SPHEROPLAST PROTOCOL

SOLUTIONS

Terrific Broth (TB) 2% glucose – for 500 mL
1,16 g of KH₂PO₄
6.27 g of K₂HPO₄
1,2% tryptone
2,4% yeast extract
0,4% glycerol

Tris-HCl solution
10 mM Tris-HCl
pH 8.0

STE solution
0.5 M sucrose
10 mM Tris-HCl
10 mM EDTA
pH 8.0

Solution A
0.5 M sucrose
20 mM MgCl₂
10 mM MOPS
pH 6.8

Lyzozyme solution (50 mg/mL)
Dissolve 50 mg of lysozyme in 1 mL of cold ddH₂O

PROCEDURE

- 5 mL O/N culture in Terrific Broth (TB) 2% glucose
- 7 mL culture in in 125 ml Δ-flask culture (1/100 dilution) for 2 hours at 37 °C
- Cool down samples 20 min at room temperature
- Centrifuge 6 mL at 14,000-16,000 rpm for 2min
- Wash 2 times with 1 mL of Tris-HCl and with centrifugation at 14,000 rpm for 1min
- Re-suspend in 1mL of STE
- Incubate at 37°C on roto-mixer for 30 min.
- Centrifuge at 14,000 rpm for 1min.
- Wash with 1mL of freshly made Solution A. Centrifuge at 14,000 rpm for 1min.
- Prepare the lysozyme solution.
- Re-suspend in 1 mL of freshly made Solution A and add 20 μL of lysozyme solution.
- Rota-incubation at 37°C for 15 min.
- Centrifuge at 14,000 rpm for 1 min.
- Pellet re-suspension in 1mL of 1XPBS.