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Plasmid DNA Extraction Midi Kit Instructions

Composition

Plasmid DNA Midi Kit	25 Preps
Cat. No.	1010025
Filter	25
Spin Columns	25
RNase A	260 μl
Buffer I	130 ml
Buffer II	130 ml
Buffer N3	130 ml
Buffer W2 (concentrate)	80 ml×2
Buffer E	60 ml
Instructions	1

Storage

1. RNase A can be transported at room temperature. After receiving the product, please store at 2~8℃.
2. After adding RNase A, Buffer I should be stored at 2~8℃. If Buffer I is stored for more than 6 months, RNase A should be re-added.
3. Other reagents and components can be stored for up to 2 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2~8℃.(the product stored at 2~8℃ should be restored to room temperature before use).

Technical Support

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Introduction

This kit is developed by alkali lysis extraction plasmid extraction and column purification technology, which is suitable for extracting up to 500 μg plasmid DNA from 40~80 ml bacteria cultured (LB medium), and is suitable for molecular biology experiments such as sequencing, in vitro transcription and translation, restriction enzyme digestion, transformation, eukaryotic cell transfection, gene therapy, DNA vaccine, etc.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, isopropanol
2. 50 ml centrifuge tube, 2 ml centrifuge tube, pipettes and tips.
3. Disposable gloves, tissues and protective equipment.
4. Centrifuge (s)(with rotors for 50 ml centrifuge tubes and 2 ml centrifuge tubes).
5. Vortexer, water bath, thermostatic incubator.
6. 70% ethanol, 3 M sodium acetate (pH 5.2) may be required.

Preparation Before Use

1. If the centrifuge has refrigeration, set the temperature to 25℃.
2. Set the incubator to 37℃.
3. Add 1 ml Buffer I to the tube containing RNase A, mix well, then transfer the solution back into the bottle containing Buffer I. Mark "RNase A Added" on the box of the label and store at 2~8℃.
4. Add absolute ethanol to Buffer W2 according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".
5. When the room temperature is lower than 15℃, check Buffer II before use for salt precipitation. Redissolve any precipitate by warming to 37℃.

Protocol

1. **Collect 40~80 ml bacterial cultures cultured overnight with your own 50 ml centrifuge tube (if using rich medium, the bacterial volume should be halved or less) and put the centrifuge tube on a paper towel for 1 minute to remove the supernatant.**
2. **Add 5 ml Buffer I with RNase A to suspend the bacteria pellet.**

* Bacteria pellet can be suspended by vortexing or pipetting. The well-suspended bacteria are a homogeneous suspension and should not be left with visible small clumps, otherwise the yield of the final plasmid will be severely affected.

3. **Add 5 ml Buffer II, gently invert 6~8 times to mix well to allow the bacteria to fully lyse.**

* Before using Buffer II, make sure that no visible precipitate exists in the solution. After applying Buffer II, tighten the cap to avoid prolonged contact with air.
* This step should not be mixed by vortexing, as this will result in genomic DNA being mixed in the final prepared plasmid.
* When the bacteria are fully lysed, the lysate should be viscous and translucent. If the above effect is not achieved, it may be caused by too much bacteria amount, increased the invert times to fully lyse bacteria. Note: This step should not take more than 5 min.

4. **Add 5 ml Buffer N3, gently invert the centrifuge tube until all the remaining blue precipitate in the solution turns to a pale-yellow precipitate, pour all the neutralized products into the Filter, close the lid, and centrifuge ≥ 4,500 ×rpm for 2 min.**

* This step should not be mixed by vortexing, as this will result in genomic DNA being mixed in the final prepared plasmid.

5. **Discard the Filter, add 5 ml isopropanol to the filtrate, close the lid, and gently invert 10 times to mix well. Transfer the mixture into a Spin Column (place the Spin Column in a 50 ml centrifuge tube), close the lid, and centrifuge ≥ 4,500 × rpm for 2 min.**
6. **Discard the filtrate, place the Spin Column back into the 50 ml centrifuge tube. Add 10 ml Buffer W2 to the Spin Column, close the lid, and centrifuge ≥ 4,500 × rpm for 2 min.**

* Ensure that absolute ethanol has been added to Buffer W2.

7. **Repeat step 6 once.**
8. **Discard the filtrate, place the Spin Column back into the 50 ml centrifuge tube, open the lid, and centrifuge ≥ 4,500 × rpm for 15 min.**

* If the maximum speed of the centrifuge can reach more than 10,000 ×rpm, the centrifugation conditions can be changed to ≥ centrifugation at 10,000 ×rpm for 5 min.

9. **Discard the 50 ml centrifuge tube, place the Spin Column in a clean 50 ml centrifuge tube (not provided), open the lid, and incubate at 37℃for 10~15 min.**

* The purpose of this step is to remove the residual ethanol on the Spin Column, if the ethanol odor can still be smelled in the Spin Column after this step, the incubation time should be extended appropriately.

10. **Add 2 ml Buffer E to the Spin Column center, open the lid, incubate at room temperature for 5 min, and centrifuge ≥ 4,500 × rpm for 5 min.**

* Do not elute DNA with less than 2 ml Buffer E, as this will affect the elution efficiency of plasmid DNA.
* Plasmid DNA can also be eluted with deionized water, but the pH of deionized water should be ensured to be 7.0~8.5, otherwise the elution efficiency of plasmid DNA will be affected.

11. **Discard the Spin Column and store the plasmid DNA at -20℃ for later use. Or follow these steps for plasmid DNA concentration:**

Plasmid concentration steps:

1. **Aliquot the plasmid DNA into 2✕2 ml centrifuge tubes, add 0.1 DNA solution volume of 3 M sodium acetate (pH 5.2) and 0.8 DNA solution volume of isopropanol to each centrifuge tube, mix well, and centrifuge at 13,000 rpm for 10 min.**
2. **Discard the supernatant and retain the DNA pellet at the bottom of the tube. Add 1.5 ml 70% ethanol to each tube, vortex to suspend the pellet, and centrifuge at 13,000 rpm for 5 min.**

* Be careful not to discard the DNA pellet from the bottom of the tube.

3. **Discard the supernatant, return the tube to the centrifuge, spin down the solution on the**

tube wall, discard the residual solution with a 200 µl tip, do not discard the pellet, open the lid, and dry the DNA pellet at room temperature on a clean bench for 10 min.

4. **Add 100 µl Buffer E or deionized pure water to each tube, vortex to fully dissolve the plasmid DNA pellet and store the plasmid DNA at -20℃ for later use.**

* If the copy number of plasmid DNA is low, reduce the volume of Buffer E added to increase the concentration of plasmid DNA.