4e™ For everyone Detection Kit B Acetics Screening

User Guide

Detection of Acetic Acid Bacteria

For research use only

Catalog #2401-15

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Section 1

4e™ For everyone Detection Kit B Acetics Screening

This test allows the quantitative detection of the members of the acetic acid bacteria group including the well known Acetobacter, Gluconacetobacter and Gluconobacter species, and also other rare occuring but also acetic acid producing representatives as Acidisphaera sp., Acidocella sp., Acidomonas sp., Asaia sp., Granulibacter, Kozakia sp. and Swaminathania sp.

All these bacteria covered by the 4e™ For everyone Detection Kit Acetics Screening test are capable to produce acetic acid. They are specifically detected together in one screening test which provides a simple and easy result read-out for the presence of acetic acid bacteria in your sample.

Section 2

Introduction to the 4e™ For everyone 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 4e™ For everyone Detection Kit system is based on DNA amplification and detection of micro-organisms 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 (494/518 nm) for detection of the target microorganisms and the HEX channel (535/556 nm) for an internal control reaction. This allows 4e™ For everyone 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:

Data analysis is optimized for use with the Chai Dual Channel Open qPCR™ real time PCR thermocycler (www.chaibio.com).

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

Section 3

Kit Components

The 4e™ For everyone Detection Kit B Acetics Screening kit contains sufficient reagents for 48 reactions.

Kit material for DNA isolation and analysis	Amount	Storage
Washing buffer A (yellow cap)	2 x 10.0 mL	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 50 µL	
PCR tubes (strips of 8) with BHQmix	6	
2 x Master mix (green cap)	1 x 850 µL	
Cap strips (strips of 8) for covering the PCR reaction tubes	6	2-8 °C or ambient

Table 1: Materials supplied

Section 4

Shelf Life and Storage

Once received, the kit must be stored at 2-8 °C. Reagents stored at this temperature can be used until the expiration date indicated on the package label.

