

Lot: 15
C2986K

NEB Turbo Electrocompetent *E. coli*



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C2986K

6 x 0.1 ml/tube

Lot: 15

Store at -80°C

CAUTION: This product contains DMSO, a hazardous material. Review the MSDS before handling.

Description: NEB Turbo Electrocompetent *E. coli* cells are suitable for high efficiency electrotransformation and rapid colony growth. These cells are ideal for DNA library constructions and all cloning purposes.

Features:

- Transformation efficiency 1×10^{10} cfu/ μ g pUC19 DNA
- Tight control of expression by *lac^h* allows potentially toxic genes to be cloned
- Highest growth rate on agar plates - visible colonies 6 hours after transformation
- 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
- EcoK^{r-}, McrBC⁻
- K12 Strain
- Isolate DNA after 4 hours of growth from a single overnight colony

Reagents Supplied:

6 x 0.1 ml/tube of NEB Turbo Electrocompetent *E. coli* cells
(Store at -80°C)

25 ml of SOC Outgrowth Medium (Store at room temperature)

0.025 ml of 50 pg/ μ l pUC19 Control DNA (Store at -20°C)

Quality Control Assays

Transformation Efficiency: 10 pg of pUC19 plasmid DNA was electroporated into 25 μ l NEB Turbo Electrocompetent *E. coli* following the protocol described below. $1-3 \times 10^{10}$ colonies formed/ μ g pUC19 after an overnight incubation on LB-ampicillin plates at 37°C.

Untransformed cells were also tested for resistance to phage ϕ 80, a standard test for resistance to phage T1. Cells are resistant to nitrofurantoin and sensitive to ampicillin, chloramphenicol, kanamycin, spectinomycin, streptomycin and tetracycline. They were shown to be suitable for blue/white screening by α -complementation of the β -galactosidase gene using pUC19.

Electroporation Protocol

1. Prepare 17 mm x 100 mm round-bottom culture tubes (e.g. VWR #60818-667) at room temperature. Place SOC recovery medium in a 37°C water bath. Pre-warm selective plates at 37°C for 1 hour.
2. Place electroporation cuvettes (1 mm) and microcentrifuge tubes on ice.
3. As a positive control for transformation, dilute the control pUC19 by 1:5 to a final concentration of 10 pg/ μ l using sterile water. Heat-denatured ligation reactions can be used for electroporation directly; however, column purification is recommended.

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.

4. Thaw NEB Turbo Electrocompetent cells on ice (about 10 min) and mix cells by flicking gently. Transfer 25 μ l of the cells (or the amount specified for the cuvettes) to a chilled microcentrifuge tube. Add 1 μ l of the DNA solution.
5. Carefully transfer the cell/DNA mix into a chilled cuvette without introducing bubbles and make sure that the cells deposit across the bottom of the cuvette. Electroporate using the following conditions for BTX ECM 630 and Bio-Rad GenePulsar electroporators: 2.1 kV, 100 Ω , and 25 μ F. The typical time constant is ~2.6 milliseconds.
6. Immediately add 975 μ l of 37°C SOC to the cuvette, gently mix up and down twice, then transfer to the 17 mm x 100 mm round-bottom culture tube.
7. Shake vigorously (250 rpm) or rotate at 37°C for 1 hour.
8. Dilute the cells as appropriate then spread 100-200 μ l cells onto a pre-warmed selective plate.
9. Incubate plates 8 hours to overnight at 37°C.

