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BAC/PAC DNA Midi Kit Instructions

Composition

BAC/PAC DNA Midi Kit Cat. No.	2 Preps 1013002	20 Preps 1013020
RNase A	28 μl	260 μl
Buffer I	14 ml	130 ml
Buffer II	11 ml	110 ml
Buffer N3	11 ml	110 ml
Buffer A	600 μl	6 ml
Buffer ETR	1 ml	10 ml
Buffer B	4 ml	40 ml
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Storage

1. RNase A and Buffer ETR can be transported at room temperature. After receiving the product, please store RNase A and Buffer ETR 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 uses alkaline lysis method to extract large fragments of plasmid DNA, which is suitable for extracting large fragments of plasmid DNA from 60~120 ml bacterial culture (LB medium). The specially designed endotoxin removal step ensures that the obtained plasmid DNA is suitable for molecular biology experiments such as sequencing, in vitro transcription and translation, restriction enzyme digestion, transformation, eukaryotic cell transfection, gene therapy, DNA vaccines, etc.

Equipment and Reagents to Be Supplied by User

1. Isopropanol, 70% ethanol, TE buffer.
2. 50 ml centrifuge tube, 2 ml centrifuge tube, pipettes and tips.
3. Disposable gloves, tissues and protective equipment.
4. Centrifuge (s)(with rotors for 50 ml centrifuge tubes and 2 ml centrifuge tubes).
5. Vortexer, ice bath, water bath, thermostatic incubator.

Preparation Before Use

1. Set the water bath to 42℃ (if you need to do the endotoxin step).
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. Cut off a portion of the 1 ml tip about 1 cm in length of the head for aspiration or transfer of large fragments of plasmid DNA solution.
4. 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 60~120 ml bacteria cultured overnight with a 50 ml centrifuge tube (not provided) (if using rich medium, the bacterial volume should be halved or less), and place the centrifuge tube on a paper towel for 1 min to remove the supernatant.**
2. **Add 5 ml Buffer I with RNase A to suspend the bacteria pellet.**

* Bacteria pellet can be suspended by vortexing or pipetting. The well-suspended bacteria are a homogeneous suspension and should not be left with visible small clumps, otherwise the yield of the final plasmid will be severely affected.

3. **Add 5 ml Buffer II, gently invert 6~8 times to mix well to allow the bacteria to fully lyse.**

* 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.
* This step should not be mixed by vortexing, as this will result in genomic DNA being mixed in the final prepared plasmid.
* When the bacteria are fully lysed, the lysate should be viscous and translucent. If the above effect is not achieved, it may be caused by too much bacteria amount, increased the invert times to fully lyse bacteria. Note: This step should not take more than 5 min.

4. **Add 5 ml Buffer N3, gently invert the centrifuge tube until all the remaining blue precipitate in the solution turns to a pale-yellow precipitate, centrifuge at 4℃, ≥ 12,000 ×g for 15 min.**

* This step should not be mixed with a vortex shaker, as this will result in genomic DNA being mixed in the final prepared plasmid.

5. **Carefully transfer the supernatant to a clean 50 ml centrifuge tube, add 10 ml isopropanol, close the lid, gently invert 10 times to mix well, and centrifuge at 4℃, ≥ 12,000×g for 10 min.**
6. **Discard the supernatant in the centrifuge tube and place the 50 ml centrifuge tube upside down on a paper towel for about 1 min to blot up the residual liquid.**
7. **Add 5 ml 70% ethanol to the 50 ml centrifuge tube, close the lid, gently invert the centrifuge tube 4~6 times, and centrifuge at 4℃, ≥ 12,000×g for 5 min.**
8. **Discard the supernatant in the 50 ml centrifuge tube, open the lid and incubate for 10~15 min at room temperature to dry the DNA pellet.**

* If any residual supernatant collects at the bottom of the tube during standing, discard it with a 200 µl pipette and do not aspirate the pellet.

9. **Add 1.4 ml Buffer I and gently pipette the DNA pellet on the bottom and the wall of the tube with a 1 ml tip with the head cut off until the pellet is completely dissolved, then divide the DNA solution evenly into two 2 ml centrifuge tubes (700 µl each) and let stand for 5 min at room temperature.**

* Large fragments of plasmid DNA are easy to be shearing and breaking, and the DNA pellet must be gently pipetted with a 1 ml tip with the head cut off (about 1 cm in length) and should not be dissolved by vortex shaking.
* High-purity DNA may not form a visible white precipitate but will be deposited on the wall the tube in the form of a mist precipitate, please pay special attention to the wall the tube on the side where the pellet is formed.

10. **Add 100 µl Buffer A to each tube and gently invert the tube to mix well.**

* If endotoxin removal is not required, skip to step 14 after this step is completed. When Buffer A is added, the solution turns yellow.

11. **Add 150 µl Buffer ETR to each tube, gently invert the tube 7~10 times to mix well, and take an ice bath for 10 min to make the solution transparent.**
12. **Incubate at 42℃ for 5 min to make the solution cloudy. Centrifuge ≥ 12,000 ×g for 3 min at room temperature.**

* If the centrifuge has a refrigeration function, please set the temperature to 30℃, otherwise it may cause the phase splitting to be unsuccessful.

13. **Carefully transfer the supernatant to two clean 2 ml centrifuge tubes (approx. 0.8 ml each).**

* The supernatant must be transferred with a 1 ml tip with the head cut off (about 1 cm in length) without transferring the lower phase, as endotoxins collect in the yellowish lower phase at the bottom of the centrifuge tube.

14. **Add 0.8 ml Buffer B, gently invert to mix well, and centrifuge ≥ 14,000×g for 10 min.**
15. **Discard the supernatant and retain the DNA pellet at the bottom of the tube. Add 1.5 ml 70% ethanol to each tube and centrifuge at 13,000 rpm for 5 min.**

* Be careful not to discard the DNA pellet from the bottom of the tube.

16. **Repeat step 15 once.**
17. **Discard the supernatant, return the centrifuge tube to the centrifuge, spin down the solution, discard the residual solution with a 200 µl pipette tip, do not discard to the pellet, open the lid, and dry the DNA pellet at room temperature on a clean bench for 10 min.**
18. **Add 50~100 µl TE buffer or deionized pure water to each tube, flick the centrifuge tube to dissolve the DNA pellet, and store the DNA at -20℃ for later use.**

* Large fragments of plasmid DNA are easy to be sheared and broken, and cannot be dissolved by vortexing, which can be shake at 37℃ and placed at 200 rpm for 10 min to help dissolve.
* If the copy number of DNA is low, reduce the volume of TE buffer added to increase the concentration of plasmid DNA.