
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 than 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°C is recommended. After addition of RNase A solution, Buffer S1 is stable for 6 months when stored at 4°C. 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°C.

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⁾	15 ml	55 ml	55 ml x 5
Buffer S2	15 ml	55 ml	55 ml x 5
Buffer S3	20 ml	80 ml	80 ml x 5
Buffer PW (conc.)	15 ml	57 ml	57 ml x 5
Buffer EB	15 ml	15 ml	15 ml x 5
RNase A (Lyophilized) ⁽²⁾	5.5 mg	5.5 mg	5.5 mg x 5
Lysis Indicator Blue ⁽³⁾	Powder	Powder	Powder x 5
Optional! Buffer AW (conc.) ⁽⁴⁾		70 ml	

(1) Buffer S1 should be stored in 4°C after additional of RNase A solution.

(2) 100 mg/ml. Before use, dissolve with 55 µl of distilled water or S1 buffer.

(3) The addition of Lysis Indicator Blue is optional. Before use, dissolve with 55 µl of absolute ethanol.

(4) Optional! Buffer AW is available to purchase.

* All Solution (Buffer) is available to purchase separately.

Using Lysis Indicator Blue reagent

Lysis Indicator Blue is color indicator which provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. This makes Lysis Indicator Blue ideal for use by researchers who have not had much experience with plasmid preparations as well as experienced scientists who want to be assured of maximum product yield.

Lysis Indicator Blue can be added to the resuspension buffer (buffer S1) bottle before use. Alternatively, smaller amounts of Lysis Indicator Blue can be added to aliquots of buffer S1, enabling single plasmid preparations incorporating visual lysis control to be performed. Lysis Indicator Blue reagent should be added to buffer S1 at a ratio of 1 : 1000 to achieve the required working concentration (e.g., 55 µl Lysis Indicator Blue into 55 ml Buffer S1). Make sufficient Lysis Indicator Blue/Buffer S1 working solution for the number of plasmid preps being performed.

Lysis Indicator Blue precipitates after addition into Buffer S1. This precipitate will completely dissolve after addition of Buffer S2. Shake Buffer S1 before use to resuspend Lysis Indicator Blue particles. The plasmid preparation procedure is performed as usual. After addition of Buffer S2 to Buffer S1, the color of the suspension. If the suspension contains localized regions of colorless solution or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

Upon addition of neutralization buffer (Buffer S3), Lysis Indicator Blue turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.

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 °C 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 250 μ l 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.

If Lysis Indicator Blue reagent has been added Buffer S1, vigorously shake the buffer bottle before use to ensure Lysis Indicator Blue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

3. Add 250 μ l 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 °C

4. Add 350 μ l 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 nuclease. *E.coli* strains with this mutation are referred to as *endA*⁻. 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 *endA*⁺. 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 α , JM103, JM105, JM106, JM107, JM108, JM109, MM294, SK1590, SRB, XL1-Blue, XLO, and etc.

***endA*⁺ strains**

7A. Apply the 500 μ l 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 750 μ l 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 750 μl 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 50 μl 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°C is recommended. When using water for elution, check the pH of water ($>\text{pH}7.0$) before elution.

Troubleshooting guide

Low or no yield of plasmid DNA

- Too many cells in sample

Cultures should be grown to $A_{600}=2\sim 4$ 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 $0.5 \mu\text{g}$ 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 thoroughly in Buffer S1.

- Buffer S2 precipitated

Dissolve Buffer S2 by warming to 37°C .

- 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 = $100 \mu\text{g}/\text{ml}$)

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.

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- 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 $\mu\text{g/ml}$)

- 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(*endA+*) or 7C(*endA-*).

Plasmid DNA degradation

- Nuclease contamination

For *endA+* strains, stick to the protocols for *endA+* 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.

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