Growing and harvesting Electro Competent E-coli Cells

Leo Blondel

Abstract

for 500ml (2x250ml)

Citation: Leo Blondel Growing and harvesting Electro Competent E-coli Cells. **protocols.io** dx.doi.org/10.17504/protocols.io.gc7bszn
Published: 09 Nov 2016

Protocol

Prepare fresh SOB media

Step 1.

for 1L

SOB

- 2% w/v tryptone (20 g)
- 0.5% w/v Yeast extract (5 g)
- 8.56mM NaCl (0.5 g) or 10mM NaCl (0.584 g)
- 2.5mM KCl (0.186 g)
- $_{dd}H_2O$ to 1000 mL[4]
- 10mM $MgCl_2$ (anhydrous 0.952 g) AND 10mM $MgSO_4$ (heptahydrate 2.467 g)[2]

Starting Cultures

Step 2.

Inoculate 1 colony from a fresh plate of the strain to be made electrocompetent into 10 ml of SOB in a 125 ml flask and incubate for 16-18 hours at 37oC and 250 rpm.

DURATION
16:00:00

Inoculate

Step 3.

Have ready 2x 1L glass flasks containing 250 ml each of SOB pre-warmed to 37°C. Add 5ml (2% inoculation) of the overnight culture to each of the flasks.

Growth phase

Step 4.

Shake at 37°C and 250 rpm until the cultures reach an **OD600 of 0.5-0.7**.

Be sure to turn on centrifuge and **cool rotor to 4°C** well in advance of harvesting cells.

Be sure to place **1 L of 10% glycerol on ice** well in advance of harvesting cells

Cool the cultures

Step 5.

Place cultures on ice for 15 minutes. **From this point on the cultures must be kept ice cold**. Pour each 250 ml culture into chilled 500 ml (or 1000 ml) centrifuge bottles.

DURATION
00:15:00

Centrifuge

Step 6.

Centrifuge at **5000 rpm for 10 min**. Pour off the supernatant and aspirate any residual broth.

DURATION
00:10:00

Wash with glycerol

Step 7.

Add 250 ml of ice cold glycerol to each of the centrifuge bottles and completely resuspend the cells by pipetting up and down.

Centrifuge

Step 8.

Centrifuge at 5000 rpm for 10 min. Pour off the supernatant, it is not necessary to aspirate.

DURATION
00:10:00

Wash

Step 9.

Completely resuspend the cells in 250 ml ice cold glycerol.

Centrifuge

Step 10.

Centrifuge at 5000 rpm for 10 min. Pour off the supernatant and resuspend the cells **in the residual glycerol** by pipetting up and down.

DURATION
00:10:00

Aliquot the culture

Step 11.

Pipet desired volume (100µl works usualy well) of the culture to ependorf tubes **on ice.**

Freeze

Step 12.

Transfer the tubes to dry ice for 10 minutes.

Once the cultures are frozen, transfer them to a -80°C freezer.

The cultures should be good for >6 months.

DURATION
00:10:00