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One-Step Plasmid DNA Extraction Mini Kit Instructions

Composition

One-Step Plasmid DNA Extraction Mini Kit	50 Preps	250 Preps
Cat. No.	1002050	1002250
Spin Columns	50	250
2 ml Collection Tubes	50	250
Lyase	180 mg	300 mg×3
Lyase Dissolve Solution	900 µl	1.5 ml×3
Buffer FL	36 ml	60 ml×3
Buffer FW2	36 ml	180 ml
Buffer E	6 ml	30 ml
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Storage

1. Lyase and Lyase Dissolve Solution can be transported at room temperature. Please store at 2~8℃ after receiving. Other reagents and items can be stored within 2 years at room temperature (0~30℃).
2. After Lyase is dissolved, it should be stored at 2~8℃ whether it is added to Buffer FL. It can be stably stored for 4 months. Buffer FL added with lyase should also be stored at 2~8℃, which will not affect the lysing effect within 4 months.

Introduction

This kit uses a novel one-step enzyme lysis technology to rapidly purify high-quality plasmid DNA from 1~5 ml overnight culture solution in 6 min. The unique buffer formula combines the three steps of bacterial resuspension, cleavage and neutralization in SDS alkaline lysis method into one step and is combined with advanced silica gel membrane adsorption technology, which can efficiently and specifically bind plasmid DNA, and effectively remove impurities such as protein and genomic DNA. The high-quality plasmid DNA obtained by elution can be directly used in molecular biology experiments such as enzyme digestion, PCR, sequencing, and transformation.

Equipment and Reagents to Be Supplied by User

1. 1.5 ml and 2 ml centrifuge tubes.
2. Pipettes and tips.
3. Disposable gloves and protective supplies and paper towels.
4. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
5. Vortexer.

Preparation Before Use

Add the Lyase Dissolve Solution to the Lyase, invert to dissolve it, then transfer it to Buffer FL, and tick the box on the label to mark "Lyase Added". After mixing, store the solution at 2~8℃.

After being stored at 2~8℃ for 4 months, the performance of lysate cells of Buffer FL after being added with lyase maybe reduced, and it can only be used normally after being re-added with Lyase.

Note

1. This kit is suitable for End A$^-$ host bacteria. Due to the presence of endogenous nuclease in end A+ host bacteria, enzyme digestion will lead to the degradation of purified plasmids. It is recommended to use the Plasmid DNA Extraction Mini Kit (Cat. No.1001050) to extract plasmids.
2. If the extracted plasmids are low-copy plasmids or plasmids larger than 10 kb, Long Fragment DNA Selection Kit (200 bp~10 kb) (Cat. No.2105050) is recommended for better extraction.

Protocol

1. **Please put Buffer FL (please check whether it has been added with Lyase) on ice, incubate to 0~4℃ before use.**

* Ensure that Buffer FL with lyase added is used at a low temperature, otherwise it will affect the subsequent lysis effect, resulting in low yield and purity.

2. **Collect 1~5 ml overnight bacteria cultured, transfer it to a 2 ml centrifuge tube (not provided), centrifuge at 12000 rpm for 1 min, discard the medium (if there is a lot of bacteria cultured, the bacterial pellet can be collected into a centrifuge tube through multiple centrifugations).**

* Do not use 1.5 ml centrifuge tube to collect bacteria. Bacteria pellet at the bottom of 1.5 ml centrifuge tube will be difficult to suspend in step 3, resulting in many small bacteria that are difficult to disperse.

3. **Add 600 µl Buffer FL with Lyase added, immediately vortex for 30 sec, completely resuspend the bacteria pellet, and incubate at room temperature (15-25℃) for 3 min. After using Buffer FL, please return it to 2~8℃ in time for storage.**

* After adding Buffer FL, immediately vortex to suspended bacteria pellet until no bacteria clumps are visible, thereby increasing yield and avoiding genomic DNA contamination.
* If the bacteria are not suspended immediately after adding Buffer FL (the effect is equivalent to adding uncooled Buffer FL), the surface of the bacteria pellet will be dissolved first, which will affect the full suspension of the bacteria pellet, resulting in many small bacteria clumps that are difficult to disperse.
* If the solution is slightly turbidity and non-viscous, it is normal and will not affect the subsequent experiment.

4. **Transfer all the solutions from step 3 to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), centrifuge at 12000 rpm for 30 sec and discard the filtrate.**
5. **Place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer FW2, centrifuge at 12000 rpm for 30 sec, discard the filtrate.**
6. **Place the Spin Column back into the 2 ml Collection Tube and centrifuged at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* The high speed of this step is to remove the remaining liquid, do not omit, otherwise there may be inhibitors in the purified plasmid, which may affect the subsequent experimental effect.

7. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube (not provided), add 50~100 µl Buffer E to the Spin Column center, and centrifuged at 12000 rpm for 30 sec.**
8. **Discard the Spin Column, the eluted plasmid DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**