Making your own electrocompetent cells

New England Biolabs

Abstract

This is the protocol for making two 250 ml cultures of electrocompetent cells

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Before start

Sterile 10% glycerol (can be autoclaved) is needed for the washes. The volume of 10% glycerol needed is 2X the culture volume (for example, a 500 ml culture requires 1L of 10% glycerol).

Protocol

Step 1.
Innoculate 1 colony from a fresh plate of the strain to be made electrocompetent into **10 ml** of SOB in a 125 ml flask

AMOUNT
10 ml Additional info:

PROTOCOL

. SOB Media

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Step 1.1.
2% tryptone

Step 1.2.
0.5% yeast extract

Step 1.3.
10 mM NaCl

Step 1.4.
2.5 mM KCl

Step 1.5.
10 mM MgCl2

Step 1.6.
10 mM MgSO4

Step 2.
Incubate for 16-18 hours at 37°C and 250 rpm

DURATION
16:00:00

Step 3.
Pre-warm 2, 1 L flasks containing **250 ml** each of SOB

AMOUNT
250 ml Additional info:

Step 4.
Add two drops of the overnight culture to each of the pre-warmed 250 ml flasks

AMOUNT
250 ml Additional info:

Step 5.
Shake at 37°C and 250 rpm until the cultures reach an OD600 of 0.5-0.7

AMOUNT
250 ml Additional info:

NOTES
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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

Step 6.
Turn on centrifuge and cool rotor to 4°C well in advance of harvesting cells

Step 7.
Place **1 L** of 10% glycerol on ice well in advance of harvesting cells

Step 8.
Place cultures on ice for 15 minutes. From this point on the cultures must be kept ice cold

DURATION
00:15:00

Step 9.
Pour each 250 ml culture into chilled 500 ml (or 1000 ml) centrifuge bottles

Step 10.
Centrifuge at 5000 rpm for 10 min

DURATION
00:10:00

Step 11.
Pour off the supernatant and aspirate any residual broth

Step 12.
Add **250 ml** of glycerol to each of the centrifuge bottles and completely suspend the cells by pipetting up and down

DURATION
00:10:00

Step 13.
Centrifuge at 5000 rpm for 10 min

DURATION
00:10:00

NOTES
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It is not necessary to aspirate

Step 14.

Pour off the supernatant, it is not necessary to aspirate

Step 15.

Add **250 ml** of glycerol to each of the centrifuge bottles and completely suspend the cells by pipetting up and down

Step 16.

Pour off the supernatant and suspend the cells in the **residual** glycerol by pipetting up and down

Step 17.

To freeze, add **100 µl** of the culture to microcentrifuge tubes on ice

AMOUNT

100 µl Additional info:

NOTES

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At this point you can electroporate or freeze the cells away.

Step 18.

Once you have used all of the culture, transfer the tubes to dry ice for 10 minutes

DURATION

00:10:00

Step 19.

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