

BILATEST Bac Kit



100 Extractions (Bacterial DNA)

Product description

The BILATEST Bac Kit is designed for the fast and easy isolation of genomic DNA from bacteria.

Included reagents

Reagent	Volume
(1) Lysis Buffer	40ml
(2) Magnetic Beads	2ml
(3) Binding Buffer	90ml
(4) Washing Buffer A	50ml
(5) Washing Buffer B	50ml
(6) Washing Buffer C	100ml
(7) Elution Buffer	10ml

The **Elution Buffer (7)** is 10mM Tris-HCl pH 8.0. It is also possible to use TE buffer pH 8.0.

This kit contains enough materials for 100 isolations from bacteria pellets or 200 µl of culture medium.

Required Materials

2 ml Lysozyme (20 mg/ml) (optional)
2 mg RNase (optional)

This kit is optimized for use with BILATEC Magnetic Separators (e.g. BILATEST magnetic separator M 12+12 for 1.5ml tubes, Order-No. 210141).

Storage conditions and Safety Informations

This kit may be stored at room temperature (15-25°C) and is stable for at least 1 year following delivery.

The kit buffers contain harmful substances. When working with chemicals, always wear suitable lab coat, disposable gloves, and protective goggles. In case liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

Samples and Protocol Adjustments / Additional Reagents

The methods described below are general protocols and optimization may be required depending on the bacteria used.

Gram-positive bacteria: For some bacteria (in particular gram positive) a special lysis treatment is recommended:

additionally add 4 µl of a fresh lysozyme solution (20 mg lysozyme/ml Tris-HCl, pH 8.0) in step 1 to lyse the substantial cell wall.

Purification Protocol

1. Place the **bacteria pellet** (e.g. from 200 µl of an overnight culture) in a micro-centrifuge tube, add **200 µl Lysis Buffer (1)** and 2 µl **RNase A** (10 mg/ml). Mix well with 8 to 10 pipetting strokes and then incubate 10 minutes at 37°C.
Note if purifying directly from the bacterial culture:
Place 200 µl of an overnight culture in a 2 ml – centrifuge tube and proceed with the protocol using 400 µl Lysis Buffer (1) (protocol step 1) and 900 µl DNA Binding Buffer (3) (protocol step 2).
2. Add **20 µl** resuspended **Magnetic Beads (2)** premixed with **300 µl DNA Binding Buffer (3)** to the tube. (*Use 900 µl Binding Buffer (3) when preparing directly from the culture*). Mix with 6-10 pipetting strokes and incubate **5 minutes** at room temperature.
3. Following incubation, place the tube in a magnetic separator to draw the Magnetic Bead/DNA complex to the side of the tube. Leave 2 minutes, then discard supernatant and remove the tube from the magnet position.
4. Add **500 µl Washing Buffer A (4)** to the tube and thoroughly resuspend the beads in the washing buffer by pipetting the bead pellet up and down 10 to 12 times.
5. Separate the **Magnetic Bead/DNA complex** in the magnetic separator, discard supernatant and remove tube from the magnet position.
6. Repeat the washing procedure (steps 4 and 5) using **Washing Buffer B (5)**. After removing all the last traces of Washing Buffer B (5), **leave the tube in the magnetic separator**.
7. With the tube in the magnetic separator, and the beads attracted to the side of the tube, gently add **1ml** (or as large a volume as possible) of **Washing buffer C (6)**, while being careful not to disturb the pellet. Leave **1 minute without resuspending the bead pellet** and then carefully remove and discard the supernatant. (Note: a longer incubation time or resuspension of the bead pellet in **Washing Buffer C (6)** may reduce the final DNA yield.)
8. Add **100 µl** (or another suitable volume) of **Elution Buffer (7)** to the tube and thoroughly resuspend the Magnetic bead/DNA complex by mixing the pellet with 10 to 15 pipetting strokes.
9. Incubate the suspension for **7 minutes at 55 °C** (occasional agitation may facilitate the complete DNA elution).
10. Following DNA elution place the tube in the magnetic separator for 2 minutes or until all the beads have separated from the eluate. Transfer the eluate containing the purified DNA into a clean tube. If there are some remaining particles in the eluate one can perform a second magnetic separation.