

Kit Contents:

	FAFTE 000-EF (2 preps_sample)	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 (50 mg/ ml)	84 µl	170 µl	430 µl
FavorFilter Maxi Cartridge	2 pcs	4 pcs	10 pcs
PM Maxi Column	2 pcs	4 pcs	10 pcs

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: 3kbp ~ 150kbp

Binding Capacity: 1.5 mg / Maxi Column

Important Notes:

1. Store RNase A at -20 °C upon receipt of kit.
2. Add 0.5 ml of PM1 Buffer to a RNase A tube, vortex the tube to mix well. Transfer the total RNase A mixture back to the PM1 bottle, mix well by vortexing 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 precipitates.
4. Pre-chill PM3 Buffer at 4 °C before starting.

Additional Requirements:

1. 50 ml tubes
2. Refrigerated centrifuge capable of $\geq 5,000 \times g$ and the centrifuge tube suitable for the centrifuge rotor
3. Isopropanol
4. 70% ethanol
5. TE buffer or ddH₂O

General Protocol:

Please Read Important Notes Before Starting Following Steps.

Harvest bacterial cells

1. Harvest the cells by centrifugation at $4,500 \sim 6,000 \times g$ at 4 °C for 10 min 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

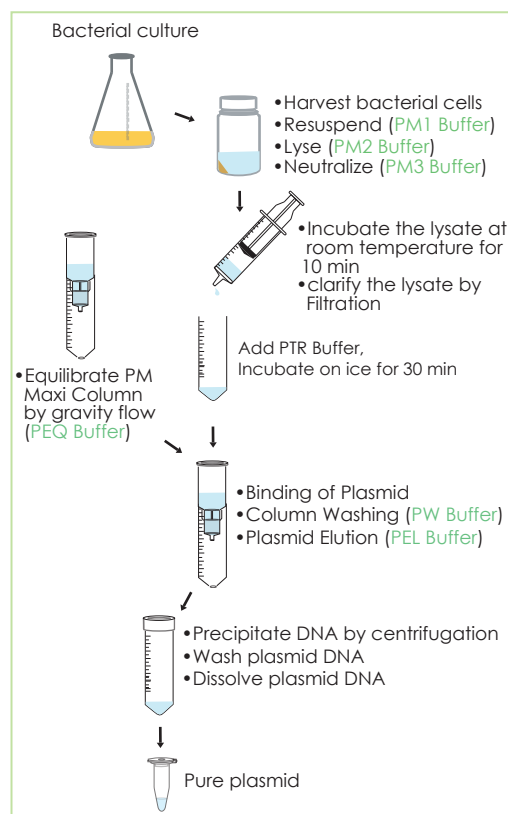
4. Add 16 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
5. Add 16 ml of PM2 Buffer and mix gently by inverting the tube 5 times.
-- Do not vortex to avoid shearing genomic DNA.
6. Incubate the sample mixture for 5 minutes at room temperature until lysate clears.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

• 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 minutes at room temperature to make the precipitate float up.

Note! To ensure filtration without clogging, 10 minutes 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 minutes. 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)
16. Centrifuge the tube at $\geq 5,000 \times g$ at 4 °C for 30 min. preferably centrifuge the tube at 15,000 ~ 20,000 $\times g$ at 4 °C for 20 minutes.

Note! Make sure that isopropanol be mixed thoroughly with eluate before centrifugation.

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 $\geq 5,000 \times g$ at 4 °C for 10 min.
19. Carefully remove the supernatant and invert the tube on paper towel for 3 minutes 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 min.)
20. Dissolve the plasmid pellet in a suitable volume ($\geq 300 \mu\text{l}$) of TE or ddH₂O.

Note! • Do not lose the DNA pellet when discard the supernatant.

- Make sure the DNA pellet adheres 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 does 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.