Pure **Plasmid Maxiprep Kit**

Cat. #: W0503-10 (10 reactions)

Storage: room temperature. After adding RNase A, store Buffer P1 at 2 ~ 8°C, and it is stable for 6 months. Other buffers and RNase A stock solution can be stored for 12 months at room temperature.

Product Description (This product is for research use only.)

This kit is based on a modified alkaline lysis method, with improved reagent formulation and silica membrane, which has high adsorption efficiency of the plasmid DNA. The proteins, genomes, RNA and other impurities can be removed after washing and an elution.

200 µg ~ 1.5 mg of plasmid DNA can be purified from 100 ~ 300 mL overnight culture of E. Coli in LB medium with this kit.

The extracted DNA can be directly used for PCR, restriction enzyme digestion, sequencing, etc. No need to precipitate, concentrate or desalt.

Product Components

Components	Amount	Storage
Buffer P1	125 mL	room temperature, store at 4°C if RNase A is added.
Buffer P2	125 mL	room temperature
Buffer P3	125 mL	room temperature
Buffer PB	50 mL	room temperature
Buffer PW (concentrate)	25 mL x 2	room temperature
Buffer EB	30 mL	room temperature
RNase A (10 mg/mL)	1 mL x 2	4°C
Spin Column (Maxi)	10	room temperature
Collection Tube	20	room temperature

Protocol

Note:

- All protocol steps should be carried out at room temperature (15 ~ 25°C)
- Add the provided RNase A to Buffer P1 before use. Store RNase added buffer P1 at 4°C.
- Add ethanol (96 ~ 100%) to Buffer PW before use (see bottle label for the volume).
- Check Buffers P2, P3 and PB before each use for salt precipitation. Dissolve any precipitate by warming the Buffers in 37°C water bath.
- Buffer P2 and Buffer P3 are irritating. Handle with care. Cover it tightly immediately after use.

1. **Harvest 150 mL overnight bacterial culture** in a centrifuge tube (not provided), and centrifuge at **13,000x g for 3 minutes** or at **6,000x g for 15 minutes** to pellet bacterial cells.

   The volume of bacterial culture needed depends on the concentration of cultured cells. For long plasmid (>10 kb) and low-copy number plasmid, centrifuge twice to harvest more bacterial cells (do not exceed 300 mL culture). Double the amount of buffer P1, P2 and P3 in the following reaction.

   The amount of the reagents must be able to fully lysis the bacteria cells, otherwise the extraction efficiency will be reduced.

2. Aspirate the supernatant completely. Invert the tubes on a paper towel to remove/drain all trances of medium.

3. Add **12 mL Buffer P1** (check if RNase A has been added) to the pelleted bacterial cells and resuspend thoroughly by vortexing or pipetting up and down till no cell clumps remain.

   Note: If bacteria are not thoroughly mixed, the plasmid yield and purity will be low.

4. Add **12 mL Buffer P2** and mix thoroughly by gently invertiny the tube 6 ~ 8 times. **Stand for 3 ~ 5 minutes** at room temperature. The solution should become clear and viscous.

   Note:
   - Close Buffer P2 bottle immediately after use.
   - **Do not allow the lysis reaction to proceed for more than 5 minutes.**
   - Do not shake vigorously or vortex, to avoid shearing of genomic DNA. If the solution is not clear, it suggests that the bacteria cells are excessive. Reduce the input amount of bacteria.

5. Add **12 mL Buffer P3** and mix immediately and thoroughly by inverting the tube 6 ~ 8 times.

   Note: Mix thoroughly and immediately after addition of Buffer P3 to avoid localized precipitation.

6. Sit at **room temperature for 5 minutes**. Centrifuge at **13,000x g for 10 minutes**.

7. Transfer the supernatant to a new centrifuge tube (not provided). Add **11 mL isopropanol (0.3 volumes) to the supernatant** and mix by inverting the tube 4 ~ 6 times.

8. Put the Spin Column in collection tube, and transfer the mixture from step 7 to the Column. **Centrifuge at 6,000 ~ 13,000x g for 2 minutes**. Discard the flow-through and put the Column back to collection tube.

   Note: The maximum volume of the Spin Column is 15 mL. Repeat this step for 4 ~ 5 times to run all the solution through the Column

9. **Add 4 mL Buffer PB** to the spin column and **centrifuge at 6,000 ~ 13,000x g for 2 minute**, Discard the flow-through and put the Column back to collection tube.

10. **Add 10 mL Buffer PW** (check if ethanol is added) to the spin column, and **centrifuge at 6,000 ~ 13,000x g for 1 minute**. Discard the flow-through.

11. Repeat step 10 once (wash twice).

12. Put the spin column back into the collection tube and centrifuged at **13,000x g for 5 minutes** to remove residual wash buffer. Discard the flow-through and air dry the Column at room temperature for a few minutes to dry thoroughly.

    Note: The residual ethanol will affect the subsequent enzymatic reaction (digestion, PCR, etc.). Residual PW buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation.

13. Place the spin column in a new collection tube. **Add 1 ~ 3 mL Buffer EB** to the center of the Column. Let **stand for 2 ~ 5 minutes** at room temperature and **centrifuge at 13,000x g for 2 minutes**. **The flow-through is purified plasmid DNA**. Use it directly or stored at -20 ℃.

    Note:

    1) In order to increase the recovery of plasmid, the obtained solution can be re-added to the spin column and repeat step 14. **Preheat Buffer EB in 65 ~ 70 ℃ water bath** and extend the adsorption and elution time will increase the yield.

    2) The pH value impacts the elution efficiency significantly. If use ddH_2O to elute the DNA, the pH value should be kept between 7.0 and 8.5 (adjust the pH of the ddH_2O with NaOH as needed). If the pH value below 7.0 the elution efficiency reduces greatly. The elution volume should not be less than 1 mL.

-- The end --