Lot: 6

C3016H

T7 Express I^q Competent *E. coli* (High Efficiency)

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20 x 0.05 ml/tube **Lot: 6**

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.

Features:

- Transformation efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA
- Enhanced BL21 derivative for T7 expression
- T7 RNA Polymerase in the *lac* operon - no lambda prophage
- Tight control of expression by *lacI^q* allows potentially toxic genes to be cloned
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (*fhuA2*)
- Does not restrict methylated DNA ($McrA^-$, $McrBC^-$, $EcoBr^-m^-$, Mrr^-)
- No Cam requirement
- B Strain
- *lacI^q* on miniF for stability
- Free of animal products

Reagents Supplied:

20 x 0.05 ml/tube of chemically competent T7 Express I^q 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 T7 Express I^q 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 tested for resistance to phage φ80, a standard test for resistance to phage T1 and sensitivity to ampicillin, kanamycin, spectinomycin, streptomycin and tetracycline. Cells are resistant to chloramphenicol.

High Efficiency Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of T7 Express I^q 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 High Efficiency Transformation Protocol above 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 Expression Using T7 Express I^q

1. Transform expression plasmid into T7 Express I^q. 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 0.4–0.6.
4. Induce with 40 µl of a 100 mM stock of IPTG (final concentration of 0.4 mM) and induce for 2 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 alone.
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 0.4–0.6. Add IPTG to 0.4 mM. Induce 2 hours at 37°C or 15°C overnight.

Transformation Protocol Variables

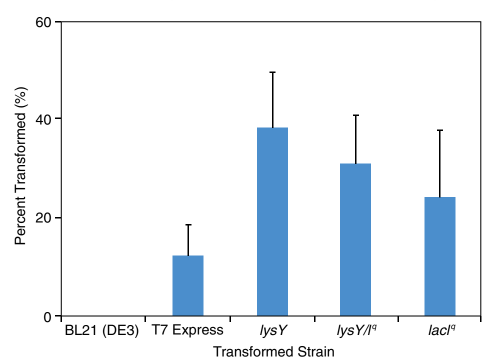
Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

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.

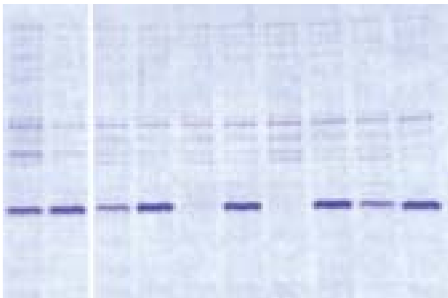
Outgrowth: Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes you shorten this step. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or 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.



Transformation of a toxic mammalian clone into E. coli hosts*. A T7 expression plasmid and the same plasmid containing a gene encoding a toxic mammalian protein were transformed into each host. Comparison of the relative transformation efficiencies demonstrates that the T7 Express hosts provide the levels of control necessary for transformation of potentially toxic clones. BL21(DE3) could not be transformed with the toxic clone.*

CERTIFICATE OF ANALYSIS



T7-controlled expression of a non-toxic protein in E. coli hosts. *A T7 expression plasmid containing a gene encoding an E. coli protein was transformed into each host, grown to 0.6 OD and induced for 3 hours. Comparison of soluble extracts from uninduced (–) and induced (+) cells shows superior control of basal expression in the T7 Express hosts while maintaining high levels of induced expression.*

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.

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 host. If toxicity of the expressed protein is likely, transformation of the expression plasmid should be carried out in one of the following strains:

- T7 Express *I^q* (NEB #C3016): over-expression of the LacI repressor reduces basal expression of the T7 RNA polymerase
- 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) combines both above effects.

Incubation at 30°C or room temperature may also 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.

- 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.
- If toxicity is the problem test the T7 Express *lysY* host to reduce basal level expression.

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 lower temperatures (as low as 12–15°C overnight)
- Reduce IPTG concentration to 0.01–0.1 mM
- Induce for less time (as little as 15 minutes)
- Induce earlier in growth (OD_{600} = 0.3 or 0.4)

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
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml

Genotype: MiniF *lacI^q*(Cam^R) */ fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10*--Tet^S*)2 [dcm] R(zgb-210::Tn10*--Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Note: Maintenance of the miniF plasmid does not require antibiotic selection. If chloramphenicol is added, use 10 µg/ml final concentration.

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 (*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.

Lac Promoter Control (*lacI^q*): The Lac repressor blocks expression from *lac*, *tac*, *trc* and *T7-lac* promoters frequently carried by expression plasmids. If the level of Lac repressor in *E. coli* cells in not sufficient to inhibit expression via these promoters during transformation or cell growth, even low levels of expression of toxic genes can reduce transformation efficiency and select against desired transformants. The extra molecules of Lac repressor in *lacI^q* strains help to minimize promoter activity until IPTG is added.

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 T7 Express 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.