T7 Express Crystal Competent *E. coli* (High Efficiency)

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

C3022I

6 x 0.2 ml/tube **Lot: 0031504**

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 high efficiency transformation and protein expression for X-ray crystallography. This strain is ideal for seleno-methionine labeling of proteins.

Features:

- Transformation efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA
- Enhanced BL21 derivative
- Methionine auxotroph (*metB1*) designed for seleno-methionine labeling of proteins
- Derivative of T7 Express with T7 RNA Polymerase on the chromosome under *lac* control
- Deficient in proteases Lon and OmpT
- Resistant to T1 phage (*fhuA2*)
- Free of animal products

Reagents Supplied:

6 x 0.2 ml/tube of chemically competent T7 Express Crystal Competent *E. coli* cells **(Store at –80°C)**

25 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 T7 Express Crystal Competent *E. coli* following the high efficiency protocol provided. 0.6–1 x 10^9 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, spectinomycin, streptomycin and tetracycline.

High Efficiency Transformation Protocol

1. Thaw a tube of T7 Express Crystal Competent *E. coli* cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
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 High Efficiency 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.

DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG*	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins* (e.g. Ligase)	Column purify or phenol/chloroform extract and ethanol precipitate

*Ideally, DNA for transformation should be purified and resuspended in water or TE. However, up to 10 µl of DNA directly from a ligation mix can be used with only a two-fold loss of transformation efficiency. Where it is necessary to maximize the number of transformants (e.g. a library), a purification step, either a spin column or phenol/chloroform extraction and ethanol precipitation should be added.

Transformation Protocol Variables

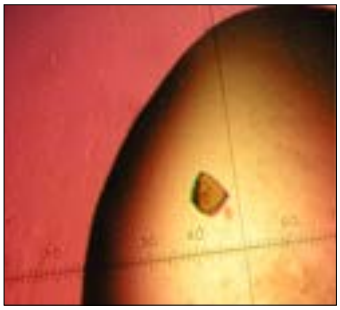
Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

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 this step is shortened.

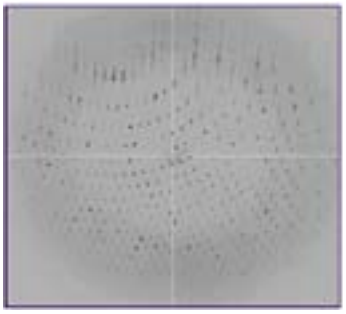
Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 10 seconds at 42°C is optimal.

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 this step is shortened. 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.



Crystal of NotI in complex with DNA substrate (Barry Stoddard and Abigail Lambert; Fred Hutchinson Cancer Research Center).



X-ray diffraction pattern of NotI.

Reference:

1. Lambert, A.R. et al. (2008) *Structure,* 16, 558–569.

Protocol for Seleno-methionine Incorporation

T7 Express Crystal is a *metB1* derivative of NEB T7 Express (NEB #C2566). 50 µg/ml L-seleno-methionine may be substituted with 100 µg/ml of D/L isomer mix. For this we recommend using Seleno-DL-methionine from Sigma (S-3875) as a 10 mg/ml stock in dH_2O. For enhanced growth, add 0.0002% ferric ammonium citrate to minimal media. For this we recommend Ferric Ammonium Citrate from Fisher (#I72-500)/as this product also provides trace metals. Filter sterilize prior to use.

Transformed cells may be plated on rich agar. To produce a starter culture, inoculate a single colony into 10 ml minimal media plus 50 µg/ml L-methionine – grow up to 24 hours to achieve saturation.

Incorporation using a regulated expression system (e.g. T7-lac promoter):

1. Add 10 ml starter culture to 1L minimal media supplemented with 50 µg/ml L-methionine. Grow to mid-log phase (O.D. 0.6–0.8).
2. Pellet cells and resuspend in fresh, pre-warmed minimal expression media without methionine or seleno-methionine.
3. Grow for 2.5 hours at 37°C to deplete cells of methionine (longer if using a lower temperature).
4. Add 50 µg/ml L-seleno-methionine or 100 µg/ml D/L isomer mix.
5. Incubate for 30 minutes at 37°C.
6. Add inducer and incubate 3 hours to overnight.

Incorporation with a constitutive expression system:

To produce a starter culture, inoculate a single colony into 10 ml minimal media plus 50 µg/ml L-methionine – grow up to 24 hours to achieve saturation.

