

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 μ L resuspension buffer, resuspend the bacterial pellet properly by vortexing.
6. Add 200 μ L 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 viscous.
7. Add 300 μ L neutralization solution and mix contents thoroughly by inverting the tube 4-6 times.
8. Centrifuge at 14.000 rpm for 5 min.
9. Take the supernatant and transfer to a new 1.5 mL falcon max 300 μ L.
10. Add equal volume of isopropanol in the supernatant (300 μ L) 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 μ L 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 μ L TE-RNAase pH 8.0. Confirm the plasmid with 5 μ L DNA solvent by Agarose Electroforesis.

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 μ M).



- 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.

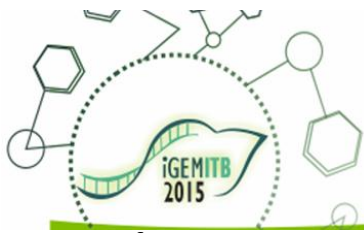
Alkaline lysis solution II	Volume
0.2 N NaOH	200 µL
1% SDS	1 mL
De-ion water	8.8 mL
Total volume	10 mL

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



PROTOCOL

ITB INDONESIA



Reference

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

