents necessary for DNA extraction and PCR together with PCR tubes, 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.

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Section 1

4everyone Detection Kit BY Typing

This test allows the quantitative detection and identification of all beer spoiling bacteria and yeast species in one single test.

The species which are proven to cause beer spoilage are

- the lactic acid bacteria species *Lactobacillus 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*
- the anaerobic bacteria species *Megasphaera cerevisiae*, *M. elsdenii*, *M. micronuciformis* and *M. paucivorans*, and *Pectinatus cerevisiiphilus, P. frisingensis, P. haikarae, and P. portalensis*
- the yeast genus *Dekkera* (*Brettanomyces*) covering *D. anomala*, *D. bruxellensis*, *D. naardenensis* (a soft drink spoiler), and the species *Saccharomyces cerevisiae var. diastaticus*

All beer spoiling species from a sample are tested side by side in 8 independent two-channel tube assays, the different species are detected and quantified in the separate reactions.

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) and the VIC channel (530/550 nm) for detection and identification of the target microorganisms. 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 BY Typing contains buffers for sample preparation and all reagents and the tubes for PCR analysis. Different package sizes are offered.

Kit material for DNA isolation and PCR analysis	2305-12-61 12-pack	2305-48-61 48-pack	2305-96-61 96-pack	Storage
Washing buffer A (yellow cap)	1 x 10.0 mL	1 x 12.0 mL	2 x 12.0 mL	dark at 2-8 °C
Lysis buffer B (blue cap)	1 x 10.0 mL	1 x 12.0 mL	2 x 12.0 mL	
Rehydration buffer B (white cap)	1 x 5.0 mL	1 x 5.0 mL	1 x 10.0 mL	
Breakable plate/s with primers&probes (OligoMix)	1	4	8	
Master mix (green cap)	1 x 1,650 µL	4 x 1,650 µL	8 x 1,650 µL	
4everyone Optical Adhesive Film for PCR	0	4	8	
Cap strips (12 strips of 8) for covering the PCR reaction tubes	1	0	0	

