LaboPass ™

Mini

Protocol Book

LaboPass ™ Plasmid Mini

Introduction

LaboPass ™ Plasmid Mini kit provides easy and rapid method for the small-scale preparation of plasmid DNA from bacterial cells. This kit can be used to isolate and purify any plasmid, but works most efficiently when the plasmid is less then 20,000 bp in size. All process to prepare pure plasmid DNA takes only about 30 min and simultaneous processing of multiple samples can be easily performed. Up to 30 μg of pure plasmid can be purified using LaboPass ™ Plasmid Mini kit and this pure plasmid DNA is ready for PCR, cloning, fluorescent sequencing, synthesis of labeled hybridization probes, cell transfection, electroporation, and enzymatic restriction without further manipulation.

Principle of method

LaboPass ™ Plasmid Mini kit utilizes glass microfiber membrane based on the modified alkaline lysis method. Alkaline lysis releases plasmid DNA from bacterial cells and RNase A removes any RNA in the lysate. In the presence of high salt, plasmid DNA binds selectively to glass microfiber membrane in the LaboPass ™ spin column. Bound plasmid DNA is purified in a series of washing steps to eliminate contamination of other bacterial components. Finally elution by low salt buffer or water releases plasmid DNA from the glass microfiber membrane. This simple method eliminates the need for organic solvent extractions and alcohol precipitation, allowing for rapid purification of many samples simultaneously.

Storage and Stability

LaboPass ™ Plasmid Mini kit is shipped at room temperature. All components except RNase A solution are stable at room temperature for 12 months from the date of manufacture that is printed on the product package. RNase A stock solution is stable at room temperature, but for longer conservation of enzyme activity, storage at 4℃ is recommended. After addition of RNase A solution, Buffer S1 is stable for 6 months when stored at 4℃. In cold ambient conditions, Buffer S2, S3 and AW may exhibit salt precipitation and this causes reduction of DNA yields. If so, heat the bottles to dissolve completely at 60℃.

Chemical Hazard

Buffer S3 and AW contain irritant which is harmful when in contact with skin, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

Quality Control

All components in LaboPass ™ Plasmid Mini kit are manufactured in particle-controlled area (class 1000 clean room), and its degree of cleanness is monitored periodically.

Kit Contents

Cat. No.	CMP0111	CMP0112	CMP0115
No. of preparation	50	200	1000
Spin column	50	200	1000
Collection tube	50	200	1000
Buffer S1(1)	15ml	55ml	55mlx5
Buffer S2	15ml	55ml	55mlx5
Buffer S3	20ml	80ml	80mlx5
Buffer AW (conc.)	17.5ml	70ml	70mlx2
Buffer PW (conc.)	15ml	57ml	57mlx5
Buffer EB	15ml	15ml	15mlx5
Rnase A solution (100mg/ml)	15µl	55µl	55µl x5

(1) Buffer S1 should be stored in 4℃ after additional of RNase A solution.
* All Solution (Buffer) is available to purchase separately.

LaboPass ™ Plasmid Mini kit Protocols

Before experiment

*- **Before first use, add appropriate volume of absolute ethanol to Buffer AW and PW as printed on the product label**.*
- All centrifugation steps should be performed at full speed (>10,000 xg or 10,000 - 14,000 rpm) in a microcentrifuge at room temperature.
- Mix Buffer S2 and S3 thoroughly by shaking before use.
- Buffer S2 or S3 may precipitate at cool ambient conditions. If so, dissolve it in 60℃ water bath.

1. Pellet the bacterial culture by centrifugation for 5 min at 10,000 xg in a tabletop centrifuge. Discard the supernatant as much as possible.

Use the appropriate volume of bacterial cultures; 1-5 ml for high copy number plasmid, 1-10 ml for low copy number plasmid. Bacterial culture should be grown for 12 to 16 hours in liquid media (eg. LB) containing a selective antibiotic to a density of A_{600} =2.0. Higher culture volumes can cause reduction of lysis efficiency and overload of a spin column, resulting in unsatisfactory yields.

2. Resuspend pelleted bacterial cells thoroughly in 250µl of Buffer S1. Transfer the suspension to a new 1.5 ml tube.

Add RNase A solution before the first use of the Buffer S1. It is essential to thoroughly resuspend the cells.

3. Add 250µl of Buffer S2 and mix by inverting the tube 4 times (do not vortex).

Incubate until the cell suspension becomes clear and viscous; approximately for 1-5 min. Do not incubate for more than 5 min. If precipitated material has formed in buffer S2, heat to dissolve at 37℃

4. Add 350µl of Buffer S3 and immediately mix by inverting the tube 4-6 times (do not vortex)

For better precipitation, mix the solution gently but completely and immediately after addition of buffer S3.

5. Centrifuge for 10 min.

6. Transfer carefully the supernatant to a spin column and centrifuge for 1 min. Remove the spin column, discard the flowthrough, and re-insert the spin column to the collection tube.

