

DetoxE™ Competent Cell

Cat No: 10111

Quantity: 2 x 0.5 ml/pkg

Transformation efficiency: $\geq 10^5$ CFU/ug pUC19

Storage: -80 °C

Genotype

F- hsdS gal ompT Lon-. An E.coli B strain with a pACYC177-derived chloramphenicol-resistant plasmid expressing medium level of lacI repressor.

Compatibility

Your plasmid should be derived from a pBR322 or pUC origin (as most commercial plasmids such as pET plasmids). It should contain an antibiotic resistant marker other than chloramphenicol.

Protocol

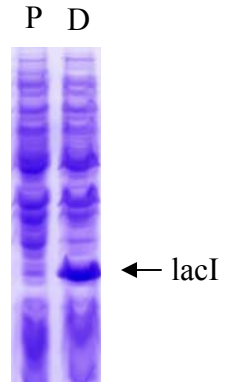
1. Thaw the competent cells on ice.
2. Add 1 to 5 μ l plasmid DNA to 50 μ l competent cell. Mix the content by brief vortexing.
3. Heat shock the contents (0 °C 2 minutes, 37 °C 3 minutes, 0 °C 5 minutes).
4. Plate all contents on a SOC agar plate containing 35 μ g/ml chloramphenicol and 50 μ g/ml ampicillin if the plasmid requires ampicillin selection. OR
(4. Use following protocol if the plasmid requires an antibiotic selection other than ampicillin. Add 940 μ l SOC to the contents. incubate it at 37 °C with 300 rpm shaking for 1 hour. Plate 100 μ l on a LB agar plate containing 35 μ g/ml chloramphenicol and appropriate amount of another antibiotic.)
5. Incubate the plate at 37 °C overnight.
6. Pick up three colonies of different sizes from the plate. Inoculate each colony into 2 ml LB with 1% glucose, 35 μ g/ml chloramphenicol and 50 μ g/ml ampicillin (or appropriate antibiotics).
7. Spin down the cells when the OD₆₀₀ of the cultures reach 0.5 to 1.0. Add 2 ml LB with 50 μ g/ml ampicillin (no glucose and chloramphenicol). Induce the cultures with 0.5 mM IPTG for 1 to 3 hours. Check the non-induced and induced samples on a SDS gel.

Fig. Expression of lacI repressor by DetoxE™ cells

E.coli lacI repressor (lacI) was consecutively over-expressed in DetoxE™ cell strain. Its parental strain BL21 express significantly lower level of the indigenous repressor.

N: parental strain BL21

D: DetoxE™.



8. The positive cultures may be scaled up to 250 to 500 ml in a two liter flask.

DetoxE™ expresses medium level of lacI repressor. For low toxic protein and higher yield, cell strains with lower level of lacI should be used. For extremely toxic protein, high lacI expressing cell strains, different vectors and protocol may be used. All tested toxic proteins can be successfully expressed in our cell strains, vectors, and media. If you have a toxic protein that cannot be expressed in our system, please contact us through above email. We will try to help with your questions.

RESEARCH USE ONLY

DetoxE™ cell strain is under patent-pending. A Non-exclusive license is granted based upon following assurance. It is for noncommercial research use only and it cannot be distributed out of the purchasing lab. This limitation applies to the cell strain, vector contained in the cell strain, their derivatives and any derivatives you may make of them. A separate license is required for any commercial use, including the use of the strain for research purposes by any commercial identity. Please contact us for any other applications. You may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this license.