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Plasmid DNA Extraction Double Mini Kit Instructions

Composition

Plasmid DNA Extraction Double Mini Kit Cat. No.	4 Preps 1007004	50 Preps 1007050
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 III	3 ml	40 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. 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 more suitable for extracting plasmid DNA from 5~15 ml of overnight bacteria cultured (LB medium), and there is no restriction on the genotype of the plasmid host bacteria. Extract up to 50 µg high-purity plasmid DNA in 20~30 min. Specially designed Buffer W1 washes away very small amounts of endonuclease residue from the Spin Column. The obtained plasmid DNA is suitable for molecular biology experiments such as restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro transcription and translation, and strong cell transfection.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 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. 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 5~15 ml overnight cultured bacteria in a 2 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.

* Recommended: Collect more than 10 ml bacterial(except rich culture medium such as 2×YT) to increase the recovery of the final plasmid DNA. Collect bacteria in 15 ml or 50 ml centrifuge tubes, then suspend bacteria in buffer I and transfer to 2 ml centrifuge tube.
* Please use a 2 ml centrifuge tube. The volume of the 1.5 ml centrifuge tube is too small to perform the follow steps.
* The pellet could be suspended completely by vortexing or pipetting until no cell clumps left. Otherwise, it would seriously affect the final plasmid DNA yield.

2. Add 500 µl Buffer II and mix thoroughly by gently inverting the tube 4~6 times.

* 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.
* Do not allow the lysis reaction to proceed for more than 5 min.
* Do not vortex, as this will result in shearing of genomic DNA.
* The lysate should be viscous and slightly clear when the bacterial is lysed sufficiently. If too many bacteria were used, continue inverting the tube until the solution becomes viscous and slightly clear.
* Do not allow this step to proceed for more than 5 min.

3. Add 700 µl Buffer III, mix gently and thoroughly by inverting the tube until the color of precipitate changes from blue to light yellow completely.

* 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 full speed (≥12,000 rpm) for 10 min.

5. Transfer 800 µl supernatant from step 4 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, centrifuge at 12,000 rpm for 30 sec.

* Transfer the supernatant into the Spin Column immediately after centrifugation, stay for a long time may cause the precipitate to float, which makes it hard to operate in following procedure.
* If precipitate was suspended in the supernatant, repeat step 4 once again.

6. Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube, transfer remaining supernatant into the Spin Column, close the lid, centrifuge at 12,000 rpm for 30 sec.

7. Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube. Add 500 µl Buffer W1 to the Spin Column. Close the lid, centrifuge at 12,000 rpm for 30 sec.

8. Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube. Add 700 µl Buffer W2 to the Spin Column. Closed the lid, centrifuge at 12,000 rpm for 30 sec.

* Ensure ethanol has been added into Buffer W2.

9. Repeat step 8 once.

10. Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube. Centrifuge at full speed (≥ 12,000 rpm) for 1 min.

* Do not omit this step, otherwise, it may cause problems in downstream applications due to the residual ethanol in the eluate.

11. Discard the 2 ml Collection Tube, place the Spin Column in a new 1.5 ml centrifuge tube. Add 60~200 µl Buffer E to the Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12,000 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.
* Plasmid 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.

12. Discard the Spin Column. The eluted plasmid DNA can be used for a variety of molecular biology experiments immediately or store at -20℃ for later use.