SIMGEN
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

Plasmid DNA Extraction Maxi Kit Instructions

Composition

Plasmid DNA Extraction Maxi Kit Cat. No.	2 Preps 1020002	25 Preps 1020025
Filters and Pistons	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	28 ml	260 ml
Buffer W2 (concentrate)	15 ml	80 ml×2
Buffer E	10 ml	90 ml
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Storage

1. The RNase A can be transported at room temperature. Please store the RNase A at 2~8°C after receiving the products.
2. Buffer I added to RNaseA should be stored at 2~8°C. If Buffer I is stored for more than 6 months, RNase A should be added again.
3. Other reagents and items can be stored within 2 years at room temperature (0~30°C). For longer storage, it is recommended to keep at 2~8°C.(the product stored at 2~8°C 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 is developed by alkaline lysis extraction of plasmid and column purification technology, which is suitable for extracting up to 1.5 mg of plasmid DNA (1.5 mg is the saturation adsorption value of the Spin Column, the actual extraction amount is determined by the amount of bacteria and the copy number of the plasmid) from 120~250 ml bacteria cultured (LB medium), and is suitable for sequencing, in vitro transcription and translation, restriction enzyme digestion, transformation, Molecular biology experiments such as eukaryotic cell transfection.

Users need to bring their own reagents and supplies

1. Absolute ethanol, isopropanol.
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, water bath, thermostatic incubator.
6. 70% ethanol, 3 M sodium acetate (pH 5.2) may be required.

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25°C.
2. Set the thermostatic incubator to 37°C.
3. Add 1 ml Buffer I to the tube containing RNaseA, mix well and then transfer the solution back into the bottle containing Buffer I. Mark "RNaseA Added" on the box of the label and store at 2~8°C.
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°C, the reagent should be observed for white precipitation before using Buffer II. If there is any precipitation, it should be incubated at 37°C until the precipitation is dissolved.

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Protocol

1. **Collect 150~250 ml bacteria cultured overnight (if using rich medium, the volume of bacteria should be halved or less) with a 50 ml centrifuge tube (not provided), invert the 50 ml centrifuge tube on a paper towel for 1 min to remove the supernatant.**
2. **Add 10 ml Buffer I RNase A added to suspend the bacteria pellet.**

   * Bacteria pellet can be suspended by vortexing or pipetting several times with a tip. Fully suspended bacteria without no visible small bacterial clumps, otherwise it will seriously affect the final plasmid yield
3. **Add 10 ml Buffer II, gently invert 6-8 times to mix well to allow the bacteria to fully lyse.**

   * Ensure that no visible precipitates are present in the solution before using Buffer II; Cap the bottle tightly after using Buffer II to avoid long-term contact with air.

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

   * When the bacteria are fully lysed, the solution should be viscous and translucent, otherwise 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 10 ml Buffer N3, gently invert the tube until all remaining blue precipitate in the solution turns to a pale-yellow precipitate, and centrifuge ≥ 4,500 rpm for 5 min.**

   * This step should not be mixed with a vortexer, as this will result in genomic DNA being mixed in the final prepared plasmid.
5. **Place the Filter into a clean 50 ml centrifuge tube (not provided), withdraw the Piston, pour all the supernatant from step 4 into the Filter, insert and gently push the Piston so that the filtrate drops all into the 50 ml centrifuge tube.**

   * The supernatant poured into the Filter is mixed with some yellow precipitate, which does not affect the filtration effect.
6. **Add 10 ml isopropanol to the filtrate, close the tube tightly and gently invert 10 times to mix well. Transfer 18 ml mixture to a Spin Column, close the lid, and centrifuge ≥ 4,500 rpm for 2 min.**
7. **Discard the filtrate, place the Spin Column back into the 50 ml centrifuge tube, pour all the remaining mixture into the Spin Column, close the lid, and centrifuge ≥ 4,500 rpm for 2 min.**
8. **Discard the filtrate, place the Spin Column back into the 50 ml centrifuge tube. Add 10 ml Buffer W2 to the Spin Column, close the lid, and centrifuge ≥ 4,500 rpm for 2 min.**

   * Ensure that absolute ethanol has been added to Buffer W2 in the volume indicated on the reagent bottle.
9. **Repeat step 8 once.**
10. **Discard the filtrate, place the Spin Column back into the 50 ml centrifuge tube, open the lid, and centrifuge ≥ 4,500 rpm for 15 min.**

    * If the maximum speed of the centrifuge can reach more than 10,000 rpm, the centrifugation conditions can be changed to centrifuge ≥ 10,000 rpm for 5 min.
11. **Discard the 50 ml centrifuge tube, place the Spin Column in a clean 50 ml centrifuge tube (self-provided), open the lid, and let stand at 37°C in a thermostatic incubator for 10-15 min.**

    * The purpose of this step is to remove the residual ethanol in the column, and if the ethanol smell can still be smelled in the column after this step, the standing time can be extended appropriately.
12. **Add 2 ml Buffer E to the center of the membrane of the Spin Column, open the lid, incubate at room temperature for 5 min, and centrifuge at full speed for 5 min.**

    * If the centrifugation speed cannot exceed 5000 rpm, only 1.2~1.5 ml plasmid DNA may be obtained, and a second elution should be performed according to step 13 to improve the recovery efficiency of plasmid DNA.

    * Plasmid DNA can also be eluted with deionized water, but the pH of the water should be greater than 7.0, otherwise the elution efficiency of the plasmid DNA will be affected.
13. **If the measured plasmid DNA concentration is greater than 200 ng/μl, a secondary elution is recommended to improve the recovery efficiency of plasmid DNA: place the eluted plasmid DNA Spin Column in another clean 50 ml centrifuge tube (not provided), add 1 ml Buffer E to the Spin Column, open the lid, incubate at room temperature for 5 min, and centrifuge at full speed for 5 min.**
14. **Discard the Spin Column and pool the plasmid DNA eluted twice and store at -20°C for later use. Or follow these steps for concentration:**

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Plasmid concentration steps:

1. **Aliquot the plasmid DNA into 3×2 ml centrifuge tubes, add 0.1 DNA solution volume of 3 M sodium acetate (pH 5.2) and 0.8 DNA solution volume of isopropanol to each centrifuge tube, mix well, and centrifuge at 13,000 rpm for 10 min.**
2. **Discard the supernatant and retain the DNA pellet at the bottom of the tube. Add 1.5 ml 70% ethanol to each tube, suspend the pellet with vortexer, and centrifuge at 13,000 rpm for 5 min.**

   * Be careful not to discard the DNA pellet from the bottom of the tube.
3. **Discard the supernatant, spin down several seconds, discard the residual liquid with a 200 µl tip (do not discard to the pellet), open the cap, and dry the DNA pellet at room temperature on a clean bench for 10 min.**
4. **Add 100 µl Buffer E or deionized purified water to each tube, vortex to fully dissolve the plasmid DNA pellet, and store the plasmid DNA at -20°C for later use.**

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