

ONE-STEP PREPARATION OF COMPETENT *E. coli*

1. Grow cells in LB to early exponential phase (OD=0.3-0.4).
2. Pellet cells by centrifugation at 1000g for 10 min. at 4C.
3. Resuspend cells in one tenth their original volume in ice-cold TSS solution.
4. For storage, freeze immediately in liquid nitrogen and store at -70 C.

Transformation and storage buffer (TSS)

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LB	For 100mL LB
10% PEG	10 g PEG
5% DMSO	5 mL DMSO
20-50mM MG ⁺² (MgSO ₄ or MgCl ₂)	1.23 g MgSO ₄ 7H ₂ O

Final pH should be 6.5

Reference:

Chung et. al., Proc. Natl. Acad. Sci. USA Vol. 86 pp. 2172-2175, April 1989.