FavorFilter™ Plasmid DNA Extraction Maxi Kit

User Manual

Cat. No.: FAFTE 001 (4 preps)
FAFTE 001-1 (10 preps)

For Research Use Only

v.1005-1

Introduction

The FavorFilter Plasmid DNA Extraction Maxi Kit is designed for rapid and efficient extraction of high quality plasmid DNA. With provided filter cartridges the bacteria lysates will be removed without centrifugation. Following a gravity-flow procedure, the plasmid DNA is bound to the resin, 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

	FAFTE001 (4 preps)	FAFTE001-1 (10 preps)
PEQ Buffer	55 ml	135 ml
PM1 Buffer	85 ml	215 ml
PM2 Buffer	85 ml	215 ml
PM3 Buffer	85 ml	215 ml
PW Buffer	130 ml	165 ml x 2
PEL Buffer	65 ml	215 ml
RNase A (50mg/ml)	170 µl	430 µl
FavorFilter Maxi Cartridge	4 pcs	10 pcs
PM Maxi Column	4 pcs	10 pcs

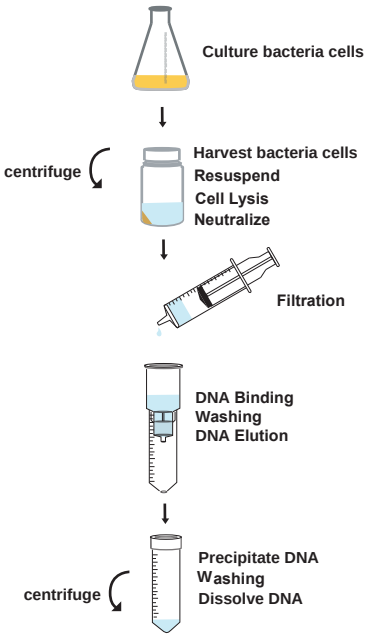
Important Notes:

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.
3. Pre-chill PM3 Buffer at 4 °C before use.

Additional Requirements:

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

Brief Procedure:



General Protocol:

1. **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.

2. **Place a PM Maxi Column onto a 50 ml centrifuge tube.**

3. **Equilibtate a PM Maxi column by applying 12.5 ml of PEQ Buffer. Allow the column to empty by gravity flow.**

4. **Discard the filtrate.**

5. **Apply 20 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.**

6. **Add 20 ml of PM2 Buffer and mix gently by inverting the tube 15 times. Do not vortex, and avoid shearing genomic DNA.**

7. **Incubate for 5 minutes at room temperature until lysate clears.**

8. **During the incubation prepare the FavorFilter Cartridge.**
   - Remove the cap from the tip of the cartridge and pull out the plunger.
   - Place the cap back to the tip of the cartridge and stand the cartridge vertically in a suitable rack.

9. **Add 20 ml of PM3 Buffer and mix immediately by inverting the tube 10 times (Do not vortex !). Proceed directly to step 10.**

10. **Pour the lysate into the barrel of the FavorFilter Cartridge. Incubate the lysate for 10 minutes at room temperature.**
    Important Step ! To ensures filtration without clogging, 10 minutes incubation is essential to make the precipitate float up .

11. Remove the cap from the tip of the FavorFilter Cartridge. Gently insert the plunger into the FavorFilter Cartridge and filter the lysate into the equilibrated PM Maxi column then allow it to flow through by gravity flow.

12. Discard the filtrate.

13. Wash the PM Maxi column by applying 30 ml of PW Buffer. Allow the column to empty by gravity folw.

14. Discard the filtrate.

15. Place PM Maxi column onto a clean 50 ml centrifuge tube (not provided) and add 15 ml of PEL Buffer to elute DNA by gravity flow.

16. Precipitate DNA by adding 11 ml of isopropanol to the eluted DNA from Step 15. Mix well by inverting the tube 10 times.

17. Centrifuge at 20,000 x g for 30 minutes at 4 °C.
- Centrifuge speed should not be less than 20,000 x g.

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

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

20. 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.)

21. 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 to ensure higher yield.

Purified DNA dose not perform well in downstream application

RNA contamination

- Prior to using PM1 Buffer, ensure that RNase A was added. 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.