Section 5

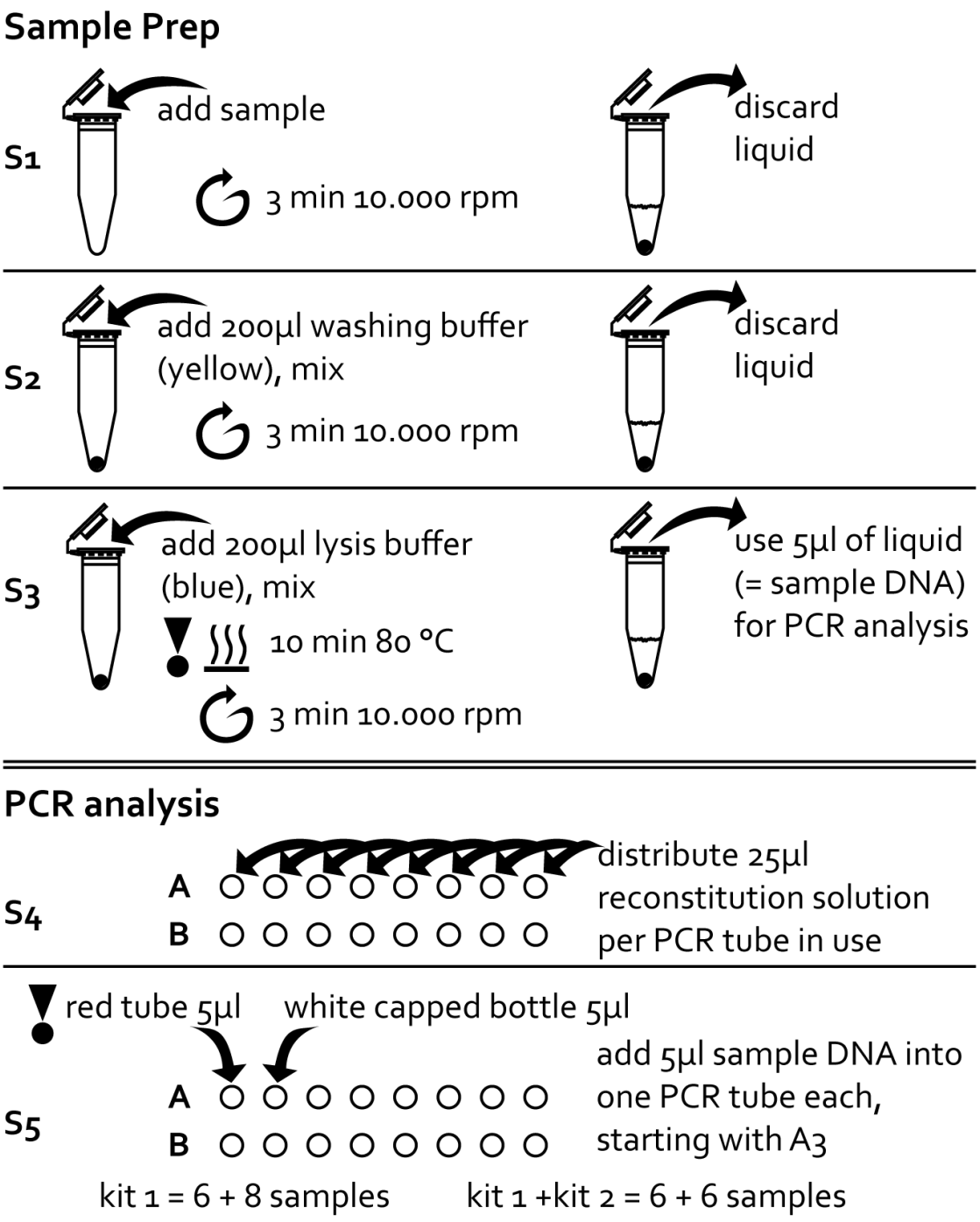
Materials Required but Not Supplied

Equipment	Supplies
Real time PCR thermocycler for 0.1 mL tubes with detection channels for FAM (520 nm emission) and 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 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)	Ideally 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 materials 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 www.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 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

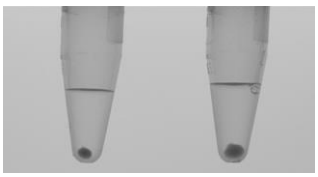


Fig. 1: recommended pellet sizes
Left: maximum pellet size for turbidity from bacteria
Right: maximum pellet size for yeast containing samples

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 BHQmix which delivers primers and probes for the detection of the target species plus the reagents for the internal positive control (IPC).

The number of PCR tubes and cap strips can be adjusted according to the number of samples to be analyzed by cutting the needed amount with sterilized (flamed) scissors or a knife. Remember to always add 2 tubes and caps for the positive and negative controls.

Preparation and Distribution of the Reconstitution Solution

1. Prepare one PCR reaction tube for each sample. Additionally one positive and one negative control reaction are always neccessary per run. Take care **NOT** to touch caps or tubes when not wearing gloves!
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 1 for the volumes.
    - ✓ When using table 1, consider only the number of samples you want to analyze. Do **NOT** count the control reactions in your calculation
    - ✓ The volume needed for the 2 control reactions per run are already accounted for in the table
    - ✓ 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 (number of samples + 2 controls)

5. Prepare the control reactions:
   - ✓ Positive control: Pipet 5.0 µL of the provided DNA into the first PCR tube. Do not add any sample.
   - ✓ Negative control: Pipet 5.0 µL Rehydration buffer into the second PCR tube. 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 3
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

For instrument and software setup, follow instructions in the Open qPCR instrument software.

