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Preparation of chemically competent *E. coli* cells

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

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ABSTRACT

Standard protocol to prepare cells for highly efficient DNA uptake using $CaCl_2$ (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 2001).

MATERIALS

NAME	CATALOG #	VENDOR
LB-Medium (Lennox) vegetal	0155.1	Carl Roth

MATERIALS TEXT

- 10 mM $CaCl_2$
- sterile glycerole

1 Inoculate a 5 ml overnight-culture in LB-medium without antibiotics.

2 Inoculate 100 ml LB-medium with 1/100 volume of the overnight culture and incubate to an OD_{600} = 0.5 - 0.6 at 37 °C and 230 rpm.

3 Transfer cells to two 50 ml sterile falcon tubes and incubate on ice for 20 min.

From this step on, keep cells always cold.

00:20:00

4 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.

00:05:00

5 Carefully suspend each pellet in 20 ml of icecold 100 m CaCl2 and incubate on ice for 1 h.

01:00:00

6 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.

00:05:00

7 Carefully suspend each pellet in 2 ml icecold 85 mM CaCl2 containing 15% Glycerol.

Citation: Alice Pawlowski (03/25/2020). Preparation of chemically competent E. coli cells. https://dx.doi.org/10.17504/protocols.io.gtcbwiw

8 Pipette 100 to 200 µl aliquots in 1.5 ml tubes (work on ice) and store at -80 °C. (you can shock-freeze the cells in liquid nitrogen or in dry ice prior to storage at -80°C, but it is not required)

For DNA transformation, thaw 100 µl of the competent cells on ice.

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