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INSTRUCTION MANUAL

High-Speed Plasmid Mini Kit

IB47100, IB47101, IB47102

Advantages

Sample: 1-5 ml of cultured bacterial cells

Binding Capacity: up to 30 µg of plasmid

DNA Format: spin column

Operation Time: within 15 minutes

Elution Volume: 30-100 µl

Storage: dry at room temperature (15-25ºC)

Introduction

The High-Speed Plasmid Mini Kit was designed for rapid isolation of plasmid DNA from 1-5 ml of cultured bacterial cells. A 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. Plasmid DNA can be purified in 15 minutes without DNA phenol extraction or alcohol precipitation. The purified plasmid DNA is then 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 obtained and the A260/A280 ratio is between 1.7 - 1.9. The purified plasmid (1 µg) is used in EcoRI digestion and analyzed by electrophoresis.

Kit Contents

Component	IB47100	IB47101	IB47102
PD1 Buffer*	1 ml	25 ml	65 ml
PD2 Buffer**	1 ml	25 ml	75 ml
PD3 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	100 µl	260 µl
PD Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	4 pcs	100 pcs	300 pcs

* For IB47101 and IB47102 add provided RNase A to PD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Store the PD1 and RNase A mixture at 2-8ºC for up to 6 months. For IB47100 samples, RNase A was already added to PD1.

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

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

Order Information

Product	Package Size	Cat. Number
High-Speed Plasmid Mini Kit	100/300 preps	IB47101/102
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Mini Plasmid Endo Free Kit	100 preps	IB47176
I-Blue Midi Plasmid Kit	25 preps	IB47181
I-Blue Midi Plasmid Kit (Endotoxin Free)	25 preps	IB47191
Midi Fast Ion Plasmid Kit	25 preps	IB47111
Midi Fast Ion Plasmid Kit (Endotoxin Free)	25 preps	IB47113
Maxi Fast Ion Plasmid Kit	10/25 preps	IB47121/122
Maxi Fast Ion Plasmid Kit (Endotoxin Free)	10/25 preps	IB47124/125
96 Well Plasmid Kit	4/10 x 96 preps	IB47151/152

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

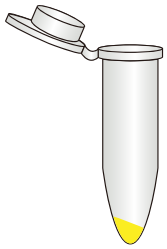
I-Blue Midi Plasmid Kit Protocol

IMPORTANT BEFORE USE!

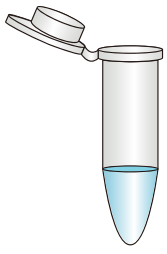
1. For IB47101 and IB47102 add provided RNase A to PD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Store the mixture at 2 — 8°C for up to 6 months.
2. For IB47100 samples, RNase A was already added to PD1.
3. If precipitates have formed in PD2 Buffer, warm in a 37°C water bath, followed by gentle shaking to dissolve.
4. Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use.

Harvesting	• Transfer 1.5 ml of cultured bacterial cells to a microcentrifuge tube. • Centrifuge at 14-16,000 x g for 1 minute then discard the supernatant. • If using more than 1.5 ml of cultured bacterial cells, repeat the Harvesting Step.
Step 1 Re-suspension	• Add 200 µl of PD1 Buffer (make sure RNase A was added) to the tube. • Re-suspend the cell pellet by vortex or pipetting.
Step 2 Lysis	• Add 200 µl of PD2 Buffer (make sure any precipitates are dissolved). • Mix gently by inverting the tube 10 times. NOTE: Do not vortex to avoid shearing the genomic DNA. • Let stand at room temperature for at least 2 minutes (do not exceed 5 minutes). NOTE: This will ensure the lysate is homogeneous.

Quick Protocol

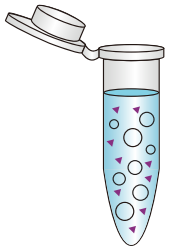


Harvesting

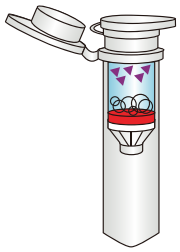


Lysis

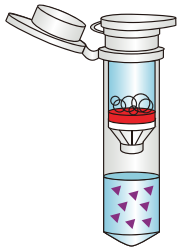
Step 3 Neutralization	• Add 300 µl of PD3 Buffer and mix immediately by inverting the tube 10 times. NOTE: Do not vortex to avoid shearing the genomic DNA.
Step 4 DNA Binding	• Centrifuge at 14-16,000 x g for 3 minutes. • Place a PD Column in a 2 ml Collection Tube. • Add the supernatant from Step 3. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the PD Column back in the 2 ml Collection Tube.
Step 5 Wash	Optional: For Sequencing 1. Add 400 µl of W1 Buffer into the PD Column. 2. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. 3. Place the PD Column back in the 2 ml Collection Tube. 4. Proceed with Wash Buffer addition.
	• Add 600 µl of Wash Buffer (make sure ethanol was added) into the PD Column then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow through. • Place the PD Column back in the 2 ml Collection Tube. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix. • Place the PD Column in a new 1.5 ml microcentrifuge tube.
Step 6 DNA Elution	If a higher DNA concentration is required, use 30 µl of Elution Buffer. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). • Add 50 µl of Elution Buffer or TE into the CENTER of the PD Column matrix. • Let stand for at least 2 minutes to allow the Elution Buffer or TE to be completely absorbed. • Centrifuge at 14-16,000 x g for 2 minutes to elute the purified DNA. NOTE: Performing a second elution step will increase yield between 10 - 30%. • Transfer the flow-through back into the CENTER of the PD Column matrix. • Centrifuge at 14-16,000 x g for 2 minutes to elute the purified DNA.



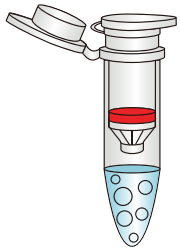
Neutralization



DNA Binding



Wash



Elution

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 PD2 Buffer addition, invert the tube 10 times then let stand at room temperature for 2 minutes to ensure the sample is lysed completely. **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 pre-heated Elution Buffer (60~70ºC) in the Elution step.
Eluted DNA does not perform well in downstream application	**Residual ethanol contamination** • Following the Wash step, dry the PD Column with additional centrifugation at 14-16,000 x g 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. Centrifuge the PD Column at 14-16,000 x g for 30 seconds and proceed with the standard Wash step.

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