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Endo-free Plasmid DNA Extraction Double Mini Kit Instructions

Composition

Endo-free Plasmid DNA Extraction Double Mini Kit Cat. No.	4 Preps 1006004	50 Preps 1006050
Spin Columns	4	50
2 ml Collection Tubes	4	50
RNase A	*	56 μl
Buffer I	2.1 ml	28 ml
Buffer II	2.1 ml	28 ml
Buffer N3	1.5 ml	16 ml
Buffer ETR	1.5 ml	16 ml
Buffer W1	3 ml	28 ml
Buffer W2 (concentrate)	3 ml	24 ml
Buffer E	1 ml	12 ml
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*4 Preps of RNase A has been added into Buffer I.

Storage

1. The RNase A and Buffer ETR can be transported at room temperature. Please store the RNase A and Buffer ETR at 2~8℃ after receiving the products.
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 two 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 developed by alkaline lysis extraction plasmid and column purification of nucleic acid. It is suitable for extracting up to 50 μg endotoxin-free high-purity plasmid DNA from 5~15 ml of bacterial culture (LB medium), especially suitable for molecular biology experiments such as eukaryotic cell transfection, in vitro transcription, and translation.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. Endotoxin-free, clean 1.5 ml and 2 ml centrifuge tubes, pipettes, and tips.
3. Protective equipment such as disposable latex gloves and paper towels.
4. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
5. Vortexer.

Preparation Before Use

1. If the centrifuge has a refrigeration function, set the temperature to 25℃.
2. Prepare an ice bath and a water bath for 42℃.
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 of 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℃.

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Protocol

1. **Collect 5-15 ml of overnight cultured bacteria in a 2 ml centrifuge tube (not provided) by centrifuging at 12000 rpm for 30 sec, discard the medium. Add 500 µl Buffer I (RNase A added) to fully suspend the bacterial pellet.**

* The bacterial pellet can be suspended by vortexer or pipette several times. Fully suspended with no visible small bacterial clumps left, otherwise it will seriously affect the final plasmid yield.

2. **Add 500 µl Buffer II and gently invert the tube 4-6 times to mix thoroughly.**

* Before using Buffer II, ensure that there is no salt precipitation in the solution; After using Buffer II, the bottle should be closed tightly to avoid long-term contact with air.
* This step CANNOT be mixed with a vortexer, otherwise genomic DNA will be mixed in the final prepared plasmid.
* When the bacteria have lysed sufficiently, the solution should be thick and translucent; If the above effect is not achieved, it may be too many bacteria used, and can increase the invert number to achieve the fully bacterial lysing.
* This step should NOT take more than 5 min.

3. **Add 250 µl Buffer N3 and gently invert the tube to mix thoroughly until all remaining blue precipitates disappear and change to a pale-yellow precipitate.**
4. **Centrifuge at 13000 rpm for 10 min.**
5. **Transfer the supernatant from Step 4 to a clean 1.5 ml centrifuge tube (not provided), add 250 µl Buffer ETR, mix thoroughly, and incubate in an ice bath for 10 min to make the mixture transparent.**
6. **Place the tube to a water bath incubate at 42°C for 5 min to give the mixture a cloudy appearance.**
7. **Centrifuge at 13000 rpm for 3 min and endotoxins will collect in the pale-yellow lower phase at the bottom of the tube.**
8. **Transfer the supernatant (about 1.2 ml) from step 7 to a clean 2 ml centrifuge tube (not provided), add 600 µl absolute ethanol, and mix well.**

* It is better to transfer less supernatant than to bring pale-yellow in the lower phase with endotoxin at the bottom.

9. **Transfer 600 µl mixture in step 8 to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid and centrifuge at 12000 rpm for 30 sec. Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.**
10. **Repeat Step 9 twice to filter all the mixture from step 8 through the Spin Column.**
11. **Add 500 µl Buffer W1 to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**
12. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 700 µl Buffer W2 to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

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

13. **Repeat step 12 once.**
14. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube and centrifuge at 14000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* This step is to remove the residual ethanol on the Spin Column. Please do not omit it, otherwise, the subsequent experimental effect may be affected by the residual ethanol in the extracted plasmid.

15. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 100-200 µl Buffer E or endotoxin-free ultra-pure water in the Spin Column membrane, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**
16. **Discard the Spin Column, the eluted plasmid DNA could be immediately used in various molecular biology experiments or stored at -20°C for later use.**