HiPure Plasmid EF Maxi Kit

Introduction

The HiPure Plasmid EF Maxi Kit combine the power of HiPure technology with Magen's innovative Endotoxin Removal Technology (ETR) to deliver high-quality plasmid DNA with low endotoxin levels for use in eukaryotic transfection, and in vitro experiments. Up to 1000 μg high copy number plasmid DNA or 200 μg low copy number plasmid DNA can be purified from 150 mL overnight culture.

Kit Contents

Product Number	P115602	P115603
Purification Times	10 Preps	50 Preps
RNase A	30 mg	150 mg
Buffer P1	100 ml	500 ml
Buffer P2	100 ml	500 ml
Buffer LN3	50 ml	250 ml
Buffer PW1	33 ml	180 ml
Buffer PW2*	20 ml	100 ml
Elution Buffer	15 ml	120 ml
Buffer CL	33 ml	180 ml
HiPure DNA Maxi Columns C	10	50
Lysate Clear Midi Syringe	10	50
50 ml Collection Tubes C	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. 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 (10 preps) or 400ml (50 preps) 100% ethanol to the bottle of Buffer PW2 and store at room temperature
- Add the vial of 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
- Isopropanol

Protocol

1. **Transfer 100-200ml overnight culture to an appropriate centrifuge bottle (not provided).** Centrifuge at 8000 rpm for 5 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 300. For example, if the OD600 of a culture is 4.0, the optimal culture volume should be 75 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. 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 9 ml 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 9 ml Buffe P2. Invert and rotate the tube gently 10-12 times to obtain a cleared lysate.** This may require a 5 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 4.5 ml Buffer LN3. Mix immediately and thoroughly by inverting the tube 10–15 times.** Addition of Buffer LN3 neutralizes the solution and precipitates proteins and cellular debris, creating a white flocculent.To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer LN3. Bacs or large plasmid (>10Kb) may be shaken to help release the large template from the flocculent.

5. Centrifuge at 8000rpm for 5 min.

6. 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. **Immediately transfer the supernatan from Step 5 into the barrel of the Lysate Clear Midi Syringe.**

7. Hold the Lysate Clear Midi Syringe barrel over a 50mL 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 50 ml centrifuge tube.**

8. **Measure the volume of cleared lysate and add 0.3 volume of isopropanol to the lysate.** Mix by inverting the tube 6~8 times.

9. Insert a HiPure DNA Maxi Column C into a 50ml Collection Tube C (provided).

10. **Apply 3 ml of Buffer CL to the Maxi Column. Incubate at room temperature for 5min and centrifuging at 8000rpm for 3min.** Discard the filtrate and reuse the collection tube.

11. **Apply 9 ml of the mixture from step 8 to the HiPure DNA Maxi column C by pipetting.** Centrifuge at 8000rpm for 2 min.

12. Discard the filtrate and reuse the collection tube. **Repeat Steps 11 until all of the mixture has been transferred to the HiPure DNA Maxi Column C.**

13. **Wash the Column by adding 3ml Buffer PW1 and centrifuging at 8000rpm for 2min.** Discard the flow through.

14. **Wash the column by adding 8ml Buffer PW2 and centrifuging at 8000rpm for 2min.** Discard the flow through.

15. **Discard the flow through, and centrifuge at 8000rpm 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.

16. **Place the Column in a clean 50 ml microcentrifuge tube C. To elute DNA, add 800~1000µl Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of each Column, let stand for 2 min, and centrifuge for 2min.**

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

18. 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.