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

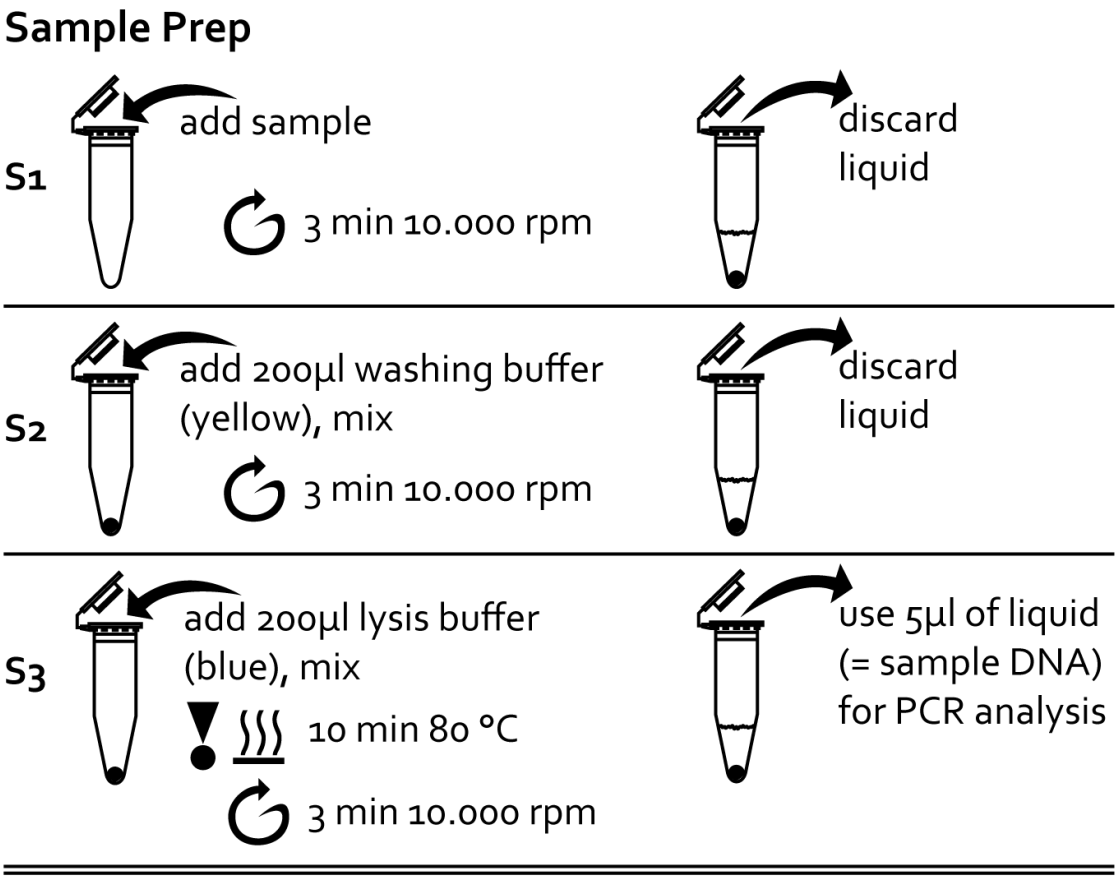
Material Required but Not Supplied

Equipment	Supplies
Real time PCR thermocycler for 0.2 mL tubes with detection channels for FAM (520 nm emission) and VIC (550 nm emission)	1.5 mL reaction tubes, 2 per sample
Benchtop microcentrifuge for 1.5 mL reaction tubes 10,000 rpm (RCF: 10,000 x g) minimum	Tube for preparation of reconstitution solution / tank for multichannel stepper pipet
Centrifuge for microtiter plates	Pipette tips with filters
Reaction tube mixer (Vortexer), optional	Gloves, powder free
Thermoincubator, dry bath or water bath set to 80 °C ± 5 °C	
Microliter pipettes for DNA extraction 100-1,000 µL variable volume and 200 µL fix volume (optional)	Use different pipettes for DNA extraction and PCR set-up
Microliter pipettes for PCR setup 100-1,000 µL variable volume 25 µL volume, or stepper / multichannel stepper pipet 5 µL volume, or multichannel stepper pipet	

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 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.

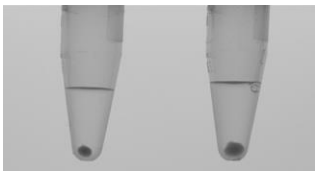


Fig. 1: recommended pellet sizes
Left: maximum pellet size for turbidity from bacteria
Right: maximum pellet size for yeast turbid samples

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

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 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 or two target species as shown in table 3.

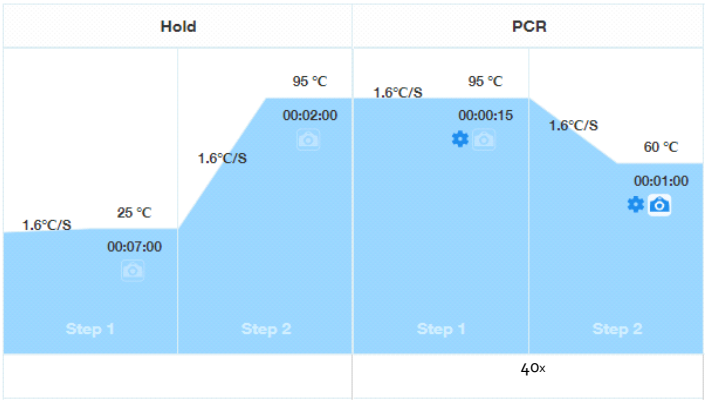
Preparation and Distribution of the Reconstitution Solution

1. Prepare one strip of 8 reaction tubes for each sample.
    - ✓ Take care **NOT** to touch caps , cover foils or tubes when not wearing gloves!
    - ✓ Be careful about the orientation of the PCR tube strips, as each tube contains a different primer/probe mix which is specific for the species detection. For easy orientation, there are numbers shown on the tube strips from 1 to 12 which is always top/first tube of a strip.
    - ✓ In the strip of 8, tubes different spoiler species are identified in each tube,refer to table 3 for details.
2. Prepare the reconstitution solution by pipetting the required amounts of Rehydration buffer and Master mix into a fresh tube which can be a rection tube or another sterile plastic tube. The size is depending on the final volume and also on the pipet type which you are going to use for the distribution into the PCR tubes.
    - ✓ Refer to table 4 for the volumes needed for certain sample numbers.
    - ✓ An overage volume to account for pipetting losses is also included in the total calculation.