1. Prepare minimal expression media with a 10:50 ratio of L-Met to L-SeMet to achieve maximal growth rate (e.g. 10 µg/ml L-methionine to 100 µg/ml D/L-selenomethionine). This ratio will result in approximately 83% SeMet labeling of expressed protein.
2. Use the same expression conditions previously optimized for your protein of interest.
3. If a higher level of SeMet labeling is desired, spike with 100 µg/ml D/L-seleno-methionine at mid-log to late-log phase of growth.

Minimal media (per liter):

200 ml 5X M9 salts (Sambrook et al.)

20 ml 20% glucose (0.4% final conc)

0.1 ml 1 M $CaCl_2$ (0.1 mM final conc)

2 ml 1 M $MgSO_4$ (2 mM final conc)

0.2 ml 1% ferric ammonium citrate (0.0002% final conc)

Met, SeMet or Met/SeMet mix

Antibiotic for plasmid selection

Sterilized dH_2O to 1 L

Solutions/Recipes

5X M9 salts (per liter):

33.9 g	Na_2HPO_4
15.0 g	KH_2PO_4
2.5 g	NaCl
5.0 g	NH_4Cl

dissolve in 1 L distilled water.
autoclave for 15 min at 121°C.

Troubleshooting T7 Protein Expression

No colonies or no growth in liquid culture: Even though T7 expression is tightly regulated, there may be a low level of basal expression in the T7 Express Crystal host. If toxicity of the expressed protein is likely, incubate at 30°C or room temperature to alleviate toxicity issues. In addition, check antibiotic concentration (test with control plasmid).

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. Check for maintenance of 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.

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. Potential solutions for this are:

- Induce at lower temperatures (as low as 15°C overnight)
- Reduce IPTG concentration to 0.1 mM and 0.01 mM
- Induce for less time (as little as 15 minutes)
- Induce earlier in growth (OD_{600} = 0.3 or 0.4)

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 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 metB1 Δ(mcrC-mrr)114::IS10*

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.

Methionine Auxotroph (*metB1*): Strains with mutations at the *metB* locus are unable to synthesize methionine and therefore require supplementation with L-methionine for growth in minimal media. Methionine auxotrophs are ideal for seleno-L-methionine labeling of proteins for X-ray crystallographic studies.

T7 RNA Polymerase (*lacZ::T7 gene1*): T7-Express has the T7 RNA polymerase gene inserted into the *lac* operon on the *E. coli* chromosome and is expressed under the control of the *lac* promoter. This configuration provides controlled induction of the polymerase and consequently, inducible control of transcription of genes downstream of the T7 promoter. This system provides potential advantages over strains such as BL21(DE3), that carry the T7 RNA polymerase on a lysogenic prophage. 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.

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. Mutations of other genes can help to ameliorate the sometimes-deleterious effects of these protease defects (e.g. *sulA*, below).

Recovery from DNA Damage (*sulA11*): *E. coli* cells can tolerate a substantial amount of chronic DNA damage as long as repair is allowed to proceed. This capacity is compromised if the cells are unable to divide following repair. In *lon*$^-$ cells, SulA, a cell division inhibitor, accumulates and causes cells to become hypersensitive to DNA damage. The *sulA* mutation introduced into the T7 Express strain allows cells to divide more normally in the absence of Lon protease.

Endonuclease I Deficient (*endA1*): The periplasmic space of wild type *E. coli* cells contains a nonspecific endonuclease. Extreme care must be taken to avoid degradation of plasmids prepared from these cells. The *endA* mutation deletes this endonuclease and can significantly improve the quality of plasmid preparations.

Restriction Deficient (Δ*(mcrC-mrr)114::IS10*): Wild type *E. coli* B strains carry a Type I restriction endonuclease which cleaves DNA with the site TGA(N8)TGCT. While *E. coli* DNA is protected from degradation by a cognate methyl-transferase, foreign DNA will be cut at these sites. The deletion described above eliminates both the methylase and the endonuclease.

Methyl Restriction Deficient (Δ*((mcrC-mrr)114::IS10 and R(mcr-73::miniTn10--*Tet^S*)2*): *E. coli* has a system of enzymes encoded by *mcrA*, *mcrBC* and *mrr* which will cleave DNA with methylation patterns found in higher eukaryotes, as well as some plant and bacterial strains. All three Mcr enzymes and Mrr have been inactivated in this strain allowing the introduction of eukaryotic DNA of genomic origin (e.g. primary libraries) if desired.

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.

Reference:

2. Sambrook, J. et al. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.), (Appendix A.3). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.