

CaCl₂ for Competent Bugs

SOB Medium:

(2% (w/v) Bacto Tryptone
0.5% (w/v) Yeast extract
10 mM NaCl
2.5 mM KCl
10 mM MgCl ₂
10 mM MgSO ₄)

To make 500 ml:

Bacto Tryptone	10 g
Yeast Extract	2.5 g
NaCl	0.29 g
KCl	0.09 g

- dissolve in 500 ml of H₂O
- autoclave

Magnesium Stock Solution

1 M MgCl ₂	2 g
1 M MgSO ₄	2.5 g

- dissolve in 10 ml H₂O
- filter sterilize

Before use, add 1/100 vol of Magnesium stock solution to medium (i.e. 2.5 ml to 250 ml medium).

Buffer TB:

- 10 mM Hepes
- 15 mM CaCl₂
- 250 mM KCl
- 55 mM MnCl₂

To make 100 ml:

Hepes (M.W. 238.3)	0.24 g
CaCl ₂ (M.W. 147.02)	0.22 g
KCl (M.W. 74.55)	1.86 g

- dissolve in 80 ml of H₂O
- adjust pH to 6.7 with KOH
- then add 1.09 g of MnCl₂ (M.W. 197.9) and adjust the final volume to 100 ml with H₂O
- filter sterilize the solution