HiPure Plasmid EF Maxi Kit B

Introduction

The HiPure Plasmid EF Maxi Kit combines 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, animal injection 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 100~200 ml overnight culture.

Kit Contents

Product Number	P115601B	P115602B	P115603B
Purification Times	2 Preps	10 Preps	50 Preps
RNase A	6 mg	30 mg	150 mg
Buffer P1	20 ml	100 ml	500 ml
Buffer P2	20 ml	90 ml	450 ml
Buffer NS3	20 ml	90 ml	450 ml
Buffer ER2	6 ml	30 ml	150 ml
Buffer PW1	12 ml	60 ml	270 ml
Buffer PW2*	6 ml	20 ml	100 ml
Elution Buffer	5 ml	20 ml	120 ml
Lysate Clear Midi Syringe	2	10	50
HiPure DNA Maxi Columns RC7	2	10	50
50 ml Collection Tube	4	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°C 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 24ml (2 Preps), 80ml (10 preps) or 400ml (50 preps) 100% ethanol to the bottle of Buffer PW2 and store at room temperature
- Add 0.2~0.5ml Buffer P1 to the RNase A vial, pipet to mix and then transfer all the reagent to the bottle of Buffer P1.It ca be store at 2-8°C for 6 months.
- Heat Elution Buffer to 70°C if plasmid DNA is >10kb
- Isopropanol
- Vacuum protocol: vacuum filtration device (QIAVAC 24 plus / Magen MagVac 20 System)
- Centrifugation protocol: Horizontal barrel centrifuge recommend at 4,000~5,000 rpm, or Angle centrifuge recommend at 6,000~8,000 rpm.

Protocol

1. **Transfer 100-200ml overnight culture to an appropriate centrifuge bottle (not provided).** Centrifuge at 8000 rpm for 5 minutes. 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 Maxi 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 end A negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®. HiPure DNA Maxi Column RC7 binding yield can reach to1500ug.

2. **Remove the culture and 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 8 ml Buffer NS3. Mix immediately and thoroughly by inverting the tube 10–15 times.**

Buffer NS3 neutralizes the solution and precipitates proteins and cellular debris, creating a white flocculent.To avoid localized precipitation, mix the solution thoroughly, immediately after adding Buffer NS3. Bacs or large plasmid (>10Kb) may be shaken to help release the large template from the flocculent.

5. Centrifuge at 4,000~8,000rpm for 10 min.

6. Take out the plunger from Lysate Clear Midi Syringe.**Transfer the supernatan from Step 5 into the the Lysate Clear Midi Syringe immediately.** Place the syringe on a tube rack to keep upright. Make sure the end cap is attached to the syringe tip.

7. Hold the Lysate Clear Midi Syringe 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 lysate and add 0.1 volume of Buffer ER2 to the lysate.** Mix by inverting the tube10~15 times, then stay for 2~3 minutes.
Choice: for sensitive applications, it can go with additional process to remove endotoxin clearly. Add 0.1 volume of Buffer ER2 to the lysate, invert the tube10~15 times to mix and stay for 10 minutes. Add 0.1 volume of chloroform or Buffer BCP (not provide, Magen Cat# C496), vortex 10 seconds to mix. Centrifuge at 8,000 rpm for 3 minutes, transfer the supernatant to a new 50ml centrifuge tube. Then follow step 9. e.g. for 22ml lysate, add 2.2ml Buffer ER2 and 2.2ml chloroform or Buffer BCP. Most cell transfection down stream application does not require this additional endotoxin remove process.

9. A**dd 0.3 volume of isopropanol to the mixture** (end mixture volume after step 8), invert 10~15 times to mix.

Centrifuge protocol

10. **Insert a HiPure DNA Maxi Column RC7 into a 50ml Collection Tube (provided), transfer half volume of mixture (do not exceed 15ml) to the column,** centrifuge at 4,000~6,000 rpm for 3 minutes. Discard the filtrate and reuse the collection tube.

11. **Transfer the balance mixture to the column,** centrifuge at 4,000~6,000 rpm for 3 minutes. Discard the filtrate and reuse the collection tube.

12. **Wash the Column by adding 5ml Buffer PW1,** centrifuge at 4,000~6,000 rpm for 3 minutes. Discard the filtrate and reuse the collection tube.

13. **Wash the Column by adding 5ml Buffer EWB,** centrifuge at 4,000~6,000 rpm for 3 minutes. Discard the filtrate and reuse the collection tube.

14. **Wash the Column by adding 5ml Buffer PW2,** centrifuge at 4,000~6,000 rpm for 3 minutes. Discard the filtrate and collection tube.

15. Centrifuge at 8,000rpm for additional 10 minutes to remove residual wash buffer. For Horizontal barrel centrifuge, centrifuge at maxi speed (>4000rmp) for 15 minutes. **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 new 50 ml Collection Tube, open the cap and dry at room temperature for 10 minutes. **Add 0.8~1.5ml Elution Buffer (10 mM Tris·Cl, pH 8.5) or sterile water to the center of each Column.** Stay for 3 minutes, and centrifuge at 4,000~8,000 rpm for 3 minutes.
    - When plasmid DNA or cosmids are >10 kb, preheat Eluiton Buffer (or water) to 70°C prior to elute DNA from the HiPure membrane.
    - As the membrane has hydroscopicity, when process with angle centrifuge at 8,000 rpm, it will lost ~0.1ml Elution Buffer. Elution volume should be >0.6ml. When process with horizontal barrel centrifuge at 4,000~5,000 rpm, it will lost ~0.3ml Elution Buffer. Elution volume should be >1.0 ml.
    - A second elution (0.3ml) step can be proceed to increase Plasmid DNA yield.

17. Discard the column, transfer Plasmid DNA in a new 2ml centrifuge tube and store at -20°C.

Vacuum protocol

10. Insert a HiPure DNA Maxi Column RC7 to Vacuum Manifold. Transfer the mixture fro step 9 to the Column and apply the vacuum. Switch off the vacuum when all liquid filtered.

11. **Wash the Column by adding 5ml Buffer PW1 and apply the vacuum.** Switch off the vacuum when all liquid filtered.

12. **Wash the Column by adding 5ml Buffer EBW and apply the vacuum.** Switch off the vacuum when all liquid filtered.

13. **Wash the Column by adding 5ml Buffer PW2 and apply the vacuum.** Switch off the vacuum when all liquid filtered.

14. Insert the Column to a 50ml Collection Tube. Then follow Step 15~17 in above Centrifuge Protocol.