**SHuffle® T7 Express Competent E. coli**

**C3029H**

- **6 x 0.05 ml/tube**
- **Lot: 0081408**
- **Store at –80°C**

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**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 x 10^7 cfu/µg 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)

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**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 x 10^10 colonies formed/µg 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 bonded bands. 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 q80, 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.

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**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.

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**Protocol for Expression Using SHuffle**

**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 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.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl 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 µl 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 OD₆₀₀ reaches 0.4–0.8.
4. Add the appropriate inducer, e.g. 40 µl 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.
Cells were harvested and crude cell lysates were prepared. vtPA was assayed using a chromogenic substrate (CalBioChem #474550) and standardized to protein concentration using Bradford reagent.

**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 (CalBioChem #474550) and standardized to protein concentration using Bradford reagent. E. coli wT cells are DHB4, which is the parent of FA113 (Origami®).

**Figure 2.** PCHT1 chitinase activity assayed from crude lysates: Plasmidum falciparum chitinase (PCHT1) 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. PCHT1 was assayed using a chromogenic substrate (CalBioChem #474550) and standardized to protein concentration using Bradford reagent.

**DNA Contaminants to Avoid**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Removal Method</th>
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</thead>
<tbody>
<tr>
<td>Detergents</td>
<td>Ethanol precipitate</td>
</tr>
<tr>
<td>Phenol</td>
<td>Extract with chloroform and ethanol precipitate</td>
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<tr>
<td>Ethanol or Isopropanol</td>
<td>Dry pellet before resuspending</td>
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<tr>
<td>PEG* (e.g. Ligase)</td>
<td>Column purity or phenol/chloroform extract and ethanol precipitate</td>
</tr>
<tr>
<td>DNA binding proteins*</td>
<td>Column purity or phenol/chloroform extract and ethanol precipitate</td>
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</tbody>
</table>

*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**

<table>
<thead>
<tr>
<th>SOB:</th>
<th>SDC:</th>
</tr>
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<tbody>
<tr>
<td>2% Vegetable peptone (or Tryptone)</td>
<td>SOB: 20 mM Glucose</td>
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<tr>
<td>0.5% Yeast Extract</td>
<td>LB agar:</td>
</tr>
<tr>
<td>10 mM NaCl</td>
<td>1% Tryptone</td>
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<tr>
<td>2.5 mM KCl</td>
<td>Yeast Extract</td>
</tr>
<tr>
<td>10 mM MgCl₂</td>
<td>0.17 M NaCl</td>
</tr>
<tr>
<td>10 mM MgSO₄</td>
<td>1.5% Agar</td>
</tr>
</tbody>
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**Antibiotics for plasmid selection**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Working Concentration</th>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>33 µg/ml</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 µg/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>25 µg/ml</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>15 µg/ml</td>
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</tbody>
</table>

**Genotype:** faHu2 ΔacZ::T7::gene1 [lon] ompT aphA gal λatt::phNBE1-r1-DsbC (Spec**, lacI*) ΔtrxB suAI1 R(mcr-73::miniTn10-Te12) [dcm] R(traD-26::Tn10-Te1] endA1 Δgor Δmcr-rr1)Δ14::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 reduces 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 (Agor 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 (aphA**). 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 misfolded proteins, and to 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 contains a nonspecific endonuclease. Extreme care must be taken to avoid degradation of plasmids prepared from these cells. The endA1 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. suA, below).

T1 Phage Resistant (faHu2): 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 Shuffl Express strains for best performance"