

High Purity Rapid Plasmid Mini Kit

Cat. No.: H1521033 | Pack size: 100T | Storage: Room temperature

Overview

Product 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 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

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.

The unique protein removal solution formula efficiently removes residual nucleases, making it easy to purify plasmids even from nuclease-rich strains such as the JM series and HB101. This effectively prevents plasmid degradation by nucleases.

Fast and convenient; no need for toxic reagents like phenol or chloroform, nor for ethanol precipitation. The obtained plasmid DNA is of high yield and purity, suitable for various molecular biology experiments such as restriction enzyme digestion, transformation, PCR, in vitro transcription, and sequencing.

Contents & Storage

Cat. No.	Component	100T	Storage
H1521033A	RNase A (powder)	1 EA	RT
H1521033B	Solution P1	30 mL	RT
H1521033C	Solution P2	30 mL	RT
H1521033D	Solution P3	40 mL	RT
H1521033E	Protein Removal Solution PE	31.5 mL	RT
H1521033F	Wash Buffer WB	25 mL	RT

Cat. No.	Component	100T	Storage
H1521033G	Elution Buffer EB	10 mL	RT
H1521033H	Spin Column AC	1 EA × 100	RT
H1521033I	Collection Tube (2 mL)	1 EA × 100	RT

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

Materials Required

- Anhydrous ethanol.

Preparation Before Use

1. Before first use, add 100 mL of anhydrous ethanol to Wash Buffer WB and 18.5 mL of anhydrous ethanol to Protein Removal Solution PE. Mix thoroughly. Mark the bottles immediately after adding ethanol to avoid double addition.
2. Pour the RNase A (powder) into Solution P1, using P1 to rinse the vial. Mix well. Store at 2-8°C after each use.
3. Pre-cool Solution P3 on ice to improve yield.

Protocol (For Reference Only)

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 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. Carefully transfer the supernatant to a new tube. Mix immediately after adding Solution P3 to avoid localized SDS precipitation. If small white precipitates remain in the supernatant, centrifuge again and transfer the supernatant.
5. Transfer the supernatant from the previous step to a Spin Column AC (placed in a Collection Tube). Centrifuge at 12,000 rpm for 30-60 seconds. Discard the flow-through.
6. Add 500 µL of Protein Removal Solution PE. Centrifuge at 12,000 rpm for 30-60 seconds. Discard the flow-through.

Note: 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.

7. Add 500 μ L of Wash Buffer WB (ensure that anhydrous ethanol has been added). Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
8. Repeat step 7.
9. 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.
10. Transfer the Spin Column AC to a clean centrifuge tube. Allow it to stand at room temperature for a few minutes.
11. Add 50-100 μ L 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. A larger elution volume increases efficiency. For a higher concentration, reduce the elution volume, but do not use less than 30 μ L, as smaller volumes reduce elution efficiency and yield. If using ddH₂O for elution, ensure its pH is within the range of 7.0-8.5; pH values below 7.0 reduce elution efficiency.

Precautions

1. For first-time use, add the entire vial of RNase A (powder) to Solution P1 (final concentration 100 μ g/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. Solutions P3 and Protein Removal Solution PE 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 μ g 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 μ g/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. Elution Buffer EB does not contain the chelator EDTA and does not affect downstream reactions such as restriction enzyme digestion or ligation. Water can also be used for elution, but ensure the pH is >7.5, as lower pH reduces elution efficiency. Plasmids eluted with water should be stored at -20°C. For long-term storage, plasmids can be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). However, EDTA may affect downstream restriction enzyme reactions, so appropriate dilution is recommended when necessary.
8. For research use only. Not for clinical diagnosis or other uses.

Specifications

Attribute	Value
Storage Conditions	Room temperature
Shipped In	Normal
Stability And Storage	Store at room temperature long term (18 months).

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Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.

- 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.
- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
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