Pure **Plasmid 96 Miniprep Kit**

Cat. #: W0506-496 (4 x 96 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 extract high purity plasmid DNA in 96-well plate format from cultured bacterial cells.

3 ~ 10 µg of plasmid DNA can be purified from one well in 96-well plate (1 ~ 1.3 mL) of overnight culture of E. Coli in LB medium with this kit.

The extracted DNA can be directly used for PCR, restriction enzyme digestion, and sequencing. 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 N3	160 mL	room temperature
Buffer PS	100 mL	room temperature
Buffer PB	240 mL	room temperature
Buffer PW (concentrate)	50 mL x 2	room temperature
Buffer EB	60 mL	room temperature
RNase A (10 mg/mL)	1.25 mL	4°C
Filter-96-Plate	4	room temperature
Bind-96-Plate	4	room temperature
Collection Plate (96-well)	8	room temperature
Sealing Film	16	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 and Buffer N3 before each use for salt precipitation. Dissolve any precipitate by warming the Buffers in 37°C water bath.
- Buffer P2 and Buffer N3 are irritating. Handle with care. Cover it tightly immediately after use.
- When using multi-channel pipet, avoid spilling and cross contamination among the wells.
- All centrifugation steps are performed at **2,600x g (~3,600 rpm)** in a 96-well plate centrifuge at room temperature.

1. **Harvest 1 ~ 1.3 mL overnight bacterial culture per well in Collection Plate (96-well),** seal with the **Sealing Film**, and centrifuge **for 10 minutes** to pellet bacterial cells.

2. Remove and discard the Sealing Film from the plate. Discard the supernatant by decanting, and invert the plate on a paper towel to remove/drain all trances of medium. If the bacterial cells concentration is low, centrifuge twice to harvest more bacterial cells

3. Add **250 µL Buffer P1** (check if RNase A has been added) to each well, seal with a new Sealing Film, and resuspend the pellet thoroughly by vortexing till no cell clumps remain.

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

4. Remove and discard the Sealing Film. Add **250 µL Buffer P2 to each well,** seal with a new Sealing Film, and gently invert the plate 6 ~ 8 times to mix. Quick spin the plate. **Stand for 3 minutes** at room temperature. The solution should become clear and viscous.

   Note:
   Do not allow the entire lysis reaction (including quick spin time) 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. Remove and discard the Sealing Film. Add **350 µL Buffer N3 to each well,** seal with a new Sealing Film, and mix immediately and thoroughly by inverting the tube 6 ~ 8 times.

   Note: Mix thoroughly and immediately after addition of Buffer N3 to avoid localized precipitation. The solution should become cloudy.

6. Place a Filter-96-Plate on the top of a new Collection Plate. **Transfer 750 µL lysate** per sample from previous step to the Filter-96-Plate (the capacity of each well of the Filter-96-Plate is 750 µL). **Centrifuge for 5 minutes.**

7. **Bind-96-Plate Equilibrate:** Place the Bind-96-Plate on the top of a 96-well Collection Plate. Add **200 µL Buffer PS** to each well, and centrifuge for 3 minutes. Discard the liquid waste and put the Bind-96-Plate back to the Collection Plate.

8. Transfer the filtered lysate from step 6 to the equilibrated Bind-96-Plate. **Centrifuge for 5 minutes**. Discard the flow-through.

9. **Recommended: Add 500 µL Buffer PB** to each well of Bind-96-Plate and **centrifuge for 5 minutes**, 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.).

10. **Add 600 µL Buffer PW** (check if ethanol is added) to each well of Bind-96-Plate, and **centrifuge for 5 minutes**. Discard the flow-through.

11. **Repeat step 10 (wash twice).**

12. Put the Bind-96-Plate back into the Collection Plate and centrifuge for 10 minutes to remove all residual wash buffer.

    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 Bind-96-Plate on a new Collection Plate. **Add 80 ~100 µL Buffer EB (or ddH_2O)** to the center of each well. Let **stand for 2 ~ 5 minutes** at room temperature and **centrifuge for 10 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.

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