

High-Speed Plasmid Mini Kit (10-50 Kb)

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 (10-50 Kb) was designed for rapid isolation of plasmid or cosmid DNA from 1-4 ml of cultured bacterial cells and is optimized for isolating DNA from 10-50 Kb plasmid. 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 (10-50 Kb) is tested on a lot-to-lot basis, by isolating plasmid DNA from a 4 ml overnight *E. coli* (DH10β) 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

Order Information

Name	PDL04	PDL100	PDL300
PDL1 Buffer*	1 ml	25 ml	65 ml
PDL2 Buffer**	1 ml	25 ml	75 ml
PDL3 Buffer	1.5 ml	45 ml	100 ml
WL1 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 (10 mM Tris-HCl, pH 8.5 at 25°C)	1 ml	6 ml	30 ml
RNase A (50 mg/ml)	Added	50 µl	130 µl
LP Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	4 pcs	100 pcs	300 pcs

Product Name	Package Size	Cat. No.
High-Speed Plasmid Mini Kit	100/300 preps	PD100/300
High-Speed Plasmid Mini Kit (10-50 Kb)	100/300 preps	PDL100/300
High-Speed Plasmid Advance Kit (50-100 ml bacterial culture)	25 preps	PA025
Geneaid Plasmid Midi Kit	25 preps	PI025
Geneaid Plasmid Midi Kit (Endotoxin Free)	25 preps	PIE25
Geneaid Plasmid Maxi Kit	10/25 preps	PM010/025
Geneaid Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	PME10/E25
96-Well Plasmid Kit	2/4/10 x 96 Wells	PDA02/04/10
Vacuum Manifold (Accessories)	1 Set	ZVF01

*Add provided RNase A to the PDL1 Buffer and store at 4°C

** If precipitates have formed in the PDL2 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve

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

Caution

PDL3 Buffer and WL1 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 (10-50) Protocol

- Add provided RNase A to the PDL1 Buffer and store at 4°C
- If precipitates have formed in the PDL2 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve
- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute ethanol

Step 1 Harvesting	<ul style="list-style-type: none"> ● Transfer 1.5 ml of cultured bacterial cells to a microcentrifuge tube. ● Centrifuge at 14-16,000 x g 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	<ul style="list-style-type: none"> ● Add 200 µl of PDL1 Buffer (RNase A added) to the tube and re-suspend the cell pellet by vortex or pipetting.
Step 3 Lysis	<ul style="list-style-type: none"> ● Add 200 µl of PDL2 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 at least 2 minutes to ensure the lysate is homologous. ● At this time, pre-heat the required Elution Buffer to 70°C (for Step 5 DNA Elution).
Step 4 Neutralization	<ul style="list-style-type: none"> ● Add 300 µl of PDL3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex. ● Centrifuge at 14-16,000 x g for 3 minutes.
Step 5 DNA Binding	<ul style="list-style-type: none"> ● Place a LP Column in a 2 ml Collection Tube. ● Add the supernatant from Step 4 to the LP Column and centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow-through and place the LP Column back in the 2 ml Collection Tube.
Step 6 Wash	<ul style="list-style-type: none"> ● Add 400 µl of WL1 Buffer into the LP Column. ● Centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow-through and place the LP Column back in the 2 ml Collection Tube. ● Add 600 µl of Wash Buffer (ethanol added) into the LP Column. ● Centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow through and place the LP Column back in the 2 ml Collection Tube. ● Centrifuge at 14-16,000 x g again for 3 minutes to dry the column matrix.
Step 7 DNA Elution	<ul style="list-style-type: none"> ● Transfer the dried LP Column to a new microcentrifuge tube. ● Add 50 µl of pre-heated Elution Buffer or TE into the center of the column matrix. ● Let stand for at least 2 minutes to ensure the Elution Buffer or TE is absorbed by the matrix. ● Centrifuge at 14-16,000 x g for 2 minutes to elute the DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	<p>Bacterial cells were not lysed completely</p> <ul style="list-style-type: none"> ● If more than 10 OD₆₀₀ units of bacterial culture are used, dilute into multiple tubes. ● Following PDL3 Buffer addition, pipetting or inverting will help to ensure the sample is homologous. <p>Incorrect DNA Elution Step</p> <ul style="list-style-type: none"> ● Ensure that Elution Buffer is added into the center of the LP Column matrix and is completely absorbed.
Eluted DNA does not perform well in downstream applications	<p>Residual Ethanol contamination</p> <ul style="list-style-type: none"> ● Following the Wash step, dry the LP Column with additional centrifugation at 14-16,000 x g for 5 minutes. <p>RNA contamination</p> <ul style="list-style-type: none"> ● Prior to using PDL1 Buffer, be sure RNase A is added. <p>Genomic DNA contamination</p> <ul style="list-style-type: none"> ● Do not use overgrown bacterial cultures. ● During PDL2 and PDL3 Buffer addition, mix gently to prevent genomic DNA shearing. <p>Nuclease contamination</p> <ul style="list-style-type: none"> ● Following the DNA Binding step, add 400 µl of WL1 Buffer into the LP Column and Incubate for 2 minutes at room temperature. Centrifuge the LP Column at 14-16,000 x g for 30 seconds and proceed with the standard wash step.