T7 Express Competent E. coli (High Efficiency)

C2566H

20 x 0.05 ml/tube Lot: 0221411
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: 6.6–1 x 10^8 cfu/µg pUC19
- Enhanced BL21 derivative for T7 expression
- T7 RNA Polymerase in the lac operon - no lambda prophage
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (fhuA2)
- Does not restrict methylated DNA (McrA, McrBC, EcoBr m, Mrr)
- B Strain
- Free of animal products

Reagents Supplied:
- 20 x 0.05 ml/tube of chemically competent T7 Express 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 Competent E. coli following the high efficiency protocol provided. 6.6–1 x 10^8 colonies formed/µg after an overnight incubation on LB-ampicillin plates.

Untransformed cells were also tested for resistance to phage 80, a standard test for resistance to phage T1, and sensitivity to ampicillin, chloramphenicol, kanamycin, spectinomycin, streptomycin and tetracycline.

High Efficiency Transformation Protocol
Perform steps 1–7 in the tube provided.
1. Thaw a tube of T7 Express 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 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.

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
1. Transform expression plasmid into T7 Express. 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 OD600 reaches 0.4–0.8.
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 OD600 reaches 0.4–0.8. 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 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, 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 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.

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

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

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 must be carried out in one of the following strains:

- T7 Express pL: over-expression of the LacI repressor reduces basal expression of the T7 RNA polymerase
- T7 Express lysY: 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/p 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 above pL and lysY hosts 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. Potential solutions for this are:

- Induce at lower temperatures (as low as 15°C overnight)
- Reduce IPTG concentration to 0.1 mM and 0.01 mM
- Induce for less time (as little as 15 minutes)
- Induce earlier in growth (OD300 = 0.3 or 0.4)