eCube Plasmid DNA Mini Kit

*** Things to do before starting**

1. Add 1ml of XPPD1 Buffer into RNase A tube and mix. Transfer the mixture into XPPD1 Buffer bottle and store at 2-8°C.
2. For 50preps, add 6.5ml ethanol (96-100%) to Wash Buffer 1 and add 40ml ethanol (96-100%) to Wash Buffer 2.
   For 200preps, add 26ml ethanol (96-100%) to Wash Buffer 1 and add 160ml ethanol (96-100%) to Wash Buffer 2.
   For 300preps, add 36ml ethanol (96-100%) to Wash Buffer 1 and add 200ml ethanol (96-100%) to Wash Buffer 2.
3. All centrifuge steps are done at full speed (**14,000 rpm or 10,000 x g**) in a microcentrifuge.

Well-grown bacterial culture

1. Harvest the bacterial culture by centrifugation

2. Add **250ul of XPPD1 Buffer (RNase A added)** to resuspend by vortexing or pipetting.
3. Add **250ul of XPPD2 Buffer** and mix gently by inverting 5-10 times.
4. Add **350ul of XPPD3 Buffer** and mix immediately by inverting 5-10 times.

5. Centrifuge for **10 min**

6. Transfer the supernatant carefully to **XPPD column**.

7. Centrifuge for **1 min** then discard the flow-through

8. Add **400ul of Wash Buffer 1** to XPPD Column.

9. Centrifuge for **1 min** then discard the flow-through

10. Add **750ul of Wash Buffer 2** to XPPD Column.

11. Centrifuge for **1 min** then discard the flow-through

12. Centrifuge for **3 min** to dry column

13. Add **50ul – 100ul of Elution Buffer or ddH_2O** (pH 7.0 – 8.5) to the membrane center of XPPD Column. Stand the column for 1 min.

14. Centrifuge for **1 min**

15. You can get pure plasmid DNA.

BENCH-TOP PROTOCOLS for eCube Plasmid DNA Mini Kit

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Please consult the eCube Plasmid DNA Mini Kit Handbook before using these protocols for the first time.

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