# FavorPrep™ Plasmid DNA Extraction Mini Kit

(Cat.: FAPDE 100, FAPDE 300)

(For Research Use Only)

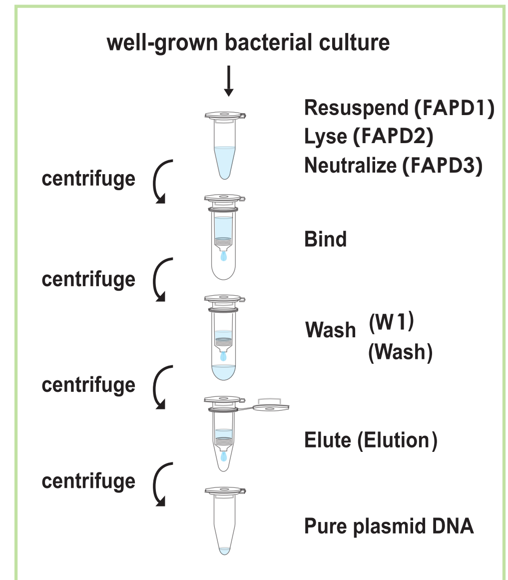
## Kit Contents:

| | FAPDE100 (100 preps) | FAPDE300 (300 preps) |
|---|---|---|
| FAPD1 Buffer | 25 ml | 65 ml |
| FAPD2 Buffer | 25 ml | 75 ml |
| FAPD3 Buffer | 35 ml | 100 ml |
| W1 Buffer | 45 ml | 130 ml |
| Wash Buffer* | 25 ml | 25 ml x 2 |
| Elution Buffer | 15 ml | 35 ml |
| RNase A (50mg/ml) | 50 µl | 130 µl |
| FAPD Column | 100 pcs | 300 pcs |
| 2 ml Collection Tube | 100 pcs | 300 pcs |

*Add 1ml of FAPD1 Buffer into provided RNase A tube and mix well. Transfer the mixture into FAPD1 Buffer bottle and store at 4 °C.

**Warm FAPD2 Buffer in a 55 °C waterbath to dissolve precipitates if any precipitate formed. Don't shake FAPD2 Buffer vigorously.

***Add 100 ml ethanol (96-100%) to Wash Buffer when first open.




## Specification:

Sampling: 1~4 ml overnight culture
Plasmid Size: < 12Kb
Yield: 20~30 µg of high-copy plasmid
Handing time: about 25 min

## Important Notes:

1. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffer.
2. To avoid acidification of FAPD2 Buffer from $CO_2$ in the air, close the bottle immediately after use.
3. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

## Genenal Protocol:

1. Transfer 1-4 ml of well-grown bacteria culture to a microcentrifuge tube (not provided).

2. Descend the bacteria by centrifuging for 1 min and discard the supernatant completely.

3. Add 200 µl of FAPD1 Buffer (RNase A added) to the pellet and resuspend the cells completely by pipetting.
   - Make sure that RNase A has been added into FAPD1 Buffer when first open.
   - No cell pellet should be visible after resuspension of the cells.

4. Add 200 µl of FAPD2 Buffer and gently invert the tube 10 times to lyse the cells and incubate at room temperature for 2 min.
   - Do not vortex, vortex may shear genomic DNA. If necessary, continue inverting the tube until the lysate become clear.
   - Do not proceed this step over 5 min.

5. Add 300 µl of FAPD3 Buffer and invert the tube 10 times immediately but gently.
   - Invert immediately after adding FAPD3 Buffer will avoid asymmetric precipitation.

6. Centrifuge for 5 min at full speed. During centrifuging, place a FAPD Column in a Collection Tube.

7. Transfer the supernatant carefully to FAPD Column. Centrifuge for 30 seconds then discard the flow-through and place the FAPD Column back in the Collection Tube.
   - Do not transfer any white pellet into the column.

8. Add 400 µl of W1 Buffer to FAPD Column. Centrifuge for 30 seconds then discard the flow-through and place the FAPD Column back in the Collection Tube.

9. Add 600 µl of Wash Buffer (ethanol added) to FAPD Column. Centrifuge for 30 seconds then discard the flow-through and place the FAPD Column back in the Collection Tube.
   • Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.

10. Centrifuge again for an additional 3 min to dry the column.
   • Important step ! This step will remove the residual liquid completely that will inhibit subsequent enzymatic reaction.

11. Place 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 FAPD Column. Stand the column for 2 min.
   • Important step ! For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.

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

13. Centrifuge for 1 min to elute plasmid DNA.

14. Store plasmid DNA at 4 °C or -20 °C.

## Troubleshooting

### Low yield

*Bacterial cells were not lysed completely*
•Too many bacterial cells were used ($OD_{600}$ > 10). Separate the bacteria 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 FAPD Column Martix.

*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 Wash Buffer*
•Ensure that Ethanol was added to Wash Buffer pior to use.

### Eluted DNA does not perform well

*Residual ethanol contamination*
•After Wash Step, dry FAPD Column with additional centrifugation at top speed for 5 minutes or incubation at 60°C for 5 minutes.

### Genomic DNA Contaminates

*Lysate prepared improperly.*
•Gently invert the tube after adding 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 50µg/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 this Optional Wash Step to remove residuary nuclease.
•After DNA Binding Step, add 400µl of W1 Buffer into FAPD column and column and incubate for 2 minutes at room temperature.
•Centrifuge at full speed (14,000 rpm or 10,000 xg) for 30 seconds.
•Followed using standard Wash Step.

### 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 longer than 5 minute in FAPD2 Buffer