Rapid Plasmid Mini Kit

P1521035

Storage: Room Temperature.
Shipping: Normal.

Introduction:

This kit employs a modified SDS-alkaline lysis method to lyse cells. The silica membrane within the centrifugal spin column selectively binds plasmid DNA in the solution under high-salt, low-pH conditions. Impurities and other bacterial components are then removed using a wash solution. Finally, the purified plasmid DNA is eluted from the silica membrane using a low-salt, high-pH elution buffer.

Product Features:

1. The silica membrane inside the centrifugal spin column is made entirely of imported specialty membrane, ensuring minimal variation in binding capacity between columns and excellent reproducibility. This overcomes the instability issues associated with the membrane quality of domestic kits.
2. Fast and convenient; no need for toxic reagents such as phenol or chloroform, nor ethanol precipitation. The obtained plasmid DNA has high yield and purity, making it suitable for various molecular biology experiments including restriction enzyme digestion, transformation, PCR, in vitro transcription, and sequencing.

Component List:

P1521035	Component	100T	Storage
P1521035A	RNase A (powder)	1 EA	RT.
P1521035B	Solution P1	30 mL	RT.
P1521035C	Solution P2	30 mL	RT.
P1521035D	Solution P3	40 mL	RT.
P1521035E	Wash Buffer WB	25 mL	RT.
P1521035F	Elution Buffer EB	10 mL	RT.
P1521035G	Spin Column AC	1 EA×100	RT.
P1521035H	Collection Tubes (2 ml)	1 EA×100	RT.

The kit can be stored at room temperature for 18 months without affecting performance.

Precautions:

1. First-time use: Add the entire vial of RNase A (powder) to Solution P1 (final concentration 100 μg/mL) and store at 2-8°C. If RNase A in Solution P1 becomes inactivated, trace RNA residues may appear in the extracted plasmid; add additional RNase A to Solution P1 to remedy this.
2. When ambient temperature is low, SDS in Solution P2 may precipitate, causing cloudiness. Warm at 37°C for a few minutes until clear. Do not shake vigorously to avoid excessive foaming.
3. Avoid prolonged exposure of reagents to air to prevent volatilization, oxidation, and pH changes.
4. This kit is suitable for endA-deficient strains such as XL-1Blue and DH5α. For endA+ strains like JM series, HB101, or wild-type strains which contain high levels of nucleases, please use our [H1521033] High Purity Rapid Plasmid Miniprep Kit.
5. Solution P3 contains irritating compounds. Wear latex gloves during operation. Avoid contact with skin, eyes, and clothing. In case of contact, rinse thoroughly with copious amounts of water or saline.
6. The amount of extracted plasmid depends on factors such as bacterial culture concentration and plasmid copy number. For high-copy plasmids, inoculate a single colony into 1.5-4.5 mL of LB medium containing the appropriate antibiotic and culture overnight for 14-16 hours; up to 20 μg of pure plasmid can be extracted. For low-copy plasmids or large plasmids >10 kb, increase the amount of bacterial culture (use 5-10 mL of overnight culture) and proportionally increase the volumes of P1, P2, and P3, while keeping other steps the same.
7. The yield and purity of the extracted plasmid DNA can be analyzed by agarose gel electrophoresis and UV spectrophotometry. An OD260 value of 1 corresponds to approximately 50 μg/mL DNA. Electrophoresis may show a single band, or two or more DNA bands, primarily due to different migration positions of various supercoiled plasmid conformations, influenced by factors like culture duration and handling intensity during extraction. Under normal operating conditions with this product, supercoiled forms typically exceed 90%.
8. Elution Buffer EB does not contain the chelator EDTA and does not interfere with downstream enzymatic reactions like restriction digestion or ligation. Deionized water can also be used for elution, but ensure the pH is >7.5; low pH reduces elution efficiency. Plasmids eluted with water should be stored at -20°C. For long-term storage, TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) can be used for elution; however, EDTA may affect downstream enzyme reactions, so dilution may be necessary when used.
9. For research use only. Not intended for clinical diagnosis or other applications.

Materials Required but Not Supplied:

- Anhydrous ethanol.

Protocol:

Notes:

- Before first use, add 100 mL of anhydrous ethanol to the Wash Buffer WB bottle. Mix thoroughly. Immediately mark the bottle to indicate ethanol has been added to avoid adding it multiple times!
- Pour the RNase A (powder) into Solution P1, rinse the RNase A vial with P1 to ensure complete transfer, and mix well. Store at 2-8°C after each use.
- Pre-cool Solution P3 on ice before use to improve yield.

1. Harvest bacteria: Centrifuge 1.5 - 4.5 mL of overnight bacterial culture at 12,000 rpm for 30 seconds. Remove the supernatant as completely as possible.
2. Resuspend: Resuspend the bacterial pellet thoroughly in 250 μL of Solution P1 by vortexing until no clumps remain.
3. Lyse: Add 250 μL of Solution P2. Gently invert the tube 4-7 times to lyse the cells completely.
4. Neutralize: Add 350 μL of Solution P3. Immediately and gently invert the tube 4-7 times until a white flocculent precipitate forms. Centrifuge at 12,000 rpm for 5 minutes. Carefully transfer the supernatant to a new tube/column.
5. Bind: Transfer the supernatant from the previous step into the Spin Column AC (placed in a collection tube). Centrifuge at 12,000 rpm for 30-60 seconds. Discard the flow-through in the collection tube.
6. Wash 1: Add 500 μL of Wash Buffer WB (ensure ethanol has been added). Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
7. Wash 2: Repeat step 6.
8. Dry: Place the Spin Column AC back into the empty collection tube. Centrifuge at 12,000 rpm for 2 minutes to remove residual wash buffer, preventing ethanol carryover that could inhibit downstream reactions.
9. Air dry: Transfer the Spin Column AC to a clean 1.5 mL microcentrifuge tube. Allow the column to air dry for several minutes at room temperature.
10. Elute: Add 50-100 μL of Elution Buffer EB (pre-heating the buffer to 65-70°C improves elution efficiency) directly onto the center of the silica membrane. Let stand at room temperature for 2 minutes. Centrifuge at 12,000 rpm for 1 minute. For higher yield, re-apply the eluate to the spin column and repeat the centrifugation step. A larger elution volume increases yield. To obtain a higher concentration of plasmid, reduce the elution volume, but do not use less than 30 μL, as smaller volumes reduce elution efficiency and yield. If using ddH_2O for elution, ensure the pH is within the range of 7.0-8.5; pH below 7.0 will reduce elution efficiency.