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Endo-free Plasmid DNA Extraction Maxi Kit Instructions

Composition

Endo-free Plasmid DNA Extraction Maxi Kit Cat. No.	2 Preps 1017002	25 Preps 1017025
Filter and Plunger	2	25
Spin Columns	2	25
RNase A	56 μl	520 μl
Buffer I	28 ml	260 ml
Buffer II	28 ml	260 ml
Buffer N3	15 ml	130 ml
Buffer ETR	15 ml	130 ml
Buffer W2 (concentrate)	15 ml	80 ml×2
Buffer E	10 ml	90 ml
Instructions	1	1

Storage

1. 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 is developed by alkaline lysis extraction plasmid and nucleic acid column purification technology. It is suitable for extracting up to 1.5 mg of plasmid DNA from 120~250 ml bacteria cultured (LB medium). It is suitable for molecular biology experiments such as sequencing, in vitro transcription and translation, digestion by restriction endonuclease, transformation, and eukaryotic transfection.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, isopropanol.
2. 50 ml centrifuge tube, pipettes, and tips.
3. Protective equipment such as disposable latex gloves and paper towels.
4. Centrifuge(s) (with rotor for 50 ml centrifuge tubes).
5. Vortexer, ice water bath, water bath, incubator.

Preparation Before Use

1. If the centrifuge has a refrigeration function, set the temperature to 30℃.
2. Set the water bath to 42℃ and the incubator to 37℃.
3. Add 1 ml Buffer I to the tube containing RNase A, mix well and 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 of 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 150~250 ml of overnight cultured bacteria in a 50 ml centrifuge tube (if rich medium is used, the bacteria cultured volume should be halved or less), discard the medium, place the centrifuge tube upside down on a paper towel for 1 min.**
2. **Add 10 ml Buffer I (RNase A added) to fully suspend the bacteria pellet.**

* The bacteria pellet can be suspended by vortexing or pipette several times. Fully suspended bacteria without no visible small bacteria clumps, otherwise it will seriously affect the final plasmid yield.

3. **Add 10 ml Buffer II, gently invert 6~8 times to mix evenly and lyse the bacteria.**

* Ensure that no salt precipitation in the solution before using Buffer II; Cap the bottle tightly after using Buffer II 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 due to the excessive number of bacteria and can increase the invert number to lyse sufficiently. Note that this step should not take more than 5 min.

4. **Add 5 ml Buffer N3 and gently invert the tube until all remaining blue precipitates in the solution have changed to pale-yellow precipitates. Centrifuge ≥12000×g for 5 min.**

* This step should NOT be mixed with a vortexer; otherwise, genomic DNA will be mixed in the final prepared plasmid.

5. **Place the Filter into a clean 50 ml centrifuge tube (not provided), draw out the Plunger, pour all the supernatant from step 4 into the filter, insert and gently push the Plunger so that all the filtrate drops into the 50 ml centrifuge tube.**

* The supernatant may drip directly after being added to the filter, be careful to always keep the filter in the 50 ml centrifuge tube.
* The supernatant poured into the filter mixed with some yellow precipitate will not affect the filtration effect.

6. **Add 5 ml Buffer ETR to the filtrate, cover tightly, invert to mix, incubate at ice bath for 10-15 min. Invert mixing several times during the ice bath to make the mixture transparent.**
7. **Incubate at 42℃ for 5 min, so that the mixture becomes turbid, ≥12000×g centrifuge for 10 min.**

* The centrifuge temperature must be set at 30℃, and if it is not found that the phase separation, it can be centrifuged again for 15 min.
* If a small yellow liquid drop is found suspended in the supernatant when the tube is removed at the end of centrifugation, it may be brought up by the fluctuation when the centrifuge stops rotating, and it can be left for about 1 min.

8. **Transfer the supernatant to a clean 50 ml centrifuge tube (not provided) without the yellow deposits containing endotoxins at the bottom.**
9. **Add 14 ml isopropanol to the supernatant, close the lid tightly, and gently invert 10 times to mix well. Transfer 20 ml of the mixture into a Spin Column, cover the lid, and centrifuge ≥12000×g for 1 min.**
10. **Discard the filtrate, place the Spin Column back into the 50 ml collection tube, transfer all the remaining mixture to the Spin Column, close the lid, centrifuge ≥12000×g for 1 min.**
11. **Discard the filtrate, place the Spin Column back into the 50 ml collection tube. Add 10 ml Buffer W2 to the Spin Column, close the lid and centrifuge ≥12000×g for 1 min.**

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

12. **Repeat Step 11 once.**
13. **Discard the filtrate, place the Spin Column back into the 50 ml collection tube, and centrifuge ≥12000×g for 5 min.**
14. **Discard the 50 ml collection tube and place the Spin Column in a clean 50 ml centrifuge tube (not provided). Open the lid and incubate at 37℃ for 10-15 min.**

* This step is to remove the residual ethanol on the Spin Column. If the taste of ethanol can still be smelled in the Spin Column after this step is finished, the incubate time should be extended appropriately.

15. **Add 2 ml Buffer E into the Spin Column membrane, open the lid, stand for 5 min at room temperature, close the lid and centrifuge at full speed for 5 min.**

* Plasmid DNA can also be eluted with deionized water but ensure that the pH of the water is ＞7.0, otherwise it will affect the efficiency of plasmid DNA elution.
* If the concentration of the eluted DNA is more than 200 ng/μl, it is recommended to elute the Spin column once more to improve DNA recovery: transfer the DNA solution from step 15 to a container, place the Spin Column back into the 50 ml centrifuge tube, add 1 ml Buffer E to the Spin Column membrane and stand for 5 min. Close the lid and centrifuge at ≥12,000×g for 5 min.

16. **Discard the Spin Column, store the elute plasmid DNA at -20℃ for later use.**