Enzymax LLC

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Isolation of plasmid DNA using a centrifuge or Vacuum manifold

1. Centrifuge 150-300ml of fresh bacterial culture at >3,500xg for 10 minutes. Discard the supernatant.
2. Add 10ml P1 buffer (add RNase A before use) to the pellet and resuspend completely with pipette.
3. Add 10ml P2 buffer and mix immediately by inverting the tube 4-6 times. Leave at room temperature for 5 minutes to lyse the cells completely.
4. Add 14.4 ml N3 and mix gently by inverting the tube 4-6 times and incubate on ice for 5 minutes.
   Note: Add Triton X-114 to remove the endotoxin if necessary (see Appendix below).
5. Transfer the mixture to a 50 ml conical tube and centrifuge at > 3,500xg for 5 -10 minutes to pellet the cell debris. Carefully transfer the supernatant (clear cell lysate) to a clean 50 ml conical tube.
6. Place the maxi column (EZC111) onto a vacuum manifold or into a 50ml conical tube. Add clear lysate to the column (either on the vacuum manifold or in 50ml conical tube).
7. Centrifuge (>3,500xg, for 2-5 minutes) or apply the vacuum. Discard the flow through.
8. Add 10ml 1x PE Wash buffer (ethanol added) or 1x CWB wash buffer (ethanol added) to the column. Centrifuge (>3,500xg, for 2-5 minutes) or apply the vacuum. Discard the flow through.
9. After the wash, centrifuge or leave the vacuum on for additional 5 minutes to remove all residual wash buffer. This step is critical, because the residual ethanol in the wash buffer may affect the downstream applications.
10. Transfer the column into a clean 50 ml conical tube and add 2-3 ml of TE or ddH_2O (or EB elution buffer) to the center of the column. Incubate at RT for one minute, then centrifuge at >3,500xg for 1 minute. Note: pre-warm TE or ddH_2O at 65*C if the size of the DNA is >4kb, which will help to elute the DNA from column.

Solutions: For Plasmid DNA Preparations (mini, midi, and maxi prep):

P1 (resuspension buffer): 50 mM Tris-HCl, 10 mM EDTA, pH 8.0 (25^{o}C), 50-100 ug/ml RNase A (or buy bulk from Qiagen Cat# 19051, 500ml)

P2 (lysis buffer): 200 mM NaOH, 1% SDS (or buy bulk from Qiagen Cat#19052, 500ml)

N3 (neutralization buffer for DNA binding): 4 M guanidine hydrochloride (GuHCl), and 0.5 M potassium acetate, pH 4.2 (or buy bulk from Qiagen Cat#19064, 500 ml)

5x PE Buffer (5x concentrated wash buffer, dilute it into 1x wash buffer with 100% ethanol before use): 80 mM NaCl, 8 mM Tris-HCl, pH 7.5 (25^{o}C) (Qiagen Cat#19065, 100ml for making 500ml 1x PE Buffer)

1.4x Column Wash Buffer (CWB): 80mM potassium acetate, 8.3mM Tris-HCl (pH 7.5), 40µM EDTA: Add 70ml 95% Ethanol to 50ml 1.4x column wash buffer. ***NOTE:** Some customers claimed this wash buffer will increase the DNA yield.

EB Buffer (DNA elution): 10 mM Tris-HCl, pH 8.0 or ddH_2O.

Appendix: Procedures to remove endotoxin

- Add 0.1 x volume of 10% Triton X-114 to the cold and crude DNA solution (Step 4).
- Incubate on ice and mix occasionally by inversion the tubes.
- Incubate at 37*C for 20-30 minutes or till the phase separate.
- Spin for 5 minutes at low speed (3000xg) at room temperature.
- Transfer the upper aqueous phase to an endotoxin free tube or load it directly to DNA mini (EZC101), Tin (EZC106) or Maxi (EZC111) spin columns.
- Proceed with step 6 for the rest of the DNA purification. Use endotoxin-free buffers and tubes.