User Manual

Cat. No.: FAFTE 000-EF (2 Preps)
FAFTE 001-EF (4 Preps)
FAFTE 001-1-EF (10 Preps)

FavorFilter™ Endotoxin-Free Plasmid Extraction Maxi Kit

For Research Use Only

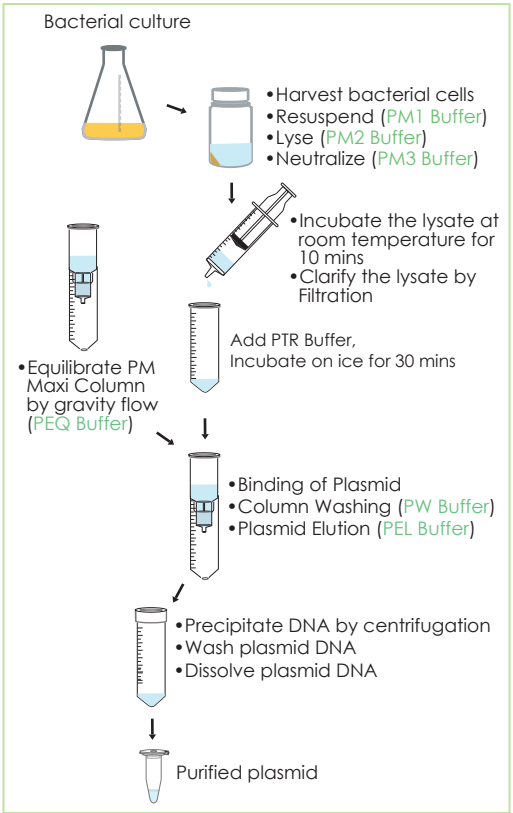
Kit Contents:

	FAFTE 000-EF (2 preps)	FAFTE 001-EF (4 preps)	FAFTE 001-1-EF (10 preps)
PEQ Buffer	30 ml	55 ml	135 ml
PM1 Buffer	42 ml	85 ml	215 ml
PM2 Buffer	42 ml	85 ml	215 ml
PM3 Buffer	42 ml	85 ml	215 ml
PTR Buffer	12 ml	25 ml	55 ml
PW Buffer	65 ml	130 ml	270 ml + 60 ml
PEL Buffer	32 ml	65 ml	215 ml
RNase A Solution	100 µl	200 µl	480 µl
FavorFilter Maxi Cartridge	2 pcs	4 pcs	10 pcs
PM Maxi Column	2 pcs	4 pcs	10 pcs

* Preparation of PM1 Buffer for first use:			
Cat. No:	FAFTE 000-EF (2 preps)	FAFTE 001-EF (4 preps)	FAFTE 001-1-EF (10 preps)
Volume of RNase A Solution for PM1 Buffer	84 µl	170 µl	430 µl

Specification:

Technology: Anion-exchange chromatography (gravity-flow column)
Lysate clarification: Filtration
Sample Size: 120~240 ml of bacteria for high-copy number or low -copy number plasmid
Plasmid or constructs range: 3 kbp~150 kbp
Binding Capacity: 1.5 mg/Maxi Column



Important Notes:

1. Store RNase A Solution at -20°C upon recipit of kit.
2. Add indicated volume of RNase A Solution into PM1 buffer, mix well and store the PM1 buffer at 4°C.
3. If precipitates have formed in PM2 Buffer, warm the buffer in 37°C waterbath to dissolve preciptates.
4. Pre-chill PM3 Buffer at 4°C before starting.

Additional Requirements:

1. 50 ml tubes
2. Refrigerated centrifuge capable of ≥5,000 xg and the centrifuge tube suitable for the centrifuge rotor
3. Isopropanol
4. 70% ethanol
5. TE buffer or ddH_2O

General Protocol:

Please Read Important Notes Before Starting Following Steps.

Harvest bacterial cells

1. Harvest the cells by centrifugation at 4,500~6,000 xg at 4°C for 10 mins and discard the supernatant.

Equilibrate PM Maxi Column

2. Place a PM Maxi Column onto a 50 ml tube.
3. Equilibrate the PM Maxi column by applying 10 ml of PEQ Buffer. Allow the column to empty by gravity flow and discard the filtrate.

