Calcium Competent Cell Production

Buffers Needed

1. Wash Buffer

5 mM Tris-HCl, pH 7.6

10 mM MgCl2

50 mM NaCl

1. Calcium Buffer

10 mM Tris-HCl, pH 7.6

10 mM MgCl2

100 mM CaCl2

1. Storage Buffer

43 mL Calcium Buffer mixed with 7 mL glycerol

Procedure

1. Preheat Luria-Bertani medium to 37° C. Inoculate with a 1% volume of overnight culture. (eg: 10 mL into 1.0L)
2. Grow to an optical density at 600nm of 0.4. Place culture on ice in the cold room for 15 minutes. Before proceeding, be certain that the culture is ice-cold!
3. Transfer to a sterile centrifuge bottle and pellet the cells for 10 minutes at 10000x g.
4. Resuspend the cells VERY GENTLY in 50 mL of Wash Buffer and spin as before.
5. Repeat step 4.
6. Resuspend the pellet in 25 mL of Calcium Buffer. Store on ice for 30 minutes.
7. Spin as before and resuspend the cells in 4 mL of storage buffer.
8. Dispense 100 μL aliquots on ice and store at -80**°**C for up to 3 months.