

Geneaid Plasmid Maxi Kit (Endotoxin Free)

For research use only

- Sample** : 100 ml of cultured bacterial cells for high-copy number plasmid
250 ml of cultured bacterial cells for low-copy number plasmid
- Yield** : up to 500 µg of plasmid DNA
- Format** : gravity flow
- Operation time:** 150 minutes

Geneaid



CERTIFICATE NO. 0604722840
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Introduction

The Geneaid Plasmid Maxi Kit (Endotoxin Free) uses pre-packed anion-exchange resin columns to purify plasmid DNA from 100-250 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 2.5 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 Maxi Kit (Endotoxin Free) is tested on a lot-to-lot basis by isolating plasmid DNA from a 100 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (A600 > 2 U/ml). More than 400 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	PME02	PME10	PME25
PM 1 Buffer*	25 ml	110 ml	275 ml
PM 2 Buffer**	25 ml	110 ml	275 ml
PM 3 Buffer	25 ml	110 ml	275 ml
PER Buffer	8 ml	40 ml	100 ml
PEQ Buffer	25 ml	130 ml	275 ml
PW Buffer	65 ml	360 ml	790 ml
PEL Buffer	25 ml	130 ml	350 ml
RNase A (50 mg/ml)	Added	200 µl	550 µl
Plasmid Maxi Column	2 pcs	10 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 Maxi Kit (Endotoxin Free) Protocol

- Use 100 ml of cultured bacterial cells for high-copy number plasmid and 250 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 Maxi Column in a 50 ml centrifuge tube. ☛ Equilibrate the Plasmid Maxi Column by adding 10 ml of PEQ Buffer; allow the column to empty by gravity flow. ☛ Discard the filtrate and place the Plasmid Maxi Column back in the 50 ml centrifuge tube.
Step 2 Re-suspension	<ul style="list-style-type: none"> ☛ Add 10 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 10 ml of PM2 Buffer and mix gently by inverting the tube 15 times (be sure to 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 10 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 3 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 Maxi Column and allow it to flow through by gravity flow. ☛ Discard the filtrate and place the Plasmid Maxi Column back in the 50 ml centrifuge tube.
Step 7 Wash	<ul style="list-style-type: none"> ☛ Wash the Plasmid Maxi Column by adding 30 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 Maxi Column in a clean 50 ml centrifuge tube and add 12 ml of PEL Buffer to elute the DNA by gravity flow.
Step 9 DNA Precipitation	<ul style="list-style-type: none"> ☛ Precipitate the DNA by adding 9 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 pellet 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, mix completely 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.