ISO 9001/14001 Certified Company

DNA-midi™ GT Plasmid DNA Purification Kit

Cat. No. 17254 25 Columns

DESCRIPTION

DNA-Midi™ GT Plasmid DNA Purification Kit is designed for efficient purification of high quality plasmid DNA from bacterial cells. This kit provides alkaline lysis reagents and columns packed with anion-exchanger resins. The entire midi prep process can be completed within 120 minutes through gravity-flow procedure.

The plasmid DNA is free from protein, genomic DNA and RNA contaminants. This pure plasmid DNA is suitable for downstream applications such as transfection, *in vitro* transcription and translation, and all enzymatic modifications.

KIT CONTENTS

- CPE Buffer 135 ml
- M1 Buffer (Resuspension Buffer) 215 ml
  : Briefly spin the dissolved RNase A solution and add the RNase A solution to M1 Buffer. Store M1 Buffer at 4℃ after adding RNase A solution.
- M2 Buffer (Lysis Buffer) 215 ml
  : If precipitates have formed in M2 Buffer, warm the buffer in 37℃ waterbath to dissolve precipitates.
- M3 Buffer (Neutralization Buffer) 215 ml
- Washing Buffer 270 ml + 60 ml
- Elution Buffer 215 ml
- RNase A (Lyophilized powder) 50mg/ml
  : Dissolve the RNase A in 430 ul of pure D.W. to vial. The lyophilized RNase A can be stored at room temperature (15-25℃) until the expiration date without affecting performance. The lyophilized RNase A only can be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20℃ for up to 24 months. DO NOT refreeze aliquots after thawing.
- Binding Column 25 pcs

STORAGE

DNA-Midi™ GT Plasmid DNA Purification Kit should be stored at room temperature (15-25℃).

ADDITIONAL REQUIREMENTS

- 50ml Centrifuge tube
- isopropanol
- 70% ethanol

APPLICATIONS

Plasmid DNA prepared using the DNA-Midi™ GT Plasmid DNA Purification Kit is suitable for a variety of routine applications including

- Restriction enzyme digestion
- Library screening
- *in vitro* translation
- Sequencing
- Ligation and transformation
- Transfection of robust cells

SPECIFICATION

Sample Size : up to 60 ml of bacteria for high-copy number plasmid
up to 120 ml of bacteria for low-copy number plasmid
Binding capacity : 650 ug / column

SAFETY INFORMATION

All chemicals should be considered as potentially hazardous chemicals. When working with chemicals, always wear a suitable lab coat and disposable gloves. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water.

If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only person trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

• DO NOT add bleach or acidic solutions directly to the sample preparation waste.

