eCube Plasmid DNA Midi Kit

*** Things to do before starting**

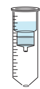
1. Brief spin RNase A tube and add the RNase A to XPD1 Buffer and mix well.
   Store XPD1 Buffer at 2-8°C after adding RNase A.
2. If precipitates have formed in XPD2 Buffer, warm the buffer in 37°C waterbath to dissolve preciptates.

Well-grown bacterial culture

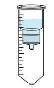
1. Harvest the bacterial culture
   Centrifugation at **6,000 x g** for **15 min**

2. Add **8ml of XPD1 Buffer (RNase A added)** to resuspend by vortexing or pipetting.
3. Add **8ml of XPD2 Buffer** and mix gently by inverting 10-15 times.
4. Add **8ml of XPD3 Buffer** and mix immediately by inverting 10-15 times.

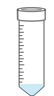
5. Centrifuge at **15,000 x g** for **20 min** at **4°C**



* Place a XPD Midi Column into a 50 ml centrifuge tube. Add 5 ml of XEQ Buffer to equilibrate the XPD Midi column by gravity flow.

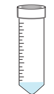


6. Transfer the supernatant to the **XPD Midi Column** and allow the column to empty by gravity flow. Discard the filtrate.
7. Add **12ml of Wash Buffer** to the XPD Midi column and allow the column to empty by gravity flow. Discard the filtrate.
8. Place the XPD Midi column into a new 50ml centrifuge tube (not provided) and add **8ml of Elution Buffer** to elute DNA by gravity flow.



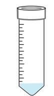
9. Precipitate DNA by adding **6ml of isopropanol** to the eluted DNA.
   Mix well by inverting the tube 10 times.

10. Centrifuge at **20,000 x g** for **30 min** at **4°C**



11. Carefully remove the supernatant and wash the DNA pellet with **5ml of room temperature 70% ethanol.**

12. Centrifuge at **20,000 x g** for **10 min** at **4°C**



13. Carefully remove the supernatant.
    Then air-dry the DNA pellet until the tube is completely dry.
    (Or incubate the DNA pellet at 70 °C for 10 min.)
14. Dissolve the DNA pellet in **300ul or a suitable volume of TE buffer or ddH$_2$O.**

15. You can get pure plasmid DNA.

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

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BENCH-TOP PROTOCOLS for eCube Plasmid DNA Midi Kit

eCube Plasmid DNA Midi Kit

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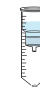

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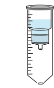

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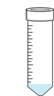

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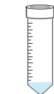

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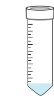

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