DNA Plasmid Isolation Using Alkaline Lysis Method

Buffers and Solutions

Alkaline lysis solution I: 50 mM glucose, 25 mM Tris-Cl (pH 8.0), 10 mM EDTA (pH 8.0), de-ion water

Alkaline lysis solution II : 0.2 N NaOH, 1% (w/v) SDS, de-ion water

Alkaline lysis solution III : 5 M potassium acetate, glacial acetic acid, de-ion water

Ethanol 70% (v/v)

Isopropanol

TE-RNAase pH 8.0

Method

1. Pour overnight grown culture to 1.5 mL labeled falcon tube.
2. Centrifugate at 14.000 rpm for 1 min.
3. Remove the supernatant from the tube.
4. Repeat step 1-3, until leaves bacterial pellet as dry as possible.
5. Add 150 µL resuspension buffer, resuspend the bacterial pellet properly by vortexing.
6. Add 200 µL lysis solution to bacterial suspension (freshly made), close the tube tightly and mix contents thoroughly by inverting the tube 4-6 times until the solution becomes viscious.
7. Add 300 µL neutralization solution and mix contents thoroughly by inverting the tube 4-6 times.
8. Centrigufe at 14.000 rpm for 5 min.
9. Take the supernatant and transfer to a new 1.5 mL falcon max 300 µL.
10. Add equal volume of isopropanol in the supernatant (300 µL) and mix it by inverting the tube couple of times.
11. Incubate in -80°C for 30 min.
12. Centrifuge at 14.000 for 5 min.
13. Remove the supernatant and add 600 µL EtOH 70%.
14. Centrifuge at 14.000 for 5 min.
15. Remove the supernatant and dry the pellet for 10-30 min.
16. Dissolve the pellet in 20-50 µL TE-RNAase pH 8.0. Confirm the plasmid with 5 µL DNA solvent by Agarose Electroforessis.

Recipes

Alkaline lysis solution I

1. 1 M glucose stock solution (50 mL)
   a. Dissolve 9 gram of glucose in 50 mL sterilized de-ion water.
   b. Filter sterilize using membrane millipore (0.20 µM).

c. Glucose solution is ready to use or store at 4°C cabinet for preservation.

2. 1 M Tris-Cl stock solution (50 mL)
   a. Dissolve 6.057 gram of Tris base in 50 mL sterilized de-ion water.
   b. Adjust the pH to the desired value by adding concentrated HCl.
3. 0.5 M EDTA stock solution (100 mL)
   a. Dissolve 14.612 gram of EDTA in 100 mL sterilized de-ion water.
   b. Adjust the pH to 8.0 with NaOH.

Prepare Solution I from standard stocks in batches of approx. 100 ml, autoclave for 15 minutes at 15 psi and store at 4°C.

Alkaline lysis solution I	Volume
1 M Glucose	5 mL
1 M Tris-Cl	2.5 mL
0.5 M EDTA	1 mL
De-ion water	90.5 mL
Total volume	100 mL

Alkaline lysis solution II

1. 10 N NaOH stock solution (50 mL)
   Dissolve 20 gram of NaOH in 50 mL sterilized de-ion water.
2. 1% (w/v) SDS stock solution (30 mL)
   Dissolve 0.3 gram of SDS in 30 mL sterilized de-ion water.

Prepare Solution II fresh and use at room temperature.

Alkaline lysis solution II	Volume
0.2 N NaOH	200 µL
1% SDS	1 mL
De-ion water	8.8 mL
Total volume	10 mL

Alkaline lysis solution III

1. 5 M potassium acetate stock solution (100 mL)
   Dissolve 49.071 gram of potassium acetate in 100 mL sterilized de-ion water.

Store the solution at 4°C and transfer it to an ice bucket just before use.

Alkaline lysis solution III	Volume
5 M Potassium acetate	60 mL
Glacial acetic acid	11.5 mL
De-ion water	28.5 mL
Total volume	100 mL

Reference

Sambrook, J., Fritsch, E.F., dan Maniatis, T. 1989. Molecular Cloning A Laboratory Manual 2nd edition. New York : Cold Spring Harbor Laboratory Press.