DNA-midi™ GT Plasmid DNA Purification Kit

RUO *For research purpose only.*

REF 17254   Σ 25   15℃–25℃

INTRODUCTION

- DNA-Midi™ GT Plasmid DNA Purification Kit is designed for efficient purification of high quality plasmid DNA from bacterial cell.
- 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.

CHARACTERISTICS

- Room Temperature Storage : Can store 2 years at room temperature (15-25 °C).
- Application : Restriction enzyme digestion, Sequencing, Library screening, Ligation and transformation, in vitro translation, Transfection of robust cells.
- Technology : Anion-exchange chromatography (gravity-flow column)
- Lysate clarification : Centrifugation
- Sample Size: up to 60 ml of bacteria for high-copy number plasmid, up to 120 ml of bacteria for low-copy number plasmid
- Plasmid or construct range : 3 kbp ~ 150 kbp
- Binding Capacity : 650 ug/Midi Column
- Shortening the experiment time : Experiment can be completed in 100 ~ 120 minutes.

KIT CONTENTS

Label	Contain
CPE Buffer	**135 ml**
M1 Buffer[1] (Resuspension Buffer)	**215 ml**
M2 Buffer[2] (Lysis Buffer)	**215 ml**
M3 Buffer[3] (Neutralization Buffer)	**215 ml**
Washing Buffer	**270 ml + 60 ml**
Elution Buffer	**215 ml**
RNase A Solution	**480 ul**
Binding Column	**25 pcs**

1. Briefly spin the RNase A solution and add the RNase A solution to M1 Buffer. And Store the M1 Buffer at 4℃ after adding RNase A solution .
2. If precipitates have formed in M2 Buffer, warm the buffer in 37℃ water bath to dissolve precipitates.
3. Pre-chill M3 Buffer at 4℃ before starting.

INTENDED TO USE

- This product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.
- Bacterial cell culture extraction and detection of its research.

MATERIALS REQUIRED BUT NOT PROVIDED

- 50 ml tubes
- Isopropanol
- Refrigerated centrifuge
- 70 % ethanol
- TE buffer or ddH_20

STORAGE AND STABILITY

- All buffers of DNA-Midi™ GT Plasmid DNA Purification Kit should be stored at room temperature (15–25 °C) for up to 24 months without showing any reduction in performance and quality.
- Exceptionally, RNase A Solution should be stored at -20 °C from receipt to use.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

- All products are undergone extensive quality control test and are warranted to perform as described when used correctly.
- Immediately any problems should be reported.
- Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

NOTICE

1. All chemicals should be considered as potentially hazardous chemicals.
2. When working with chemicals, always wear a suitable lab coat and disposable gloves.
3. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water.
4. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant.
5. 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.**

PROTOCOLS

※ Before You Begin

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.**
   [Note] : If you use ampicillin as an antibiotic for culture (OD600 1.5 ~ 2.0), we recommend to increase of your working ampicillin concentration to 200 ~ 300 μg/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.**
   [Note] : Centrifuge speed should not be less than 20,000 × g.
   [Note] : Gently remove the isopropanol after centrifugation step.
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℃.**
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℃.**
   [Note] : Centrifuge speed should not be less than 20,000 × g.
   [Note] : 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 ddH2O.**

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Belief & Relief, LiliF Diagnostics MDx Kit
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TROUBLE SHOOTING GUIDE

Problem	Possible Cause	Recommendation
Purified DNA does not perform well in downstream application	RNA Contamination	• Make sure that RNase A has been added in M1 Buffer when first using. If added M1 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 and M3 Buffer addition, mix gently to prevent genomic DNA shearing.
		• Lysis time was too long. (over 5 minutes)
Low yield	M2 Buffer is precipitated	• If precipitates are formed in M2 Buffer, warm the buffer in 37℃ water bath to dissolve precipitates
	Cell resuspension incomplete	• Pelleted cells should be completely resuspended in M1 Buffer. Do not add M2 Buffer until an even suspension is obtained.
	Bacterial cells were not lysed completely	• Too many bacterial cells were used.
		• After M3 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.
	Column was overloaded with DNA	• Check the culture volume and yield for use, and reduce the culture volume accordingly.
	Too much salt residual in pellet	• Wash the pellet twice with 70% ethanol.

TECHNICAL INFORMATIONS

※ General Protocol

- Ensure that RNase A solution has been added to M1 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.
- It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps.
- If the M2 Buffer is stored under the cold condition, SDS precipitation may occur. (It may cause the poor cell lysis.)
- Before using the M2 Buffer, warm it in 37 ℃ water bath to dissolve the SDS.

※ Growth of Bacterial Cultures

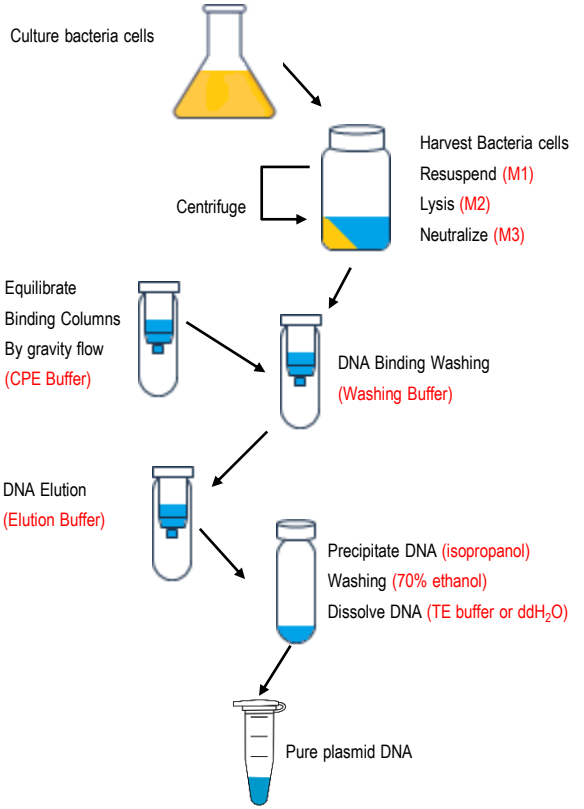
- Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic.
- The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic, and type of culture medium.
- High-copy number plasmids 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
Plasmid			
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

BRIEF PROCEDURE



RELATED PRODUCTS

Product Name	Cat. No.
DNA-spin™ Plasmid DNA Purification Kit	17096 / 17098
DNA-maxi™ SV Plasmid DNA Purification Kit	17253
MEGAquick-spin™ Plus Total Fragment DNA Purification Kit	17290
Maxime™ PCR PreMix (i-Taq)	25025 / 25026
Maxime™ PCR PreMix (i-StarTaq)	25165 / 25167
Maxime™ PCR PreMix (i-Pfu)	25185
Maxime™ PCR PreMix (i-MAX II)	25265

LiliF Diagnostics

EXPLANATION OF SYMBOLS

Manufactured by | Attention | Manufacturing date | Expire date | Keep away from sunlight | Sufficient for tests | Consult Instructions For Use | Storage temperature limitation | Batch number (LOT) | Research use only (RUO) | Product number (REF)

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