NEB 5-alpha F’Iq
Competent E. coli
(High Efficiency)

C2992H

20 x 0.05 ml/tube  Lot: 1231502
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 10^9 cfu/µg pUC19 DNA
- Efficient transformation of unmethylated DNA derived from PCR, cDNA
  and many other sources (hsdR)
- Tight control of expression by lacIq allows potentially toxic genes to be
  cloned
- Activity of nonspecific endonuclease I (endA1) eliminated for highest
  quality plasmid preparations
- Resistance to phage T1 (thuA2)
- Suitable for blue/white screening by β-galactosidase gene
- F’ allows cells to be infected with bacteriophage M13 for ssDNA
  production
- Reduced recombination of cloned DNA (recA1)
- K12 Strain
- Free of animal products

Reagents Supplied:
- 20 x 0.05 ml/tube of chemically competent NEB 5-alpha F’Iq 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 NEB 5-alpha F’Iq Competent E. coli following the high efficiency
protocol provided. 1–3 x 10^9 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; cells are resistant to tetracycline,
and sensitivity to ampicillin, chloramphenicol, kanamycin, nitrofurantoin,
spectinomycin and streptomycin. The cells were shown to be suitable for
blue/white screening by β-galactosidase gene

High Efficiency Transformation Protocol
Perform steps 1–7 in the tube provided.
1. Thaw a tube of NEB 5-alpha F’Iq 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 2 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
    25°C for 48 hours.

5 Minute Transformation Protocol
The following protocol results in only 10% efficiency compared to the
High Efficiency Transformation Protocol. Perform steps 1–6 in the tube
provided.
1. Remove cells from –80°C freezer and thaw in your hand.
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.
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 or 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 * (e.g. Ligase)</td>
<td>Column purity or phenol/chloroform extract and ethanol precipitate</td>
</tr>
</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.

Calculation of Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) which would be produced by transforming 1 µg of plasmid into a given volume of competent cells. The term is somewhat misleading in that 1 µg of plasmid is rarely actually transformed. Instead efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. If you plan to calculate efficiency to compare cells or ligations, keep in mind the many variables which affect this metric.

Transformation efficiency (TE) equation:

$$\text{TE} = \frac{\text{Colonies}}{\mu g \cdot \text{Dilution}}$$

Colonies = the number of colonies counted on the plate

$\mu g$ = the amount of DNA transformed expressed in $\mu g$

Dilution = the total dilution of the DNA before plating

TE calculation example:

Transform 2 µl (100 pg) of control pUC19 DNA into 50 µl of cells, out-grow by adding 250 µl of SOC and dilute 10 µl up to 1 ml in SOC before plating 30 µl. If you count 150 colonies on the plate, the TE is:

Colonies = 150

$\mu g$ DNA = 0.0001

Dilution = 10/300 x 30/1000 = 0.001

$$\text{TE} = \frac{150/0.0001/0.001}{1.5 \times 10^9 \text{ cfu}/\mu g}$$

Solutions/Recipes

SOB:

<table>
<thead>
<tr>
<th>2%</th>
<th>Vegetable peptone (or Tryptone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>Yeast Extract</td>
</tr>
<tr>
<td>10 mM</td>
<td>NaCl</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>KCl</td>
</tr>
<tr>
<td>10 mM</td>
<td>MgCl$_2$</td>
</tr>
<tr>
<td>10 mM</td>
<td>MgSO$_4$</td>
</tr>
</tbody>
</table>

SOC:

| SOB + 20 mM Glucose |

LB agar:

<table>
<thead>
<tr>
<th>1%</th>
<th>Tryptone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>Yeast Extract</td>
</tr>
<tr>
<td>0.17 M</td>
<td>NaCl</td>
</tr>
<tr>
<td>1.5%</td>
<td>Agar</td>
</tr>
</tbody>
</table>

Blue/White Screening:

<table>
<thead>
<tr>
<th>X-gal</th>
<th>80 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPTG*</td>
<td>0.3 mM</td>
</tr>
</tbody>
</table>

*pOmit IPTG for potentially toxic genes

Antibiotics for Plasmid Selection

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

Genotype: F$^\prime$ proA$^B$ lacI$^q$ Δ(lacZ)M15 zff::Tn10 (Tet$^R$) / fhuA2Δ(argF-lacZ) U169 phoA glnV44 o805(λacZ)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 (F$^\prime$ Δ(lacZ)M15): makes $\omega$-fragment of $\beta$-gal; Δ(lac-proAB) deletes the $\beta$-gal gene on the chromosome. pUC19 and similar plasmids code for the $\alpha$-peptide of $\beta$-galactosidase ($\beta$-gal). The $\alpha$-peptide can combine with the $\omega$-fragment of $\beta$-galactosidase which is carried on the F$^\prime$ (α-complementation). When $\beta$-galactosidase is reconstituted in this manner it can cleave X-gal and results in blue colonies on an X-gal plate. Insertions cloned into the plasmid polylinker disrupt the $\alpha$-peptide gene and the colonies are white.

Recombination Deficient (recA1): E. coli has a repair system that will recombine homologous sequences. Genomic clones often have duplicated regions, and RecA mediated rearrangements can be problematic, particularly when regions of homology are longer than 50 bp. Strains that have the RecA function deleted tend to grow more slowly than recA strains.

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.

Restriction Deficient (hsdR17): Wild type E. coli K12 strains carry a restriction endonuclease which cleaves DNA with sites (AAC(N6)GTGC and GCAC(N6)GT). While E. coli DNA is protected from degradation by a cognate methyl-transferase, foreign DNA will be cut at these sites. The hsdR mutation eliminates this endonucleas activity. However, this strain has functional methyl restriction systems and may not be suitable for direct cloning of eukaryotic DNA.

M13 phage sensitive (F$^\prime$): Infection by M13 and other similar phage requires E. coli surface features conferred by the F plasmid carried by some E. coli strains. Infection by these phage allows production of single-stranded DNA and the generation of phage display libraries. The F plasmid is frequently modified to carry other useful DNA in the cell [e.g. Δ(lacZ)M15 in this cell line] and when modified is called F$^\prime$.

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.

Lac Promoter Control (lacIq): The lac repressor blocks expression from lac, tac and trc promoters frequently carried by expression plasmids. If the level of lac repressor in E. coli cells is not sufficient to inhibit expression via these promoters during transformation or cell growth, even low levels of expression can reduce transformation efficiency and select against desired transformants. The extra molecules of lac repressor in lac$^q$ strains help to minimize promoter activity until IPTG is added.

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Companion Products Sold Separately:

SOC Outgrowth Medium

#B9020S 4 x 25 ml medium