

Rubidium Chloride Competent Cells

1. Grow 2-5ml overnight culture in LB.
2. Use small culture to inoculate 200ml culture. Grow at 37C to OD595 of 0.5.
3. Cool 15 min. on ice.
4. Transfer to centrifuge bottles.
5. Spin 15 min. at 3000rpm to pellet cells.
6. Pour off supernatant.
7. Resuspend pellets in 12ml total Tfb I by pipetting up and down.
8. Combine and place on ice 15 min.
9. Spin 5 min at 3000rpm.
10. Resuspend in 2.4ml Tfb II on ice.
11. Place aliquots in crushed dry ice.

Tfb I	100 ml
RbCl (100mM)	1.45 g
MnCl ₂ ·4H ₂ O (50mM)	1.0 g
Potassium Acetate (30mM)	2.94 g
CaCl ₂ ·2H ₂ O (10mM)	0.15 g
Glycerol (15% final)	15 ml
Water	QC to 100 ml
filter sterilize, store at 4C, protect from light	
Tfb II	100 ml
0.2M MOPS (10mM final)	5 ml
RbCl (10mM)	0.12 g
CaCl ₂ ·2H ₂ O	1.1 g
Glycerol (15%)	15 ml
Water	QC to 100 ml
filter sterilize, store at 4C, protect from light	
0.2M MOPS	
10.46g in 250ml water	
pH to 6.5 with HCl	
filter sterilize, store at 4C, protect from light	