

PLASMID DNA EXTRACTION MIDI AND MAXI KIT

Description

The Plasmid Extraction Midi and Maxi Kit provides pre-packed an ion exchange resin column for high-purity plasmid DNA maxiperp from 50 - 300 ml bacterial cultures. Depending on the copy-number of plasmids, either a 50-150ml (low-copy plasmid) or a 150 - 500 ml (high-copy number) bacterial suspension could be used in the process. When using the standard protocol, the entire midi prep process can be completed in 120 minutes or less and up to 500 µg of plasmid can be expected. The purity of purified plasmid DNA is equity to that obtained by 2x CsCl-gradient centrifugation and suitable for all molecular biological applications.

Features

- Purity: equity to that obtained by 2x CsCl-gradient centrifugation .
- Safe: Eliminates the use of phenol, chloroform, ethidium bromide, and cesium chloride, minimizing exposure to, and disposal of hazardous materials.
- Time saving: Complete the process in less than 120 minutes.

Applications

The purified plasmid DNA can be immediately used in any downstream molecular biology application.

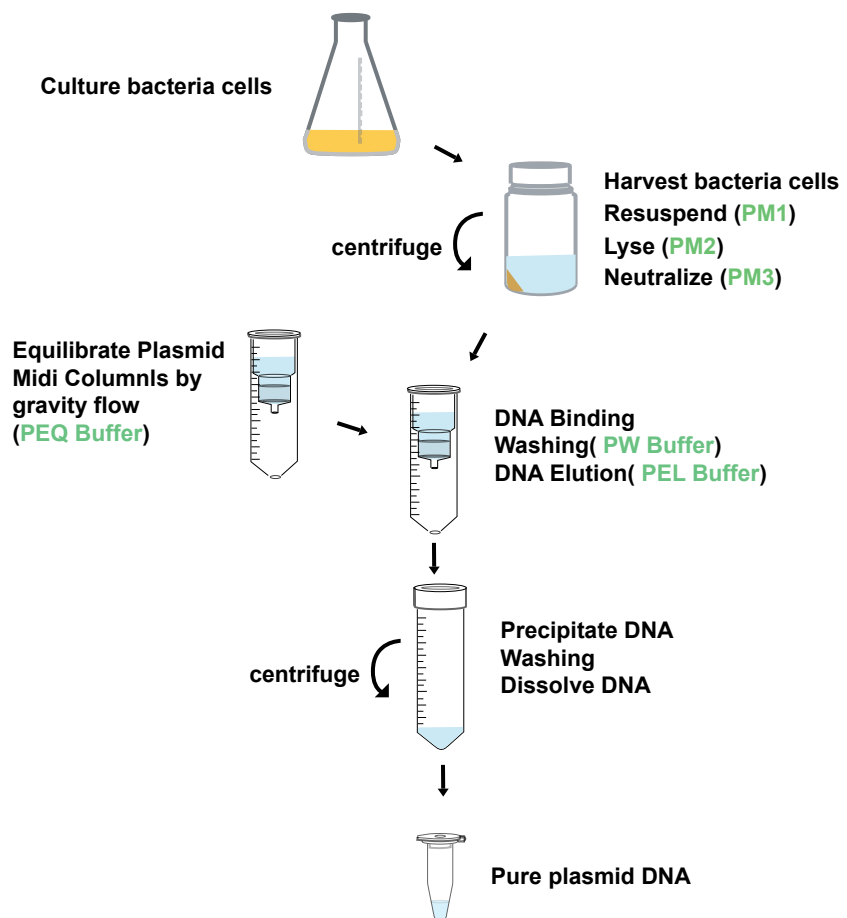
- Transfection
- Microinjection
- Sequencing
- PCR
- Restriction enzyme digestion

Operation Time

Less than 120 minutes

Operation Format

Gravity-Flow



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High-copy plasmid Low-copy plasmid



Agarose Gel Analysis Of Plasmids Purified

Agarose gel analysis of various plasmid DNA purified with Plasmid DNA Midi / Maxi Kit

- C: crude lysate
- F: flow-through
- W: wash
- E: elute
- M: 1Kb DNA Ladder

Plasmid pBluescript (high-copy) and pBR322 (low-copy) are purified with the Plasmid DNA Midi / Maxi Kit. DNA in crude lysate from alkaline lysis and fractions from each gravity-flow step was collected by isopropanol precipitation and load to each lane.

