SO DETECTION KIT B LACTOBACILLUS ACETOTOLERANS

For the identification of *Lactobacillus acetotolerans*

Cat. No. 2201-52

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.

This product is for research use only.

Product description

The PCR Kit Lactobacillus acetotolerans was developed for the detection and identification of *Lactobacillus acetotolerans*.

PCR Kit content

Materials supplied are sufficient for 48 reactions

Description	Amount	Storage*
Material for DNA isolation		
Washing buffer A (yellow cap)	1 x 10.0 mL	4°C
Lysis buffer B (blue cap)	2 x 10.0 mL	
Reagents for DNA analysis		
Rehydration buffer B (white cap)	1 x 5.0 mL	4°C
DNA (red cap) as positive control	1 x 50 µL	
PCR tubes (strips of 8) with Oligo Mix	6	
Cap strips (strips of 8) for covering the PCR reaction tubes	6	4°C or room temperature

* Kit is shipped at ambient temperature

Materials required but not supplied

Material
Instruments and equipment
Real-time PCR System in microtiter format (0.1 mL tubes) with measuring channels for FAM (520 nm emission) and VIC/HEX (550 nm emission)
Benchtop microcentrifuge for 1.5 mL reaction tubes
Plate centrifuge or adaptor for 8-tube strips
Reaction tube mixer (Vortexer)
Thermoincubator or water bath set to 80°C
Pipettors
Consumables and reagents
Powder-free gloves
1.5 mL reaction tubes, safe-lock, sterile
Filter pipette tips
2-fold concentrated Master Mix with DNA Polymerase + dNTPs + $MgCl_2$

Procedural guidelines

Part 1: Sample preparation

1. Transfer the sample into a 1.5 mL reaction tube:
   a) Liquid samples:
   - 50 µL of a turbid, bacterial sample (previously enriched sample or spoiled product)
   - 1.0 – 1.5 mL of a clear sample (even larger sample sizes can be used)
   - 50 – 200 µL of yeast slurries or other samples with sediment to reach a pellet size of app. 2 mm in diameter after centrifugation (see fig. 1)

   b) *Colonies:* single colonies as well as different colonies can be processed together as one sample
   - Transfer 200 µL Washing buffer A and cell material into a 1.5 mL reaction tube, skip step 5.
2. Centrifuge for 3 min at 14,000 rpm (25,000 x g) or alternatively 10 min at 4,000 rpm (1,500 x g)
3. Control the pellet size, it is including the cells from the sample. Pellet size should not exceed 2 mm in diameter (see fig. 1).

   If necessary, remove part of the pellet together with the liquid phase
4. Remove the liquid phase carefully and discard
5. Wash the pellet with 200 µL Washing buffer A, resuspend pellet and repeat steps 2 to 4
6. Add 200 µL of Lysis buffer B and resuspend by mixing briefly
7. Incubate sample at 80 °C ± 5 °C for 10 min in a thermoincubator or water bath
8. Centrifuge again as in step 2. The pellet contains cell walls and other particles separated from the DNA
9. Transfer 100 µL of the liquid phase containing the DNA to a new 1.5 mL reaction tube and use the liquid phase for PCR. For long-term storage, freeze at -18 to –20 °C

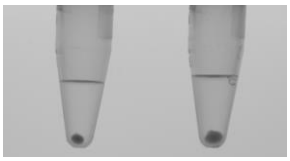


Fig. 1: recommended pellet sizes:
Left: max. pellet bacterial size
Right: max. pellet size for samples containing yeast or particles

Part 2: DNA Analysis

All reaction components except the 2-fold concentrated Master Mix are provided in a dried form in the PCR tubes. All PCR tubes contain Oligo Mix and an internal positive control (IPC).

Preparation and distribution of the Rehydration solution

Prepare one reaction for each sample. The use of a positive and a negative control is highly recommended.

1. Calculate the required amounts for the Rehydration solution according to table 1
2. Pipet all components in the shown order in a new 1.5 mL reaction tube
3. Close the reaction tube containing the Rehydration solution, mix briefly and spin down shortly

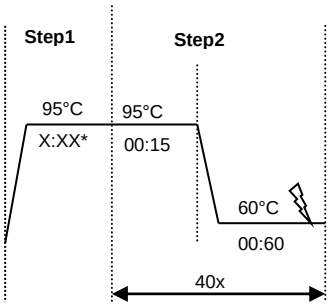
Components	Volume per PCR reaction	+ 10% pipetting reserve	Multiplied by number of samples n	***Total volume for Rehydration solution***
Rehydration buffer B	10,0 µL	1,0 µL	(n + 2)	**= 11,0 µL x (n+2)**
2-fold conc. Master Mix	15,0 µL	1,5 µL	(n + 2)	**= 16,5 µL x (n+2)**
Total volume for Rehydration solution	25,00 µL	2,5 µL	(n + 2)	**= 27,5 µL x (n+2)**

Table 1: Preparation of Rehydration solution

Preparation of PCR

1. Pipet 25 µL of the Rehydration solution in each PCR tube
2. Pipet 5.0 µL of the extracted sample (from Part 1: sample preparation) into one PCR tube from 1.
3. For the control reactions:
   - Pipet 5.0 µL of the provided DNA (positive control) instead of sample into one PCR tube
   - Pipet 5.0 µL Rehydration buffer (negative control) instead of sample in another PCR tube

1. Close the PCR tubes with the provided Cap strips. Attention: Always use gloves when touching caps and tubes!
2. Optional: spin down shortly (max. at 2,000 rpm)
3. Transfer PCR tubes in the Thermocycler and set the following profile:
   - Set volume to 30 µL
   - Set detectors for FAM (520 nm emission) and VIC/HEX (550 nm emission). The Quencher is TAMRA for all reactions.



: Measuring point

*****activation time depending on used Master Mix (ref. to manufacturer)

Evaluation

1. Verify the curves
2. Evaluation of the measured Ct values:

FAM Channel detects target organisms:

a. Ct ≤ 38: Reaction is positive

b. Ct 38 – 40: Reaction is critically low, repeat the sample preparation and/or the PCR

c. Ct >40: Reaction is negative

VIC/HEX Channel detects internal positive control:

a. For the internal positive reaction a Ct value ≤ 35 is expected

b. If the Ct value is between 38-40, the control reaction has to be assessed as inhibited/negative

c. In case of a positive sample with Ct values ≈ 20 – 25, the internal positive control may show higher Ct values or fail completely

Detection of target (FAM dye)	Control reaction (VIC/Hex dye)	Result
+	+	DNA of Lactobacillus acetotolerans is present
+	-	DNA of Lactobacillus acetotolerans is present
-	+	DNA of Lactobacillus acetotolerans is not detected
-	-	Result is not evaluable: • Either: Repeat the DNA extraction with a smaller amount of sample • or: Dilute extracted sample with Rehydration buffer (1:100 to 1:1000) and repeat PCR

Table 2: Evaluation of PCR results

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