Column-Pure Plasmid Miniprep Kit

Cat. No. G4003

Store all components at 18-25°C.

Product Description

abm's **Column-Pure Plasmid Miniprep Kit** combines the standard alkaline lysis method with silica spin column technology to rapidly extract and purify plasmid DNA from bacterial cells. The extracted high quality plasmid DNA is primarily in supercoiled form and is ready for downstream applications such as PCR, restriction digest and sequencing.

Product Component	Quantity
RNase A	10 mg
Buffer P1	80 ml
Buffer P2	80 ml
Buffer P3	100 ml
Buffer PW1	140 ml
Buffer PW2	50 ml
Elution Buffer	30 ml
Spin Columns	250
Collection Tubes	125 x 2

Protocol

Before use, add 200 ml of 95% Ethanol to Buffer PW2 and 10 mg of RNase A into Buffer P1. Perform all centrifugation steps at 12,000 rpm (~12,000-14,000 x g).

1. Pellet 1-5 ml of overnight bacterial culture in a 1.5 ml microcentrifuge tube using a tabletop microcentrifuge for 2 min. Discard the supernatant completely by aspiration or pipette.

2. Add 250 µl of Buffer P1 (with added RNase A) to the bacterial pellet and mix by pipette or vortex to completely resuspend so that there are no visible cell clumps.

3. Add 250 µl of Buffer P2 and thoroughly mix by gentle inversion 8-10 times. Do not allow this reaction to proceed longer than 5 min.

4. Add 350 µl of Buffer P3 and thoroughly mix by gentle inversion 8-10 times. The solution should become cloudy.

5. Centrifuge for 5 min.

6. Assemble a Spin Column into a Collection tube.

7. Transfer 800 µl of the supernatant from Step 5 into the assembled Spin Column and centrifuge for 30 s. Discard the flow-through.

8. Recommended: Add 500 µl of Buffer PW1 to the Spin Column and centrifuge for 30 s. Discard the flow-through.
   •This step is necessary to remove trace nuclease activity when using endA+ strains, such as the JM series, HB101 derivatives, or any wild-type strain. Host E. coli *strains such as DH5α and XL-1 Blue do not require this step.*

9. Add 750 µl of Buffer PW2 (with added ethanol) to the Spin Column and centrifuge for 30 s. Discard the flow-through. Centrifuge for an additional 1 min to remove residual wash buffer.

10. Discard the Collection Tube and transfer the Spin Column to a new 1.5 ml microcentrifuge tube.

11. Add 50-100 µl of Elution Buffer to the center of the Spin Column. Incubate for 1 min at room temperature and then centrifuge for 1 min. Store purified plasmid DNA at -20°C.
    •Elution Buffer can be preheated to 70°C prior to Step 11 to allow for efficient elution of large plasmid DNA (>10 kb).

General Notes

- Buffer P1 with added RNase A is stable at 4°C for 6 months.
- Buffer P2 may form a precipitate upon storage – warm to 37°C to dissolve.

Troubleshooting

1. Low DNA yields
   •Ensure ethanol is added to Buffer PW2 before use.
   •Poor cell lysis due to inadequate cell dispersion at Step 2. Vortex to resuspend.
2. Plasmid DNA does not settle in agarose gel well
   Ethanol was not completely removed from column following wash step. Perform additional 1 min dry spin at Step 9.
3. Plasmid DNA contains high molecular weight contaminant DNA
   •Sample may have been mixed too aggressively during Step 3. Do not shake, vortex or incubate longer than 5 min.
   •Overgrown cultures contain lysed cells and degraded DNA. Do not overgrow culture.
4. Abnormal Plasmid DNA absorbance (A260/A280)
   •Sample may be contaminated with RNA due to insufficient RNase A treatment. Add RNase A to Buffer P1 and ensure this buffer is stored optimally at 4°C.

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