HiPure FastFilter Plasmid Midi Kit

Introduction

The HiPure FastFilter Plasmid Midi Kits combine the power of HiPure technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality DNA. HiPure DNA columns facilitate the binding, washing and elution steps thus enabling multiple samples to be simultaneously processed. This system also includes a special filter cartridge which replaces the centrifugation step following alkaline lysis. Plasmid DNA purified by this system is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations. Up to 250 µg high copy number plasmid DNA or 10-75 µg low copy number plasmid DNA can be purified from 15-50 mL overnight culture.

Principle

The HiPure Plasmid procedure is based on alkaline lysis of bacterial cells followed by adsorption of DNA onto silica in the presence of high salt. The unique silica membrane used in the kit completely replaces glass or silica slurries for plasmid DNA minipreps.The procedure consists of 3 basic steps: Preparation and clearing of a bacterial lysate by alkaline method, then transfer the superantan to column to bind DNA. After washing proteins and other impurities, Nucleic acid was finally eluted with low-salt buffer (10mm Tris,pH9.0, 0.5mm EDTA).

Kit Contents

Product Number	P101302	P101303
Purification Times	25 Preps	100 Preps
RNase A	10 mg	40 mg
Buffer P1	80 ml	300 ml
Buffer P2	80 ml	300 ml
Buffer LEN3	40 ml	150 ml
Buffer GBT	60 ml	250 ml
Buffer PW1	60 ml	250 ml
Buffer PW2*	20 ml	100 ml
Elution Buffer	30 ml	120 ml
HiPure DNA Midi Columns III	25	100
Lysate Clear Midi Syringe	25	100
15 ml Collection Tubes	50	200

Storage and Stability

The Kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. If any precipitates form in the buffers,warm at 37℃ to dissolve. After addition of RNase A, Buffer P1 is stable for 6 months when stored at 2–8°C.

Materials and Equipment to be Supplied by User

- Add 80ml (25 preps) or 400ml (100 preps) 100% ethanol to the bottle of Buffer PW2 and store at room temperature
- Add the RNase A to the bottle of Buffer P1 and store at 2-8°C
- Heat Elution Buffer to 70°C if plasmid DNA is >10kb

Protocol

1. **Transfer 25-50 ml overnight culture to a 50 ml centrifuge tube (not provided). Centrifuge at 3000~5000 x g for 10 minute.** Decant or aspirate and discard the culture media.
   The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD600 x mL culture) for the HiPure Midi Column is 100. For example, if the OD600 of a culture is 4.0, the optimal culture volume should be 25 mL. If excess culture cell mass is used, alkaline lysis will be inefficient, the HiPure matrix will be overloaded, and the performance of the system will be decreased. HiPure Midi protocols in this handbook can be used for preparation of low-copynumber plasmid DNA or cosmids from 50~100 ml overnight E. coli cultures grown in LB medium. When using 50~100 ml culture volume, it is recommended to double the volumes of Buffers P1, P2, LEN3 and Buffer GBT used. It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5a and JM109.

2. **Resuspend pelleted bacterial cells in 2.5ml Buffer P1.**
   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

3. **Add 2.5mL Buffe P2. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate.**
   This may require a 2 minute incubation at room temperature with occasional mixing.
   Mix gently by inverting the tube. Do not vortex, because this will result in shearing of genomic DNA and contamination of plasmid. If continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

4. **Add 1.3ml Buffer LEN3. Mix immediately and thoroughly by inverting the tube 8–10 times.**

To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer LEN3.

5. Prepare a Lysate Clear Midi Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip. **Transfer the lysate from Step 4 into the barrel of the Lysate Clear Midi Syringe.**

6. **Add 2.0ml Buffer GBT to the barrel of the lysate Clear Midi Syringe, let sit at room temperature for 1 minutes.** Any white precipitates should float to the top.

7. Hold the Lysate Clear Midi Syringe barrel over a 15 ml centrifuge tube (not provided) and remove the end cap from the syringe tip. **Gently insert the plunger into the barrel to expel the cleared lysate into the 15 ml centrifuge tube. Mix the cleared lysate by inverting the tube 4~6 times.**

8. Insert a HiPure DNA Midi Column III into a 15ml Collection Tube (provided).

9. **Apply 3.5~4ml of the cleared Lysate from step 7 to the HiPure DNA Midi Column III by pipetting.** Centrifuge at 2000~3000 x g for 3 min.

10. **Discard the filtrate and reuse the collection tube. Repeat Steps 9 until all of the lysate has been transferred to the HiPure DNA Midi Column III**.

11. **Wash the Column by adding 2.0 ml Buffer PW1 and centrifuging at 3,000~5,000 x g for 3min.** Discard the flow through.

12. **Wash the column by adding 3.5 ml Buffer PW2 and centrifuging at 3,000~5,000 x g for 3min.** Discard the flow through.

13. Discard the flow through, and centrifuge at full speed for an additional 15 min to remove residual wash buffer.
    Residual wash buffer will not be completely removed unless the flow through is discarded before this additional centrifugation. Residual ethanol from Buffer PW2 may inhibit subsequent enzymatic reactions.

14. **Place the Column in a clean 15 ml microcentrifuge tube. To elute DNA, add 400µl Elution Buffer or water to the center of each Column, let stand for 2 min, and centrifuge for 3min.**

15. **Add 300µl Elution Buffer or water to the center of each column.** Let it stand for 2 min and centrifuge for 3min.
    When plasmid DNA or cosmids are >10 kb, preheat Eluiton Buffer (or water) to 70°C prior to eluting DNA from the HiPure membrane.
16. Discard the column and store Plasmid DNA at -20°C.

Troubleshooting Guide

1. Low DNA yields

- **Buffer PW2 did not contain ethanol:** Ethanol must be added to Buffer PW2 before used.
- **Poor cell lysis:** Cells may not have been dispersed adequately prior to the addition of Buffer P2. Vortex to completely resuspend the cells.
- **Column matrix lost binding capacity during storage:** Follow the Optional Protocol for Column Equilibration prior to transferring the cleared lysate to the Column. Add 500μL 3M NaOH to the column prior to loading the sample. Centrifuge at 3000~5000 x g for 3minutes. Discard the filtrate.

2. Plasmid DNA floats out of well while loading agarose gel

Ethanol was not completely removed from column following wash steps, centrifuge column as instructed to dry the column before elution.

3. High molecular weight DNA contamination of product

Do not vortex or mix aggressively after adding Buffer P2. Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.

4. Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A260/A280 ratio is high or low)

- **Plasmid DNA is contaminated with RNA:** RNase A treatment is insufficient Confirm that the RNase A Solution was added to Buffer P1 prior to first use. The RNase A solution may degrade due to high temperatures (>65 °C) or prolonged storage (> 6 months at room temperature).
- **Background reading is high due to silica fine particulates:** Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.