Pure **Plasmid Miniprep Kit**

Cat. #: W0500-50 (50 reactions); W0500-200 (200 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.

3 ~ 30 µg of plasmid DNA can be purified from 1 ~ 5 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, and transfection. No need to precipitate, concentrate or desalt.

Product Components

Components	Amount		Storage
	Cat. #: **W0500-50**	Cat. #: **W0500-200**	
Buffer P1	15 mL	60 mL	room temperature, store at 4°C if RNase A is added.
Buffer P2	15 mL	60 mL	room temperature
Buffer N3	20 mL	80 mL	room temperature
Buffer PB	30 mL	120 mL	room temperature
Buffer PW (concentrate)	10 mL	40 mL	room temperature
Buffer EB	10 mL	30 mL	room temperature
RNase A (10 mg/mL)	150 µL	600 µL	4°C
Spin Column (CM)	50	200	room temperature
2 mL Collection Tube	50	200	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.
- All centrifugation steps are performed at **13,000 rpm** in a table-top microcentrifuge.
- Add ethanol (96 ~ 100%) to Buffer PW before use (see bottle label for the volume).

1. **Harvest 1.5 ~ 2 mL overnight bacterial culture** in microcentrifuge tube(s) (not provided), and centrifuge **for 1 minute** 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 plasmid, centrifuge twice to harvest more bacterial cells (do not exceed 5 mL culture). Double the amount of buffer P1, P2 and N3 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. Discard the supernatant by decanting. Invert the tubes on a paper towel to remove/drain all trances of medium.

3. Add **250 µL 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 **250 µL Buffer P2** and gently invert the tube 4 ~ 6 times. **Stand for 3 ~ 5 minutes** at room temperature. The solution should become clear and viscous.

   Note:
   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 **350 µL Buffer N3** and mix immediately and thoroughly by inverting the tube 4 ~ 6 times.

   Note: Mix thoroughly and immediately after addition of Buffer N3 to avoid localized precipitation. Large culture volumes (e.g. ≥ 5 mL) may require inverting up to 10 times. The solution should become cloudy.

6. Sit at room temperature for 5 minutes. Centrifuge **for 10 minutes**. A compact white pellet will form.

7. Transfer the supernatant obtained in step 6 to a Spin Column CM (sit in collection tube). **Centrifuge for 1 minute**. Discard the flow-through. The capacity of the Spin Column CM is 750 µL. If the total volume is more than 750 µL, repeat this step.

8. **Recommended: Add 500 µL Buffer PB** to the spin column and **centrifuge for 1 minute**, Discard the flow-through.

Note: This step is recommended for endA+ host strain (TG1 is, BL21, HB101, JM101, ET12567, etc.), which contain high levels of nucleic acid enzymes causing degradation of plasmid DNA. This step can be omitted for endA- host strain (DH5α, TOP10, XL-1 blue etc.).

9. **Add 750 µL Buffer PW** (check if ethanol is added) to the spin column, and **centrifuge for 1 minute**. Discard the flow-through.

10. Put the spin column back into the collection tube and centrifuged an additional 2 minutes to remove residual wash buffer. (Optional) place it at room temperature for a few minutes to dry thoroughly with lid opened.

    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.

11. Place the spin column in a clean 1.5 mL microcentrifuge tube (not provided). **Add 50 ~100 µL Buffer EB (or ddH_2O)** to the center of the film of each column. Let **stand for 2 minutes** at room temperature and **centrifuge 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 13. **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 50 µL.

Related Products

Category	Extracted DNA application	Cat.# / Product Name
Pure Plasmid Extraction	PCR, enzyme digestion, sequencing, transfection into cell lines	W0500 **Pure Plasmid Miniprep Kit**
		W0503 **Pure Plasmid Maxiprep Kit**
Endotoxin-Free Plasmid Extraction	Transfection into all kind of cells, PCR, enzyme digestion, sequencing,	W2106 **Endotoxin-Free Plasmid Miniprep Kit**
		W2581 **Endotoxin-Free Plasmid Midiprep Kit**
		W2104 **Endotoxin-Free Plasmid Maxiprep Kit**
High-throughput Plasmid Extraction	PCR, enzyme digestion, sequencing, transfection into cell lines	W0506 **Pure Plasmid 96 Miniprep Kit**

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