User Manual:

FavorPrep™ Plasmid Extraction Mini Kit

Cat. No.: FAPDE 000-Mini (4 preps)
FAPDE 001 (100 preps)
FAPDE 001-1 (300 preps)
(For Research Use Only)

Kit Contents:

Cat. No:	FAPDE 000-Mini (4 preps_sample)	FAPDE 001 (100 preps)	FAPDE 001-1 (300 preps)
FAPD1 Buffer	1.5 ml	30 ml	90 ml
FAPD2 Buffer	1.5 ml	30 ml	90 ml
FAPD3 Buffer	1.5 ml	40 ml	120 ml
W1 Buffer (concentrate)[a]	1.3 ml	35 ml	98 ml
Wash Buffer (concentrate)[b]	1.0 ml	20 ml	50 ml
Elution Buffer	0.5 ml	15 ml	35 ml
FAPD Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
RNase A (Lyophilized)	0.15 mg	3 mg	9 mg
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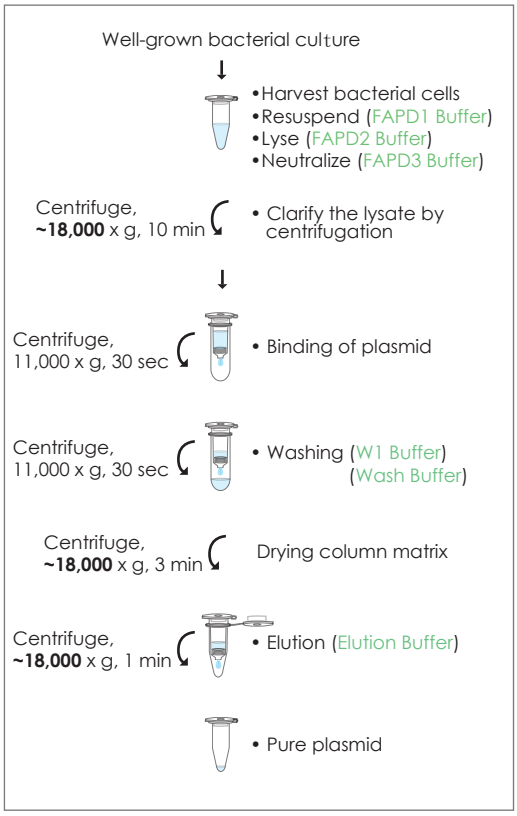
Preparation of W1 Buffer and Wash Buffer by adding ethanol (96 ~ 100%)			
Ethanol volume for W1 Buffer[a]	0.5 ml	13 ml	36 ml
Ethanol volume for Wash Buffer[b]	4 ml	80 ml	200 ml

Brief procedure:



Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1 ~ 5 ml
Size of plasmid or construct:	< 15 kb
Operation time:	< 25 minutes
Typical Yield:	25 ~ 40 µg
Binding capacity:	60 µg/ column
Column applicability:	centrifugation and vaccum

Important Notes:

1. Store RNase A at -20 °C upon recipit of kit.
2. Add 0.5 ml of FAPD1 Buffer to a RNase A tube, Dissolve the RNase A by vortexing. Briefly spin the tube and transfer the total RNase A mixture back to the FAPD1 bottle, mix well by vortexing and store the FAPD1 buffer at 4 °C.
3. If precipitates have formed in FAPD2 Buffer, warm the buffer in 37°C waterbath to dissolve precipitates.
4. Preparation of W1 Buffer and Wash Buffer by adding 96 ~100% ethanol (not provided) for first use.
5. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~1,8000 x g.

General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Transfer 1~ 5 ml of well-grown bacterial culture to a centrifuge tube (not provided).
2. Centrifuge the tube at 11,000 x g for 1 minute to pellet the cells and discard the supernatant completely.
3. Add 250 µl of FAPD1 Buffer (RNase A added) to the cell pellet and resuspend the cells completely by pipetting.
- Make sure that RNase A has been added into FAPD1 Buffer when first use.
- No cell pellet should be visible after resuspension of the cells.

4. Add 250 µl of FAPD2 Buffer and gently invert the tube 5 ~ 10 times. Incubate the sample mixture at room temperature for 2 ~ 5 minutes to lyse the cells.
- Do not vortex, vortex may shear genomic DNA. If necessary, continue inverting the tube until the lysate become clear.
- Do not proceed the incubation over 5 minutes.

