



FavorPrep[™] Plasmid DNA Extraction Midi Kit

User Manual

For Research Use Only

Cat. No.: FAPDE 002 (25 Preps)
FAPDE 002-1 (50 Preps)

Introduction

The FavorPrep™ Plasmid DNA Extraction Midi Kit is designed for efficient extraction of high quality plasmid DNA from 50~100 ml of 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 remove 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: 25-50 ml of bacteria for high-copy number plasmid
50-100 ml of bacteria for low-copy number plasmid
Yield: up to 200 µg of DNA
Handling Time: about 2 hrs

Kit Contents

	FAPDE002 (25 preps)	FAPDE002-1 (50 preps)
PEQ Buffer	135 ml	270 ml
PM1 Buffer	110 ml	220 ml
PM2 Buffer	110 ml	220 ml
PM3 Buffer	110 ml	220 ml
PW Buffer	270+60 ml	540+120 ml
PEL Buffer	270 ml	270 ml x2
RNase A (50 mg/ ml)	220 µl	440 µl
PM Midi Column	25 pcs	50 pcs

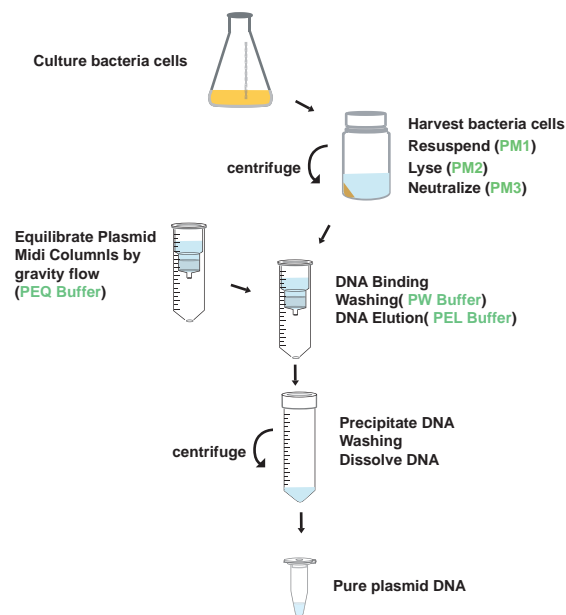
Important Notes:

1. Brief spin the RNase A tube and adding the RNase A to PM1 Buffer.
Store the 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. 75% ethanol

Brief Procedure:



General Protocol:

1. Place a PM Midi Column into a 50 ml centrifuge tube, add 5 ml of PEQ Buffer to equilibrate the PM Midi column and allow the column to empty by gravity flow. Discard the filtrate.
2. Harvest the bacterial culture by centrifugation at 6,000 x g for 15 minutes.
3. Add 4 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
4. Add 4 ml of PM2 Buffer and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing genomic DNA.
5. Incubate for 3 minutes at room temperature until lysate clears.
6. Add 4 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.
8. Transfer the supernatant to the equilibrated PM Midi 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.

10. Place the PM Midi column into a clean 50 ml centrifuge tube (not provided) and add 8 ml of PEL Buffer to elute DNA by gravity flow.

11. Precipitate DNA by adding 6 ml (0.75 volumes) of isopropanol to the eluted DNA from Step 12.

12. Mix gently and centrifuge at 20,000 g for 30 minutes at 4 °C.

13. Carefully remove the supernatant and wash the DNA pellet with 5 ml of room temperature 75% ethanol.

14. Centrifuge at 20,000 g for 10 minutes at 4 °C.

15. Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.

16. Dissolve the DNA pellet in 300 ul or a suitable volume of TE or ddH₂O.

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 RNase A 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.