

Plasmid DNA Isolation with Alkaline Lysis and Glassmilk

1. Spin down a 1.5 mL overnight bacterial culture for 10 sec in a microcentrifuge (max speed). Remove the supernatant by aspiration.
2. Resuspend bacterial pellet in 50 μ L of resuspension buffer by vortexing.
3. Add 50 μ L of cell lysis solution and invert 4 times (**do not vortex**).
4. Add 50 μ L of neutralization solution and invert 4 times (**do not vortex**).
5. Spin tube at max speed in microcentrifuge for 10 min and transfer supernatant to a new 1.5 mL tube. Avoid pelleted material.
6. Add 500 μ L of 6 M NaI binding solution to transferred supernatant and invert. Then add 20 μ L of glassmilk suspension. Mix by inversion and let set at room temp for 5 – 7 minutes.
7. Pellet glassmilk by spinning tube at 10 sec at max speed in microcentrifuge.
8. Remove as much supernatant as possible, spin a second time and remove remaining NaI from bottom of tube with pipetter.
9. Add 1 mL of New Wash solution and resuspend glassmilk by pipetting.
10. Spin down glassmilk for 10 sec and remove supernatant. If plasmid is for diagnostic digests proceed to next step. If plasmid is for sequencing or other operations requiring clean DNA, repeat wash step 2 more times before going to step 11.
11. Remove last traces of wash solution by spinning down and removing the remaining wash solution with a pipette. Resuspend pellet in 15 μ L of sterile water and place at 37 C for 2 minutes.
12. Spin down glassmilk for 1 min and transfer eluted DNA to a clean tube. Resuspend the glassmilk a second time with 15 μ L of sterile water and place at 37 C for 2 min and spin down for 1 min, and add supernatant to previous DNA elution from step 11.
13. Quantify plasmid concentration by spectrophotometer. Typical yields are 2 – 3 μ g of clean plasmid DNA from high copy number plasmids.

Solutions:

Resuspension Solution:

50 mM Tris-HCl, pH 7.5
10 mM EDTA, pH 8.0
100 µg/mL RNase A

Cell Lysis Solution:

0.2 M NaOH
1% SDS

Neutralization Solution:

4.09 M Guanidine Hydrochloride
0.76 M Potassium Acetate
2.12 M Glacial Acetic Acid

6 M NaI Binding Solution for Glassmilk

NaI	90 g
Sodium sulfite	1 g
water	to 100 mL

Filter through Whatmann filter paper and store in an opaque or smoky bottle.

New Wash Solution:

50% Ethanol
20 mM Tris-HCl (pH 7.6-8.0)
0.1 M NaCl