



chemagic DNA Bacteria Kit *for general purposes* *for the isolation of genomic DNA from bacteria*

Kit Components

Magnetic Beads	2 ml
Lysis Buffer 1	40 ml
DNA Binding Buffer 2	90 ml
Wash Buffer 3	50 ml
Wash Buffer 4	50 ml
Wash Buffer 5	100 ml
Elution Buffer 6	10 ml

The **Elution Buffer 6** is 10 mM Tris-HCl pH 8.0; one can also use TE buffer pH 8.0.

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

Required Materials

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

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

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

Gram-positive bacteria: Lysis treatment: additionally add 4 μ l of a fresh lysozyme solution (20 mg lysozyme/ml Tris-HCl, pH 8.0) in step 1 to lyse the substantial cell wall.

Before You Start

Ensure that you have a proper magnetic separator. This kit is optimized for use with **chemagic** Magnetic Separators.



Purification Protocol for Bacteria Pellets

1. Place the **bacteria pellet** (e.g. from 200 μ l of an overnight culture) in a micro-centrifuge tube and add 200 μ l **Lysis Buffer 1** and 2 μ l RNaseA (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 μ l of an overnight culture in a 2 ml - centrifuge tube and proceed with the protocol using 400 μ l **Lysis Buffer 1** (protocol step 1) and 900 μ l **DNA Binding Buffer 2** (protocol step 2).*

2. Add 20 μ l resuspended **Magnetic Beads**, premixed with 300 μ l **DNA Binding Buffer 2**, to the tube. Mix with 6 - 10 pipetting strokes and incubate **5 minutes** at room temperature.
3. Following incubation, place the tube in a **chemagic** 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 μ l **Wash Buffer 3** to the tube and thoroughly resuspend the beads in the wash 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 the tube from the magnet position.
6. Repeat the washing procedure (steps 4 and 5) using **Wash Buffer 4**. After removing all the last traces of **Wash Buffer 4**, **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 1 ml (or as large a volume as possible) of **Wash Buffer 5**, 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 **Wash Buffer 5** may reduce the final DNA yield.)
8. Add 100 μ l (or another suitable volume) of **Elution Buffer 6** to the tube and thoroughly resuspend the **Magnetic Bead / DNA Complex** by pipetting the pellet up and down 10 to 15 times.
9. Incubate the suspension for **7 minutes** at 55 °C (or alternatively 10 - 15 minutes at room temperature), with occasional agitation, e.g. using a thermomixer, to facilitate complete DNA elution.
10. Following DNA elution place the tube in the Magnetic Separator for 2 minutes or until all the **Magnetic Beads** have separated from the eluate. Transfer the **eluate** containing the purified DNA to a clean tube. If there are some remaining particles in the eluate one can perform a second magnetic separation.