

# SHuffle® T7 Express Competent *E. coli*



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



## C3029H

6 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* B cells engineered to form disulfide bonded proteins in the cytoplasm. Suitable for T7 promoter driven protein expression.

### Features:

- Transformation efficiency:  $1 \times 10^7$  cfu/ $\mu$ g pUC19 DNA
- Engineered *E. coli* B strain to promote disulfide bond formation in the cytoplasm
- Expresses constitutively a chromosomal copy of the disulfide bond isomerase DsbC
- DsbC promotes the correction of mis-oxidized proteins into their correct form (1,3)
- The cytoplasmic DsbC is also a chaperone that can assist in the folding of proteins that do not require disulfide bonds (4)
- Enhanced BL21 derivative
- Expresses a chromosomal copy of T7 RNAP
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Deficient in proteases Lon and OmpT
- Resistance to phage T1 (*fhuA2*)

### Reagents Supplied:

6 x 0.05 ml/tube of chemically competent SHuffle T7 Express Competent *E. coli* cells (Store at **-80°C**)

### Quality Control Assays

**Transformation Efficiency:** 100 pg of pUC19 plasmid DNA was used to transform one tube of SHuffle® T7 Express Competent *E. coli* following the high efficiency protocol provided.  $1 \times 10^7$  colonies formed/ $\mu$ g after an overnight incubation on LB-ampicillin plates at 37°C.

**Disulfide bond formation:** The *Serratia marcescens* extracellular nuclease NucA requires disulfide bonds for its stability. When expressed cytoplasmically at 37°C in *E. coli*, NucA is toxic to cells only in its oxidized disulfide-bonded state. Transformation of a plasmid that expresses a MBP-NucA fusion in the cytoplasm was used to test the ability of SHuffle strains to form cytoplasmic disulfide bonds. 100 pg pMBP-NucA was used to transform SHuffle, resulting in no transformants. Empty pMAL vector was used to calculate transformation efficiency and the wild type parent of SHuffle was used as a control.

Untransformed cells were tested for resistance to phage  $\phi$ 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, chloramphenicol, kanamycin and tetracycline. Cells are resistant to streptomycin\* and spectinomycin.

\*Resistance to low levels of streptomycin may be observed.

### High Efficiency Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of SHuffle Competent *E. coli* cells on ice for 10 minutes.
2. Add 1–5  $\mu$ l 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 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950  $\mu$ l of room temperature SOC into the mixture.
7. Place at 30°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 30°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  $\mu$ l of each dilution onto a selection plate and incubate overnight at 30°C. Alternatively, incubate at 25°C for 48 hours.

### 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 SHuffle

1. Transform expression plasmid into SHuffle. Plate on antibiotic selection plates and incubate 24 hours at 30°C.
2. Resuspend a single colony in 10 ml liquid medium with antibiotic.
3. Incubate at 30°C until OD<sub>600</sub> reaches 0.4–0.8.
4. Add the appropriate inducer, e.g. 40  $\mu$ l of a 100 mM stock of IPTG. Incubate for 4 hours at 30°C or 16°C overnight.
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 30°C until reaches 0.4–0.8. Add the appropriate inducer, e.g. IPTG to 0.4 mM. Induce 4 hours or 16°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, 30 seconds at 42°C is optimal.

**Outgrowth:** Outgrowth at 30°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.

**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.

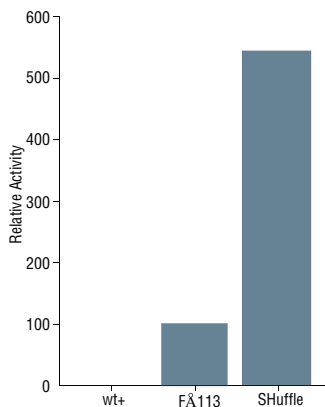
## DNA Contaminants to Avoid

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

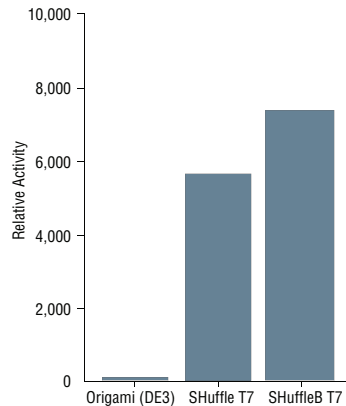
\*Ideally, DNA for transformation should be purified and resuspended in water or TE. However, up to 10 µl of DNA directly from a ligation mix can be used with only a two-fold loss of transformation efficiency. Where it is necessary to maximize the number of transformants (e.g. a library), a purification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

## Solutions/Recipes

SOB:		SOC:	
2%	Vegetable peptone (or Tryptone)	SOB + 20 mM Glucose	
0.5%	Yeast Extract	LB agar:	
10 mM	NaCl	1%	Tryptone
2.5 mM	KCl	0.5%	Yeast Extract
10 mM	MgCl <sub>2</sub>	0.17 M	NaCl
10 mM	MgSO <sub>4</sub>	1.5%	Agar



**Figure 1, vtPA activity assayed from crude lysates:** Truncated tissue plasminogen activator (vtPA), which contains nine disulfide bonds when folded and oxidized correctly, was expressed from a pTrc99a plasmid in the cytoplasm of *E. coli* cells. After induction, cells were harvested and crude cell lysates were prepared. vtPA was assayed using a chromogenic substrate Chromozym t-PA (Roche #11093037001) and standardized to protein concentration using Bradford reagent. *E. coli* wt+ cells are DHB4, which is the parent of FÅ113 (Origami™).



