

User Manual



FavorPrep™ Plasmid Fast Extraction Mini Kit

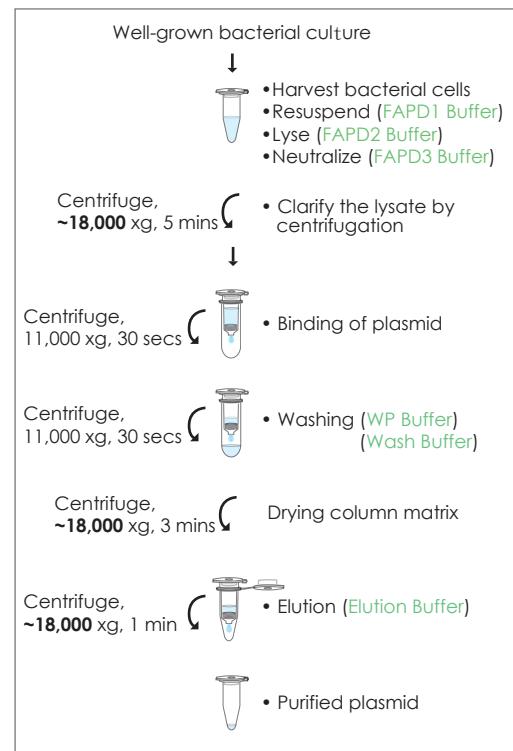
Kit Contents:

	Cat. No:	FSPD2020 (4 preps)	FSPD2024 (100 preps)	FSPD2026 (300 preps)
FAPD1 Buffer		1 ml	25 ml	75 ml
FAPD2 Buffer		1 ml	25 ml	75 ml
FAPD3 Buffer		1.5 ml	35 ml	105 ml
WP Buffer		2 ml	45 ml	135 ml
Wash Buffer (Concentrate) ^a		1 ml	20 ml	50 ml
Elution Buffer		0.5 ml	15 ml	35 ml
FAPD Columns		4 pcs	100 pcs	300 pcs
Collection Tubes		4 pcs	100 pcs	300 pcs
RNase A Solution ^b		10 µl	130 µl	360 µl
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Preparation of FAPD1 and Wash Buffer.				
Ethanol volume for Wash Buffer ^a		4 ml	80 ml	200 ml
Volume of RNase A Solution for FAPD1 Buffer ^b		4 µl	100 µl	300 µl

Specification:

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

Brief procedure:



Important Notes:

1. Store RNase A Solution at -20°C upon receipt of kit.
2. Add indicated volume of RNase A Solution into FAPD1 buffer, mix well 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) when first open.
5. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000~1,8000 xg.

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 xg for 1 min 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 at the 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 mins.
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 xg) for 5 mins 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 xg for 30 secs. 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 WP Buffer to the FAPD Column and centrifuge at 11,000 xg for 30 secs. Discard the flow-through and place the column back to the Collection Tube.

9. Add 700 μ l of Wash Buffer to the FAPD Column and centrifuge at 11,000 xg for 30 secs. 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 at the first use.
10. Centrifuge at full speed (~18,000 xg) for an additional 3 mins 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 50~100 μ l of Elution Buffer or ddH₂O to the membrane center of the FAPD Column. Stand the column for 1 min.
 - 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 (50 μ l). It will lower the final yield.

13. Centrifuge at full speed (~18,000 xg) for 1 min 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>1). 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 hrs.

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 elution step to improve the elution efficiency.

Incorrect preparation of WF Buffer and Wash Buffer

- Ensure that the correct volume of ethanol (96~100%) was added to Wash Buffer prior to using.

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 xg) for 5 mins or incubation at 60°C for 5 mins.

Genomic DNA Contaminates

Lysate prepared improperly.

- Gently invert the tube after adding the FAPD2 Buffer. And the incubation time should not longer than 5 mins.
- 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.
- RNase A is not properly preserved.
- 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 μ l of WP Buffer into the FAPD Column and incubate for 2 mins at room temperature.
 - b. Centrifuge at full speed (~18,000 xg) for 30 secs.
 - 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 mins (Step 10).

Denatured Plasmid DNA migrate faster than supercoiled form during electrophoresis

Incubation time of FAPD2 Buffer is too long

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