GeneBio Systems, Inc.™

Magen

HiPure Plasmid DNA Mini Kit

Introduction

The HiPure Mini system provides a fast, simple, and cost-effective plasmid DNA miniprep method for routine molecular biology laboratory applications. HiPure Plasmid Mini 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.

Principle

The HiPure Plasmid procedure is based on alkaline lysis of bacterial cells followed by adsorption of DNA onto silica in the presence of high salt. The unique silica membrane used in the kit completely replaces glass or silica slurries for plasmid DNA minipreps.The procedure consists of 3 basic steps: Preparation and clearing of a bacterial lysate by alkaline method, then transfer the superantan to column to bind DNA. After washing proteins and other impurities, Nucleic acid was finally eluted with low-salt buffer (10mm Tris,pH9.0, 0.5mm EDTA).

Kit Contents

Product Number	P100101	P100103
Number of Preps	50 Preps	250 Preps
RNase A	2 mg	10 mg
Buffer P1	15 ml	80 ml
Buffer P2	15 ml	80 ml
Buffer P3	20 ml	100 ml
Buffer PW1	25 ml	140 ml
Buffer PW2	10 ml	50 ml
Elution Buffer	6 ml	30 ml
HiPure DNA Mini Columns II	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

- Dilute Buffer PW2 with 200mL, 160mL or 40mL (for 250prep, 200prep,or 50prep kits respectively) 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 (1-5ml)

1. **Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic.** Incubate for 12~16 hours at 37°C with vigorous shaking. Centrifuge at 13000rpm for 1 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®. HiPure Mini protocols in this handbook can be used for preparation of low-copy number plasmid DNA or cosmids from 1–10 ml overnight E. coli cultures grown in LB medium. When using 10 ml culture volume, it is recommended to double the volumes of Buffers P1, P2, and P3 used.

2. **Resuspend pelleted bacterial cells in 250μl Buffer P1 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 250μl Buffer P2 and mix thoroughly by inverting the tube 8–10 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 350 µl Buffer P3. Mix immediately and thoroughly by inverting the tube 8–10 times.

   To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer P3. Large culture volumes (e.g., ⩾5 ml) may require inverting up to 10 times. The solution should become cloudy.

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

6. Insert a HiPure DNA Mini Column II into a 2.0mL Collection Tube(provided). Apply 800µl of the supernatant from step 5 to the column by pipetting. Centrifuge for 30~60s. Discard the flow through.

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

   This step is necessary to remove trace nuclease activity when using endA+ strains, such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains, such as XL-1 Blue and DH5 α , do not require this additional wash step.

8. Wash the column by adding 0.75 ml Buffer PW2 and centrifuging for 30–60s.

9. Discard the flow through, and centrifuge at full speed for an additional 1 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.

10. Place the Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50~100µl Elution Buffer or water 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.

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.