High-Speed Plasmid Advance Kit

For research use only

Sample: 50-100 ml of cultured bacterial cells

Yield: up to 500 µg of plasmid/cosmid DNA

Format: spin column

Time: within 40 minutes

Elution volume: 2 ml

Storage: dry at room temperature (15-25ºC) for up to 2 years

CERTIFICATE NO. QAIC/TW/50077

ISO 9001:2008 QMS

Introduction

The High-Speed Plasmid Advance Kit was designed for rapid isolation of plasmid or cosmid DNA from 50-100 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/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, TE Buffer or water. Typical yields are 200-350 µg for high-copy number plasmid or 30-100 µg for low-copy number plasmid from 50 ml of cultured bacterial cells. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 1 hour. 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 Advance Kit is tested on a lot-to-lot basis, by isolating plasmid DNA from a 50 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (A600 > 2 U/ml). Following the purification process, a yield of more than 200 µg is obtained and the ratio of A260/A280 is between 1.7-1.9. The purified plasmid (1 µg) is used in *Eco*R I digestion, and analyzed by electrophoresis.

Kit Contents

Name	PA002	PA025
PD 1 Buffer*	10 ml	110 ml
PD 2 Buffer**	10 ml	110 ml
PD 3 Buffer	15 ml	160 ml
W1 Buffer	20 ml	220 ml
Wash Buffer*** (Add Ethanol)	5 ml (20 ml)	50 ml x 2 (200 ml)
Elution Buffer	6 ml	60 ml
RNase A (50 mg/ml)	40 µl	400 µl
PA Column	2 pcs	25 pcs

Order Information

Product Name	RXNS	Cat. No.
High-Speed Plasmid Mini Kit	100/300	PD100/300
Presto™ Mini Plasmid Kit	100/300	PDH100/300
HS Plasmid Mini Kit (10-50 Kb)	100/300	PDL100/300
HS Plasmid Advance Kit (50-100 ml)	25	PA025
Geneaid™ Midi Plasmid Kit	25	PI025
Geneaid Midi Plasmid Kit (EF)	25	PIE25
Presto™ Midi Plasmid Kit	25	PIF025
Presto™ Midi Plasmid Kit (EF)	25	PIFE25
Presto™ Plasmid DNA Concentration Kit	25/50/100	PC0250/500/1000
Geneaid™ Maxi Plasmid Kit	10/25	PM010/025
Geneaid™ Maxi Plasmid Kit (EF)	10/25	PME10/E25
Presto™ 96 Well Plasmid Kit	4/10 x 96	96PDC04/10 96PDV04/10

*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 to dissolve

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

Caution

PD3 Buffer and W1 Buffer contain guanidine hydrochloride. 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 Advance Kit Protocol

- Add provided RNase A to the PD1 Buffer and store at 4ºC.
- If precipitates have formed in PD2 Buffer, warm the buffer in a 37ºC water bath to dissolve
- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: 50 ml microcentrifuge tubes, absolute ethanol

Harvesting	• Transfer **50 ml of cultured bacterial cells** to a 50 ml centrifuge tube. • Centrifuge at 14-16,000 x g for 5 minutes then discard the supernatant. • Repeat for samples > 50 ml using the same tube.
Step 1 Resuspension	• Add **4 ml of PD1 Buffer** (make sure RNase A was added) to the tube. • Resuspend the cell pellet by vortex or pipetting.
Step 2 Lysis	• Add **4 ml 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 at least 2 minutes to ensure the lysate is homologous.
Step 3 Neutralization	• Add **6 ml of PD3 Buffer** and mix immediately by shaking the tube vigorously for 10 seconds. Do not vortex to avoid shearing the genomic DNA. • Centrifuge at 14-16,000 x g for 10 minutes then transfer the supernatant to a new 50 ml centrifuge tube.
Step 4 DNA Binding	• Place a **PA Column** in a 50 ml microcentrifuge tube. • Add **6 ml of supernatant** to the **PA Column** then centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through then place the **PA Column** back in the 50 ml microcentrifuge Tube. • Add the **remaining supernatant** to the **PA Column** then centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through then place the **PA Column** back in the 50 ml microcentrifuge Tube.
Step 5 Wash	• Add **6 ml of W1 Buffer** into the **PA Column** then centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through then place the **PA Column** back in the 50 ml microcentrifuge tube. • Add **6 ml of Wash Buffer** (make sure ethanol was added) into the **PA Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow through then place the **PA Column** back in the 50 ml microcentrifuge tube. • Add **6 ml of Wash Buffer** (make sure ethanol was added) into the **PA Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow through then place the **PA Column** back in the 50 ml microcentrifuge tube. • Centrifuge at 14-16,000 x g again for 3 minutes to dry the column matrix.
Step 6 DNA Elution	• Transfer the dried **PA Column** to a new 50 ml microcentrifuge tube. • Add **2 ml of Elution Buffer**, TE Buffer or water into the CENTER of the column matrix. • Let stand for 2 minutes. • Centrifuge at 14-16,000 x g 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 PA 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 applications	**Residual ethanol contamination** • Following the Wash step, dry the PA 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 8 ml of W1 Buffer into the PA Column and incubate for 2 minutes at room temperature. • Centrifuge the PA Column at 14-16,000 x g for 5 minutes and proceed with the standard wash step.