

Competent Cell Growth

1. Streak LB plate (antibiotic free or CM if Roseta) with cells from the manufacturer and grow overnight at 37°C.
2. Inoculate a single colony into 8ml of LB and grow overnight at 37°C.
3. Inoculate 200ml S.O.B. with 0.5-1ml overnight culture and grow at 37°C until $O.D._{600} = 0.45-0.55$.
4. Spin at 4K rpm for 15min at 4°C.
5. Gently resuspend cells in 20ml ice-cold TFB and incubate on ice for 10 min.
6. Slowly add 700 μ l fresh DMSO while swirling cells and incubate on ice for 10 min.
7. Add another 700 μ l fresh DMSO while swirling cells and incubate for another 5 min on ice.
8. Aliquot 220 μ l into chilled 500 μ l tubes and flash-freeze in liquid nitrogen. Store at -80°C.

S.O.B. medium (200ml)

Bactotryptone	4g
Bacto-yeast extract	1g
NaCl	0.1g
dH ₂ O	200ml
250mM KCl	2ml

TFB Buffer (500ml) – store wrapped in foil at 4°C for up to 1 year

		<u>Final concentration</u>
1M MES (pH 6.3)*	5ml	1 mM
MnCl ₂ · 4H ₂ O	4.46g	45mM
CaCl ₂ · 2H ₂ O	0.735g	10mM
KCL	3.73g	100mM
Hexamminecobalt(II) Cl	0.4g	3mM
dH ₂ O	to 500ml	

*1M MES is prepared by dissolving 9.76g of MES in 40ml dH₂O, adjust pH to 6.3 with 5-10M NaOH, then adjust final volume to 50ml. Sterile filter solution and store at 4°C

MES (4-Morpholineethanesulfonic acid): Sigma – M361
Hexamminecobalt(II) chloride: Aldrich – 481521