GENARAL PROTOCOL

1. Inoculate LB medium containing the appropriate antibiotic either with a single colony of transformed bacteria or with 0.2 ~ 1.0 ml of a small-scale LB culture grown from a single colony.
2. Incubate the culture at 37℃ with vigorous shaking (approx. 300 rpm) for 12-16 hrs.
   - If you use ampicillin as an antibiotic for culture (OD_{600} 1.5 ~ 2.0), we recommend to increase of your working ampicillin concentration to 200 ~ 300 ㎍/mL to sustain selective antibiotic pressure for obtaining higher plasmid yield.
3. Place a Binding column into a 50 ml centrifuge tube for equilibration. Add 5 ml of CPE Buffer to equilibrate the Binding column and allow the column to empty by gravity flow. Discard the filtrate.
4. Harvest the bacterial culture by centrifugation at 6,000 × g for 15 minutes.
5. Add 8 ml of M1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
6. Add 8 ml of M2 Buffer and mix gently by inverting the tube 15 times. Do not vortex to avoid shearing genomic DNA.
7. Incubate for 3 minutes at room temperature until lysate clears.
8. Add 8 ml of M3 Buffer and mix immediately by inverting the tube 10 times (Do not vortex).
9. Centrifuge at 15,000 × g for 20 minutes at 4℃. Remove supernatant containing plasmid DNA promptly.
   - Centrifuge speed should not be less than 15,000 x g.
   - If the white debris remains in supernatant, centrifuge again for 5 minutes to avoid applying suspended or particulate material to the Binding column.
10. Transfer the supernatant to the equilibrated Binding Column and allow the column to empty by gravity flow. Discard the filtrate.
11. Add 12 ml of Washing Buffer to wash the Binding Column and allow the column to empty by gravity flow. Discard the filtrate.
12. Place the Binding Column into a clean 50 ml centrifuge tube (not provided) and add 8 ml of Elution Buffer to elute DNA by gravity flow.
13. Precipitate DNA by adding 6 ml of isopropanol to the eluted DNA from previous step. Mix well by inverting the tube 10 times.
14. Centrifuge at 20,000 x g for 30 minutes at 4℃.
    - Centrifuge speed should not be less than 20,000 x g.
    - Gently remove the isopropanol after centrifugation step.
15. Carefully remove the supernatant and wash the DNA pellet with 5 ml of room temperature 70% ethanol.
16. Centrifuge at 20,000 x g for 10 minutes at 4℃.
    - Centrifuge speed should not be less than 20,000 x g.
    - Gently remove the 70% ethanol after centrifugation step. If the centrifuge tube is left to stand for too long, the pellet of DNA will become detached from the wall.
17. Carefully remove the supernatant. Then air-dry the DNA pellet until the tube is completely dried. (Or incubate the DNA pellet at 70℃ for 10 min.)
18. Dissolve the DNA pellet in 300 ul or a suitable volume of TE buffer or ddH_2O.

TROUBLESHOOTING

Purified DNA dose not perform well in downstream application

RNA Contamination

- Make sure that RNase A has been added in M1 Buffer (Resuspension Buffer) when first using. If RNase A added M1 Buffer (Resuspension Buffer) is overdue, add RNase A again.
- Too many bacterial cells were used, reduce the sample volume.

Genomic DNA contamination

- Do not use overgrown bacterial culture.
- During M2 Buffer (Lysis Buffer) and M3 Buffer (Neutralization Buffer) addition, mix gently to prevent genomic DNA shearing.
- Lysis time was too long (over 5 minutes).

Low yield

M2 Buffer (Lysis Buffer) is precipitated

- If precipitates are formed in M2 Buffer (Lysis Buffer), warm the buffer in 37℃ waterbath to dissolve precipitates.

Cell resuspension incomplete

- Pelleted cells should be completely resuspended in M1 Buffer (Resuspension Buffer). Do not add M2 Buffer (Lysis Buffer) until an even suspension is obtained.

Bacterial cells were not lysed completely

- Too many bacterial cells were used.
- After M3 Buffer (Neutralization Buffer) addition, break up the precipitate by inverting.
- DNA pellet was insufficiently redissolved.
- DNA failed to precipitate or DNA pellet was lost after precipitation.

Plasmid did not propagate

- Check the bacterial culture conditions (age of culture, antibiotics, culture volume and bioreactor).

