



chemagic Plasmid Mini Kit

for general purposes

Kit Components

Magnetic Beads I	8 ml
Magnetic Beads II	4 ml
Suspension Buffer 1	14 ml
Lysis Buffer 2	14 ml
Neutralization Buffer 3	20 ml
Binding Buffer 4	40 ml
Wash Buffer 5	70 ml
Wash Buffer 6	2 x 35 ml
Elution Buffer 7	10 ml

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

This kit contains enough material for 100 isolations from 1.5 ml *E.coli* overnight culture and is optimized for use with **chemagic** Magnetic Separators.

Required Materials

- 70 % Ethanol
- Magnetic Particle Separator
- RNase A

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.



Plasmid 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 A (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 the chromosomal 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 **chemagen** 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 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 on 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 **Wash Buffer 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 **Wash Buffer 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 55 °C 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.