



Plasmid Extraction Mini Kit

Cat. No.:PDE-100
(For Research Use Only)

Kit Contents:

Cat. No:	PDE-100 (100preps)
FAPD1 Buffer	25 ml
FAPD2 Buffer	25 ml
FAPD3 Buffer	35 ml
W1 Buffer	45 ml
Wash Buffer (concentrate) ^a	20 ml
Elution Buffer	15 ml
FAPD Column	100 pcs
Collection Tube	100 pcs
RNase A	2.5 mg
User Manual	1
Preparation of Wash Buffer by adding 80ml ethanol (96 ~ 100%)	

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1 ~ 3 ml
Size of plasmid or construct:	< 15 kb
Operation time:	< 25 minutes
Typical Yield:	20 ~ 30 µg
Binding capacity:	60 µg/ column
Column applicability:	centrifugation and vaccum

Important Notes:

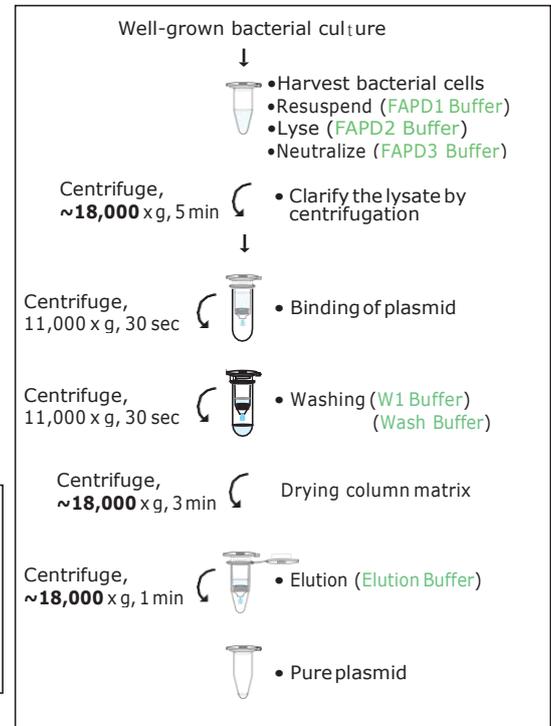
1. Store RNase A at -20 °C upon receipt of kit.
2. Add 0.5 ml of FAPD1 Buffer to a RNase A tube, vortex the tube to mix well. Briefly spin the tube and transfer the total RNase A mixture back to the FAPD1 bottle, mix well by vortexing and store the FAPD1 buffer at 4 °C.
3. If precipitates have formed in FAPD2 Buffer, warm the buffer in 37°C waterbath to dissolve precipitates.
4. Preparation of 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 ~ 3 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 200 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 200 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 300 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 5 min to clarify the lysate. During centrifugation, place a FAPD Column in a Collection Tube.
 7. Transfer the supernatant 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.
 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 ddH₂O 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.

Brief procedure:



Troubleshooting

Low yield

Bacterial cells were not lysed completely

- Too many bacterial cells were used ($OD_{600} > 10$). 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 ($OD_{600} > 1$) 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 ($60\sim 70^{\circ}\text{C}$) on slution step to improve the elution efficiency.

Incorrect preparation of Wash Buffer

- Ensure that the correct volume of ethanol (96 ~ 100 %) was added to and Wash Buffer prior 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 ($\sim 18,000 \times g$) 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 $50 \mu\text{g}/\text{ml}$ 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 $400 \mu\text{l}$ of W1 Buffer into the FAPD Column and incubate for 2 minutes at room temperature.
 - b. Centrifuge at full speed ($\sim 18,000 \times g$) 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 supercoiled form during electrophoresis

Incubation in FAPD2 Buffer too long

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