Cell lysis and lysate neutralization

4. Add 16 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
5. Add 16 ml of PM2 Buffer and mix gently by inverting the tube 5 times.
   -Do not vortex to avoid shearing genomic DNA.
6. Incubate the sample mixture for 5 mins at room temperature until lysate clears.

7. **Add 16 ml of chilled PM3 Buffer and mix immediately by inverting the tube 10~15 times to neutralize the lysate. (Do not vortex!)**
   -Note: • Make sure the density of cultured cell is optimal, the buffers volume (PM1, PM2, PM3) should be increased proportionally to the culture volume.
   (ex. culture volume, 120~240 ml: PM1, 16 ml; PM2, 16 ml; PM3, 16 ml
   culture volume, 240~480 ml: PM1, 32 ml; PM2, 32 ml; PM3, 32 ml)
   • When processing 240~480 ml bacteria where additional buffer volumes of PM1, PM2, and PM3 are needed, the buffers may be purchased separately.
   • Make sure cell pellet be suspended completely within Buffer PM1.
   • Mix the sample mixture completely after adding Buffer PM2 and Buffer PM3.

Lysate filtration and endotoxin removal

8. **Pour the lysate into a cartridge barrel. Incubate the lysate for 10 mins at room temperature to make the precipitate float up.**
   -Note! To ensure filtration without clogging,10 mins incubation is essential to make the precipitate float up.
9. **Remove the cap from cartridge tip. Gently insert the plunger into cartridge to filter the lysate and load the filtrate into a clean 50 ml tube.**
10. **Add 5 ml of PTR Buffer to the filtrate and mix gently by pipetting, incubate it on ice for 30 mins. After the incubation, the sample mixture will become clear.**

Binding of plasmid

11. **Transfer the half of the sample mixture from step 10 to the equilibrated PM Maxi Column. Allow sample mixture to flow through the PM Maxi Column by gravity flow and discard the filtrate.**
12. **Repeat step 11 for the rest of the sample mixture.**

Wash PM Maxi Column

13. **Wash the PM Maxi column by applying 30 ml of PW Buffer. Allow PW Buffer to flow through the PM Maxi Column by gravity flow and discard the filtrate.**

Elution

14. **Place the PM Maxi column onto a clean 50 ml centrifuge tube (not provided). Add 15 ml of PEL Buffer to the PM Maxi Column to elute the plasmid by gravity flow.**

Precipitate plasmid DNA

15. **Transfer the eluate from step 14 to a centrifuge tube. Add 0.75 volume of room temperature isopropanol to the eluate and mix well by inverting the tube 10 times. (ex: add 11.25 ml isopropanol to 15 ml eluate)**
    -Note! Make sure that isopropanol be mixed thoroughly with eluate before centrifugation.
16. **Centrifuge the tube at ≥5,000 xg at 4°C for 30 mins. Preferably centrifuge the tube at 15,000~20,000 xg at 4°C for 20 mins.**

Wash and dissolve plasmid DNA

17. **Carefully remove the supernatant and wash the plasmid pellet with 5 ml of room temperature 70% ethanol.**
18. **Centrifuge the tube at ≥5,000 xg at 4°C for 10 mins.**
19. **Carefully remove the supernatant and invert the tube on paper towel for 3 mins to remove residual ethanol. Air-dry the plasmid pellet until the tube is completely dry. (Or incubate the plasmid pellet at 70°C for 10 mins.)**
20. **Dissolve the plasmid pellet in a suitable volume (≥300 µl) of TE or ddH_2O.**
    -Note! • Do not lose the DNA pellet when discard the supernatant.
    • Make sure the DNA pellet adhesive lightly on the centrifuge tube.
    • If the DNA pellet loose from tube, repeat the precipitation step again.
    • Make sure the DNA is dissolved completely before measure the concentration.

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 applications

RNA contamination

- **Make sure that that RNase A was has been added in PM1 Buffer at the first use.**
- **RNase A is not properly preserved.**
- **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 mins).**

Too much salt residual in DNA pellet

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