**NEB 5-alpha Competent E. coli (High Efficiency)**

**C2987I**

6 x 0.2 ml/tube  
Lot: 222  
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 in a wide variety of applications.

**Features:**
- DH5α™ derivative
- Transformation efficiency: 1–3 x 10⁹ cfu/µg pUC19 DNA
- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (hsdR)
- Activity of nonspecific endonuclease I (endA1) eliminated for highest quality plasmid preparations
- Resistance to phage T1 (thuA2)
- Suitable for blue/white screening by α-complementation of the β-galactosidase gene
- Reduced recombination of cloned DNA (recA1)
- K12 Strain
- Free of animal products

**Reagents Supplied:**
- 6 x 0.2 ml/tube of chemically competent NEB 5-alpha 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 NEB 5-alpha Competent E. coli following the high efficiency protocol provided. 1–3 x 10⁹ 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. The cells were shown to be suitable for blue/white screening by α-complementation of the β-galactosidase gene using pUC19.

**High Efficiency Transformation Protocol**

1. Thaw a tube of NEB 5-alpha 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 2 minutes. Do not mix.
5. Place on ice for 2 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 25°C for 48 hours.

**5 Minute Transformation Protocol**

The following protocol results in only 10% efficiency compared to the High Efficiency Transformation Protocol.

1. Thaw a tube of NEB 5-alpha 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 2 minutes. Do not mix.
5. Place on ice for 2 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37–42°C. **NOTE:** Selection using antibiotics other than ampicillin may require some outgrowth before plating on selective media. Colonies develop faster at temperatures above 37°C, however some constructs may be unstable at elevated temperatures.

**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, 30 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.

**DNA Effects on Transformation Efficiency and Colony Output:** The optimal amount of DNA to use in a transformation reaction is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

**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.
**Antibiotics for Plasmid Selection**

- Ampicillin 100 µg/ml
- Carbenicillin 100 µg/ml
- Chloramphenicol 33 µg/ml
- Kanamycin 30 µg/ml
- Streptomycin 25 µg/ml
- Tetracycline 15 µg/ml

**DNA Contaminants to Avoid**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Removal Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergents</td>
<td>Ethanol precipitate</td>
</tr>
<tr>
<td>Phenol</td>
<td>Extract with chloroform and ethanol precipitate</td>
</tr>
<tr>
<td>Ethanol/Isopropanol</td>
<td>Dry pellet before resuspending</td>
</tr>
<tr>
<td>PEG*</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>
</tr>
</tbody>
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*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.

**Blue/White Screening**

1. 1.5% Agar
2. 0.17 M NaCl
3. 0.5% Yeast Extract
4. 1% Tryptone

**LB agar**

- SOB + 20 mM Glucose

**SOC Outgrowth Medium**

- #B9020S 4 x 25 ml medium

**Solutions/Recipes**

- **SOB:**
  - 2% Vegetable peptone (or Tryptone)
  - 0.5% Yeast Extract
  - 10 mM NaCl
  - 2.5 mM KCl
  - 10 mM MgCl₂
  - 10 mM MgSO₄

- **SOC:**
  - SOB + 20 mM Glucose

**LB agar:**

- 1% Tryptone
- 0.5% Yeast Extract
- 0.17 M NaCl
- 1.5% Agar

**Blue/White Screening:**

- X-gal 80 µg/ml
- IPTG* 0.3 mM

*Omit IPTG for potentially toxic genes

**Calculation of Transformation Efficiency**

Transformation efficiency (TE) equation:

\[
\text{TE} = \frac{\text{Colonies}}{\mu g \text{DNA}} / \text{Dilution}
\]

**Genotype:**

- *thuA2 Δ(argF-lacZ)U169 phoA glnV44 ß0Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

**Strain Properties**

The properties of this strain that contribute to its usefulness as a cloning strain are described below. The genotypes underlying these properties appear in parentheses.

**Blue/White Screening**

(ß0Δ(lacZ)M15): makes the ß-protein of ß-galactosidase (ß-gal). (argF-lacZ) deletes the ß-gal gene on the chromosome. pUC19 and similar plasmids code for the ß-protein of ß-gal (ß2).

**Companion Products Sold Separately:**

SOC Outgrowth Medium

DH5α™ is a trademark of Invitrogen Corporation.