FavorPrep™ Plasmid Extraction Mini Kit

Cat. No. FAPDE 000-Mini, FAPDE 001, FAPDE 001-1

Additional Material Provided by User:

• Lysozyme for molecular biology.
Add proper ddH_2O to lysozyme tube to make a **20 mg/ml** stock solution. Vortex and make sure that lysozyme has been completely dissolved. Aliquot the lysozyme stock into small fractions and store the unused portions at -20°C.

For Gram-Positive Bacteria Sample

Please Read Important Notes of General Protocol 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) and 15 µl of **lysozyme solution** 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. Incubate the sample mixture at 37°C for 30 mins.
5. 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.
6. 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.
7. Centrifuge at full speed (~18,000 xg) for 10 mins to clarify the lysate. During centrifugation, place a **FAPD Column** in a **Collection Tube**.
8. 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.
9. 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.

10. 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.
11. 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.
12. Place the FAPD Column to a new 1.5 ml microcentrifuge tube (not provided).
13. Add 50 µl~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 use less than suggested volume (50 µl). It will lower the final yield.
14. Centrifuge at full speed (~18,000 xg) for 1 min to elute plasmid DNA and store the DNA at -20°C.