

**Kit Contents:**

	FAPDE 000-Maxi (2 preps)	FAPDE 003 (10 preps)	FAPDE 003-1 (20 preps)
PEQ Buffer	30 ml	135 ml	270 ml
PM1 Buffer	42 ml	215 ml	215 ml × 2
PM2 Buffer	42 ml	215 ml	215 ml × 2
PM3 Buffer	42 ml	215 ml	215 ml × 2
PW Buffer	65 ml	270 ml + 60 ml	270 ml × 2 + 120 ml
PEL Buffer	32 ml	215 ml	215 ml × 2
RNase A Solution	180 µl	900 µl	900 µl × 2
PM Maxi Column	2 pcs	10 pcs	20 pcs

**\* Preparation of PM1 Buffer for the first use:**

Cat. No:	FAPDE 000-Maxi (2 preps)	FAPDE 003 (10 preps)	FAPDE 003-1 (20 preps)
Volume of RNase A Solution for PM1 Buffer	168 µl	860 µl	860 µl

**Specification:**

Technology: Anion-exchange chromatography (gravity-flow column)

Lysate clarification: Centrifugation

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 receipt 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 water bath to dissolve precipitates.
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<sub>2</sub>O

**General Protocol:**

Please Read Important Notes Before Starting Following Steps.

**Harvest bacterial cells**

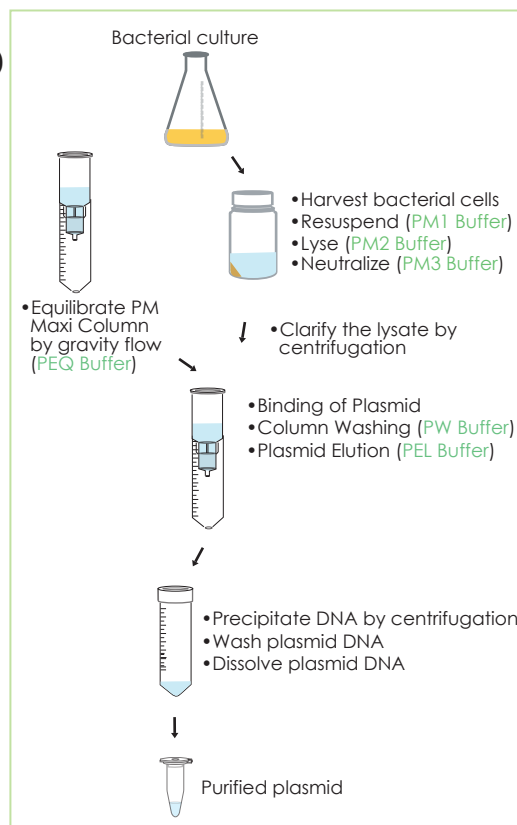
1. Harvest the cells by centrifugation at 4,500~6,000 x g at 4°C for 10 mins and discard the supernatant.

**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.

**Clarify lysate by centrifugation**

**8. Centrifuge the tube at  $\geq 5,000$  xg at 4°C for 20 mins. (Preferably centrifuge the tube at 15,000~20,000 xg at 4°C for 15 mins.)**

-If the supernatant still contains suspended matter, transfer the supernatant to a clean centrifuge tube and repeat this centrifugation step.

**9. Transfer the supernatant to a clean 50 ml tube.**

**Binding of plasmid**

**10. Transfer the half of the supernatant from step 9 to the equilibrated PM Maxi column. Allow it to flow through the PM Maxi Column by gravity flow and discard the filtrate.**

**11. Repeat step 10 for the rest of the supernatant.**

**Wash PM Maxi Column**

**12. 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**

**13. 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**

**14. Transfer the eluate from step 13 to a centrifuge tube. Add 0.75X 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.

**15. Centrifuge the tube at  $\geq 5,000$  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**

**16. Carefully remove the supernatant and wash the plasmid pellet with 5 ml of room temperature 70% ethanol.**

**17. Centrifuge the tube at  $\geq 5,000$  xg at 4°C for 10 mins.**

**18. 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.)**

**19. Dissolve the plasmid pellet in a suitable volume ( $\geq 300$   $\mu$ l) of TE or ddH<sub>2</sub>O.**

-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 RNase A had 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.