Lot: 37

C2925H

dam⁻ / *dcm*⁻ Competent *E. coli*

NEW ENGLAND BioLabs Inc.

1-800-632-7799
info@neb.com
www.neb.com

C2925H

20 x 0.05 ml/tube **Lot: 37**

Store at –80°C

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

Description: Methyltransferase deficient chemically competent *E. coli* cells suitable for growth of plasmids free of Dam and Dcm methylation. Note that *dam*⁻ strains are not recommended as a host for primary cloning/ligation. The *dam* mutation can result in an increased mutation rate in the cell and a reduction in the transformation efficiency. DNA should be maintained in a *dam*⁺ strain unless there is a specific need for DNA free of Dam or Dcm methylation.

Features:

- Transformation efficiency 1–3 x 10^6 cfu/µg pUC19 DNA
- Allows for growth of plasmids free of Dam and Dcm methylation
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Phage T1 resistant (*fhuA31*)
- K12 Strain
- Free of animal products

Reagents Supplied:

20 x 0.05 ml/tube of chemically competent *dam*⁻/*dcm*⁻ 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: 1 ng of pUC19 plasmid DNA was used to transform *dam*⁻/*dcm*⁻ Competent *E. coli* following the high efficiency protocol provided. Greater than 1–3 x 10^6 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, kanamycin, spectinomycin and tetracycline. Cells are resistant to chloramphenicol and streptomycin,

Transformation Protocol

1. Thaw a tube of *dam*⁻/*dcm*⁻ 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.
4. Heat shock at exactly 42°C for exactly 30 seconds. 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.

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.

5 Minute Transformation Protocol

The following protocol results in only 10% efficiency compared to the 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 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.
4. Heat shock at exactly 42°C for exactly 30 seconds. 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

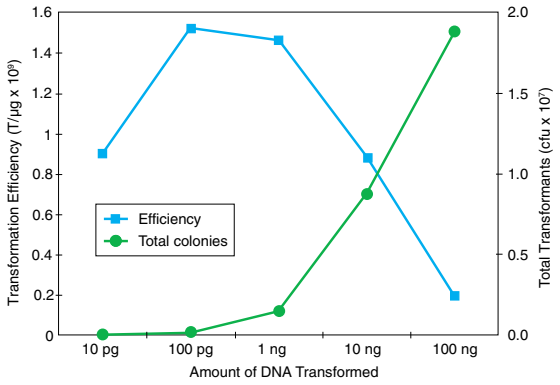
Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

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

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.

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 2 µl (100 pg) of control pUC19 DNA into 50 µl of cells, outgrow by adding 950 µl of SOC before plating 100 µl. If you count 20 colonies on the plate, the TE is:
Colonies = 20
µg DNA = 0.0001
Dilution = 100/1000 = 0.1
TE = 20/0.0001/0.1 = 2 x 10^6 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

Antibiotics for Plasmid Selection

Antibiotic	Working Concentration
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Kanamycin	30 µg/ml
Tetracycline	15 µg/ml

Genotype: *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)* Tet^S *endA1 rspL136 (*Str^R*) dam13::Tn9 (*Cam^R*) xylA-5 mtl-1 thi-1 mcrB1 hsdR2*

Strain Properties

The properties of this strain that contribute to its usefulness as a protein subcloning strain are described below. The genotypes underlying these properties appear in parentheses.

dam and *dcm* Methylation Deficient (*dam13::Tn9* (Cam^R), *dcm-6*): Most laboratory strains of *E. coli* contain both Dam methylase and Dcm methylase. Dam methylase transfers a methyl group to the adenine in the sequence GATC. Dcm methylase methylates the internal cytosine residues in the sequences CCAGG and CCTGG. Several restriction endonucleases will not cleave sites with these modified bases. The *damdcm* strain allows growth and purification of DNA free of Dam and Dcm methylation

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 (*hsdR2*): 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 *hsdR2* mutation described above eliminates the endonuclease.

Partially Methyl Restriction Deficient (*mcrA, mcrB1*): *E. coli* has a system of enzymes, *mcrA, mcrB* and *mrr* which will cleave DNA with methylation patterns found in higher eukaryotes, as well as some plant and bacterial strains. 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 a functional Mrr endonuclease and may not be suitable for direct cloning of eukaryotic DNA.

T1 Phage Resistant (*fhuA31*): 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