FavorPrep™ Endotoxin-Free Plasmid Extraction Mini Kit

■ Kit Contents

Cat. No.	FSPD302-004 (4 Preps)	FSPD302-100 (100 Preps)
PM1 Buffer	1 ml	25 ml
PM2 Buffer	1 ml	25 ml
PM3 Buffer	1 ml	25 ml
FAER Buffer	1 ml	25 ml
WP Buffer	6 ml	135 ml
Wash Buffer (Concentrate) ▲	1 ml	20 ml
Elution Buffer	0.5 ml	15 ml
RNase A Solution ■	10 µl	75 µl
FAPD Mini Columns	4 pcs	50 pcs x 2
Collection Tubes	4 pcs	100 pcs
Elution Tubes	4 pcs x 2	100 pcs x 2
User Manual	1	1
Preparation of **PM1 Buffer** and **Wash Buffer** for the first use.		
RNase A Solution volume for PM1 Buffer ■	2 µl	50 µl
Ethanol volume for Wash Buffer ▲	4 ml	80 ml

1. All kit components are shipped at room temperature and should be stored at room temperatures between 15~25°C upon receipt, **except RNase A Solution**.
2. Store RNase A Solution at -20°C upon receipt.

■ Specification

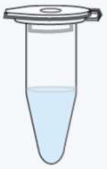
Format/Principle	Spin column (Silica matrix)
Binding Capacity	≤60 µg DNA/column
Operation Time	<30 mins
Sample Size	1~5 ml of bacteria for high-copy number or low-copy number plasmid
Plasmid or Constructs Range	<150 kbp
Minimum Elution Volume	50 µl

■ Procedure Overview

Well-grown bacterial culture

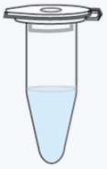
Centrifuge
1,100 xg, 1 min

- Harvest bacterial cells.



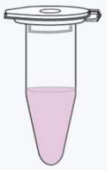
- Resuspend (200 µl PM1 Buffer)
- Lyse (200 µl PM2 Buffer)
- Neutralize (200 µl PM3 Buffer)

Centrifuge
18,000 xg, 5 mins



- Clarify the lysate by centrifuge.
- Transfer the supernatant.

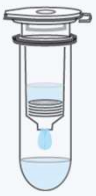
Centrifuge
18,000 xg, 5 mins



- Add 200 µl FAER Buffer, vortex thoroughly.
- Incubation on ice for 5 mins.
- Centrifugation.
- Transfer the supernatant into an Elution Tube .

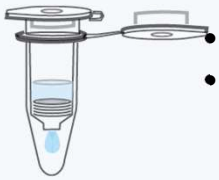
FAPD Mini Column

Centrifuge
18,000 xg, 1 min



- Add 650 µl WP Buffer, vortex thoroughly.
- Binding DNA.

Centrifuge
18,000 xg, 1 min



- Add 400 µl WP Buffer.
- Add 700 µl Wash Buffer (ethanol contained).

Centrifuge
18,000 xg, 3 mins

- Drying the column membrane.

Centrifuge
18,000 xg, 1 min

- Elution (50~100 µl Elution Buffer).
- Obtain purified endotoxin-free plasmid DNA.

■ Preparation Before Starting

1. Add indicated volume of **RNase A Solution** into **PM1 Buffer**, mix well and store at 4℃.
2. Add indicated volume of ethanol (96~100%) into **Wash Buffer**, mix well and store at room temperature.
3. Check **PM2 Buffer** for precipitates before use. If precipitates are observed, warm-up PM2 Buffer at 37˚C in a water bath until precipitates are dissolved.
4. All centrifugation steps should be performed between 11,000~18,000 xg at room temperature.
5. Preheat the Elution Buffer to 65˚C for elution step.

■ General Protocol

♦ Harvest Bacterial cell

1. Pellet 1~5 ml of well-grown bacterial culture by centrifuge at 11,000 xg for 1 min. Discard the supernatant completely.

♦ Resuspend Cells, Lyse and Neutralize

2. Add 200 µl of **PM1 Buffer** (RNase A added) to the cell pellet and resuspend the cells completely by vortexing or pipetting until no cell pellet remain.
3. Add 200 µl of **PM2 Buffer** and gently invert the tube for 5~10 times. Incubate the sample mixture at room temperature for 3~5 mins to lyse the cells.
   - Do not vortex to avoid shearing genomic DNA.
   - Do not proceed the incubation more than 5 mins.
4. Add 200 µl of **PM3 Buffer** and gently invert the tube for 5~10 times immediately to neutralize the lysate.
   - Invert immediately after adding PM3 Buffer to avoid asymmetric precipitation.

♦ Lysate Clarification

5. Centrifuge at 18,000 xg for 5 mins to clarify the lysate. Transfer the supernatant carefully to a new 1.5 ml microcentrifuge tube without disrupting the white precipitate.
   - Avoid disturbing or transferring the white precipitate.
   - If the supernatant is not clear, repeat this step.

♦ Endotoxin Removal

6. Add 200 µl of **FAER Buffer** and vortex for 10 secs. The mixture will turn turbid. Incubate the sample mixture on ice for 5 mins then vortex for 10 secs.
   - FAER Buffer is a viscous solution, pipetting the FAER Buffer at slower speeds.
   - If room temperature is below 10°C, heat the mixture at 65°C for 5 mins after the ice cooling step.
7. Centrifuge at 18,000 xg for 5 mins. Transfer the clear supernatant carefully to an **Elution Tube** without disrupting the red bottom phase.
   - If the endotoxin contained red bottom phase was absorbed, repeat step 7.

♦ Binding of Plasmid

8. Add the 650 µl **WP Buffer** to the clear supernatant and mix thoroughly.
9. Place a **FAPD Mini Column** in a **Collection Tube**. Transfer the supernatant carefully to the FAPD Mini Column and centrifuge at 18,000 xg for 1 min. Discard the flow-through.

♦ Wash Column

10. Add 400 µl of **WP Buffer** to the FAPD Mini Column and centrifuge at 18,000 xg for 1 min. Discard the flow-through.
11. Add 700 µl of **Wash Buffer** (ethanol contained) to the FAPD Mini Column and centrifuge at 18,000 xg for 1 min. Discard the flow-through.
12. Centrifuge at 18,000 xg for an additional 3 mins to dry the FAPD Mini Column.

♦ Elution

13. Place the FAPD Mini Column to a new **Elution Tube**.
14. Add 50~100 µl of **Elution Buffer** or endotoxin-free water (pH 7.5~9.0) to the membrane center of the FAPD Mini Column. Stand the column for 1 min.
15. Centrifuge at 18,000 xg for 1 min to elute plasmid DNA and store the DNA at -20°C.