5. Add 350 µl of FAPD3 Buffer and invert the tube 5 ~ 10 times immediately to neutralize the lysate.
- Invert immediately after adding FAPD3 Buffer will avoid asymmetric precipitation.

6. Centrifuge at full speed (~18,000 x g) for 10 min to clarify the lysate. During centrifugation, place a FAPD Column in a Collection Tube.
7. Transfer the suspernatant carefully to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.
- Do not transfer any white pellet into the column.

8. Add 400 µl of W1 Buffer to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.
- Make sure that ethanol (96-100 %) has been added into W1 Buffer when first use.

9. Add 700 µl of Wash Buffer to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.

• Make sure that ethanol (96-100 %) has been added into Wash Buffer when first use.

10. Centrifuge at full speed (~ 18,000 x g) for an additional 3 minutes to dry the FAPD Column.

• **Important step !** The residual liquid should be removed thoroughly on this step.

11. Place the FAPD Column to a new 1.5 ml microcentrifuge tube (not provided).

12. Add 50 µl ~ 100 µl of Elution Buffer or ddH_2O to the membrane center of the FAPD Column. Stand the column for 1 minute.

• Important step ! For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.

> • **Note !** Do not Elute the DNA using less than suggested volume (50ul). It will lower the final yield.

13. Centrifuge at full speed (~ 18,000 x g) for 1 minute to elute plasmid DNA and store the DNA at -20 °C.

Troubleshooting

Low yield

Bacterial cells were not lysed completely

- Too many bacterial cells were used (OD600 > 10). Separate the bacterial culture into multiple tubes.
- After FAPD3 Buffer addition, break up the precipitate by inverting to ensure higher yield.

Overgrown of bacterial cells

- Incubation time should not longer than 16 hours.

Bacterial cells were insufficient

- Ensure that bacterial cells have grown to an expected amount (OD600 > 1) after incubation under suitable shaking modes.

Incorrect DNA elution step

- Ensure that Elution Buffer was added and absorbed to the center of the FAPD Column matrix.

Incomplete DNA Elution

- If size of DNA fragments is larger than 10 kb, use preheated Elution Buffer (60~70°C) on slution step to improve the elution efficiency.

Incorrect preparation of W1 Buffer and Wash Buffer

- Ensure that the correct volume of ethanol (96 ~ 100 %) was added to W1 Buffer and Wash Buffer pior to use.

Eluted DNA does not perform well

Residual ethanol contamination

- After Wash Step, dry the FAPD Column with an additional centrifugation at top speed (~18,000 x g) for 5 minutes or incubation at 60°C for 5 minutes.

Genomic DNA Contaminates

Lysate prepared improperly.

- Gently invert the tube after adding the FAPD2 Buffer. And the incubation time should not longer than 5 minutes.
- Do Not use overgrown bacterial culture.

RNA Contaminates Plasmid DNA

Insufficiency of RNase A activity in FAPD1 Buffer because of long-term storage

- Prior to using FAPD1 Buffer, ensure that RNase A was added. If RNase A added FAPD1 Buffer is out of date, add additional RNase A into FAPD1 Buffer to a concentration of 50 µg/ ml then store 4°C.
- Too many bacterial cells were used, reduce sample volume.

Smearing or degrading of Plasmid DNA

Nuclease contamination

- If used host cells have high nuclease activity (e.g., *enA*$^+$ strains), perform the following optional Wash Step to remove residuary nuclease.
  a. After DNA Binding Step, add 400 µl of W1 Buffer into the FAPD Column and incubate for 2 minutes at room temperature.
  b. Centrifuge at full speed (~18,000 xg) for 30 seconds.
  c. Proceed to step 9.

Plasmid DNA is not adequate for enzymatic digestions

Eluted plasmid DNA contains residual ethanol

- Make sure you have discarded the flow-through after washing with Wash Buffer (Step 9) and centrifuged for an addition 3 minutes (Step 10).

Denatured Plasmid DNA migrate faster than supercoilded form during electrophoresis

Incubation in FAPD2 Buffer too long

- Do not incubate the sample longer than 5 minute in FAPD2 Buffer