User Manual

Cat. No.: FSPD1020 (4 preps)
FSPD1024 (100 preps)
FSPD1026 (300 preps)

For Research Use Only

FavorPrep™ Plasmid Extraction Mini Kit

Kit Contents:

Cat. No:	FSPD1020 (4 preps)	FSPD1024 (100 preps)	FSPD1026 (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
WF Buffer (Concentrate)[a]	1.3 ml	35 ml	98 ml
Wash Buffer (Concentrate)[b]	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 [c]	10 µl	130 µl	420 µl
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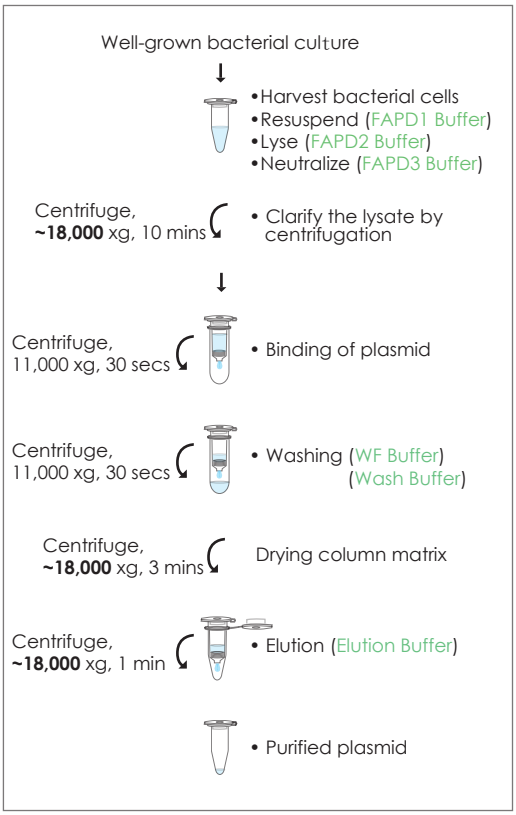
Preparation of FAPD1 Buffer, WF Buffer and Wash Buffer.			
Ethanol volume for WF Buffer [a]	0.5 ml	13 ml	36 ml
Ethanol volume for Wash Buffer [b]	4 ml	80 ml	200 ml
Volume of RNase A Solution for FAPD1 Buffer [c]	6 µl	120 µl	360 µl

Specification:

Principle:	Mini spin column (silica matrix)
Sample size:	1~5 ml
Size of plasmid or construct:	<15 kb
Operation time:	<25 mins
Typical Yield:	25~40 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 1~5 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 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 at the 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 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 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 xg) for 10 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.**
   -Make sure that ethanol (96~100%) has been added into WF Buffer at the first use.

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 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 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