Endo-Free High Purity Rapid Plasmid Mini Kit

Cat. No.: E1521050 | Pack size: 50T | Storage: Room temperature

Overview

This kit employs a modified SDS-alkaline lysis method to lyse cells. The crude lysate is passed through a filter column to remove endotoxins. Subsequently, 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 removed using a protein removal solution and a wash buffer. Finally, the purified plasmid DNA is eluted from the silica membrane with a low-salt, high-pH elution buffer.

Product Features

1. The silica membrane in the centrifugal spin column is a specially imported adsorption membrane, ensuring minimal variation in adsorption capacity between columns and excellent reproducibility. This overcomes the instability issues associated with membrane quality in domestic kits.
2. The unique protein removal solution formula efficiently removes nucleases. Even nuclease-rich strains such as the JM series and HB101 are easily addressed, effectively preventing plasmid degradation by nucleases.
3. A unique endotoxin removal method provides effective endotoxin clearance, typically resulting in less than <0.1 EU/μg.

Kit Contents & Storage

Cat. No.	Component	50T	Storage
E1521050A	RNase A (powder)	1 EA	RT.
E1521050B	Solution P1	15 mL	RT.
E1521050C	Solution P2	15 mL	RT.
E1521050D	Solution P3	20 mL	RT.
E1521050E	Protein Removal Solution PD	15 mL	RT.
E1521050F	Wash Buffer WB	15 mL	RT.

Cat. No.	Component	50T	Storage
E1521050G	Filter Column E	1 EA×50	RT.
E1521050H	Elution Buffer EB	10 mL	RT.
E1521050I	Spin Column AC	1 EA×50	RT.
E1521050J	Collection Tube (2 mL)	1 EA×50	RT.

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

Precautions

1. For 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 contamination may appear in the extracted plasmid. This can be remedied by supplementing Solution P1 with additional RNase A.
2. When ambient temperature is low, SDS in Solution P2 may precipitate, causing cloudiness or sediment. Warm the solution in a 37°C water bath for a few minutes to restore clarity. Do not shake vigorously to avoid excessive foaming.
3. Avoid prolonged exposure of reagents to air to prevent volatilization, oxidation, and pH changes.
4. Solution P3 and Protein Removal Solution PD contain irritating compounds. Wear latex gloves during operation and avoid contact with skin, eyes, and clothing. In case of contact, rinse thoroughly with plenty of water or saline.
5. The amount of plasmid extracted 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. This can yield up to 20 μg of pure plasmid. For low-copy plasmids or large plasmids (>10 kb), increase the bacterial amount by using 5–10 mL of overnight culture and proportionally increase the volumes of P1, P2, and P3, while keeping other steps unchanged.
6. The concentration and purity of the extracted plasmid DNA can be assessed by agarose gel electrophoresis and UV spectrophotometry. An OD260 of 1 corresponds to approximately 50 μg/mL of DNA. Electrophoresis may reveal a single band, or two or more bands, which is primarily due to different migration positions of various supercoiled plasmid conformations and depends on factors like culture duration and the vigor of operation. Under normal operating conditions with this product, supercoiled plasmids typically exceed 90%.
7. To determine the exact molecular size of the plasmid DNA, it must be linearized by restriction enzyme digestion and then compared to a DNA molecular weight marker. Circular or supercoiled plasmids have variable migration positions, making it impossible to determine their exact size by electrophoresis alone.
8. For research use only. Not for clinical diagnosis or other uses.

Materials Required but Not Provided

Anhydrous ethanol.

Procedure

Notes:

- Before first use, add 60 mL of anhydrous ethanol to Wash Buffer WB. Mix thoroughly. Mark the bottle immediately after adding ethanol to avoid double addition.
- Pour the RNase A (powder) into Solution P1, using P1 to rinse the vial. Mix well. Store at 2–8°C after each use.

1. Centrifuge 1.5–4.5 mL of overnight bacterial culture at 12,000 rpm for 30 seconds. Remove the supernatant as completely as possible to collect the bacterial pellet.
2. Resuspend the bacterial pellet in 250 µL of Solution P1. Vortex until thoroughly suspended.
3. Add 250 µL of Solution P2. Gently invert the tube 4–7 times (lysis time should not exceed 5 minutes) to lyse the cells completely.
4. Add 350 µL of Solution P3, and immediately and gently invert the tube 4–7 times. A white flocculent precipitate should appear upon thorough mixing. Centrifuge at 12,000 rpm for 5 minutes.
5. Transfer the supernatant to a Filter Column E (placed in a 1.5 mL or 2 mL centrifuge tube). Centrifuge at 12,000 rpm for 2 minutes to collect the liquid. If the supernatant volume is large, centrifuge in two separate batches.
6. Transfer the collected liquid to a Spin Column AC (placed in a Collection Tube). Centrifuge at 12,000 rpm for 30–60 seconds. Discard the flow-through.
7. Add 500 µL of Protein Removal Solution PD. Centrifuge at 12,000 rpm for 30–60 seconds. Discard the flow-through.

This step removes trace nucleases and other impurities. It is necessary for nuclease-rich strains such as JM series, HB101 (endA+), or wild-type strains. For nuclease-deficient strains such as XL-1Blue and DH5α, this step can be omitted.

8. Add 500 µL of Wash Buffer WB (ensure that anhydrous ethanol has been added). Centrifuge at 12,000 rpm for 30–60 seconds. Discard the flow-through.
9. Repeat step 8.
10. Place the Spin Column AC back into the empty Collection Tube. Centrifuge at 12,000 rpm for 2 minutes to remove residual wash buffer, which could inhibit downstream reactions due to residual ethanol.
11. Transfer the Spin Column AC to a clean centrifuge tube. Allow it to stand at room temperature for a few minutes.
12. Add 50–100 µL of Elution Buffer EB (pre-heating the buffer in a 65–70°C water bath improves efficiency) to the center of the silica membrane. Let it stand at room temperature for 2 minutes.

Centrifuge at 12,000 rpm for 1 minute. To increase the yield, reload the eluate onto the column and centrifuge again for 1 minute. If using ddH_2O for elution, ensure its pH is within the range of 7.0–8.5; pH values below 7.0 reduce elution efficiency. The elution buffer volume should not be less than 30 µL, as a smaller volume affects recovery efficiency. The DNA product should be stored at – 20°C to prevent degradation.

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- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
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