3. After adding both solutions, mix briefly by either pipetting up and down or by vortexing or inverting the closed tube a couple times. If vortexing or inverting, follow up with a quick spin down to collect the liquid at the bottom of the tube.
4. If you use less than 12 samples, break a segment of PCR tube strips corresponding to the sample number from the PCR breakable plate and cut the cover foil along the breaking line. Leave all tube strips which are intended for use connected to each other! - don't break them into single strips!
5. Pipet 25 µL of the reconstitution solution into each of the PCR tubes of all strips in use
   - ✓ It is preferable to use a stepper pipet or multi channel stepper pipet for the distribution of the reconstitution solution
6. Pipet 5.0 µL of the extracted DNA from one sample (from part: Sample Preparation) into each of the 8 tubes of **one** PCR strip (A-H)
   - ✓ Again, it is preferable to use a multichannel stepper pipet for the addition of sample DNA from multiple samples into the different tubes of the PCR plate strips
7. Follow up with all other samples, using one full PCR strip per sample, if not using a multichannel pipet for 6.
8. Close all PCR tubes of the plate (segment) with the provided Optical Adhesive Film, cut the film into an appropriate size before you tear off the cover foil and mount it on the plate (segment).
   - ✓ **CAUTION:** For PCR, NEVER use the cover foil which sticks on the PCR plate when it is packaged. This foil is for protection of the PCR tubes during storage only and might melt and irreversibly damage the thermocycler if used for PCR!
   - ✓ If using the 12 samples size package, cover strips of 8 for closing the PCR strip tubes are packaged instead of PCR film
9. Spin down the PCR plate (segment) shortly (10-15 seconds) to collect all liquid at the bottom (max. 2,000 rpm), and check for trapped air bubbles
10. If trapped bubbles are still present, repeat step 9
11. Transfer the PCR plate (segment) 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 program of thermocycler

Data Analysis

1. Follow the user manual of your thermocycler instrument
2. Evaluate the thermocycler results manually as follows:
    i. Verify the curves if their shape is typical for real time PCR
    ii. Evaluate the measured Cq/Ct values: Refer to table 3 for specific species detection
3. A result is regarded as
    i. **positive** if the result gives a value Cq/Ct < 38.5 and the curve shows the typical shape of a real time PCR
    ii. **signal at detection limit** which is not a 100% safe result if Cq/Ct is between 38.5 and 39.5
    iii. **negative** if no Cq/Ct is detected after 40cycles
4. When using a QuantStudio™ thermocyler instrument, there is a software available for automated plus-minus and quantitative readout of results (DA2)

Position	Channel 1 (FAM) targets	Channel 2 (VIC) targets
A	Negative Control - should show no signal until 40 cycles	Inhibition Control - should give a positive signal at Cq/Ct 31 ± 2.5
B	Lactobacillus brevis	channel not in use
C	Lactobacillus lindneri	Pectinatus Screening
D	Pediococcus damnosus & Pediococcus inopinatus	channel not in use
E	Lactobacillus parabuchneri ("frigidus") & Lactobacillus rossiae	Dekkera Screening
F	Lactobacillus Spoiler Group Common detection of the Lactobacillus species L. casei, L. collinoides, L. coryniformis, L. perolens, L. plantarum	Saccharomyces cerevisiae var. diastaticus
G	Lactobacillus acetotolerans & Lactobacillus backii	channel not in use
H	Positive Control - should give a positive signal at Cq/Ct 31 ± 2.5	Megasphaera Screening

Table 3: PCR results per spoiler target in the different tubes A-H of a PCR 8-tube strip.

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

Per sample, one full strip of 8 tubes is needed (8 reactions per sample).

To find the correct volumes for a certain number of samples, prepare the reconstitution solution as follows: Check the number of samples to be analyzed and find the corresponding volumes of Rehydration Buffer and Master Mix in the table. A pipetting reserve is already included in the total.

Number of samples	Rehydration Buffer volume in µL	Master Mix volume in µL	Reconstitution Solution volume in µL
1	90	135	225
2	180	270	450
3	275	410	685
4	365	545	910
5	455	685	1.140
6	550	820	1.370
7	640	960	1.600
8	730	1.100	1.830
9	820	1.230	2.050
10	915	1.370	2.285
11	1.005	1.505	2.510
12	1.100	1.645	2.745

Table 4: Volumes needed for preparation of reconstitution solution according to sample number

Note

The bags containing the OligoMix breakable plate are hermetically sealed. This is a packaging technique for storable products. A bag protects the product from environmental influences. A bag that is not one hundred per cent vacuum-sealed is due to the regular production process and does not represent a quality defect.

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Use of product:

4everyone Detection Kit is to be used for *in vitro* research purposes only.

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