**Figure 2, PfCHT1 chitinase activity assayed from crude lysates:** *Plasmodium falciparum* chitinase (PfCHT1) with three cysteines were expressed from a plasmid under the regulation of T7 promoter. After induction, cells were harvested and crude cell lysates were prepared. PfCHT1 was assayed using a chromogenic substrate (CalBioChem #474550) and standardized to protein concentration using Bradford reagent.

## 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 ahpC gal λatt::pNEB3-r1-cDsbC (Spec<sup>R</sup>, lac<sup>R</sup>) ΔtrxB sulA11 R(mcr-73::miniTn10--Tet<sup>S</sup>)2 [dcm] R(zgb-210::Tn10 --Tet<sup>S</sup>) endA1 Δgor 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.

**Disulfide bond formation in the cytoplasm:** Normally reductases in the *E. coli* cytoplasm keep cysteines in their reduced form, thereby reducing any disulfide bond that may form in this compartment. SHuffle has deletions of the genes for glutaredoxin reductase and thioredoxin reductase (*Δgor ΔtrxB*), which allows disulfide bonds to form in the cytoplasm. This combination of mutations is normally lethal, but the lethality is suppressed by a mutation in the peroxiredoxin enzyme (*ahpC\**). In addition, SHuffle expresses a version of the periplasmic disulfide bond isomerase DsbC which lacks its signal sequence, retaining it in the cytoplasm. This enzyme has been shown to act on proteins with multiple disulfide bonds, to correct mis-oxidized bonds and promote proper folding. The gene for the cytoplasmic DsbC is present on the chromosome.

**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.

**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).

**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.

**Usage Note:** NEB recommends using SHuffle Express strains for best performance"

## References

- Bessette, P.H. et al. (1999) *Proc. Natl. Acad. Sci. USA*, 96, 13703–13708.
- Qiu, J., Swartz, J.R. and Georgiou, G. (1998) *Appl. Environ. Microbiol.*, 64, 4891–4896.
- Levy, R. et al. (2001) *Protein Expr. Purif.*, 23, 338–347.
- Chen, J. et al. (1999) *J. Biol. Chem.*, 274, 19601–19605.
- Boyd, D. et al. (2000) *J. Bacteriol.*, 182, 842–847.

New England Biolabs, Inc.: U.S. Patent No. 6,569,669

**RESEARCH USE ASSURANCE STATEMENT:** The buyer and user have a non-exclusive sub-license to use this system or any component thereof for RESEARCH PURPOSES ONLY, based upon agreement to the following assurances.

Transfer of the host cells that contain the cloned copy of the T7 gene 1 to third parties is explicitly prohibited. This limitation applies to *E. coli* ER2566, ER2833, ER3011, ER3012, ER3013 and ER3021, SHuffle T7, SHuffle T7 LysY, SHuffle T7 Express, SHuffle T7 Express LysY and their competent derivatives, C2566, C2833, C3010, C3013, C3016, C3022, C3026, C3027, C3029 and C3030 when provided separately or when provided in combination with appropriate vectors for said systems.

A license to use this system or any components thereof for commercial purposes may be obtained from New England Biolabs, Inc.

**COMMERCIAL LABORATORY BUYER AND USER:** Use of the host cells ER2566, C2566, C2833, C3010, C3013, C3016, C3022, SHuffle T7, SHuffle T7 LysY, SHuffle T7 Express, SHuffle T7 Express LysY or their competent derivatives that contain the cloned copy of T7 gene 1, the gene for T7 RNA polymerase for any purpose other than in combination with either a T7/MAL or T7/IMPACT vector is explicitly prohibited.

Use of host cells that may contain the cloned copy of the T7 gene 1, the gene for T7 RNA polymerase with any other vector(s) containing a T7 promoter to direct the production of RNA or protein requires a license from Brookhaven National Laboratory. Information about research-use or commercial-use license agreements may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 475D, P.O. Box 5000, Upton, New York, 11973-5000; telephone: 631-344-7134; fax: 631-344-3729.

You may refuse this non-exclusive research license agreement by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this sub-license.

## ACADEMIC AND NON-PROFIT LABORATORY ASSURANCE LETTER

The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patent applications assigned to Brookhaven Science Associates, LLC (BSA). BSA will grant a non-exclusive license for use of this technology, including the enclosed materials, based upon the following assurances:

- These materials are to be used for noncommercial research purposes only. A separate license is required for any commercial use, including the use of these materials for research purposes or production purposes by any commercial entity. Information about commercial licenses may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 475D, P.O. Box 5000, Upton, New York 11973-5000, telephone (631) 344-7134.
- No materials that contain the cloned copy of T7 gene 1, the gene for T7 RNA polymerase, may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this license and agrees to be bound by its terms. This limitation applies to strains ER2566, C2566, C3010, C3013, C3016, C3022, BL21(DE3), BL21(DE3)pLysE, BL21(DE3)pLysE, SHuffle T7, SHuffle T7 LysY, SHuffle T7 Express, SHuffle T7 Express LysY their competent derivatives, and any derivatives you may make of them, including such strains containing recombinant vectors.

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.