# Preparation of chemically competent *Escherichia coli* cells

*Materials*

0.5 or 1.5-ml microfuge tubes
50-ml Falcon tubes

*Chemicals*

DMSO

**Procedure**

1. Inoculate 5 ml LB medium with the appropriate antibiotic(s) with the *E. coli* strain of which you want to make competent cells and incubate overnight at 37°C.
   *When preparing DH5α competent cells it is better to use SOB medium instead of LB.*
2. Use the overnight culture to inoculate 500 ml LB medium and incubate at 30°C until the absorbance at 600 nm is between 0.4-0.6.
   Optional: add 2.5 ml 2M $MgCl_2$ to the medium (to a final concentration of 10 mM) at the start of the cultivation.
   *In the original paper (Inoue et al, 1990) the culture was incubated at 18°C but similar results were obtained when the cultures were incubated at higher temperatures (25-30°C). SOB medium for DH5α competent cells.*
3. Chill the culture for at least 10 min on ice.
   *In the following steps the cell suspension should be kept on ice as much as possible.*
4. Spin the cell suspension for 10 min at 6000 rpm (Sorvall GSA rotor) or 4000 rpm when harvested in 50-ml Falcon tubes.
5. Gently resuspend the pellet in 100 ml ice-cold CC buffer in 50-ml Falcon tubes.
   *Resuspend with a 10-ml serological pipette and avoid introducing bubbles.*
6. Incubate the cell suspension on ice for at least 10 min.
7. Spin for 10 min at 4000 rpm at 4°C.
8. Gently resuspend the pellet in 18.6 ml ice-cold CC buffer and add 1.4 ml DMSO.
9. Incubate the cell suspension on ice for at least 10 min.
10. Distribute the cell suspension in 100-200 µl aliquots in 0.5 or 1.5-ml microfuge tubes.
11. Flash freeze the cell suspension in liquid nitrogen and store the tubes at -80°C.
    *At -80°C the cell will be competent for at least 6 months.*

**CC buffer**

| | | |
|---|---|---|
| 10 mM | Hepes | 2.38 g/L |
| 15 mM | $CaCl_2$ | 2.21 g/L |
| 55 mM | $MnCl_2.4H_2O$ | 10.89 g/L |
| 250 mM | KCl | 18.64 g/L |

Dissolve all components except $MnCl_2$ and adjust the pH to 6.7 with KOH. Then add the $MnCl_2$ and filter sterilize the solution over a 0.22 µm filter.

*In some protocols Pipes is used instead of Hepes.*

**2 M $MgCl_2$**

| | |
|---|---|
| 40.66 g/100mL | $MgCl_2.6H_2O$ |

Dissolve 40.66 g $MgCl_2.6H_2O$ in 74 ml water. Sterilize by autoclaving and store at room temperature.

**LB medium**

| | |
|---|---|
| 10 g/L | tryptone |
| 5 g/L | yeast extract |
| 10 g/L | NaCl |

Sterilize by autoclaving and store at room temperature.

**SOB medium**

| | |
|---|---|
| 20 g/L | tryptone |
| 5 g/L | yeast extract |
| 0.5 g/L | NaCl |
| 0.186 g/L | KCl |

Adjust the pH to 7.0 with NaOH.
Sterilize by autoclaving and store at room temperature.

**Reference**

Inoue *et al.* (1990) *Gene 96*, 23-28.

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