Midiprep Spin Column Plasmid Kit

For research use only

Sample: 50-100 ml of cultured bacterial cells

Binding Capacity: up to 400 µg of plasmid DNA

Format: midiprep spin column

Operation Time: within 40 minutes

Elution Volume: 1 ml

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

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Midiprep Spin Column Plasmid Kit was designed for rapid isolation of plasmid or cosmid DNA from 50-100 ml of cultured bacterial cells. A modified alkaline lysis method 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. 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 40 minutes. The purified plasmid DNA is ready for use in restriction enzyme digestion, ligation, PCR, and sequencing reactions.

Quality Control

The quality of the Midiprep Spin Column Plasmid Kit it 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*RI digestion, and analyzed by electrophoresis.

Kit Contents

Component	PA002	PA025
PD1 Buffer*	10 ml	110 ml
PD2 Buffer**	10 ml	110 ml
PD3 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	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
Large Plasmid DNA Extraction Kit	100/300 preps	PDL100/300
Midiprep Spin Column Plasmid Kit	25 preps	PA025
Geneaid™ Plasmid Midi Kit	25 preps	PI025
Geneaid™ Plasmid Midi Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Plasmid Maxi Kit	10/25 preps	PM010/25
Geneaid™ Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10

*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.
**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.

Caution

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Midiprep Spin Column Plasmid Kit Test Data

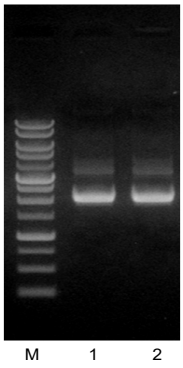

Figure 1. Plasmid DNA was extracted using the Midiprep Spin Column Plasmid Kit. 5 µl aliquots of purified supercoiled plasmid DNA [100 ml overnight *E. coli* (DH5α) culture, containing a 3 kb plasmid pBluescript] from a 1 ml eluate were analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid™ 1 Kb DNA Ladder
Lane 1-2: pBluescript

Sample	ng/µl	260/280	260/230	Yield (µg)
1	383.5	1.94	1.94	383.5
2	384.8	1.92	1.94	384.8

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IMPORTANT BEFORE USE!

1. 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.
2. If precipitates have formed in PD2 Buffer, warm in a 37ºC water bath, followed by gentle shaking to dissolve.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use.

Additional requirements: 50 ml centrifuge tubes, absolute ethanol

Harvesting	• Transfer **50 ml of cultured bacterial cells** to a 50 ml centrifuge tube then centrifuge at 6,000 x g for 5 minutes. • Discard the supernatant then repeat for 100 ml samples 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. 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).
Step 3 Neutralization	• Add **6 ml of PD3 Buffer** and mix immediately by shaking the tube vigorously for 10 seconds. NOTE: Do not vortex to avoid shearing the genomic DNA. • Centrifuge at 6,000 x g for 10 minutes.
Step 4 DNA Binding	• Place a **PA Column** in a 50 ml centrifuge tube then transfer the supernatant to the PA Column. • Centrifuge at 6,000 x g for 3 minutes. • Discard the flow-through then place the **PA Column** back in the 50 ml centrifuge tube.
Step 5 Wash	**Optional Endonuclease Removal Wash Step** • Add 6 ml of W1 Buffer into the PA Column then centrifuge at 6,000 x g for 3 minutes. • Discard the flow-through then place the PA Column back in the 50 ml centrifuge tube. NOTE: This optional wash step is recommended if using EndA+ bacterial strains such as *E. coli* HB 101, JM, or wild-type strains. However, plasmid DNA yield will be reduced by 20%. **For Standard Plasmid DNA Purification** • Add **12 ml of Wash Buffer (make sure ethanol was added)** into the **PA Column**. • Centrifuge at 6,000 x g for 3 minutes, discard the flow-through then place the **PA Column** back in the 50 ml tube. • Centrifuge for another 5 minutes at 6,000 x g to dry the column matrix.
Step 6 DNA Elution	• Transfer the dry **PA Column** to a new 50 ml centrifuge tube. • Add **1 ml of Elution Buffer**, TE Buffer or H_2O into the CENTER of the column then let stand for 2 minutes. • Centrifuge at 6,000 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 6 OD_{600} units of bacterial culture are used, dilute in multiple tubes. • Following PD3 Buffer addition, pipetting or inverting will help to ensure the sample is homogenous.
	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 6,000 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 6,000 x g for 5 minutes and proceed with the standard wash step.