User Manual

Cat. No.: FAPDE 004 (4 preps)
FAPDE 050 (50 preps)
FAPDE 200 (200 preps)

For Research Use Only

FavorPrep™ Plasmid Extraction Mini Kit

Kit Contents:

Cat. No:	FAPDE 004 (4 preps)	FAPDE 050 (50 preps)	FAPDE 200 (200 preps)
FAPD1 Buffer	1 ml	13 ml	50 ml
FAPD2 Buffer	1 ml	13 ml	50 ml
FAPD3 Buffer	1.5 ml	18 ml	70 ml
WF Buffer (Concentrate)[a]	2 ml	23 ml	90 ml
Wash Buffer (Concentrate)[b]	1 ml	10 ml	40 ml
Elution Buffer	0.5 ml	7 ml	30 ml
FAPD Column	4 pcs	50 pcs	200 pcs
Collection Tube	4 pcs	50 pcs	200 pcs
RNase A Solution [c]	10 µl	50 µl	170 µl
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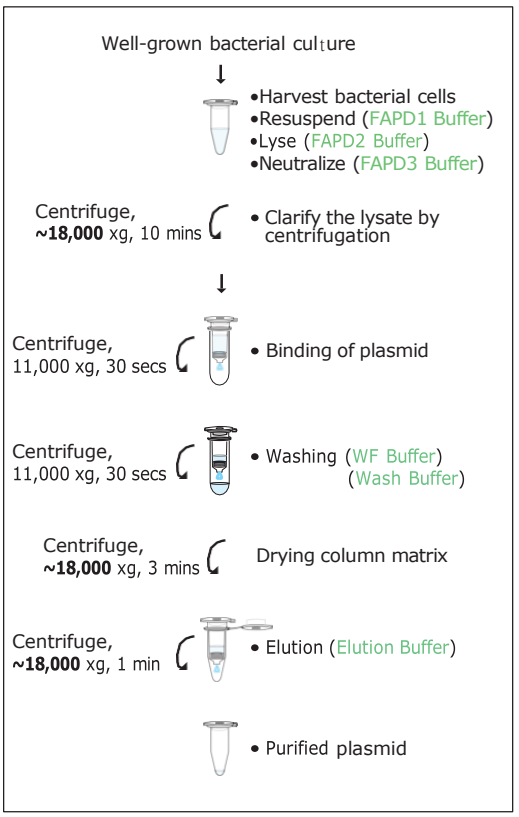
Preparation of FAPD1 Buffer, WF Buffer and Wash Buffer.			
Ethanol volume for WF Buffer [a]	0.5 ml	13 ml	36 ml
Ethanol volume for Wash Buffer [b]	4 ml	80 ml	200 ml
Volume of RNase A Solution for FAPD1 Buffer [c]	6 µl	120 µl	360 µl

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:	20~43 µg
Binding capacity:	60 µg/column
Column applicability:	Centrifugation and vacuum

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 water bath to dissolve precipitates.
4. Preparation of WF Buffer and 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 mins 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 WF 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 µl 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 µl of Elution Buffer or ddH_2O 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 µl). 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 WF Buffer and 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 µl of WF 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 supercoilded form during electrophoresis

Incubation time of FAPD2 Buffer is too long
- •Do not incubate the sample longer than 5 mins in FAPD2 Buffer