

# BILATEST Plasmid Mini Kit



100 extractions from 1.5 ml *E.coli* overnight cultures

## Product Description

The Bilatest Plasmid Mini Kit is designed for the fast and easy isolation of Plasmid DNA from bacteria.

This kit contains enough materials for 100 isolations from 1.5 ml *E.coli* overnight culture.

## Kit Components

Reagent	Volume
Magnetic Beads I	8 ml
Magnetic Beads II	4 ml
(1) Suspension Buffer	14 ml
(2) Lysis Buffer	14 ml
(3) Neutralization Buffer	20 ml
(4) Binding Buffer	40 ml
(5) Washing Buffer A	70 ml
(6) Washing Buffer B	70 ml
(7) Elution Buffer	10 ml

The **Elution Buffer** is 5 mM Tris-HCl, pH 8.0; one can also use TE buffer pH 8.0.

## Required Materials

70 % Ethanol

Magnetic Particle Separator

RNase A

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

Stored at room temperature (15-25°C), the kit is stable for at least 12 months following delivery.

## UV Measurements

In some cases there may be some traces of the magnetic beads left in the eluate after removal from the tube. Such particles will not interfere with PCR and most downstream applications but may increase the background in UV measurements. In such a case, prior to UV analysis, we recommend an additional application of the magnet to the eluate for 3 minutes in order to separate any traces of particles. For pure DNA the expected  $A_{260}/A_{280}$  ratio is between 1.8 - 1.9. The  $A_{260}$  value should fall between 0.1 and 1.0 for accurate readings.

### **Purification Protocol for 1.5 ml *E.coli* overnight culture**

1. Harvest the bacterial cells by centrifugation at 4000 g for 5 minutes and discard the supernatant. Resuspend the bacterial pellet in **140 µl Suspension Buffer (1)** and add **5 µl RNase** (10 mg/ml).
2. Add **140 µl Lysis Buffer (2)** and mix gently by inverting the tube 4 times. Do not vortex in order to avoid shearing of genomic DNA. Incubate at room temperature for not more than 4 minutes.
3. Premix **200 µl Neutralization Buffer (3)** with **80 µl** resuspended **Magnetic Beads I**. Following incubation add **Neutralization Buffer (3)/Magnetic Beads I** to the tube. Mix by inverting and incubate 2 minutes at room temperature with another mix inbetween.
4. Place the tube in a Magnetic Separator to draw the **Magnetic Bead/Debris-Complex** to the side of the tube. Leave 2 minutes, then transfer the supernatant to a clean tube. **(Take care not to disturb the pellet and transfer parts of the Magnetic Bead/Debris-Complex.)**
5. Add **400 µl Binding Buffer (4)** and **40 µl** resuspended **Magnetic Beads II** to the tube and thoroughly resuspend the beads by pipetting. Incubate the tube 2 minutes at room temperature (pipette up and down one more time inbetween).
6. Separate the **Magnetic Bead/DNA Complex** in the magnetic separator, discard supernatant and remove tube from the magnet position.
7. Wash the bead pellet by adding **700 µl Washing Buffer A (5)** to the tube and thoroughly resuspend the beads by pipetting up and down 7 times. Separate the **Magnetic Bead/DNA Complex** in the magnetic separator and discard supernatant.
8. Add **700 µl 70% EtOH** to the tube and thoroughly resuspend the beads in the wash buffer by pipetting the bead pellet up and down 5 times. Separate the **Magnetic Bead/DNA Complex** in the magnetic separator and discard supernatant.
9. Add **700 µl** cold **Washing Buffer B (6)** without disturbing the pellet. Leave 30 seconds and discard supernatant.
10. Add **100 µl** (or another suitable volume) of **Elution Buffer (7)** to the tube and thoroughly resuspend the **Magnetic Bead/DNA Complex** by pipetting.
11. Incubate the suspension for **5 minutes at RT** with occasional agitation.
12. Following DNA elution place the tube in the Magnetic Separator until all the **Magnetic Beads II** have separated from the eluate. If there are particles left in the eluate a second separation step is recommended.