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Feb 10, 2022

Making your own electrocompetent cells V.2

New England Biolabs[1]

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dx.doi.org/10.17504/protocols.io.bd2zi8f6

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This protocol explains methods for making two 250 ml cultures of electrocompetent cells.

An Electroporation Protocol can be found here.

DOI

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https://www.neb.com/protocols/2012/06/21/making-your-own-electrocompetent-cells

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Electrocompetent cells

protocol ,

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MATERIALS

Magnesium sulfate heptahydrate **Sigma Aldrich Catalog #M2773**

NaCl **Sigma Aldrich Catalog #53014**

Tryptone **Fisher Scientific Catalog #BP1421-500**

Glucose **Sigma Aldrich Catalog #G8270**

Magnesium chloride hexahydrate **Sigma Aldrich Catalog #M2670**

Potassium chloride **Sigma Aldrich Catalog #P9333**

Glycerol **Thermo Fisher Catalog #17904**

Yeast Extract **Thermo Fisher Catalog #211930**

Media

SOB:
2% tryptone
0.5% yeast extract
10 mM NaCl
2.5 mM KCl
10 mM MgCl2
10 mM MgSO4

SOC (for Electroporation Protocol)**:**
SOB + 20 mM glucose

Appropriate Antibiotics for Your Application
Antibiotics for Plasmid selection

Antibiotic	**Working Concentration**
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Chloramphenicol	33 µg/ml
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml

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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).

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

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

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

2 

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

3 Pre-warm two 1 L flasks containing **250 mL each of SOB** pre-warmed to **37 °C** .

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

5 Shake at **37 °C** and **250 rpm** until the cultures reach an OD_{600} of 0.5-0.7.

IMPORTANT:
Be sure to turn on centrifuge and cool rotor to 4°C well in advance of harvesting cells.
Be sure to place **1 L 10% glycerol** **On ice** well in advance of harvesting cells.

6 

Place cultures **On ice** for **00:15:00** . **From this point on the cultures must be kept**

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ice cold.

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

8 

Centrifuge at **5000 rpm, 00:10:00** .

9 Pour off the supernatant and aspirate any residual broth.

10 

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

11 

Centrifuge at **5000 rpm, 00:10:00** .

12 Pour off the supernatant.

It is not necessary to aspirate any residual broth.

13 Completely suspend the cells in **250 mL glycerol** and re-centrifuge.

14 

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

15 

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

To freeze, add **100 µL culture** to microcentrifuge tubes **On ice** .

16 Once you have used all of the culture, transfer the tubes to dry ice for **00:10:00** .

17

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

The cultures should be good for >6 months.

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