User Manual

FavorPrep™ Plasmid Extraction Midi Kit

Cat. No.: FAPDE 000-Midi (2 Preps)
FAPDE 002 (25 Preps)
FAPDE 002-1 (50 Preps)

(For Research Use Only)

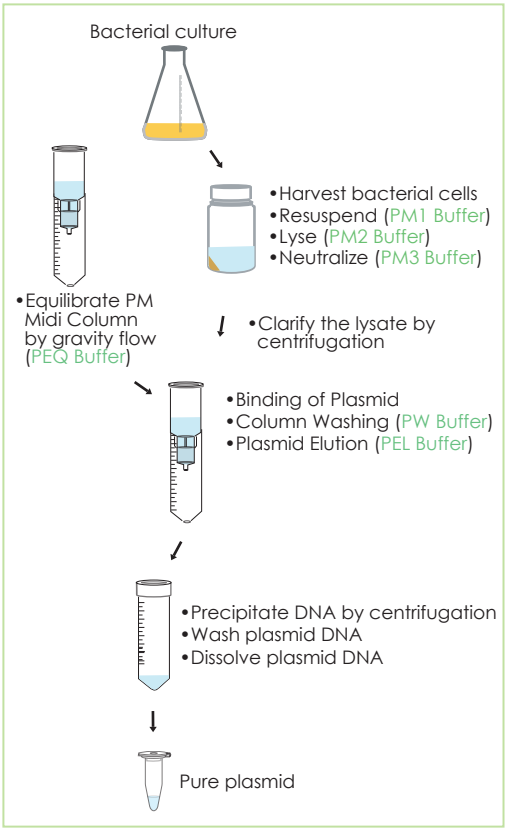
Kit Contents:

	FAPDE 000-Midi (2 preps_sample)	FAPDE 002 (25 preps)	FAPDE 002-1 (50 preps)
PEQ Buffer	12 ml	135 ml	270 ml
PM1 Buffer	20 ml	215 ml	215 ml x 2
PM2 Buffer	20 ml	215 ml	215 ml x 2
PM3 Buffer	20 ml	215 ml	215 ml x 2
PW Buffer	30 ml	270 ml + 60 ml	270 ml x 2 + 120 ml
PEL Buffer	20 ml	215 ml	215 ml x 2
RNase A (lyophilized)	2 mg	21.5 mg	* 21.5 mg x 2
PM Midi Column	2 pcs	25 pcs	50 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: 60 ~ 120 ml of bacteria for high-copy number or low -copy number plasmid
Plasmid or constructs range: 3kbp ~ 150kbp
Binding Capacity: 650 µg / Midi Column



Important Notes:

1. Store RNase A at -20 °C upon recipit of kit.
2. Adding the provided RNase A to PM1 Buffer: add 1 ml of PM1 Buffer to a RNase A tube, vortex the tube to dissolve the RNase A completely. 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 preciptates.
4. Pre-chill PM3 Buffer at 4 °C before starting.

Additional Requirements:

1. 50 ml tubes
2. Refrigerated centrifuge capable of ≥ 5,000 x g 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 Midi Column

2. Place a PM Midi Column onto a 50 ml tube.
3. Equilibtate the PM Midi column by applying 5 ml of PEQ Buffer. Allow the column to empty by gravity flow and discard the filtrate.

Cell lysis and lysate neutralization

4. Add 8 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
5. Add 8 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 8 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, 60 ~ 120 ml: PM1, 8 ml; PM2, 8 ml; PM3, 8 ml
culture volume, 120~ 240 ml: PM1, 16 ml; PM2, 16 ml; PM3, 16 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 x g 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.

Binding of plasmid

9. **Transfer the supernatant from step 8 to the equilibrated PM Midi column. Allow it to flow through the PM Midi Column by gravity flow and discard the filtrate.**

Wash PM Maxi Column

10. **Wash the PM Midi column by applying 12.5 ml of PW Buffer. Allow PW Buffer to flow through the PM Midi Column by gravity flow and discard the filtrate.**

Elution

11. **Place the PM Midi column onto a clean 50 ml centrifuge tube (not provided). Add 8 ml of PEL Buffer to the PM Midi Column to elute the plasmid by gravity flow.**

Precipitate plasmid DNA

12. **Transfer the eluate from step 11 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 6 ml isopropanol to 8 ml eluate)**
    Note! Make sure that isopropanol be mixed thoroughly with eluate before centrifugation.
13. **Centrifuge the tube at ≥ 5,000 x g at 4 °C for 30 min. preferably centrifuge the tube at 15,000 ~ 20,000 x g at 4 °C for 20 minutes.**

Wash and dissolve plasmid DNA

14. **Carefully remove the supernatant and wash the plasmid pellet with 5 ml of room temperature 70% ethanol.**
15. **Centrifuge the tube at ≥ 5,000 x g at 4 °C for 10 min.**
16. **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.)**
17. **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 complelety 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 insufficiiently 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.**