Recommended	Culture	Expeted yield
High Copy plasmid	Low Copy plasmid	
Midi 50 ml	100 ml	up to 200µg
Maxi 100 ml	250 ml	up to 500µg

Procedure

In the process, the modified alkaline lysis method and RNase treatment are used to get cleared cell lysate plasmid DNA in crude lysate has been bound to the column, the contaminants can be washed off with wash buffer. Finally, 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 120minutes without ultracentrifuges and HPLC or other toxic reagents.

Storage Conditions

Plasmid DNA Extraction Kit can be stored at room temperature (15-25°C). After adding RNase A, PM1 Buffer, should be stored at 4°C and is stable for six months. Other buffers and columns can be stored dry for up to 1 year at room temperature (15-25°C).

Ordering Information

PLASMID DNA MIDI PREP KIT

Cat. No.	Product Name	Size	Kit Components	Store at
DE-007	Plasmid DNA Extraction Midi Kit	25 preps.	<ol style="list-style-type: none"> 1. PM1 Buffer 2. PM2 Buffer 3. PM3 Buffer 4. PEQ Buffer 5. PW Buffer 6. PEL Buffer 7. RNase A (50mg/ml) 8. PM Midi columns 	PM1 Buffer with RNaseA at 4°C , others at room temperature.
DE-008	Plasmid DNA Extraction Midi Kit	50 preps.	<ol style="list-style-type: none"> 1. PM1 Buffer 2. PM2 Buffer 3. PM3 Buffer 4. PEQ Buffer 5. PW Buffer 6. PEL Buffer 7. RNase A (50mg/ml) 8. PM Midi columns 	PM1 Buffer with RNaseA at 4°C , others at room temperature.

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Ordering Information

PLASMID DNA MAXI PREP KIT

Cat. No.	Product Name	Size	Kit Components	Store at
DE-009	Plasmid DNA Extraction Maxi Kit	10 preps.	1. PM1 Buffer 2. PM2 Buffer 3. PM3 Buffer 4. PEQ Buffer 5. PW Buffer 6. PEL Buffer 7. RNase A (50mg/ml) 8. PM Maxi columns	PM1 Buffer with RNaseA at 4°C , others at room temperature.
DE-010	Plasmid DNA Extraction Maxi Kit	20 preps.	PM1 Buffer PM2 Buffer PM3 Buffer PEQ Buffer PW Buffer PEL Buffer RNase A (50mg/ml) PM Maxi columns	PM1 Buffer with RNaseA at 4°C , others at room temperature.

Distributed by:



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PLASMID DNA EXTRACTION MAXI KIT

Introduction

Plasmid DNA Extraction Maxi Kit is designed for efficient extraction of high quality plasmid DNA from bacterial culture. This kit provide the alkaline lysis reagents and the columns packed with anion-exchanger resin. After the cells lysis, the plasmid DNA is bound to the resin insided the column by a gravity-flow procedure, and the contaminants can be removed with wash buffer. After using this convenient kit, the purified plasmid DNA is suitable for downstream application such as transfection, in vitro transcription and translation, and all enzymatic modification.

Specification

Sample Size: 60-240 ml of bacteria for high-copy number plasmid
 200-480 ml of bacteria for low-copy number plasmid
 Binding Capacity: up to 1.5 mg of DNA

Kit Contents:	DE-009 (10 preps)	DE-010 (20 preps)
PEQ Buffer	135 ml	135 ml x 2
PM1 Buffer	215 ml	215 ml x 2
PM2 Buffer	215 ml	215 ml x 2
PM3 Buffer	215 ml	215 ml x 2
PW Buffer	165 ml x 2	165 ml x 4
PEL Buffer	215 ml	215 ml x 2
RNase A (50 mg/ ml)	430 µl	430 µl x 2
PM Maxi Column	10 pcs	20 pcs

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Important Notes:

