HiPure Plasmid EF Midi Kit

Introduction

The HiPure Midi system provides a fast, simple, and cost-effective plasmid DNA midiprep method for routine molecular biology laboratory applications. HiPure Midiprep Kits use silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Plasmid DNA purified with Mini Kits is immediately ready for use. Phenol extraction and ethanol precipitation are not required, and high quality plasmid DNA is eluted in a small volume of Tris buffer or water.

Kit Contents

Product Number	P123102	P123102B
Purification Times	50 Preps	50 Preps
RNase A	30 mg	30 mg
Buffer E1	150 ml	150 ml
Buffer E2	150 ml	150 ml
Buffer E3	150 ml	150 ml
Buffer E4	150 ml	150 ml
Buffer E5	150 ml	150 ml
Buffer PW2*	50 ml	50 ml
Elution Buffer	30 ml	30 ml
MaxPure EF Mini Column	50	50
2ml Collection Tubes	50	50
Lysate Clear Midi Syringe	50	50
Extend Tubes	50	50
50ml Centrifuge Tubes	-	50
Support Tubes	-	50

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 and optional LyseBlue reagent, Buffer P1 is stable for 6 months when stored at 2–8°C.

Materials and Equipment to be Supplied by User

- Dilute Buffer PW2 with 200ml 100% ethanol 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

Protocol

1. Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 25~50 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 DH5a® and JM109®. HiPure Midiprep protocols in this handbook can be used for preparation of low-copynumber plasmid DNA or cosmids from 50–100 ml overnight E. coli cultures grown in LB medium. When using 100 ml culture volume, it is recommended to double the volumes of Buffers E1, E2, E3 and E4 used.

2. Resuspend pelleted bacterial cells in 2.5ml Buffer E1 and 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 2.5ml Buffer E2 and mix thoroughly by inverting the tube 4–6 times.
   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 2.5ml Buffer E3. Mix immediately and thoroughly by inverting the tube 4–6 times.
   To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer E3. Large culture volumes (e.g., ⩾5 ml) may require inverting up to 10 times. The solution should become cloudy.

5. Centrifuge at 3,000 ~5,000 x g for 5min.

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 supernatant from Step 5 into the barrel of the Lysate Clear Midi Syringe.

7. Gently insert the plunger into the barrel to expel the cleared lysate into the 15 mL centrifuge tube. Add 1/3 volume of Buffer E4 to the clear Lysate. Invert 4~6 to mix.

Centrifuge Protocol (P1231B)

8. Combine MaxPure EF Mini Column with Sealing Ring, Support Tubes and Extender Tubes, then place it into 50ml collection tube. Apply the mxiture from step 7 to the Column. Centrifuge at 3,000 x g for 3min. Discard the flow through and reuse 50ml collection tube.

9. Wash the column by adding 1ml Buffer E5 and centrifuging at 3,000 x g for 3 min.

10. Wash the column by adding 2 ml Buffer PW2 and centrifuging at 3,000 x g for 3 min. Discard the flow through.

Vacuum Protocol (P1231)

11. Combine Extender Tube with MaxiPure EF Column, then insert to Vaccum Manifold. Apply the mxiture from step 7 to the Column and applying the vacuum.

12. Switch off the vacuum. Wash the Column by adding 1 ml of Buffer E5 to Column and applying the vacuum.

13. Switch off the vacuum. Wash the Column by adding 2 ml of Buffer PW2 to Column and applying the vacuum.

14. Insert a HiPure DNA Mini Column II into a 2.0mL Collection Tube(provided). Centrifuge at 10,000 x g form 2 min.

15. Place the Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 100~300µl Elution Buffer or water to the center of each column, let stand for 1 min, and centrifuge for 1 min.

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.