7. When using *endA*+ strains, continue with step 7A, 7B. If not, continue with step 7C.

The wild type *E.coli* strains produce endonuclease I, which is encoded in *endA* gene and degrades double-stranded DNA. The *E.coli* genotype *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nucelase. *E.coli* strains with this mutation are referred to as e*ndA*-. The absence of *endA1* (or *endA*) in an *E.coli* genotype denotes the presence of the wildtype gene, which expressed an active endonuclease I. The wildtype is indicated as e*ndA*+. Buffer AW of step 7A removes the trace of endonuclease when using *endA*+ strains. If you cannot ensure the *endA* genotype of *E.coli*, follow the procedure of *endA*+. (step 7A and 7B)

The genotype of various *E.coli* strains

EndA+ strains; BL21(DE3), CJ236, HB101, JM83, JM101, JM110, LE392, MC1061, NM series, P2392, PR series, RR1, TB1, TG1, BMH71-18, ES1301, wildtype, and etc

EndA- strains; BJ5183, DH1, DH20, DH21, DH5□, JM103, JM105, JM106, JM107, JM108, JM109, MM294, SK1590, SRB, XL1-Blue, XLO, and etc.

endA+ strains

7A. Apply the 500µl of Buffer AW and centrifuge for 30 sec. Remove the spin column, discard the flowthrough, and re-insert the spin column to the collection tube.

7B. Apply the 750µl of Buffer PW and centrifuge for 1 min. Remove the spin column, discard the flowthrough, and re-insert the spin column to the collection tube. Go to step 8.

endA- strain

7C. Apply the 750µl of Buffer PW and centrifuge for 1 min. Remove the spin column, discard the flowthrough, and re-insert the spin column to the collection tube.

8. Discard the flowthrough, and centrifuge for an additional 1 min to remove residual wash buffer. Transfer the spin column to a new 1.5 ml tube.

If the spin column has Buffer PW associated with it, centrifuge again for additional 1 min at full speed before transferring to the new 1.5 ml tube. Residual ethanol from Buffer PW may inhibit subsequent enzymatic reaction.

9. Add 50µl of buffer EB or deionized distilled water, let stand for 1 min, and centrifuge for 1 min.

Ensure that the buffer EB or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. For high copy number plasmids, elution volume can be increased to 200 µl maximum. It will increase the total yields of plasmid but decrease the concentration of eluate. For long-term storage, eluting in buffer EB(10mM Tris-Cl, pH 8.5) and storing at –20℃ is recommended. When using water for elution, check the pH of water (>pH7.0) before elution.

Troubleshooting guide

Low or no yield of plasmid DNA

-Too many cells in sample

Cultures should be grown to A_{600}=2~4 in proper media with antibiotics. Reduce the culture volume of sample. (For low-copy number plasmid, 10ml, for high-copy plasmid, 5ml maximum)

- Low-copy-number plasmid used
Low-copy-number plasmids may yield as little as 0.5 µg of DNA from a 5 ml overnight culture. Increase culture volume to 10 ml or use high copy-number plasmid, if possible.

- Poor resuspension of bacterial pellets in Buffer S1
Bacterial cell pellets ought to be resuspended throughly in Buffer S1.

- Buffer S2 precipitated
Dissolve Buffer S2 by warming to 37℃.

- Ethanol was not added to Buffer AW and PW
Buffer AW and PW should be diluted with appropriate volume (printed on a label) of absolute ethanol.

- Insufficient digestion with RNase A
Excess RNA can interfere the binding of plasmid DNA with glass-fiber membrane. If Buffer S1 containing RNase A is more than 6 months old, add additional RNase A solution. (Working concentration = 100µg /ml)

Low or no yield of plasmid DNA

- Inadequate elution buffer
DNA is eluted only in low salt condition. Buffer EB (10mM Tris-Cl, pH8.5) has the optimal elution efficiency, but other elution buffer can be engaged as user's need. Elution efficiency is dependent on pH and the maximum efficiency is achieved between pH 7.0 and pH 8.5. When using water for elution, make sure the pH value.

Chromosomal DNA contamination

- Mishandling of the lysate after addition of Buffer S2
Vigorous vortexing after addition of Buffer S2 can cause shearing of chromosomal DNA followed by chromosomal DNA contamination. Handle gently the lysate after addition of Buffer S2. Simple inverting and rotating tube to cover walls with viscous lysate is sufficient for mixing.

- Too long lysis time
Too long lysis time after addition of Buffer S2 can cause chromosomal DNA contamination. Lysis time should not be over 5 min. And immediate mixing after addition of Buffer S3 is strongly recommended.

RNA contamination

- RNase A omitted or old
RNase A solution should be added to Buffer S1 before first use. If Buffer S1 containing RNase A is more than 6 months old, add additional RNase solution. (work conc.=100 µg /ml)

- Too many cells in sample
Reduce the sample volume. Too many cells may not be subjected properly to RNase A digestion.

High salt concentration in eluate
- Improper wash step
Ensure the wash step in protocols. Alternatively, incubate for 5 min at room temperature after applying Buffer PW in step 7B(*end*A+) or 7C(*end*A-).

Plasmid DNA degradation

- Nuclease contamination
For *end*A+ strains, stick to the protocols for *end*A+ strains. Buffer AW ought to be used in wash step.

DNA floats out of well while loading of agarose gel
- Ethanol is not completely removed during wash steps.
Ensure that step 8 in protocols is performed well. Spin column membrane should be completely dried via additional centrifugation or air-drying. Refer the annotation of step 8 in protocols.

Contact us ;

製造元

COSMO GENETECH CO.,Ltd.
Internet : www.cosmo4.com
E-mail : LaboPass@cosmo4.com
Tel : 82-2-922-7307
Fax : 82-2-922-7307

販売元

北海道システム・サイエンス株式会社
HokkaidoSystem Science.,Ltd.

北海道システム･サイエンス株式会社
〒001-0932
札幌市北区新川西2条1丁目2-1
TEL ： 011-768-5901
FAX ： 011-768-5951
E-mail ： bio@hssnet.co.jp
URL ： http://www.hssnet.co.jp/