

# Geneaid Plasmid Midi Kit

*For research use only*

- Sample** : 50 ml of cultured bacterial cells for high-copy number plasmid  
100 ml of cultured bacterial cells for low-copy number plasmid
- Yield** : up to 250 µg of plasmid DNA
- Format** : gravity flow
- Operation time** : 120 minutes

**Geneaid**



[www.geneaid.com](http://www.geneaid.com)

## Introduction

The Geneaid Plasmid Midi Kit uses pre-packed anion-exchange resin columns to purify plasmid 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. Using an efficient gravity-flow procedure, plasmid DNA in the crude lysate is bound to the column and the contaminants are removed with the PW Buffer. The purified plasmid DNA is eluted by a high salt buffer and then precipitated with Isopropanol for desalting. The entire procedure can be completed in 2 hours without ultracentrifuges, HPLC or other toxic reagents and the purified plasmid DNA is suitable for Transfection, Sequencing Reactions, Ligation, PCR, and In-vitro Transcription, Microinjection, Restriction Enzyme Digestion and Gene Gun.

## Quality Control

The quality of the Geneaid Plasmid Midi 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). More than 120 µg of plasmid DNA is quantified with a spectrophotometer. The purified plasmid (1 µg) is used in EcoR I digestion, and checked by electrophoresis.

### Kit Contents

Name	PI002	PI025
PM 1 Buffer*	10 ml	110 ml
PM 2 Buffer**	10 ml	110 ml
PM 3 Buffer	10 ml	110 ml
PEQ Buffer	12 ml	130 ml
PW Buffer	30 ml	360 ml
PEL Buffer	25 ml	220 ml
RNase A (50 mg/ml)	Added	200 µl
Plasmid Midi 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 PM1 Buffer and store at 4°C.

\*\*If precipitates have formed in the PM2 Buffer, warm the buffer in a 37°C water bath to dissolve.

## Caution

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

## References

- (1) Birnboim, H. C., and Doly, J. (1979) *Nucleic Acids Res.* 7, 1513

## Geneaid Plasmid Midi Kit Protocol

- Use 50 ml of cultured bacterial cells for high-copy-number plasmid and 100 ml of cultured bacterial cells for low-copy number plasmid.
- Additional requirements: 50 ml centrifuge tubes, Isopropanol, 75% ethanol

Step 1 Cell Harvesting/ Column Equilibration	<ul style="list-style-type: none"> <li>● Harvest the bacterial culture by centrifugation at 6,000 x g for 15 minutes and discard the supernatant completely.</li> <li>● Place a <b>Plasmid Midi Column</b> in a 50 ml centrifuge tube.</li> <li>● Equilibrate the <b>Plasmid Midi Column</b> by adding <b>5 ml of PEQ Buffer</b>; allow the column to empty by gravity flow.</li> <li>● Discard the filtrate and place the <b>Plasmid Midi Column</b> back in the 50 ml centrifuge tube.</li> </ul>
Step 2 Resuspension	<ul style="list-style-type: none"> <li>● Add <b>4 ml of PM1 Buffer</b> (RNase A added) to resuspend the cell pellet completely by vortex or pipetting.</li> </ul>
Step 3 Lysis	<ul style="list-style-type: none"> <li>● Add <b>4 ml of PM2 Buffer</b> and mix gently by inverting the tube 10 times (be sure and mix completely). <i>Do not vortex</i>, to avoid shearing the genomic DNA.</li> <li>● Let stand at room temperature for 2 minutes or until the lysate clears.</li> </ul>
Step 4 Neutralization	<ul style="list-style-type: none"> <li>● Add <b>4 ml of PM3 Buffer</b> and mix immediately by gently inverting the tube 10 times. <i>Do not vortex</i>.</li> <li>● Centrifuge at 15,000 x g for 20 minutes at room temperature.</li> </ul>
Step 5 DNA Binding	<ul style="list-style-type: none"> <li>● Transfer the supernatant from Step 4 to the <b>Plasmid Midi Column</b> and allow it to flow through by gravity flow.</li> <li>● Discard the flow through and place the <b>Plasmid Midi Column</b> back in the 50 ml centrifuge tube.</li> </ul>
Step 6 Wash	<ul style="list-style-type: none"> <li>● Wash the <b>Plasmid Midi Column</b> by adding <b>12 ml of PW Buffer</b>; allow the column to empty by gravity flow.</li> <li>● Discard the filtrate.</li> </ul>
Step 7 DNA Elution	<ul style="list-style-type: none"> <li>● Place the <b>Plasmid Midi Column</b> in a clean 50 ml centrifuge tube and add <b>8 ml of PEL Buffer</b> to elute the DNA by gravity flow.</li> </ul>
Step 8 DNA Precipitation	<ul style="list-style-type: none"> <li>● Precipitate DNA by adding 6 ml (0.75 volumes) of Isopropanol to the eluted DNA from Step 7.</li> <li>● Mix gently and centrifuge at 15,000 x g for 30 minutes at 4°C.</li> <li>● Carefully remove the supernatant and wash the DNA pellet with 5 ml of 75% ethanol.</li> <li>● Centrifuge at 15,000 x g for 10 minutes at 4°C.</li> <li>● Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.</li> <li>● Dissolve the DNA in 2 ml (or a suitable volume) of TE or ddH<sub>2</sub>O.</li> </ul>

## Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	<p><b>Bacterial cells were not lysed completely</b></p> <ul style="list-style-type: none"> <li>● Too many bacterial cells were used.</li> <li>● Following PM3 Buffer addition, mix completely by inverting to ensure higher yield.</li> </ul>
Purified DNA does not perform well in downstream applications	<p><b>RNA contamination</b></p> <ul style="list-style-type: none"> <li>● Prior to using PM1 Buffer, check that RNase A is added. If the RNase A added PM1 Buffer is out of date, add additional RNase A.</li> <li>● If too many bacterial cells were used, reduce the sample volume.</li> </ul> <p><b>Genomic DNA contamination</b></p> <ul style="list-style-type: none"> <li>● Do not use overgrown bacterial culture samples.</li> <li>● During the PM2 and PM3 Buffer addition steps, mix the solution gently to prevent genomic DNA shearing.</li> </ul>