

Geneaid Plasmid Midi Kit (Endotoxin Free)

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 150 µg of plasmid DNA
- Format** : gravity flow
- Operation time** : 120 minutes

Geneaid



www.geneaid.com

Introduction

The Geneaid Plasmid Midi Kit (Endotoxin Free) 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 (Endotoxin Free) 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	PIE02	PIE25
PM 1 Buffer*	10 ml	110 ml
PM 2 Buffer**	10 ml	110 ml
PM 3 Buffer	10 ml	110 ml
PER Buffer	4 ml	40 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 Mini Kit (10-50 Kb)	100/300 preps	PDL100/300
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 (Endotoxin Free)

- 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, TE or ddH₂O

Step 1 Cell Harvesting/ Column Equilibration	<ul style="list-style-type: none"> ● Harvest the bacterial culture by centrifugation at 6,000 x g for 15 minutes and discard the supernatant completely. ● Place a Plasmid Midi Column in a 50 ml centrifuge tube. ● Equilibrate the Plasmid Midi Column by adding 5 ml of PEQ Buffer; allow the column to empty by gravity flow. ● Discard the filtrate and place the Plasmid Midi Column back in the 50 ml centrifuge tube.
Step 2 Re-suspension	<ul style="list-style-type: none"> ● Add 4 ml of PM1 Buffer (RNase A added) to re-suspend the cell pellet completely by vortex or pipetting.
Step 3 Lysis	<ul style="list-style-type: none"> ● Add 4 ml of PM2 Buffer and mix gently by inverting the tube 15 times (be sure and mix completely). Do not vortex, to avoid shearing the genomic DNA. ● Let stand at room temperature for at least 2 minutes to ensure the lysate is clear.
Step 4 Neutralization	<ul style="list-style-type: none"> ● Add 4 ml of PM3 Buffer and mix immediately by shaking the tube vigorously for 10 seconds. Do not vortex. ● Centrifuge at 6,000 x g for 20 minutes at room temperature.
Step 5 Endotoxin Removal	<ul style="list-style-type: none"> ● Transfer the supernatant from Step 4 to a clean 50 ml centrifuge tube and mix with 1.2 ml of PER Buffer (invert PER Buffer bottle 3-5 times before use) and incubate on ice for 30 minutes. Note: Following PER Buffer addition, the solution will become cloudy. This does not affect the plasmid purity; however, recovery may be decreased by as much as 20%.
Step 6 DNA Binding	<ul style="list-style-type: none"> ● Transfer the mixture from Step 5 to the Plasmid Midi Column and allow it to flow through by gravity flow. ● Discard the flow through and place the Plasmid Midi Column back in the 50 ml centrifuge tube.
Step 7 Wash	<ul style="list-style-type: none"> ● Wash the Plasmid Midi Column by adding 12 ml of PW Buffer; allow the column to empty by gravity flow. ● Discard the filtrate.
Step 8 DNA Elution	<ul style="list-style-type: none"> ● Place the Plasmid Midi Column in a clean 50 ml centrifuge tube and add 8 ml of PEL Buffer to elute the DNA by gravity flow.
Step 9 DNA Precipitation	<ul style="list-style-type: none"> ● Precipitate DNA by adding 6 ml (0.75 volumes) of isopropanol to the eluted DNA from Step 8. ● Mix the tube completely and then centrifuge at 15,000 x g for 30 minutes at 4°C. ● Carefully remove the supernatant and wash the DNA pellet with 5 ml of 75% ethanol. ● Centrifuge at 15,000 x g for 10 minutes at 4°C. ● Carefully remove the supernatant and air-dry the DNA pellet for at least 10 minutes in order to completely evaporate ethanol residue. ● Dissolve the DNA in 2 ml (or a suitable volume) of TE or ddH₂O.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	<p>Bacterial cells were not lysed completely</p> <ul style="list-style-type: none"> ● Too many bacterial cells were used. ● Following PM3 Buffer addition, break up the precipitate by inverting to ensure higher yield.
Purified DNA does not perform well in downstream applications	<p>RNA contamination</p> <ul style="list-style-type: none"> ● 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. ● If too many bacterial cells were used, reduce the sample volume. <p>Genomic DNA contamination</p> <ul style="list-style-type: none"> ● Do not use overgrown bacterial culture samples. ● During the PM2 and PM3 Buffer addition steps, mix the solution gently to prevent genomic DNA shearing.