High-Speed Plasmid Mini Kit

For research use only

Sample : 1-4 ml of cultured bacterial cells

Yield : up to 30 µg of plasmid/cosmid DNA

Format : spin column

Operation time: within 30 minutes

Elution volume: 50-100 µl

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Introduction

The High-Speed Plasmid Mini Kit was designed for rapid isolation of plasmid or cosmid DNA from 1-4 ml of cultured bacterial cells. Modified Alkaline Lysis method (1) and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA and RNA contaminants. In the presence of chaotropic salt, plasmid DNA in the lysate binds to the glass fiber matrix of the spin column (2). Contaminants are removed with a Wash Buffer (containing ethanol) and the purified plasmid DNA is eluted by a low salt Elution Buffer or TE. Typical yields are 20-30 µg for high-copy number plasmid or 3-10 µg for low-copy number plasmid from 4 ml of cultured bacterial cells. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 30 minutes. The purified plasmid DNA is ready for use in Restriction Enzyme Digestion, Ligation, PCR, and sequencing reactions.

Quality Control

The quality of the High-Speed Plasmid Mini Kit is tested on a lot-to-lot basis, by isolating plasmid DNA from a 4 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (A600 > 2 U/ml). Following the purification process, a yield of more than 20 µg is expected and the ratio of A260/A280 is between 1.7-1.9. The purified plasmid (1 µg) is used in EcoR I digestion, and checked by electrophoresis.

Kit Contents

Name	IB47100	IB47101	IB47102
PD 1 Buffer*	1 ml	25 ml	65 ml
PD 2 Buffer**	1 ml	25 ml	75 ml
PD 3 Buffer	1.5 ml	45 ml	100 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer*** (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	6 ml	30 ml
RNase A (50 mg/ml)	Added	50 µl	130 µl
PD Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	4 pcs	100 pcs	300 pcs

Order Information

Product Name	Package Size	Cat. No.
High-Speed Plasmid Mini Kit	100/300 preps	IB47101/102
Fast Ion Plasmid Midi Kit	25 preps	IB47111
Fast Ion Plasmid Midi Kit (Endotoxin Free)	25 preps	IB47113
Fast Ion Plasmid Maxi Kit	10/25 preps	IB47121/122
Fast Ion Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	IB47124/125
96-Well Plasmid Kit	2/4/10 x 96 Wells	IB47150/51/52
Vacuum Manifold (Accessories)	1 SET	IB47500

*Add provided RNase A to the PD1 Buffer and store at 4ºC.

**If precipitates have formed in the PD2 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve.

***Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Caution

PD3 Buffer and W1 Buffer contain guanidine hydrochloride which is a harmful irritant. During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

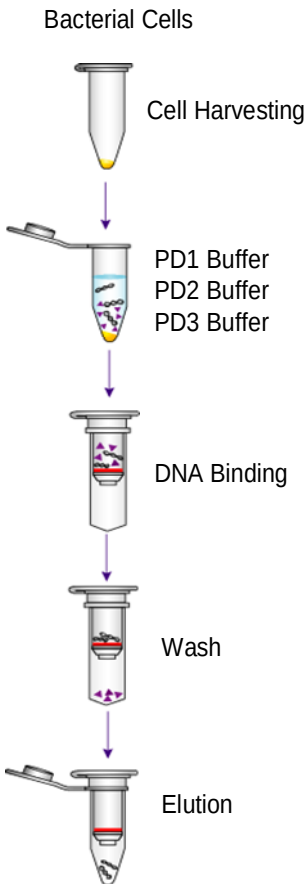
References

(1) Birnboim, H. C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513.
(2) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

High-Speed Plasmid Mini Kit Protocol

- Add provided RNase A to the PD1 Buffer and store at 4ºC.
- If precipitates have formed in the PD2 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve.
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: microcentrifuge tubes.

Step1 Harvesting	• Transfer 1.5 ml of cultured bacterial cells to a microcentrifuge tube. • Microcentrifuge for 1 minute and discard the supernatant. • If more than 1.5 ml of cultured bacterial cells is used, repeat the Harvesting Step.
Step 2 Re-suspension	• Add **200 µl of PD1 Buffer** (RNase A added) to the tube and resuspend the cell pellet by vortex or pipetting.
Step 3 Lysis	• Add **200 µl of PD2 Buffer** and mix gently by inverting the tube 10 times. ***Do not vortex*** to avoid shearing the genomic DNA. • Let stand at room temperature for 2 minutes or until the lysate is homologous.
Step 4 Neutralization	• Add **300 µl of PD3 Buffer** and mix immediately by inverting the tube 10 times. ***Do not vortex***. • Microcentrifuge for 3 minutes.
Step 5 DNA Binding	• Place a **PD Column** in a **2 ml Collection Tube**. • Add the supernatant from Step 4 to the **PD Column** and microcentrifuge for 30 seconds. • Discard the flow-through and place the **PD Column** back in the **2 ml Collection Tube**.
Step 6 Wash	• Add **400 µl of W1 Buffer** into the **PD Column**. • Microcentrifuge for 30 seconds. • Discard the flow-through and place the **PD Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (ethanol added) into the **PD Column**. • Microcentrifuge for 30 seconds. • Discard the flow through and place the **PD Column** back in the **2 ml Collection Tube**. • Microcentrifuge again for 3 minutes to dry the column matrix.
Step 7 DNA Elution	• Transfer the dried **PD Column** to a new microcentrifuge tube. • Add **50 µl of Elution Buffer** or TE into the center of the column matrix. • Let stand for 2 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. • Microcentrifuge for 2 minutes to elute the DNA.



Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Bacterial cells were not lysed completely** • If more than 10 OD_{600} units of bacterial culture are used, dilute into multiple tubes. • Following PD3 Buffer addition, pipetting or inverting will help to ensure the sample is homologous.
	Incorrect DNA Elution Step • Ensure that Elution Buffer is added into the center of the PD Column matrix and is completely absorbed.
	Incomplete DNA Elution • If plasmid DNA are larger than 10 Kb, use preheated Elution Buffer (60~70ºC) in the Elution step.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** • Following the Wash step, dry the PD Column with additional microcentrifugation for 5 minutes.
	RNA contamination • Prior to using PD1 Buffer, be sure RNase A is added.
	Genomic DNA contamination • Do not use overgrown bacterial cultures. • During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination • Following the DNA Binding step, add 400 µl of W1 Buffer into the PD Column and Incubate for 2 minutes at room temperature. Microcentrifuge the PD Column for 30 seconds and proceed with the standard Wash step.