1. Brief spin the RNase A tube and adding the RNase A to PM1 Buffer.

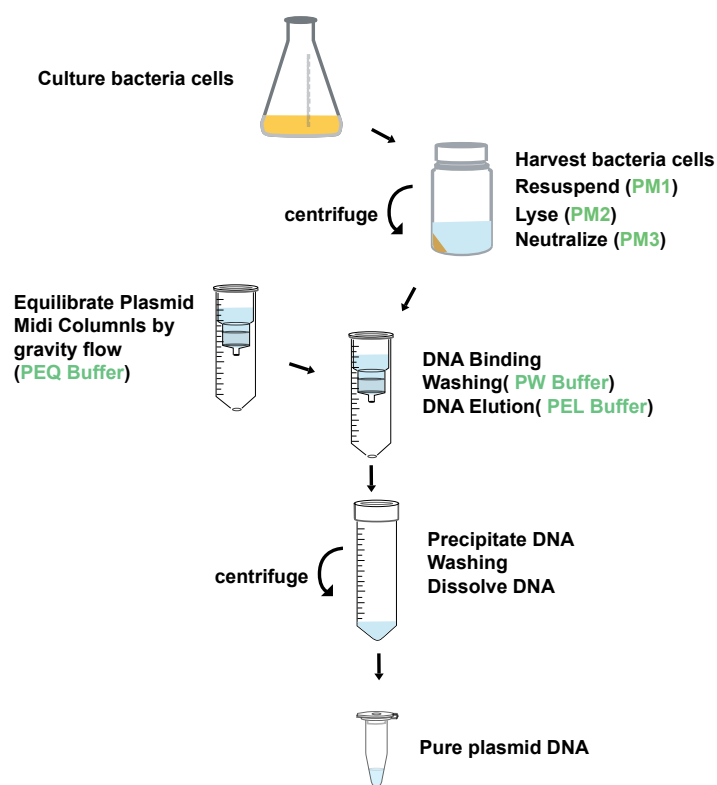
Store PM1 Buffer at 4 °C after adding RNase A.

2. If precipitates have formed in PM2 Buffer, warm the buffer in 37°C waterbath to dissolve precipitates.

Additional Requirements:

1. 50 ml centrifuge tube
2. Isopropanol
3. 70% ethanol

Brief Procedure



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• General Protocol:

Please Read Important Note Before Starting The Following Steps.

1. Place a PM Maxi Column into a 50 ml centrifuge tube. Add 12.5 ml of PEQ Buffer to equilibrate the PM Maxi column and allow the column to empty by gravity flow. Discard the filtrate.
2. Harvest the bacterial culture (up to 240 ml) by centrifugation at 6,000 x g for 15 minutes.

Note: For culture volume more than 240 ml, add twice the amount of PM1 Buffer (RNase A added), PM2 Buffer, and PM3 Buffer for the following steps.

3. Add 20 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
4. Add 20 ml of PM2 Buffer and mix gently by inverting the tube 15 times. Do not vortex to avoid shearing genomic DNA.
5. Incubate for 5 minutes at room temperature until lysate clears.
6. Add 20 ml of PM3 Buffer and mix immediately by inverting the tube 10 times (Do not vortex!).
7. Centrifuge at 15,000 x g for 20 minutes at 4°C.
 - **Centrifuge speed should not be less than 15,000 x g.**
8. Transfer the supernatant to the equilibrated PM Maxi Column and allow the column to empty by gravity flow. Discard the filtrate.
9. Add 12 ml of PW Buffer to wash the PM Midi column and allow the column to empty by gravity flow. Discard the filtrate.
9. Add 30 ml of PW Buffer to wash the PM Maxi column and allow the column to empty by gravity flow. Discard the filtrate.
10. Place the PM Maxi column into a clean 50 ml centrifuge tube (not provided) and add 15 ml of PEL Buffer to elute DNA by gravity flow.
11. Precipitate DNA by adding 11 ml of isopropanol to the eluted DNA from previous step. Mix well by inverting the tube 10 times.
12. Centrifuge at 20,000 x g for 30 minutes at 4 °C.
 - **Centrifuge speed should not be less than 20,000 x g.**
13. Carefully remove the supernatant and wash the DNA pellet with 5 ml of room temperature 70% ethanol. Then shake the tube gently.
14. Centrifuge at 20,000 x g for 10 minutes at 4 °C.
 - **Centrifuge speed should not be less than 20,000 x g.**
15. Carefully remove the supernatant. Then air-dry the DNA pellet until the tube is completely dry. (Or incubate the DNA pellet at 70 °C for 10 min.)
16. Dissolve the DNA pellet in 300 µl or a suitable volume of TE or ddH₂O.

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• Troubleshooting

Low yield

Bacterial cells were not lysed completely

- **Too many bacterial cells were used.**
- **After PM3 Buffer addition, break up the precipitate by inverting.**
- **DNA failed to precipitate or DNA pellet was lost after precipitation.**
- **DNA pellet was insufficiently redissolved.**

Purified DNA dose not perform well in downstream application

RNA contamination

- **Make sure that that RNase A was has been added in PM1 Buffer when first using.**
If RNase A added PM1 Buffer is overdue, add additional RNase A.
- **Too many bacterial cells were used, reduce the sample volume.**

Genomic DNA contamination

- **Do not use overgrown bacterial culture.**
- **During PM2 and PM3 Buffer addition, mix gently to prevent genomic DNA shearing.**
- **Lysis time was too long (over 5 minutes).**

Too much salt residual in DNA pellet

- **Wash the DNA pellet twice with 70% ethanol.**

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