HiPure Plasmid EF Mini Kit

Introduction

The HiPure Plasmid EF Mini Kit combine the power of HiPure technology with Magen's innovative Endotoxin Removal Technology to deliver high-quality plasmid DNA with low endotoxin levels for use in eukaryotic transfection, and in vitro experiments. The HiPure Plasmid Endo-Free System uses a specially formulated buffer that prevents endotoxin molecules from binding to the surface of the HiPure matrix. Endotoxin contamination lowers transfection efficiencies for endotoxin sensitive cell lines. For gene therapy, endotoxin contamination should be of major concern since endotoxins have the potential to cause fever, endotoxic shock syndrome, and interfere with in vitro transfection into immune cells.

Kit Contents

Product Number	P115402	P115403
Purification Times	50 Preps	250 Preps
RNase A	5 mg	20 mg
Buffer P1	30 ml	140 ml
Buffer P2	30 ml	140 ml
Buffer LEN3	15 ml	70 ml
Buffer LN4	50 ml	250 ml
Buffer LN5	30 ml	140 ml
Buffer PW1	30 ml	140 ml
Buffer PW2*	12 ml	50 ml
Elution Buffer	15 ml	30 ml
HiPure DNA Mini Columns III	50	250
2 ml Collection Tubes	50	250

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 48ml (50 preps) or 200ml (250 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
- HiPure DNA Mini Column III can bind up to 70μg DNA.

Protocol

1. **Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 5~15 ml LB medium containing the appropriate selective antibiotic. Incubate for 12~16 hours at 37°C with vigorous shaking.** Centrifuge at 3000~5000 x g for 10 minute at room temperature. Decant or aspirate and discard the culture media.
   It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. **Resuspend pelleted bacterial cells in 450μl Buffer P1and transfer to a microcentrifuge tube.**
   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 450μl Buffer P2 and mix thoroughly by inverting the tube 8–10 times.** 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 200μl Buffer LEN3. Mix immediately and thoroughly by inverting the tube 10–15 times.**
   Addition of Buffer LEN3 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 LEN3. Bacs or large plasmid (>10Kb) may be shaken to help release the large template from the flocculent.

5. Centrifuge for 10 min at 13,000 rpm in a table-top microcentrifuge.

6. Transfer 0.9ml of the supernatant into a 2ml microcentrifuge tube. **Add equal volume of Buffer LN4 and mix by inverting the tube 4~6 times.**
   For example, to 900µl supernatant, add 900µl Buffer LN4. When using 5~10 ml culture volume, adjust the volumes of Buffers P1(380µl), Buffer P2(380µl), and Buffer LEN3 (190µl) to reduce the volume of supernatant.

7. **Insert a HiPure DNA Mini Column III into a 2.0ml Collection Tube (provided).**

8. **Apply no more than 750µl of the mixture from step 6 to the Column by pipetting.** Centrifuge for 30~60 s. Discard the flow through.

9. Discard the filtrate and reuse the collection tube. Repeat Steps 8 until all of the mixture has been transferred to the HiPure DNA Mini Column III.

10. **Wash the Column by adding 0.5ml Buffer LN5 and centrifuging for 30–60s.** Discard the flow through.

11. **Wash the Column by adding 0.5ml Buffer PW1 and centrifuging for 30–60s.** Discard the flow through.

12. **Wash the column by adding 0.7ml Buffer PW2 and centrifuging for 30–60s.**

13. Discard the flow through, and centrifuge at full speed for an additional 2 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 1.5 ml microcentrifuge tube. To elute DNA, add 60~100µl Elution Buffer to the center of each Column, let stand for 1 min, and centrifuge for 1 min.**
    When plasmid DNA or cosmids are >10 kb, preheat Eluiton Buffer (or water) to 70°C prior to eluting DNA from the HiPure membrane.
15. 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 100µL 3M NaOH to the column prior to loading the sample. Centrifuge at 13000 rpm for 30 seconds. 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.