

## SO DETECTION KIT B LACTOBACILLUS ROSSIAE

For the identification of *Lactobacillus rossiae*

Cat. No. 2201-30

**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](http://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 rossiae* was developed for the detection and identification of *Lactobacillus rossiae*.

### PCR Kit content

#### Materials supplied are sufficient for 48 reactions

Description	Amount	Storage*
<b>Material for DNA isolation</b>		
Washing buffer A (yellow cap)	1 x 10.0 mL	4°C
Lysis buffer B (blue cap)	2 x 10.0 mL	
<b>Reagents for DNA analysis</b>		
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
<b>Instruments and equipment</b>
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
<b>Consumables and reagents</b>
Powder-free gloves
1.5 mL reaction tubes, safe-lock, sterile
Filter pipette tips
2-fold concentrated Master Mix with DNA Polymerase + dNTPs + MgCl <sub>2</sub>

## Procedural guidelines

### Part 1: Sample preparation

- Transfer the sample into a 1.5 mL reaction tube:
  - Liquid samples:**
    - 50  $\mu\text{L}$  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  $\mu\text{L}$  of yeast slurries or other samples with sediment to reach a pellet size of app. 2 mm in diameter after centrifugation (see fig. 1)
  - Colonies:** single colonies as well as different colonies can be processed together as one sample
    - Transfer 200  $\mu\text{L}$  Washing buffer A and cell material into a 1.5 mL reaction tube, skip step 5.
- Centrifuge for 3 min at 14,000 rpm (25,000 x g) or alternatively 10 min at 4,000 rpm (1,500 x g)
- 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
- Remove the liquid phase carefully and discard
- Wash the pellet with 200  $\mu\text{L}$  Washing buffer A, resuspend pellet and repeat steps 2 to 4
- Add 200  $\mu\text{L}$  of Lysis buffer B and resuspend by mixing briefly
- Incubate sample at  $80\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  for 10 min in a thermoincubator or water bath
- Centrifuge again as in step 2. The pellet contains cell walls and other particles separated from the DNA
- Transfer 100  $\mu\text{L}$  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  $-20\text{ }^{\circ}\text{C}$

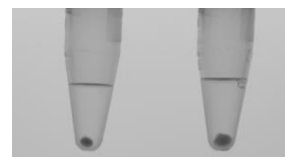


Fig. 1: recommended pellet sizes:  
Left: max. pellet bacterial size  
Right: max. pellet size for samples containing yeast or particles

### 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.

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

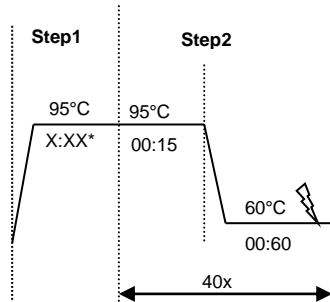
Components	Volume per PCR reaction	+ 10% pipetting reserve	Multiplied by number of samples n	<b>Total volume for Rehydration solution</b>
Rehydration buffer B	10,0 $\mu\text{L}$	1,0 $\mu\text{L}$	(n + 2)	<b>= 11,0 <math>\mu\text{L}</math> x (n+2)</b>
2-fold conc. Master Mix	15,0 $\mu\text{L}$	1,5 $\mu\text{L}$	(n + 2)	<b>= 16,5 <math>\mu\text{L}</math> x (n+2)</b>
Total volume for Rehydration solution	25,00 $\mu\text{L}$	2,5 $\mu\text{L}$	(n + 2)	<b>= 27,5 <math>\mu\text{L}</math> x (n+2)</b>

Table 1: Preparation of Rehydration solution

#### Preparation of PCR

- Pipet 25  $\mu\text{L}$  of the Rehydration solution in each PCR tube
- Pipet 5.0  $\mu\text{L}$  of the extracted sample (from Part 1: sample preparation) into one PCR tube from 1.
- For the control reactions:
  - Pipet 5.0  $\mu\text{L}$  of the provided DNA (positive control) instead of sample into one PCR tube
  - Pipet 5.0  $\mu\text{L}$  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 <i>Lactobacillus rossiae</i> is present
+	-	DNA of <i>Lactobacillus rossiae</i> is present
-	+	DNA of <i>Lactobacillus rossiae</i> is not detected
-	-	Result is not evaluable: <ul style="list-style-type: none"> <li>• <u>Either</u>: Repeat the DNA extraction with a smaller amount of sample</li> <li>• <u>or</u>: Dilute extracted sample with Rehydration buffer (1:100 to 1:1000) and repeat PCR</li> </ul>

Table 2: Evaluation of PCR results

A Screening Kit that detects *Lactobacillus backii*, *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus collinoides*, *Lactobacillus coryniformis*, *Lactobacillus parabuchneri*, *Lactobacillus lindneri*, *Lactobacillus perolens*, *Lactobacillus plantarum*, *Lactobacillus rossiae*, *Pediococcus damnosus* and *Pediococcus inopinatus* is also available (Cat. No. 2201-38).



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