MediRes Corp.

USER GUIDE **Bi2M-PlasMini**

Plasmid Mini Kit

Product #: Bi2M-PlasMini

Quantity: 100 reactions

Product Description

- Designed for rapid and cost-effectives small-scale preparation of high quality plasmid DNA from recombinant *E.coli* cultures.
- Based on modified SDS-alkali lysis method and utilizes an exclusive silica-based membrane technology in the form of a convenient spin column.
- Each column can recover up to 25 µg of plasmid DNA.
    - *The actual plasmid yield will depend on the plasmid copy number & culture density.*
- The purified plasmid DNA can be used in all molecular biology procedures such as conventional digestion with restriction enzymes, PCR, transformation and sequencing.

Storage

- Store at room temperature for up to 12 months.
- For longer storage, store at 4℃.
- IMPORTANT: After addition of RNase A, the resuspension solution **Buffer S1** should be stored at 4℃.

Kit Components

Buffer BL	50 mL
Buffer S1 (Red)	30 mL (*note: add 300 µL **RNase A**, and store at 4℃)*
Buffer S2	30 mL (*note: DO NOT shake vigorously*)
Buffer S3	40 mL
Buffer PD	60 mL
Buffer PW	2×15 mL (*note: add 60 mL 100% ethanol per bottle before use*)
Elution Buffer	12 mL (*note: pre-heat to 60℃ before use*)
RNase A	300 µL
Bind DNA Mini Column	100 columns
Collection Tube	100 tubes

Important Preparatory Notes (PLEASE READ CAREFULLY)

1. If precipitate forms in the buffers during storage, solutions should be re-dissolved by incubating at 37℃, then cooled back down to 25℃ before use.
2. Add the provided **RNase A** solution to **Buffer S1** (Red) and mix. The resuspension solution should be stored at 4℃.
3. Add **60 mL 100% ethanol** to the **Buffer PW** and mark the bottle on the label.
4. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag.
5. Proper laboratory safety practices should be employed, including the use of lab coats, gloves, and eye protection.
6. (Optional step) Column balance:
    - Put the **Bind DNA Mini Columns** into the **Collection Tubes**, add **500 µL** solution **Buffer BL** to the adsorption column, centrifuge at 10,000 g for 1 min, remove the waste liquid in the **Collection Tubes**.
    - Return the **Bind DNA Mini Columns** to the **Collection Tube** (please use the column treated on the day).
    - Note: Addition of **Buffer BL** can improve the adsorption capacity, homogeneity and stability of the **Bind DNA Mini Columns**, and eliminate the influence of high temperature, humidity or other adverse environmental factors on the **Bind DNA Mini Columns**.

Protocol

1. **Bacterial culture collection:** harvest bacterial culture from 1-5 mL of fresh bacterial culture incubated for 12-16 h by centrifugation at 10,000 rpm for 2 min at room temperature. Decant the supernatant and remove all remaining medium.
    - *Note: For **high-copy-number plasmids**, do not process more than 5 mL of bacterial culture. If more than 5 mL, the Bind DNA Mini Column capacity (25 µg) will be exceed and no increase in plasmid yield will be obtained.*

- *Note: For **low-copy-number plasmids**, it may be necessary to process larger volumes of bacterial culture (up to 10 mL) to recover sufficient quantity of DNA.*

2. Resuspend the pelleted cells in **250 µL of Buffer S1** (ensure **RNase A** has been added before using). The bacteria should be resuspended completely by vortexing or pipetting up and down until no bacterial clumps remain. Let the uniform red bacterial suspension stand at room temperature for 5 min.
   - *Note: If bacterial clumps are incompletely mixed, it will affect the lysis, resulting in low extraction amount and purity.*
3. **Lysis:** add **250 µL** of the **Buffer S2** and mix thoroughly by inverting the tube 6-8 times until the solution becomes viscous and slightly clear.
   - *Note: mix well gently, do not shake violently. At this time, the sample should appear clear and viscous. If the solution does not become clear, it may be due to too many bacteria and incomplete lysis. Therefore, the starting volume of bacteria should be reduced.*
   - *Note: After Buffer S2 is added and thoroughly mixed, the solution should appear clear and purple. If the solution appears purple mixed with turbid red, this indicates that the cell lysis is incomplete. Continue mixing until the solution is completely clear and purple.*
   - ***Note: Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.***
4. **Neutralization:** add **350 µL** of the **Buffer S3** and mix **immediately** and thoroughly by inverting the tube 6-8 times. At this time, the neutralized bacterial lysate should become appear cloudy.
   - *Note: Buffer S3 should be mixed immediately after adding, and a small amount of tiny white precipitate in the supernatant has no effect on subsequent experiments.*
   - *Note: After adding Buffer S3 and thoroughly mixing, the solution should appear clear and yellow. If there are purple particulates mixed in the yellow, this indicates incomplete renaturation. Therefore, continue to mix until the solution is completely clear yellow.*
5. Centrifuge sample at 13,000 g for 15 min at room temperature.
6. Carefully transfer the supernatant to the **Bind DNA Mini Columns**, taking care not to pipette precipitation as much as possible.

7. Centrifuge at 10,000 g for 1 min. Discard the waste liquid. Place the **Bind DNA Mini Columns** into the collection tubes.
8. Add **500 µL** of the **Buffer PD** to the **Bind DNA Mini Columns**. Centrifuge at 10,000 g for 1 min and discard the flow-through. Place the column back into the same collection tube.
9. Add **700 µL** of the **Buffer PW** (please check whether ethanol has been added before using) to the **Bind DNA Mini Columns**. Centrifuge at 10,000 g for 1 min and discard the flow-through.
10. Place the **Bind DNA Mini Columns** back into the same collection tube.
    - *Note: If the recovered DNA is used for salt-sensitive experiments, such as flat end joining experiment or direct sequencing, it is recommended that **Buffer PW** be added and let stand for 5 min before centrifugation.*
11. Repeat the wash procedure (step 9).
12. Place the **Bind DNA Mini Columns** into the collection tube and centrifuge at 10,000 g for 2 min to remove the residual liquid.
13. Dry the **Bind DNA Mini Columns** at room temperature for 5 min.
    - *Note: residual ethanol in rinse solution can affect subsequent enzyme digestion and PCR experiments.*
14. Transfer the **Bind DNA Mini Columns** into a fresh **1.5 mL microcentrifuge tube**. Add **50~100 µL** of the **Elution Buffer** or ddH_2O to the center **of Bind DNA Mini Columns** membrane to elute the plasmid DNA
    - *Note: pre-heat **Elution Buffer** or **ddH_2O** to 60℃ before use.*
    - *Note: take care not to contact the membrane with the pipette tip.*
    - *Note: minimum elution volume is 50 µL.*
15. Incubate for 2 min at room temperature and centrifuge at 10,000 g for 2 min to collect DNA solution.
16. Store DNA at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

NOTES:

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