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	P111600	P111602	P111603
Purification Times	4 Preps	10 Preps	50 Preps
RNase A	30 mg	60 mg	2 x 150 mg
Buffer E1	100 ml	220 ml	2 x 550 ml
Buffer E2	100 ml	220 ml	2 x 550 ml
Buffer E3	100 ml	220 ml	2 x 550 ml
Buffer E4	100 ml	220 ml	2 x 550 ml
Buffer E5	50 ml	120 ml	550 ml
Buffer PEW2*	20 ml	25 ml	2 x 100 ml
Elution Buffer (Endo Free)	15 ml	30 ml	120 ml
Buffer ERS1	5 ml	10 ml	40 ml
Buffer ERS2	5 ml	10 ml	40 ml
HiPure EF Maxi Columns	4	10	50
Lysate Clear Maxi Syringe	4	10	50
50 ml Collection Tubes	8	20	2 x 50

Storage and Stability

The kit components can be stored dry at room temperature (15–25°C) and are stable for at least 24 months under these conditions. If any precipitates form in the buffers,warm at 37°C to dissolve. After addition of RNase A, Buffer E1 is stable for 6 months when stored at 2–8°C.

Materials and Equipment to be Supplied by User

- Dilute Buffer PEW2 with 80ml (4 Preps) or 100ml (10 Preps) or 2 x 400ml (50 Preps) 100% ethanol and store at room temperature
- Add 0.5ml Buffer E1 to the bottle of RNase A, pipetting to mix. Then transfer all RNase A liquid 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: Low-Endotoxin Plasmid DNA Purification

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 EF Maxi 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 Buffer 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 8,000 rpm 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 8,000 rpm 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 8,000 rpm for 3 min. Discard the flow through.

12. **Wash the column by adding 10ml Buffer PEW2** and centrifuging at 8,000 rpm for 3min. Discard the flow through.

13. **Repeat step 12 once.**

14. Centrifuge the empty column at maxi speed for 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.

15. Place the Column in a clean 50 ml microcentrifuge tube(Provided). To elute DNA, add 1ml Elution Buffer (Endo-free) to the center of each Column. Incubate at room temperature for 3 min and centrifuge at 8,000 rpm for 3min.

16. Add 1ml Elution Buffer (Endo-free) to the center of each column. Incubate at room temperature for 3 min and centrifuge at 8,000 rpm for 3min.Transfer the plasmid DNA to a new 5 ml microcentrifuge tube and store at −20°C.

Additional Protocol:

Endotoxin-Free Plasmid DNA Purification (0.1 EU/μg DNA)

1. **Add Buffer E1/RNase A to the Plasmid DNA (from Step 16) to reach volume 3ml.** Mix by vortex and incubate at room temperature for 10 min.
2. **Add 0.6ml Buffer ERS1,** invert 10~15 times to mix, incubate at room temperature for 10 min during which invert few times.
3. **Add 0.6ml Buffer ERS2,** invert 10~15 times to mix, get a turbid liquid. Centrifuge at 10000 rpm for 10 min.

   Note: If the column is not dry completely, the mixture may fail to become turbid after mixing. In that case, add 0.3ml Buffer ERS2 and mix to obtain a turbid mixture. If the supernatant remains turbid after centrifugation, repeat the centrifuge step and set the deceleration (brake) parameter of the centrifuge to the slowest setting. If the centrifuge has no adjustable deceleration parameter, transfer the mixture into several 2ml centrifuge tubes and centrifuge at 13,000 rpm for 10 min at room temperature.
4. **Transfer the supernatant to a new centrifuge tube, add 0.7 volume of isopropanol to the tube and invert 10~15 times to mix.** Incubate at room temperature for 10 min, centrifuge at 10,000~13,000 rpm for 15 min to pellet the plasmid DNA.

   Note: for easier handling, aliquot the mixture into several 2ml centrifuge tubes and centrifuge in a benchtop high-speed centrifuge.
5. **Discard the supernatant carefully, add 1ml 75% ethanol to the tube,** vortex for 5 sec, and then centrifuge at 10,000~13,000 rpm for 3 min.
6. Discard the supernatant carefult, centrifuge shortly, pipette all the residual liquid carefully. Do not pipette the white pellet, air dry for 5 min.
7. **Add suitable amount of Nuclease Free Water, vortex to mix**. Incubate at room temperature for 5~10 min to dissolve Plasmid DNA thoroughly. Store Plasmid DNA at −20°C.