User Manual

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

For Research Use Only

FavorPrep™ Plasmid Extraction Midi Kit

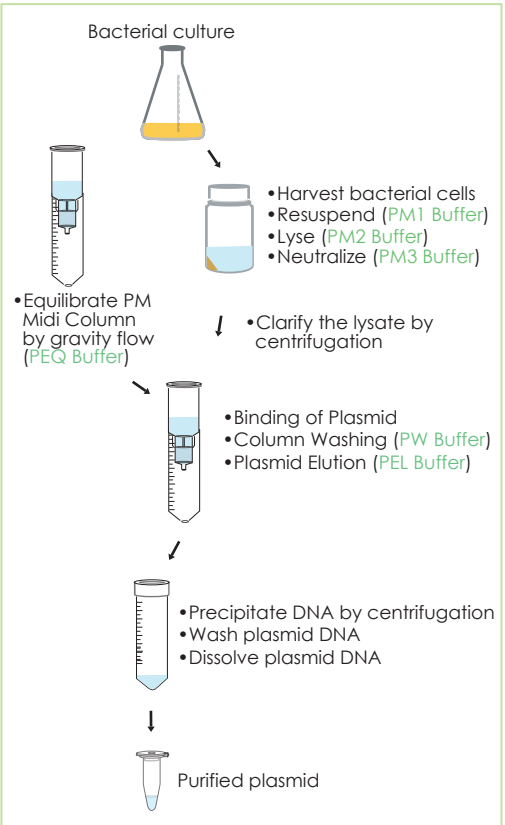
Kit Contents:

	FAPDE 000-Midi (2 preps)	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 × 2
PM2 Buffer	20 ml	215 ml	215 ml × 2
PM3 Buffer	20 ml	215 ml	215 ml × 2
PW Buffer	30 ml	270 ml + 60 ml	270 ml x 2 + 120 ml
PEL Buffer	20 ml	215 ml	215 ml × 2
RNase A Solution	50 µl	480 µl	480 µl × 2
PM Midi Column	2 pcs	25 pcs	50 pcs

* Preparation of PM1 Buffer for the first use:			
Cat. No:	FAPDE 000-Midi (2 preps)	FAPDE 002 (25 preps)	FAPDE 002-1 (50 preps)
Volume of RNase A Solution for PM1 Buffer	40 µl	430 µl	430 µl

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: 3 kbp~150 kbp
Binding Capacity: 650 µg/Midi 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_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 xg at 4°C for 10 mins 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 mins 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 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.

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 Midi 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 0.75X 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 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

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 mins.**
16. **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.)**
17. **Dissolve the plasmid pellet in a suitable volume (≥300 µl) of TE or ddH$_2$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.**