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Ultra-pure Plasmid DNA Extraction Mini Kit

Composition

Ultra-pure Plasmid DNA Extraction Mini Kit Cat. No.	5 Preps 1019005	50 Preps 1019050	250 Preps 1019250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
RNase A	*	28 μl	140 μl
Alkaline Proteinase	60 μl	0.6 ml	1.2 ml
Buffer I	1.5 ml	14 ml	70 ml
Buffer II	1.5 ml	14 ml	70 ml
Buffer III	2 ml	20 ml	100 ml
Buffer W1	3 ml	28 ml	130 ml
Buffer W2 (concentrate)	2 ml	16 ml	100 ml
Buffer E	0.6 ml	6 ml	25 ml
Instructions	1	1	1

* 5 Preps of RNase A has been added into Buffer I.

Storage

1. Alkaline Proteinase can be transported at room temperature. After receiving the product, please store at -20℃.
2. RNase A can be transported at room temperature. After receiving the product, please store at 2~8℃.
3. 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.
4. 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

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This kit combines the principle of extracting plasmid by alkaline lysis and column purification of nucleic acid technology, which is suitable for extracting up to 50 μg of high purity plasmid DNA from 1~5 ml of various wild type strains. This product is equipped with a special alkaline Proteinase, which can efficiently hydrolyze and remove nuclease, so that the obtained plasmid is more pure and more stable. It is suitable for molecular biology experiments such as sequencing, in vitro transcription and translation, restriction enzyme digestion, and bacteria transformation.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 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. 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℃.
3. 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".
4. 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 1-5 ml of overnight cultured bacteria in a 1.5 ml centrifuge tube (not provided) by centrifuging at 12000 rpm for 30 sec, discard the medium. Add 250 µl of Buffer I (RNase A added) to fully suspend the bacteria pellet.**

* The bacteria pellet can be suspended by vortex or pipette several times. Fully suspended with no visible small bacteria clumps left, otherwise it will seriously affect the final plasmid DNA yield.
* To extract plasmids from Gram-positive bacteria, add 20 µl of lysozyme solution with a concentration of 100 mg/ml at the end of this step, vortex to mix, and incubate at 37℃ for 10-30 min to lyse the bacteria cell wall.

2. **Add 250 µl Buffer II and 10 µl Alkaline Proteinase, gently invert the tube 4-6 times and incubate at room temperature for 5 min.**

* 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.
* Do not mix this step by vortexing, otherwise genomic DNA will be mixed in the final prepared plasmid DNA.
* When the bacteria have lysed sufficiently, the lysate should be thick and translucent; If the lysate does not reach the translucent effect, it may be too many bacteria were used, and the number of inverts must be increased to achieve the effect of full bacteria lysing.

3. **Add 350 µl Buffer III and gently invert the tube until all trace of blue disappears and a yellow precipitate is formed.**

* This step should not be mixed by vortexing, otherwise, genomic DNA will be mixed in the final prepared plasmid.
* When the action of this step is sufficient, there should be a loose yellow flocculent precipitate. If the sediment appears thick, it may be too many bacteria were used, and the number of inverts can be increased to make the precipitation loose.

4. **Centrifuge at 13000 rpm for 10 min.**
5. **Place a Spin Column in a 2 ml Collection Tube, decanting the supernatant from step 4 into the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**
6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer W1 to the Spin Column, close the lid, centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded. To avoid contamination of the centrifuge by the filtrate adhering to the mouth of the Collection Tube, invert the 2 ml Collection Tube and slap once on a paper towel.

7. **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.

8. **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 14000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise, it may cause problems in subsequent experiments due to the residual ethanol in the purified plasmid.

9. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube. Add 50-100 µl Buffer E to the Spin Column membrane, close the lid, incubate at room temperature for 1 min and centrifuged at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cover, change the centrifuge condition to 8000 rpm for 1 min, to avoid damage to the centrifuge by the 1.5 ml centrifuge tube cap falling off.
* DNA can also be eluted with deionized water but ensure that the pH of the deionized water is 7.0~8.5, otherwise it will affect the efficiency of DNA elution.

10. **Discard the Spin Column, the eluted plasmid DNA can be immediately used in various molecular biology experiments or store at -20℃ for later use.**