SHuffle® T7 Express Competent *E. coli*

C3029H

6 x 0.05 ml/tube Lot: 0101503
Store at –80°C

<table>
<thead>
<tr>
<th>CAUTION:</th>
<th>This product contains DMSO, a hazardous material. Review the MSDS before handling.</th>
</tr>
</thead>
</table>

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 10^8 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)

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 10^6 colonies formed/µg after an overnight incubation on LB-ampicillin plates at 37°C.

Disulfide bond formation: *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 e80, 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.

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.

CERTIFICATE OF ANALYSIS
Antibiotics for plasmid selection

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

Genotype: **tha2 lacZ::T7::gene1 [lon] ompT** 

**References**


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