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Preparation of XL1-Blue competent cells using MgCl2 and CaCl2

Rashmi Karki[1], Monica Rieth[1]

[1]Southern Illinois University-Edwardsville

1 Works for me dx.doi.org/10.17504/protocols.io.3trgnm6

labyrieth

Monica Rieth
Southern Illinois University-Edwardsville

ABSTRACT

Preparation of chemically competent Xl1-Blue cells using $MgCl_2$ and $CaCl_2$

The purpose is to prepare batches of chemically competent bacteria for the purposes of subcloning and protein expression. Competent bacteria are able to readily and passively take up foreign DNA due their compromised cell walls as a result of exposure to divalent metal ions.

This protocol is based on current methods reported in:
Sambrook, Joseph et al. (2001). Molecular cloning : a laboratory manual. Cold Spring Harbor, N.Y. :Cold Spring Harbor Laboratory Press

GUIDELINES

This protocol must be carried out under sterile conditions. Wherever possible working benchtop and spaces should be wiped down with 70% EtOH solution. Sterilized, gloved hands are a must at all times.

MATERIALS

NAME	CATALOG #	VENDOR
CaCl2		
MgCl2		
DMSO		
Micropipettes and tips		
Eppendorf tubes (1.5 & 2.0 ml)		
Paper towels		
LB Broth		
XL1-Blue cells		
sterile culture tubes (17 x 100 mm)		
high-speed preparatory centrifuge		

SAFETY WARNINGS

Proper clothing is required as it needs to be done in cold room (4 °C)
Keep the DMSO at room temperature
Wear gloves at all times

Citation: Rashmi Karki, Monica Rieth (04/13/2020). Preparation of XL1-Blue competent cells using MgCl2 and CaCl2. https://dx.doi.org/10.17504/protocols.io.3trgnm6

BEFORE STARTING

Autoclave 100 ml LB- broth in a 1000 ml flask

Sterilize and pre-chill the 50-mL conical tubes

Prepare 30 ml of ice cold MgCl2-CaCl2 solution (80mM $MgCl_2$, 20 mM $CaCl_2$)

Prepare ice cold 0.1 $CaCl_2$

Pre-warm the LB plates

Day 1

1 Inoculate a 5 mL culture of liquid LB media from a glycerol stock (-80ºC) of XL1-Blue competent cells

2 Incubate it for 16-20 hours at 37°C at 220-225 rpm

Day 2

3 Remove the culture tubes from incubator

4 Make four dilutions of the culture in liquid broth

4.1 Dilution 1: 20µl of the culture into 980µl LB

4.2 Dilution 2: 20µl from dilution 1 into 980µl LB

4.3 Dilution 3: 20µl from dilution 2 into 980µl LB

4.4 Dilution 4: 20µl from dilution 3 into 980µl LB

4.5 Plate 200µl of dilution 4 in LB plate and let it sit for some time until all the culture has been soaked

4.6 Keep the plates inverted in incubator for 16-20 hours at 37ºC.

Day 3

5 Remove the plates from the incubator.

6 Pick a single bacterial colony (2-3mm in diameter) from a plate (dilution 4) that has been incubated for 16-20 hours at 37ºC.

7 Transfer into 100 ml sterilized LB broth or SOB medium in a 1-liter flask.

8 Incubate for 6 hr at 37°C with shaking at 220-250 rpm.

9 Measure OD_{600} of culture every 15-20 minutes to ensure that the culture does not grow to a higher density. The transformation is efficient at OD_{600} 0.45.

10 Harvest the cell by arresting the growth when OD_{600} 0.45. Immediately put the flask in the ice.

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11 Transfer the bacterial cells to pre sterilized and ice-cold 50 ml conical tube. Cool the cultures to 0°C by storing the tubes on the ice for 10 minutes.

12 Centrifuge at 2700g (4100 rpm in a Sorvall GSA rotor)or (RC-3B refrigerated centrifuge) for 10 minutes at 4 °C

13 Decant the medium from the cell pellets and keep the tubes in an inverted position over the paper towel for 1 minute to allow the traces of media to drain away

14 Resuspend the pellets by swirling or gentle vortexing in 30 ml of ice-cold MgCl2-CaCl2 solution(80mM $MgCl_2$, 20 mM $CaCl_2$) and mix until you get rid of all visible chunks

15 Centrifuge at 2700g (4100 rpm in a Sorvall GSA rotor) for 10 minutes at 4 °C

16 Decant the medium from the cell pellets and keep the tubes in the inverted position over the paper towel for 1 minute to allow the traces of media to drain away

17 Resuspend the pellets by swirling or gentle vortexing in 2 ml of ice-cold 0.1 $CaCl_2$ solutions for each 50 ml of the original culture and leave the tubes on ice for 15 minutes.

18 Add 140 µl DMSO (cryo-protectant) per 4ml. Swirl gently to mix and let it sit for 15 minutes.

19 Add additional 140 µl DMSO per 4 ml. Mix gently and return the tube to the icebox.

20 Transfer and store in aliquots of 100 µl in sterile 1.5 mL Eppendorf tube.

21 Freeze at -80 °C.

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