

Rubidium Chloride Competent Cells

Autoclave:

- 250ml centrifuge bottles (2)
- 500ml LB in 2L flask
- microfuge tubes for aliquats

1. Grow 5-10ml overnight culture in LB
2. Use small culture to inoculate 500ml culture. Grow at 37°C to OD₅₉₅ of 0.5
3. Cool 15 min. on ice
4. Transfer to two 250ml centrifuge bottles
5. Spin 10min at 4500rpm in GSA rotor to pellet cells
6. Pour off supernatant
7. Resuspend pellets in 30ml total of Tfb I by pipetting up and down
8. Combine and ice 15min
9. Spin 5min at 4000rpm
10. Resuspend in 6ml Tfb II on ice
11. Place aliquats in crushed-up dry ice

Tfb I	500 ml
RbCl (100mM)	7.25g
MnCl ₂ -4H ₂ O (50mM)	5.0g
Potassium Acetate (30mM)	14.7g
CaCl ₂ -2H ₂ O (10mM)	0.75g
Glycerol (15% final)	75.0 ml
Water	425ml

-filter sterilize, store at 4°C, protect from light

Tfb II	400ml
0.2M MOPS (10mM final)	20ml
RbCl (10mM)	0.48g
CaCl ₂ -2H ₂ O	4.4g
Glycerol (15%)	60ml

-filter sterilize, store at 4°C, protect from light

0.2M MOPS
20.93g in 500ml water
pH to 6.5 with HCl

-filter sterilize, store at 4°C, protect from light