HiPure Plasmid EF Mega Kit

Introduction

The HiPure Plasmid DNA Mega 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. 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 10 mg high copy number plasmid DNA can be purified from 500 mL overnight culture.

Kit Contents

Product Number	P111602	P111603
Purification Times	10 Preps	50 Preps
RNase A	60 mg	2 x 150 mg
Buffer E1	220 ml	2 x 550 ml
Buffer E2	220 ml	2 x 550 ml
Buffer E3	220 ml	2 x 550 ml
Buffer E4	220 ml	2 x 550 ml
Buffer E5	120 ml	550 ml
Buffer PW2*	25 ml	2 x 100 ml
Elution Buffer	30 ml	120 ml
HiPure EF Maxi Columns	10	50
Lysate Clear Maxi Syringe	10	50
50 ml Collection Tubes	20	100

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.

Materials and Equipment to be Supplied by User

- Dilute Buffer PW2 with 100ml (10 Preps) or 2 x 400ml (50 Preps) 100% ethanol and store at room temperature
- Add RNase A to the bottle of Buffer E1 and store at 2-8°C
- Heat Elution Buffer to 70°C if plasmid DNA is >10kb
- Isopropanol

Protocol

1. Transfer ~500 mL overnight culture to an appropriate centrifuge bottle (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 DNA Midi Column is 1000. For example, if the OD600 of a culture is 4.0, the optimal culture volume should be 250mL. 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. 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 20 ml Buffer E1.
   Ensure that RNase A has been added to Buffer E1. 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 20 mL Buffe E2. Invert and rotate the tube gently 10-12 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 20 ml Buffer E3. Mix immediately and thoroughly by inverting the tube 10–15 times. To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer E3. The solution should become cloudy.

5. Centrifuge at 3000~5000 x g for 10 min.

6. Prepare a Lysate Clear Maxi 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. Immediately transfer the supernatant from Step 5 into the barrel of the Lysate Clear Maxi Syringe.

7. Hold the Lysate Clear Maxi Syringe barrel over a bottle (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 bottle.

8. Measure the volume of cleared lysate and add 1/3 volume of Buffer E4 to the lysate. Mix by inverting the tube 4~6 times.

9. Insert a HiPure EF Maxi Column into a 50mL Collection Tube (provided). Apply 20ml of the cleared Lysate from step 8 to the HiPure DNA EF Maxi column by pipetting. Centrifuge at 3000 x g for 3 min.

10. Discard the filtrate and reuse the collection tube. Repeat Steps 9 until all of the mixture has been transferred to the HiPure EF Maxi Column.

11. Wash the Column by adding 10 ml Buffer E5 and centrifuging at 3000 x g for 3 min. Discard the flow through.

12. Wash the column by adding 10ml Buffer PW2 and centrifuging at 3000 x g for 3min. Discard the flow through.

13. Discard the flow through, and centrifuge at full speed for an additional 10 min to remove residual wash buffer.
    Important: 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 50 ml microcentrifuge tube(Provided). To elute DNA, add 1ml Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of each Column, let stand for 3 min, and centrifuge for 3min.

15. Add 500~1000µl Elution Buffer or water to the center of each column. Let it stand for 3 min and centrifuge for 3min.

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 E2. 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 E2. 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 E1 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.