

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
Operation time: within 60 minutes
Elution volume: 2 ml

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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. 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 or TE. 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 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	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	Package Size	Cat. No.
High-Speed Plasmid Mini Kit	100/300 preps	PD100/300
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 PD1 Buffer and store at 4°C.

**If precipitates have formed in the PD2 Buffer, warm the buffer in a 37°C water bath 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 Advance Kit Protocol

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

Step 1 Harvesting	<ul style="list-style-type: none"> ● Transfer 50 ml of cultured bacterial cells to a microcentrifuge tube. ● Centrifuge at 14-16,000 x g for 5 minutes and discard the supernatant. ● If more than 50 ml of cultured bacterial cells is used, repeat the Harvesting Step.
Step 2 Re-suspension	<ul style="list-style-type: none"> ● Add 4 ml of PD1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortex or pipetting.
Step 3 Lysis	<ul style="list-style-type: none"> ● 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 2 minutes or until the lysate is homologous.
Step 4 Neutralization	<ul style="list-style-type: none"> ● Add 6 ml of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex. ● Centrifuge at 14-16,000 x g for 10 minutes.
Step 5 DNA Binding	<ul style="list-style-type: none"> ● Place a PA Column in a 50 ml microcentrifuge tube. ● Add the supernatant from Step 4 to the PA Column (be sure and twist the 50 ml microcentrifuge tube lid on tightly) and centrifuge at 14-16,000 x g for 3 minutes. ● Discard the flow-through and place the PA Column back in the 50 ml microcentrifuge Tube.
Step 6 Wash	<ul style="list-style-type: none"> ● Add 8 ml of W1 Buffer into the PA Column (be sure and twist the 50 ml microcentrifuge tube lid on tightly). ● Centrifuge at 14-16,000 x g for 3 minutes. ● Discard the flow-through and place the PA Column back in the 50 ml microcentrifuge tube. ● Add 12 ml of Wash Buffer (ethanol added) into the PA Column (be sure and twist the 50 ml microcentrifuge tube lid on tightly). ● Centrifuge at 14-16,000 x g for 3 minutes. ● Discard the flow through and 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 7 DNA Elution	<ul style="list-style-type: none"> ● Transfer the dried PA Column to a new 50 ml microcentrifuge tube. ● Add 2 ml of Elution Buffer or TE into 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 <ul style="list-style-type: none"> ● If more than 10 OD₆₀₀ 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 <ul style="list-style-type: none"> ● Ensure that Elution Buffer is added into the center of the PA Column matrix and is completely absorbed.
	Incomplete DNA Elution <ul style="list-style-type: none"> ● If plasmid DNA are larger than 10 Kb, use preheated Elution Buffer (60–70°C) in the Elution step.
Eluted DNA does not perform well in downstream applications	Residual ethanol contamination <ul style="list-style-type: none"> ● Following the Wash step, dry the PA Column with additional centrifugation at 14-16,000 x g for 5 minutes.
	RNA contamination <ul style="list-style-type: none"> ● Prior to using PD1 Buffer, be sure RNase A is added.
	Genomic DNA contamination <ul style="list-style-type: none"> ● Do not use overgrown bacterial cultures. ● During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination <ul style="list-style-type: none"> ● 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.