FavorPrep™ Endotoxin-Free Plasmid DNA Extraction Maxi Kit

User Manual

Cat. No.: FAPDE 003-EF (10 Preps)

For Research Use Only

v.1005-1

Introduction

The FavorPrep™ Endotoxin-Free Plasmid DNA Extraction Maxi Kit is designed for efficient extraction of high quality plasmid DNA from 50~250 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: 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

	FAPDE003-EF (10 preps)
PEQ Buffer	135 ml
PM1 Buffer	215 ml
PM2 Buffer	215 ml
PM3 Buffer	215 ml
PTR Buffer	55 ml
PW Buffer	165 ml x 2
PEL Buffer	215 ml
RNase A (50 mg/ ml)	430 µl
PM Maxi Column	10 pcs

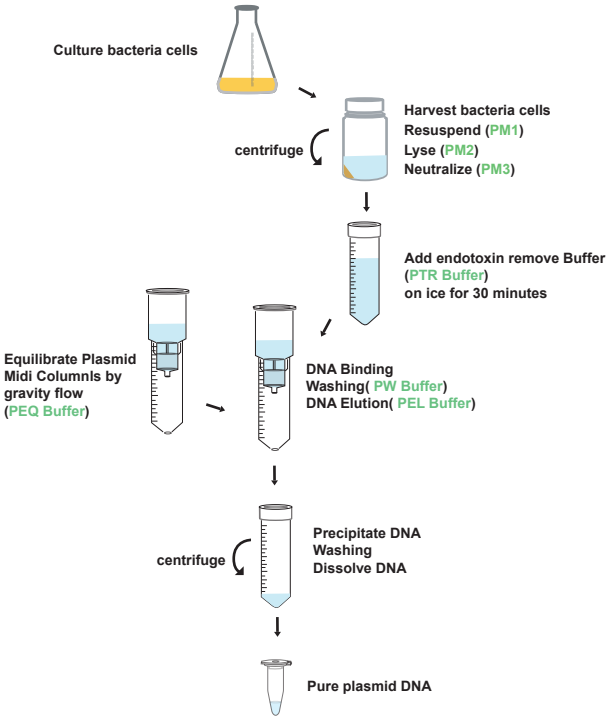
Important Notes:

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 preciptates.

Additional Requirements:

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

Brief Procedure:



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 from step 7 to a clean 50 ml centrifuge. Add 5 ml of PTR Buffer and mix gently by inverting the tube 10 times.**

9. **Incubate on ice for 30 minutes.**
   - After the incubation, the sample mixture will become clear.

10. Transfer the the sample mixture from step 9 to the equilibrated PM Maxi Column and allow the column to empty by gravity flow. Discard the filtrate.

11. Add 30 ml of PW Buffer to wash the PM Maxi column and allow the column to empty by gravity flow. Discard the filtrate.

12. 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.

13. Precipitate DNA by adding 11 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 °C.
- Centrifuge speed should not be less than 20,000 x g.

15. Carefully remove the supernatant and wash the DNA pellet with 5 ml of room temperature 70% ethanol. Then shake the tube gently.

16. Centrifuge at 20,000 x g for 10 minutes at 4 °C.
- Centrifuge speed should not be less than 20,000 x g.

17. 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.)

18. Dissolve the DNA pellet in 300 µl or a suitable volume of TE or ddH_2O.

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 insufficiiently 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.