- If you are using a different thermocycler instrument, set the following profile:

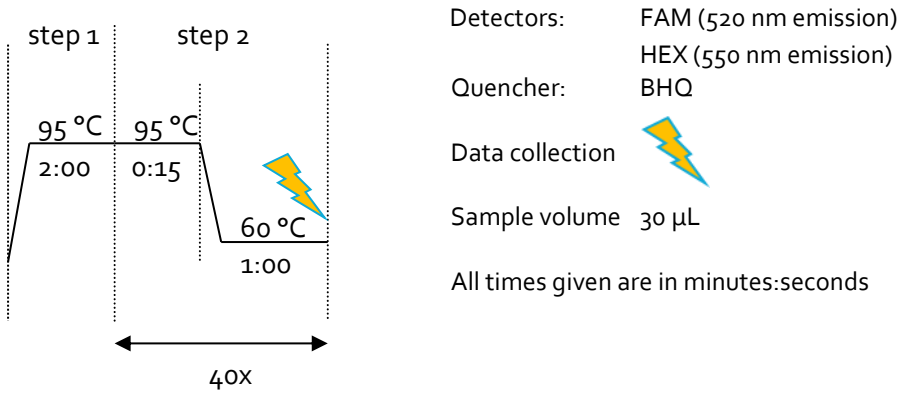


Fig. 2: Temperature scheme of thermocycler

Data Analysis

1. Chai Dual Channel Open qPCR™ real time PCR thermocycler
   - All data can be analyzed directly at the end of the PCR run or at a later time by opening the stored experiment. Follow instructions in the corresponding Open qPCR software manual for opening experiments and setting the data analysis parameters
   - Interpreting Results
     i. Once the run is complete, the software displays the full data interpretion in a table format which is the standard read-out
     ii. The full data including amplification curves can be seen under the "Full Results" view
2. Other real time PCR thermocyclers
   - Follow user manual of thermocycler instrument
   - Evaluate the thermocycler results as follows:
     i. Verify the curves
     ii. Evaluation of the measured Cq/Ct values:

- FAM channel detects target organisms
- HEX channel detects internal positive control reaction

Detection of target (FAM channel)	**Internal control reaction** (HEX channel)	**Result from analysis**
+	**+**	Positive: DNA of target (ref. Section 1) is present
+	**-**	Positive: DNA of target (ref. Section 1) is present
-	**+**	Negative: DNA of target (ref. Section 1) 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

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 22174:2005 (Microbiology of food and animal feeding stuffs Polymerase chain reaction (PCR) for the detection of food pathogens General requirements and definitions)

Section 9 Appendix

PCR Mix Calculation Guide

When using the Chai Dual Channel Open qPCR™ real time PCR thermocycler: To find the correct volumes to pipet when preparing the reconstitution solution, add the total number of samples to be analyzed, and find the corresponding volumes of Rehydration buffer and 2 x Master mix in the table.

Kit 1	**volume to pipet in µL**		total
number of samples	Rehydration buffer	2x Master mix	Volume in µL
1	33.0	49.5	82.5
2	44.0	66.0	110.0
3	55.0	82.5	137.5
4	66.0	99.0	165.0
5	77.0	115.5	192.5
6	88.0	132.0	220.0
7	99.0	148.5	247.5
8	110.0	165.0	275.0
9	121.0	181.5	302.5
10	132.0	198.0	330.0
11	143.0	214.5	357.5
12	154.0	231.0	385.0
13	165.0	247.5	412.5
14	176.0	264.0	440.0

Table 4: 1 Kit per Run

Kit 1 + Kit 2	**volume to pipet in µL**		total
sum of samples from both kits	Rehydration buffer	2x Master mix	Volume in µL
2	66.0	99.0	165.0
3	77.0	115.5	192.5
4	88.0	132.0	220.0
5	99.0	148.5	247.5
6	110.0	165.0	275.0
7	121.0	181.5	302.5
8	132.0	198.0	330.0
9	143.0	214.5	357.5
10	154.0	231.0	385.0
11	165.0	247.5	412.5
12	176.0	264.0	440.0

Table 5: 2 Kits per Run

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Use of product:

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