User Manual

Cat. No.: FAPD1040 (2 Preps)
FAPD1042 (25 Preps)
FAPD1043 (50 Preps)

FavorPrep™ Plasmid Extraction Midi Kit

For Research Use Only

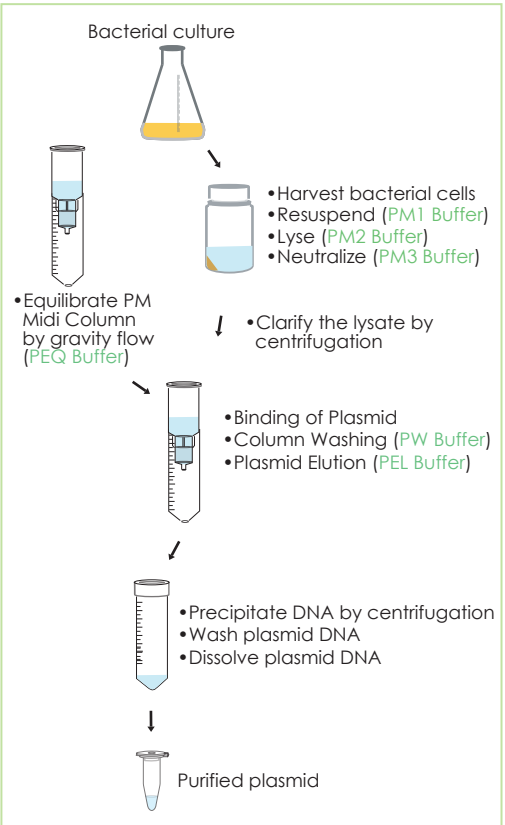
Kit Contents:

	FAPD1040 (2 preps)	FAPD1042 (25 preps)	FAPD1043 (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	100 µl	900 µl	900 µl × 2
PM Midi Columns	2 pcs	25 pcs	50 pcs

* Preparation of PM1 Buffer for the first use:			
Cat. No:	FAPD1040 (2 preps)	FAPD1042 (25 preps)	FAPD1043 (50 preps)
Volume of RNase A Solution for PM1 Buffer	80 µl	860 µl	860 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)
•When processing 120~240 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 ≥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 xg 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_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 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.**