BL21
Competent *E. coli*

1-800-632-7799
info@neb.com
www.neb.com

C2530H

20 x 0.05 ml/tube **Lot: 0171502**

Store at –80°C

CAUTION: This product contains DMSO, a hazardous material. Review the MSDS before handling.

Description: Chemically competent *E. coli* cells suitable for transformation and protein expression. This strain does not express the T7 RNA polymerase.

Features:

- Transformation efficiency: 1–5 x 10^7 cfu/µg pUC19 DNA
- Routine non-T7 expression
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (*fhu*A2)
- Free of animal products

Reagents Supplied:
20 x 0.05 ml/tube of chemically competent BL21 Competent *E. coli* cells **(Store at –80°C)**

20 ml of SOC Outgrowth Medium **(Store at room temperature)**

0.025 ml of 50 µg/ml pUC19 Control DNA **(Store at –20°C)**

Quality Control Assays

Transformation Efficiency: 100 pg of pUC19 plasmid DNA was used to transform one tube of BL21 competent *E. coli* following the protocol provided. 1–5 x 10^7 colonies formed/µg after an overnight incubation on LB-ampicillin plates at 37°C.

Untransformed cells were also tested for resistance to phage ϕ80, a standard test for resistance to phage T1 and sensitivity to ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline.

Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of BL21 Competent *E. coli* cells on ice for 10 minutes.
2. Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. **Do not vortex.**
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 10 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50–100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 20–24 hours or at 25°C for 48 hours.

STORAGE AND HANDLING: Competent cells should be stored at –80°C. Storage at –20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above –80°C, even if they do not thaw.

5 Minute Transformation Protocol

A shortened transformation protocol resulting in approximately 10% efficiency compared to the standard protocol may be suitable for applications where a reduced total number of transformants is acceptable. Follow the Transformation Protocol with the following changes:

1. Steps 3 and 5 are reduced to 2 minutes.
2. Omit outgrowth (step 7) completely for ampicillin-resistant plasmids or reduce the outgrowth time for other selective media as appropriate.

Compatible Expression Vectors/Promoters

$P_{tac,}$ $P_{trc,}$ $P_{lac,}$ PlacUV5, ParaBAD, PrhaBAD, T5-lacO
Note: Expression from vectors containing a T5-lacO promoter will not be regulated unless the expression vector encodes the *lacI* gene or a compatible vector expresses LacI or the host strain overproduces LacI (e.g. NEB #C3037 NEB Express I^q Competent *E. coli*).

Protocol for Protein Expression Using BL21

1. Transform expression plasmid into BL21. Plate on antibiotic selection plates and incubate overnight at 37°C.
2. Resuspend a single colony in liquid culture with antibiotic to produce a starter culture. Inoculate starter culture at a 1:100 dilution into expression media containing antibiotic.
3. Incubate at 37°C with shaking until OD_{600} reaches 0.4–0.8.
4. For most vector systems, induce with 40 or 400 µM IPTG and express protein for 3 hours at 37°C, 5 hours at 30°C or overnight at 16°C or 23°C.
5. For large scale, inoculate 1 Liter of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C until OD_{600} reaches 0.4–0.8. Add 40 or 400 µM IPTG and express protein using optimal time/temperature determined in a small scale trial.

Troubleshooting Protein Expression

No colonies or no growth in liquid culture: This outcome may be due to basal expression of a target gene product, which is detrimental to cell viability. If target protein toxicity is suspected, strains that overproduce LacI (e.g. NEB #C3037 NEB Express I^q Competent *E. coli*) are recommended for vectors containing a tac, trc, lac, lacUV5 or T5-lacO promoter.

No protein visible on gel or no activity:

- Check for toxicity: no protein may mean the cells have lost the expression plasmid or elements of the expression plasmid have been deleted.
- Culture cells for protein expression. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, there will be a significant difference between the number of colonies on the plates. Fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic.
- Check clone integrity by restriction enzyme analysis and/or sequencing of the ORF.

Induced protein is insoluble: High level expression of non-native genes often results in the target protein becoming insoluble. Potential solutions are:

- Induce at a lower temperature (as low as 15°C overnight)
- Reduce IPTG concentration to 40 µM
- Induce earlier in growth phase (OD_{600} = 0.3–0.4) and harvest cells earlier.
- Create an expression construct where the target protein is fused to maltose binding protein (pMAL Protein Fusion and Purification System, NEB #E8200).

(see other side)

Solutions/Recipes

SOB:

2%	Vegetable peptone (or Tryptone)
0.5%	Yeast Extract
10 mM	NaCl
2.5 mM	KCl
10 mM	$MgCl_2$
10 mM	$MgSO_4$

SOC:

SOB + 20 mM Glucose

LB agar:

1%	Tryptone
0.5%	Yeast extract
0.17 M	NaCl
1.5%	Agar

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

Genotype: *fhuA2 [lon] ompT gal [dcm] ΔhsdS*

Transformation Protocol Variables

Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes you shorten this step.

Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 10 seconds at 42°C is optimal.

Outgrowth: Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes you shorten this step. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or rotating the tube gives 2-fold higher transformation efficiency than incubation without shaking.

Plating: Selection plates can be used warm or cold, wet or dry without significantly affecting the transformation efficiency. However, warm, dry plates are easier to spread and allow for the most rapid colony formation.

Strain Properties

The properties of this strain that contribute to its usefulness as a protein expression strain are described below.

Protease Deficient (*[lon] ompT*): *E. coli* B strains are "naturally" deficient in the *lon* protease which in K-12 strains serves to degrade misfolded proteins and to prevent some cell cycle-specific proteins from accumulating. The OmpT protease resides at the surface of wild type *E. coli* in both K-12 and B strains, presumably helping the cells to derive amino acids from their external environment. Cells deficient in both these proteases are much more amenable to the production of proteins from cloned genes.

T1 Phage Resistant (*fhuA2*): T1, an extremely virulent phage requires the *E. coli* ferric hydroxamate uptake receptor for infectivity. Deletion of this gene confers resistance to this type of phage, but does not significantly affect the transformation or growth characteristics of the cell.