Lot: 29

C2527H

BL21(DE3) Competent *E. coli*

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20 x 0.05 ml/tube **Lot: 29**

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.

Features:

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

Reagents Supplied:
20 x 0.05 ml/tube of chemically competent BL21(DE3) Competent *E. coli* cells **(Store at –80°C)**

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

0.025 ml of 50 pg/µl 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(DE3) 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, nitrofurantoin, spectinomycin, streptomycin and tetracycline.

Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of BL21(DE3) 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 24–36 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.

Protocol for Protein Expression Using BL21(DE3)

1. Transform expression plasmid into BL21(DE3). Plate on antibiotic selection plates and incubate overnight at 37°C.
2. Resuspend a single colony in 10 ml liquid culture with antibiotic.
3. Incubate at 37°C until OD_{600} reaches 0.4–0.8.
4. Induce with 4 or 40 µl of a 100 mM stock of IPTG (final concentration of 40 or 400 µM) and induce for 3 to 5 hours at 37°C.
5. Check for expression either by Coomassie stained protein gel, Western Blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction only.

   *If a fraction of the target protein is insoluble, repeat expression at a lower temperature (15 to 30°C) or test expression in Lemo21(DE3) (NEB #C2528).
6. For large scale, inoculate 1 L 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 T7 Protein Expression

No colonies or no growth in liquid culture: T7 expression in BL21(DE3) is not tightly regulated and thus toxic proteins may affect cell viability, especially if the expression vector does not encode lacI. For tightly regulated expression, use a strain expressing *lysY*:

- T7 Express *lysY* (NEB #C3010): *lysY* produces mutant T7 lysozyme which binds to T7 RNA polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA polymerase titrates out the lysozyme and results in expression of the target protein
- T7 Express *lysY/I*^{q} (NEB #C3013): *lysY* expression as well as lacI overexpression to repress basal expression of the T7 RNA Polymerase.
- Lemo21(DE3) (NEB #C2528): BL21(DE3) containing the Lemo System™. *lysY* expression is modulated by L-rhamnose, making T7 protein expression tightly regulated and tunable.

No protein visible on gel or no activity: Check for toxicity - no protein may mean the cells have eliminated or deleted elements in the expression plasmid.

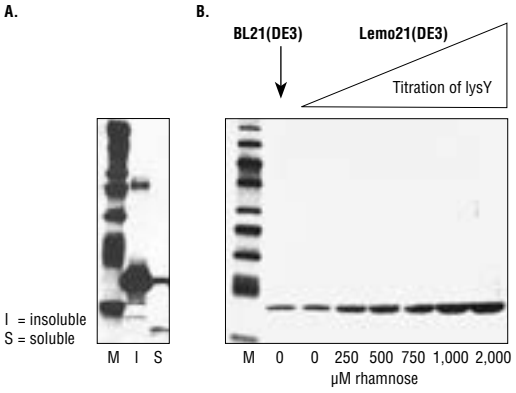
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, there will be a significant difference between the number of colonies on the plates. Fewer colonies will be seen on plates containing antibiotic (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.

  If toxicity is the problem, *lysY* expressing strains will provide clone stability.

(see other side)

Induced protein is insoluble: Check for insolubility - this is important because T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. Solutions around this 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 or 0.4)
- Test expression in Lemo21(DE3) – see example below:



Western analysis of 6-His tagged Brugia malayi protein. *A) B. malayi protein expressed at 20°C in BL21(DE3). B)* ***Soluble fractions*** *of B. malayi protein expressed at 30°C.*

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

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

Genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*

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. The genotypes underlying these properties appear in parentheses.

T7 RNA Polymerase: (*T7 gene1)* is encoded by the lambda *DE3* prophage present within the chromosome. T7 RNA polymerase is expressed from the *lacUV5* promoter, which is less sensitive to catabolite repression than the wt *lac* promoter. Thus *DE3* strains may exhibit uninduced target protein expression. Although *λDE3* is normally dormant in the host chromosome, the induction of the SOS cascade can occur as the result of expressing proteins that damage the *E. coli* chromosome, either directly or indirectly. This may lead to cell lysis. T7 Express strains do not carry the *DE3* prophage and better tolerate an SOS response.

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.