Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

Endo-free Plasmid DNA Extraction Midi Kit Instructions

Composition

Endo-free Plasmid DNA Extraction Midi Kit Cat No	25 Preps 1016025
Filters	25
Spin Columns	25
RNase A	260 μl
Buffer I	130 ml
Buffer II	130 ml
Buffer N3	65 ml
Buffer ETR	65 ml
Buffer W2 (concentrate)	80 ml×2
Buffer E	60 ml
Instructions	1

Storage

1. RNase A and Buffer ETR can be transported at room temperature, please store at 2~8℃ after receiving the product.
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

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

Introduction

This kit developed by alkaline lysis extraction plasmid and column purification of nucleic acid. It is suitable for extracting up to 500 μg of endotoxin-removed plasmid DNA from 40~80 ml bacteria cultured (LB medium) for molecular biology experiments such as sequencing, in vitro transcription and translation, restriction endonuclease digestion, transformation, eukaryotic cell transfection, gene therapy, DNA vaccine and so on.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, isopropanol.
2. Endo-free 50 ml centrifuge tubes, pipettes, and tips.
3. Protective equipment such as latex gloves, disposable masks, and paper towels.
4. Centrifuge(s) (with rotor for 50 ml centrifuge tubes and centrifugal force≥12000×g).
5. Vortexer, water bath, incubator.
6. May need 3 M NaAc (pH 5.2), 70% ethanol.

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 (42℃). Set 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 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℃.

Protocol

1. **Collect 40~80 ml 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 5 ml Buffer I (RNase A added) to fully suspend the bacteria pellet.**

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

3. **Add 5 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 increased the invert number to lyse sufficiently. Note that this step should not take more than 5 min.

4. **Add 2.5 ml Buffer N3, gently invert the tube until all remaining blue precipitates in the solution have changed to pale-yellow precipitates. decanting all the supernatant into a Filter, close the lid, and centrifuge at ≥5000×rpm for 2 min.**

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

5. **Discard the Filter column, add 2.5 ml Buffer ETR into the filtrate, close the lid, invert to mix, and incubate at ice bath for 10~15 min. Invert to mix several times during the ice bath to make the mixture transparent.**
6. **Incubate at 42℃ for 5 min, so that the mixture becomes turbid, centrifuge at ≥12000×g for 10 min.**

* Centrifuge temperature must above 25℃, if the phase cannot be effectively separated, set the temperature to 30℃, centrifuge for 15 min.
* If a small yellow liquid drop is found suspended in the supernatant 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.

7. **Transfer the supernatant to a clean 50 ml centrifuge tube (not provided), do not transfer the yellow precipitant containing endotoxin at the bottom.**
8. **Add 7 ml isopropanol to the collected supernatant, close the lid, and gently invert 10 times to mix well. Transfer the mixture into a Spin Column, close the lid and centrifuge at ≥12000×g for 1 min.**
9. **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 at ≥12000×g for 1 min.**

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

10. **Repeat step 9 once.**
11. **Discard the filtrate, place the Spin Column back into the 50 ml collection tube, and centrifuge at ≥12000×g for 5 min.**
12. **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 over, the incubate time can be extended appropriately.

13. **Add 1-2 ml Buffer E to the membrane center of the Spin Column, open the lid, stand for 5 min at room temperature, close the lid and centrifuge at ≥12000×g for 5 min.**

* Plasmid DNA can also be eluted with pure water and ensure that the pH of the water is 7.0-8.5, 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 13 to a container, place the Spin Column back into the 50 ml centrifuge tube, add 1 ml Buffer E to the Spin Column membrane. Open the lid, stand for 5 min. Close the lid and centrifuge at ≥12000×g for 5 min.

14. **Discard the Spin Column, and the elute plasmid DNA can store at -20℃ for later use. Or follow the following steps to concentrate.**

Plasmid Concentration Protocol

1. **The eluted plasmid DNA was dispensed into 2 ml microcentrifuge tubes, and 0.1 DNA solution volume of 3 M NaAc (pH 5.2) and 0.8 DNA solution volume of isopropanol were added to each tube, mixed well, and centrifuged at 13000 rpm for 10 min.**
2. **Discard the supernatant and keep the DNA precipitate at the bottom of the tube. Add 1.5 ml 70% ethanol to each tube, vortex to suspend the precipitation, centrifuge at 13000 rpm for 5 min.**

* Be careful not to discard the DNA precipitate at the bottom of the tube.

3. **Discard the supernatant, spin down the solution, and use a 200 μl pipette to absorb the residual liquid at the bottom of the tube. Do not absorb the precipitation, open the lid, stand at room temperature for 10 min.**
4. **Add 100 μl Buffer E or Endo-free water to each tube, vortex to fully dissolve plasmid DNA precipitation, and store plasmid DNA at -20℃ for later use.**

* If the copy number of plasmid DNA is low, the volume of Buffer E added can be reduced to increase the concentration of plasmid DNA.