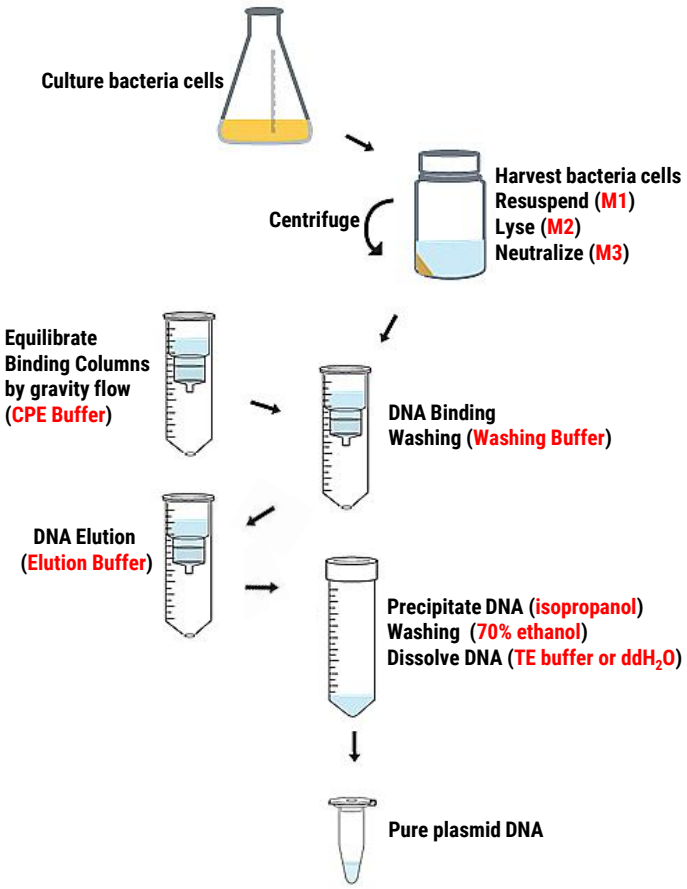
Column was overloaded with DNA

- Check the culture volume and yield for use, and reduce the culture volume accordingly.

Too much salt residual in DNA pellet

- Wash the DNA pellet twice with 70% ethanol.

BRIEF PROCEDURE



TECHNICAL ADVICE

❖ **General Protocol**

Ensure that RNase A solution has been added to M1 Buffer (Resuspension Buffer) It is essential to completely resuspend the cell pellet. It may affect the lysis efficiency. Long exposure to alkaline condition may cause the plasmid to become irreversibly denatured. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps. Do not vortex, it may cause shearing of genomic DNA. If the M2 Buffer (Lysis Buffer) is stored under the cold condition SDS precipitation may occur. It may cause the poor cell lysis. Therefore, before using the M2 Buffer, warm it in 37℃ water bath to dissolve the SDS.

❖ **Growth of Bacterial Cultures**

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic. The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic, and type of culture medium. High-copy number plasmids and large quantities of recombinant proteins can severely hamper the growth, and even the survival, of transformed cells. To prevent the emergence of bacteria from which the plasmid has been eliminated, it is important to sustain selective pressure by including the appropriate antibiotic in the culture medium at all times.

❖ **Plasmid Copy Numbers**

Plasmids vary widely in their number per cell, depending on their origin of replication (e.g., pMB1, ColE1, or pSC101) which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmids and cosmids are often maintained at very low copy numbers per cell.

DNA construct	Origin replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1	500~700	High copy
pBluescript vectors	ColE1	300~500	High copy
pGEM® vectors	pMB1	300~400	High copy
pTZ vectors	pMB1	> 1000	High copy
pBR322 and derivatives	pMB1	15~20	Low copy
pACYC and derivatives	p15A	10~12	Low copy
pSC101 and derivatives	pSC10	1~5	Very low copy
Cosmids			
SuperCos	ColE1	10~20	Low copy
pWE15	ColE1	10~20	Low copy

RELATIVE PRODUCTS

Product Name	Cat. No.
DNA-spin™ Plasmid DNA Purification Kit	17096/17097/17098
DNA-midi™ SV Plasmid DNA Purification Kit	17252
DNA-maxi™ SV Plasmid DNA Purification Kit	17253
MEGAquick-spin™ Total Fragment DNA Purification Kit	17286/17287/17288
LINKeed®Rapid DNA Ligation Kit (Version 2.0)	15023
MacCell™ DH5α (10^7)	15052
MacCell™ DH5α (10^8)	15053
MacCell™ DH5α (10^9)	15054
MacCell™ TOP10 (10^7)	15055
MacCell™ TOP10 (10^8)	15056
MacCell™ TOP10 (10^9)	15057
Maxime™ PCR PreMix (i-Taq)	25025
Maxime™ PCR PreMix (i-StarTaq)	25165/25167
Maxime™ PCR PreMix (i-Pfu)	25185
Maxime™ PCR PreMix (i-MAX Ⅱ)	25265
Genelator™ in vitro Transcription & Translation Kit	12011
α-Complementation Solution	15032