

Kit Contents:

	FAPD1050 (2 preps)	FAPD1051 (10 preps)	FAPD1052 (25 preps)
PEQ Buffer	30 ml	135 ml	330 ml
PM1 Buffer	42 ml	215 ml	500 ml
PM2 Buffer	42 ml	215 ml	500 ml
PM3 Buffer	42 ml	215 ml	500 ml
PW Buffer	65 ml	270 ml + 60 ml	500 ml × 2
PEL Buffer	32 ml	215 ml	500 ml
RNase A Solution	180 µl	900 µl	900 µl × 2 + 360 µl
PM Maxi Columns	2 pcs	10 pcs	25 pcs

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

Cat. No:	FAPD1050 (2 preps)	FAPD1051 (10 preps)	FAPD1052 (25 preps)
Volume of RNase A Solution for PM1 Buffer	168 µl	860 µl	2000 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 ddH₂O

General Protocol:

Please Read Important Notes Before Starting Following Steps.

Harvest bacterial cells

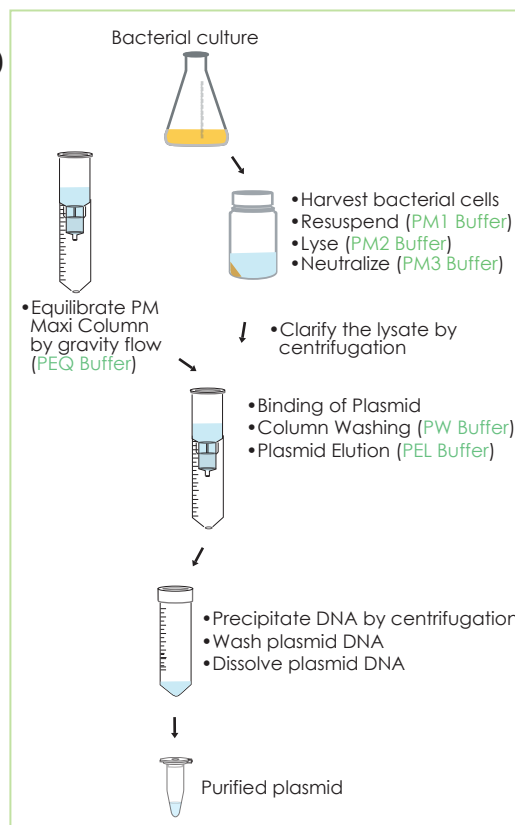
1. Harvest the cells by centrifugation at 4,500~6,000 x g at 4°C for 10 mins and discard the supernatant.

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 (≥ 300 μ l) of TE or ddH₂O.

- 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.