

Plasmid DNA minipreps (alkaline lysis method)

1. Centrifuge 1 mL of overnight bacterial culture in eppi tube for 2 min at 10,000 x g at room temperature.
2. Aspirate supernatant; add 100 µL GTE buffer and resuspend by vortex.
3. Add 200 µL of fresh lysis buffer; mix gently by inversion 5-7 times.
4. Incubate on ice 5 min (do not go longer).
5. Add 150 µL ice-cold KoAC buffer; mix gently by inversion 5-7 times.
6. Incubate on ice 5 min (or longer).
7. Centrifuge for 10 min at 16,000 x g at 4°C.
8. Transfer supernatant to new eppi tube; add equal volume PCI; mix by vortex.
9. Centrifuge for 5 min at 16,000 x g at room temperature.
10. Carefully transfer aqueous phase to new eppi tube.
11. Add equal volume 100% isopropanol or 2 volumes 95-100% ethanol; mix by vortex.
12. Incubate on ice for at least 20 min.
13. Centrifuge for 10 min at 16,000 x g at 4°C.
14. Aspirate supernatant; add 500 µL 70-80% ethanol; vortex.
15. Centrifuge for 5 min at 16,000 x g at room temperature.
16. Aspirate supernatant; dry pellet under vacuum for 10-15 min (or on bench for longer).
17. Add 50 µL TE buffer.

Buffers:

GTE buffer

1% glucose
10 mM EDTA, pH 8.0
50 mM Tris-HCl, pH 7.5

Lysis buffer*

1% SDS
0.2 M NaOH
*prepare fresh

KoAC buffer*

5M potassium acetate
*note: do not adjust pH

PCI

25:24:1 preparation of
TE-saturated phenol:chloroform:isoamyl alcohol
plus 8 mg/mL 8-hydroxyquinoline

TE buffer

10 mM Tris, pH 7.5
1 mM EDTA, pH 8.0