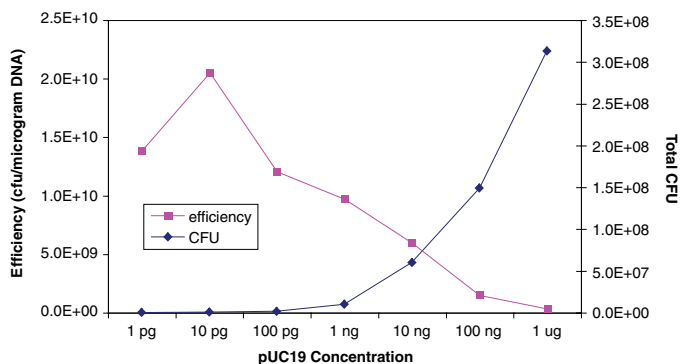
Calculation of Electrotransformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) which would be produced by electrotransforming 1 μ g of plasmid into a given volume of electrocompetent cells. For example, if a 10 pg transformation with pUC19 yields 100 colonies when 100 μ l of a 1:100 dilution is plated, then the efficiency is 1.0×10^{10} cfu/ μ g pUC19 ($100 \text{ cfu}/10 \text{ pg} \times 10^6 \text{ pg}/\mu\text{g} \times 1 \text{ ml}/0.1 \text{ ml plated} \times 100 \text{ dilution}$).

Electroporation Tips

- Electroporation cuvettes and microcentrifuge tubes should be pre-chilled on ice.
- Electrocompetent cells should be thawed on ice and suspended well by carefully flicking the tubes.
- Once DNA is added to the cells, electroporation can be carried out immediately. It is not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 μ l. Addition of a large volume of DNA decreases transformation efficiency.
- Contaminants such as salts and proteins can lower electroporation efficiency. Ideally, DNA for electroporation should be purified and suspended in water or TE. Transformation efficiency is more than 10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns are recommended for clean up of ligation reactions.
- Electroporation conditions vary with different cuvettes and electroporator. If you are using electroporators not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1 mm gap are recommended (e.g. BTX Model 610/613 and Bio-Rad #165-2089). Higher voltage and/or increased resistance is required for cuvettes with 2 mm gap.

Effect of pUC19 Concentration on Electroporation Efficiency and Total Colony Output



DNA Effects of Transformation Efficiency and Colony Output. Electrotransformation efficiency remains extremely high up to about 1 ng of input DNA, then decreases at higher DNA concentrations.

Electroporation Tips (continued)

- Arcing may occur due to high concentration of salts or air bubbles.
- It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction of efficiency.
- Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
- Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at -80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

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: F⁺ proA⁺B⁺ lacI^h ΔlacZM15 / fhuA2 Δ(lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)Tet^s endA1 thi-1 Δ(hsdS-mcrB)5

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⁺ Δ(lacZ)M15) makes ω-fragment of β-gal; Δ(lac-proAB) deletes the β-gal gene on the chromosome) pUC19 and similar plasmids code for the α-peptide of β-galactosidase (lacZ). The α-peptide can combine with the ω-fragment of β-galactosidase which is carried on the F⁺ (α-complementation). When β-galactosidase is reconstituted in this manner it can cleave 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.

Lac Promoter Control (lacI^h): 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 lacI^h strains help to minimize promoter activity until IPTG is added.

Recombination plus (recA⁺): E. coli has a repair system that will recombine homologous sequences. Although genomic clones often have duplicated regions, they are generally less than 200 bp. The recA repair system will not cause rearrangements or deletions under these circumstances. Strains which have the recA function intact tend to be more healthy and to grow faster 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 (Δ(hsdS-mcrB)5): Wild type E. coli K12 strains carry the EcoK Type I 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 deletion of hsdS eliminates both the endonuclease and methyl-transferase activities of EcoK.

Partially Methyl Restriction Deficient (Δ(hsdS-mcrB)5): E. coli has system of enzymes encoded by mcrA, mcrB and mrr which will cleave DNA with methylation patterns typical in eukaryotic cells. DNA derived from PCR fragments, cDNA or DNA previously propagated in E. coli will not be methylated at these sites and will not be cleaved. This strain has functional McrA and Mrr endonucleases and may not be suitable for direct cloning of eukaryotic DNA.

M13 phage sensitive (F⁻): 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⁻.

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.