

High-Speed Plasmid Mini Kit

PD100/ PD300
Oct. 2003

Sample: 1-4 ml of bacterial culture

Yield: up to 30 µg of plasmid/ cosmid DNA

Formate: spin column

Operation time: 20 min.

Elution volume: 50-100 µl

*for research use only
not for diagnostic or drug purposes*

Store at room temperature (15-25°C).

Introduction

High-Speed Plasmid Mini Kit is designed for rapid isolation of plasmid or cosmid DNA from 1-4 ml of bacterial cultures. In the process, the modified alkaline lysis method (1) and RNase treatment are used to get cleared cell lysate with minimal genomic DNA and RNA contaminants. In the presence of a chaotropic salt, the plasmid DNA in the lysate binds to glass fiber matrix in the spin column (2). The contaminants are washed with a ethanol-contained wash buffer and finally, the purified plasmid DNA is eluted by low salt elution buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation. Typical yields are 10-20 µg for high-copy number plasmid or 0.5-5 µg for low-copy number plasmid. The entire procedure can be completed in 20 minutes and the purified plasmid DNA is ready for restriction digestion, ligation, PCR, and sequencing reaction.

Quality Control

The quality of High-Speed Plasmid Mini Kit is tested on a lot-to-lot basis. The Kits are tested by isolation of plasmid DNA from 4 ml culture of *E. coli* DH5α which contains the plasmid pBluescript ($A_{600} > 2$ units/ ml). More than 20 µg of plasmid DNA could be quantified with spectrophotometer. 1 µg of the purified plasmid is used on restriction enzyme digestion with *Eco* RI and digested DNA is checked by agarose gel analysis.

Kit Contents

Name	PD100/ 100 prep	PD300/ 300 prep
PD1 Buffer*	25 ml	65 ml
PD2 Buffer**	25 ml	65 ml
PD3 Buffer	45 ml	125 ml
W1 Buffer	45 ml	130 ml
Wash Buffer (concentrated)***	12.5 ml	50 ml
Elution Buffer	6 ml	20 ml
RNase A (50 mg/ ml)	40 µl	120 µl
PD Columns	100 pcs	300 pcs
2 ml Collection Tubes	100 pcs	300 pcs

* add provided RNase A to PD1 Buffer and store at 4°C.

** if precipitates have formed in PD2 Buffer, warm the buffer in a 37°C waterbath to dissolve precipitates.

*** add 50 ml/ 200 ml ethanol to Wash Buffer before first use.

Caution

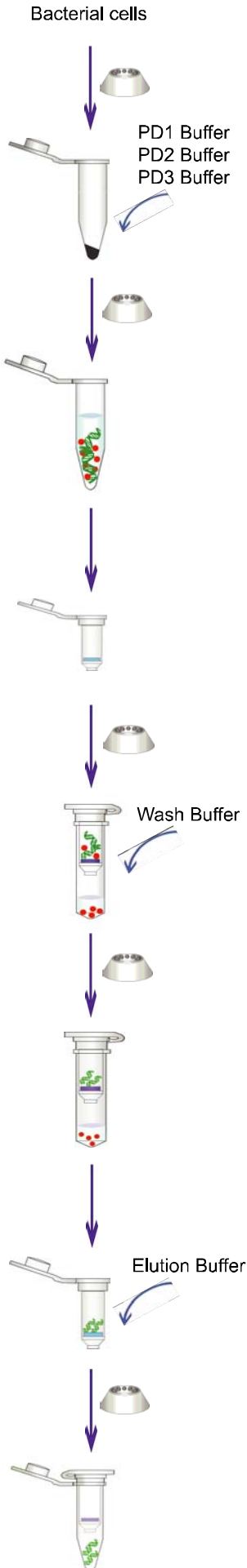
PD3 Buffer contain guanidine hydrochloride which is harmful and irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

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



Step 1 Harvesting	<ul style="list-style-type: none"> Transfer 1.5 ml of bacterial culture to a microcentrifuge tube (not provided). Centrifuge for 1 min at full speed (14,000 rpm) in a microcentrifuge and discard the supernatant. If more than 1.5 ml of bacterial culture is used, repeat the Harvesting Step.
Step 2 Resuspension	<ul style="list-style-type: none"> Add 200 μl of PD1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortexing or pipetting.
Step 3 Lysis	<ul style="list-style-type: none"> Add 200 μl of PD2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, avoid shearing genomic DNA. Stand for 2 minutes at room temperature until lysate clears.
Step 4 Neutralization	<ul style="list-style-type: none"> Add 300 μl of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex. Centrifuge for 3 minutes at full speed.
Step 5 DNA Binding	<ul style="list-style-type: none"> Place a PD Column in a Collection Tube. Apply the clear lysate (supernatant) from Step 4 to the PD Column. Centrifuge at 6000 x g (8,000 rpm) for 30 seconds. Discard the flow-through and place the PD Column back in the Collection Tube.
Step 6 Wash	<ul style="list-style-type: none"> Add 400 μl of W1 Buffer in the PD column. Centrifuge at 6000 x g (8,000 rpm) for 30 seconds. Discard the flow-through and place the PD Column back in the Collection Tube. Add 600 μl of Wash Buffer (ethanol added) in the PD column. Centrifuge at 6000 x g (8,000 rpm) for 30 seconds. Discard the flow-through and place the PD Column back in the Collection Tube. Centrifuge again for 2 minutes at full speed to dry the column matrix.
Step 7 DNA Elution	<ul style="list-style-type: none"> Transfer dried PD Column on a clean microcentrifuge tube (not provided). Add 50 μl of Elution Buffer or water in the center of the column matrix. Stand for 2 minutes until Elution Buffer or water absorbed by the matrix. Centrifuge for 2 minutes at full speed to elute purified DNA.

Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	Bacterial cells were not lysed completely <ul style="list-style-type: none"> Too many bacterial cells were used. If use more than 10 A₆₀₀ units of bacterial culture, separate it into multiple tubes. After PD 3 Buffer addition, break up the precipitate by inverting to ensure higher yield.
	Incorrect DNA Elution Step <ul style="list-style-type: none"> Ensure that Elution Buffer was added and absorbed to the center of PD Column matrix
	Incomplete DNA Elution <ul style="list-style-type: none"> If plasmid DNA are larger than 10 kb, use preheat Elution Buffer (60-70°C) on Elution Step to improve the elution efficiency.
Eluted DNA does not perform well in downstream applications	Residual ethanol contamination <ul style="list-style-type: none"> After wash step, dry PD Column with additional centrifugation at top speed for 5 minutes or incubation at 60°C for 5 minutes.
	RNA contamination <ul style="list-style-type: none"> Prior to using PD1 Buffer, ensure that RNase A was added. If RNase A added PD1 Buffer is out of date, add additional RNase A. Too many bacterial cells were used, reduce sample volume.
	Genomic DNA contamination <ul style="list-style-type: none"> Do not use overgrown bacterial culture. During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing
	Nuclease contamination <p>If used host cells have high nuclease activity (e.g., <i>endA</i>⁺ strains), perform this Optional Wash Step to remove residuary nuclease.</p> <ul style="list-style-type: none"> After DNA Binding Step, add 200 μl of PD3 Buffer into PD column and Incubate for 2 minutes at room temperature. Centrifuge at 6000 x g (8,000 rpm) for 30 seconds. Followed using standard Wash Step.