User Manual

FavorPrep™ Plasmid Extraction Maxi Kit

Cat. No.: FAPDE 000-Maxi (2 Preps)
FAPDE 003 (10 Preps)
FAPDE 003-1 (20 Preps)

For Research Use Only

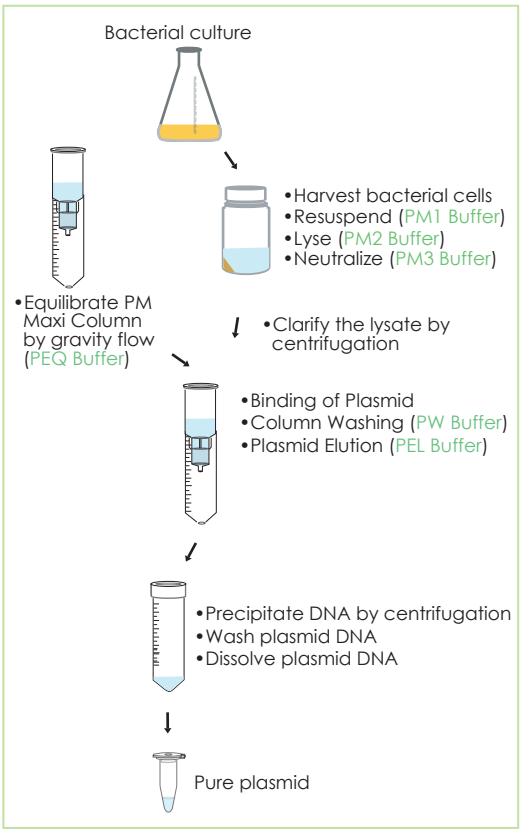
Kit Contents:

	FAPDE 000-Maxi (2 preps_sample)	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 x 2
PM2 Buffer	42 ml	215 ml	215 ml x 2
PM3 Buffer	42 ml	215 ml	215 ml x 2
PW Buffer	65 ml	270 ml + 60 ml	270 ml x 2 + 120 ml
PEL Buffer	32 ml	215 ml	215 ml x 2
RNase A (lyophilized)	4.2 mg	21.5 mg	* 21.5 mg x 2
PM Maxi Column	2 pcs	10 pcs	20 pcs

* Each tube of RNase A is for adding to each PM1 Buffer individually.

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 at -20°C upon receipit of kit.
2. Add 0.5 ml of PM1 Buffer to a RNase A tube and vortex the tube to mix well. Transfer the total RNase A mixture back to the PM1 bottle and mix well by vortexing. 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_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 x 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. Equilibtate 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

Clarify lysate by centrifugation

8. **Centrifuge the tube at ≥ 5,000 x g at 4°C for 20 min. preferably centrifuge the tube at 15,000 ~ 20,000 xg at 4°C for 15 minutes.**
   -- 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 0.75 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.
15. **Centrifuge the tube at ≥ 5,000 x g at 4°C for 30 min. preferably centrifuge the tube at 15,000 ~ 20,000 xg at 4°C for 20 minutes.**

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 ≥ 5,000 x g at 4 °C for 10 min.**
18. **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.)**
19. **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 application

RNA contamination

- **Make sure that that RNase A was 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.**