Protocol: Plasmid Miniprep for Plasmid Isolation - Jena Bioscience

Last updated: 2026-05-29

Reviewed by: Patrik

Materials

- *E. coli* culture (1-5 mL) Max
- Microcentrifuge tubes (1.5 mL and 2.0 mL)
- Ethanol (96-99%)
- Centrifuge capable of 10,000 × g
- Jena Bioscience Plasmid Mini-Prep Kit
    - Lysis Buffer
    - Neutralization Buffer
    - RNase A – store at -20 °C!
    - Activation Buffer
    - Washing Buffer
    - Elution Buffer
- Nanodrop or spectrophotometer (for DNA quantification)

Instructions

Preparation:

- Add RNase A (1x4 mg) into 16 mL of Neutralization Buffer. After addition, store the Neutralization Buffer at 4 °C.
- Add 64 mL Ethanol to the Washing Buffer (final volume should be 80 mL).

Cell Harvest and Lysis:

1. Harvest the bacterial cell culture (1-3 mL) by centrifugation.
2. Resuspend pelleted bacterial cells in 300 µL Lysis Buffer by pipetting or vortexing for 1 min.

Neutralization:

3. Add 300 µL of Neutralization Buffer (containing RNase A) to sample and mix gently by inverting the tube 4-6 times (do NOT VORTEX!)
4. Centrifuge at 10,000 g for 5 min at room temperature in a micro-centrifuge.
   The color of the binding mixture should change to bright yellow indicating a pH of 7.5 required for optimal DNA binding. An orange or violet color shows a pH >7.5 and indicates an inefficient DNA adsorption. In this case, it is recommended to adjust the pH of the mixture by addition of a small volume of 3M sodium acetate, pH 5.0 before proceeding.

Column Activation:

5. Place a Binding Column into a 2 mL collection tube.
6. Add 100 µL of Activation Buffer into the Binding Column.
7. Centrifuge at 10,000 g for 30 seconds in a micro-centrifuge.

Column Loading:

8. Apply the supernatant from Step 4 into the activated Binding Column by decanting or pipetting.
9. Centrifuge at 10,000 g for 30 seconds.
10. Discard the flow-through.

Column Washing:

11. Place the DNA loaded Binding Column into the used 2 mL tube.
12. Apply 500 µL of Washing Buffer (containing Ethanol) to the Binding Column.
13. Centrifuge at 10,000 g for 30 seconds and discard the flow-through.

Optional **secondary washing**: Recommended only for DNA > 200 bp, if highly purified DNA (for DNA sequencing, transfection etc.) is required.

- Add 700 µL of Washing Buffer to the Binding Column.
- Centrifuge at 10,000 g for 30 seconds and discard the flow-through.
- Centrifuge again for 2 min to remove residual Washing Buffer.

Elution:

14. Place the Binding Column into a clean 1.5 mL microtube (not provided in the kit).
15. Add 30-50 µL (better is 50 µL) Elution Buffer or dd-water to the center of the column membrane.
16. Incubate for 1 minute at room temperature.
17. Centrifuge at 10,000 g for 1 min to elute DNA.
18. Measure concentration using Nanodrop.
19. Store the purified plasmid DNA at –20 °C.
