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

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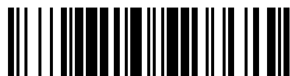

C2987P

1 x 96 well plate (20 µl/well) **Lot: 3311410**

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-throughput, high efficiency transformation in a wide variety of applications. The cells are prepacked in 20 µl aliquots in a 96-well divisible polypropylene plate and sealed with aluminum foil film. The plate can be snapped into four separate 24-well segments when running smaller-scale transformations.

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)*
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Resistance to phage T1 (*fhuA2*)
- 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:

1 x 96 well plate (20 µl/well) 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)**

Additional Included Components:

1 plate cover

1 bag of cap strips (8 x 12 strips)

Quality Control Assays

Transformation Efficiency: 50 pg of pUC19 plasmid DNA was used to transform NEB 5-alpha 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 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.

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.

High Efficiency Transformation Protocol

1. Chill a metal 96-well block on ice.
2. Remove the plate from -80°C freezer, and place in chilled metal 96-well block (or directly on ice) for 2 minutes to thaw the competent cells. (Alternatively, a 24-well segment could be separated from the plate by placing the 96-well plate on dry ice, bending the plate at the connections, and cutting the foil seal between the sections. The unused sections could be returned to -80°C freezer.)
3. Carefully remove the aluminum foil seal from the plate or pierce holes through the foil seal with pipette tips.
4. Add 1-2 µl containing 1 pg-100 ng of plasmid DNA to the cell mixture using a multichannel pipette. Carefully swirl the tips to mix cells and DNA.
5. Seal the plate with plate cover, or cap strips, or tapes.
6. Incubate the plate in the chilled metal block (or on ice) for 20 minutes.
7. Heat shock the cells at exactly 42°C for exactly 10 seconds by transferring the plate to a pre-warmed thermal block or water bath.
8. Place in the chilled metal block (or on ice) for 2 minutes.
9. Pipette 180 µl of room temperature SOC into each well.
10. Place at 37°C for 60 minutes. Shaking is not necessary.
11. Warm selection plates to 37°C.
12. Mix the cells thoroughly by pipetting, then perform several 10-fold serial dilutions in SOC.
13. Spread 50-100 µl of each dilution onto a selection plate and incubate overnight at 37°C.

Transformation Protocol Variables

Thawing: Cells are best thawed in chilled metal 96-well block and DNA added as soon as cells thawed (less than 2 minutes).

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 20 minutes. Expect a 2-fold loss in transformation efficiency when this step is shortened to 2 minutes.

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 plate 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. Shaking is not necessary.

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 Contaminants to Avoid

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

*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.

(see other side)

CERTIFICATE OF ANALYSIS

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:

TE = Colonies/µg/Dilution
Colonies = the number of colonies counted on the plate
µg = the amount of DNA transformed expressed in µg
Dilution = the total dilution of the DNA before plating

TE calculation example:

Transform 1 µl (50 pg) of control pUC19 DNA into 20 µl of cells, outgrow by adding 180 µl of SOC and dilute 10 µl up to 1 ml in SOC before plating 50 µl. If you count 250 colonies on the plate, the TE is:
Colonies = 250
µg DNA = 0.00005
Dilution = 10/200 x 50/1000 = 0.0025
TE = 250/0.00005/0.0025 = 2 x 10^9 cfu/µg

Solutions/Recipes

SOB:

2% Vegetable peptone (or Tryptone)
0.5% Yeast Extract
10 mM NaCl
2.5 mM KCl
10 mM $MgCl_2$
10 mM $MgSO_4$

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

Antibiotics for Plasmid Selection

Antibiotic	Working Concentration
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Chloramphenicol	33 µg/ml
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml

Genotype: *fhuA2* Δ*(argF-lacZ)U169 phoA glnV44* φ*80*Δ*(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 (φ*80*Δ(*lacZ*)M15): makes the ω-fragment of β-galactosidase (β-gal); *(argF-lacZ)* deletes the β-gal gene on the chromosome. pUC19 and similar plasmids code for the α-peptide of β-gal (*lacZ*). The α-peptide can combine with the ω-fragment of β-gal that is carried on φ*80* (α-complementation). When β-gal is reconstituted in this manner it can cleave 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-gal) and results in blue colonies on an X-gal plate. Inserts cloned into the plasmid polylinker disrupt the α-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 which 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)GTT. 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 endonuclease activity. However, this strain has functional methyl restriction systems and may not be suitable for direct cloning of eukaryotic DNA.

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.

Companion Products Sold Separately:

SOC Outgrowth Medium
#B9020S 4 x 25 ml medium

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