



Product Insert
dam-/dcm- Chemically Competent Cells
Bacteriophage T1-Resistant

Catalogue Numbers:
 BIO-85044 $\geq 10^7$ cfu/ μ g of pUC19

Features

- Lacks *dam* and *dcm* methylases
- Bacteriophage T1-Resistant
- $\geq 10^7$ transformation efficiency
- Convenient 100 μ l aliquots

Applications

- Generate plasmid DNA devoid of *dam* and *dcm* methylation
- Enable restriction digestion of plasmid DNA by methylation-sensitive endonucleases

Description

dam-/dcm- Chemically Competent Cells are an ideal host to generate plasmid DNA lacking in *dam* and *dcm* methylation. The absence of *dam* and *dcm* methylases in this strain prevents methylation at GATC and CC(A/T)GG sites respectively. Plasmid DNA propagated and purified from this host can be digested by the many restriction enzymes otherwise inhibited by *dam* or *dcm* methylation. pUC19 DNA is also provided as a positive control. The transformation efficiency of the dam-/dcm- Chemically Competent Cells is 10^7 cfu/ μ g of pUC19.

Product Specifications:

Efficiency	Pack Size	Control DNA
$\geq 10^7$ cfu/ μ g of pUC19	1ml (10 x 100 μ l)	pUC19 (10pg/ μ l)

Genotype:

F *dam*-13: *Trn9*(Cam^R) *dcm*-6 *ara*-14 *hisG4* *leuB6* *thi*-1 *lacY1* *galK2* *galT22* *glnV44* *hsdR2* *xyIA5* *mtf*-1 *rpsL* 136(Str^R) *ribD1* *tonA31* *tsx78* *mcrA* *mcrB1*

Storage Conditions:

dam-/dcm- Chemically Competent Cells can be stored for 6 months at -80°C.

Shipping Conditions:

On Dry Ice

Associated Products:

Product Name	Pack Size	Cat No
SureClean	1 x 5ml	BIO-37042
Quick-Stick Ligase	50 Reactions	BIO-27027
IPTG	5g	BIO-37036
X-GAL	1g	BIO-37035

Notes

1. This product insert is a declaration of analysis at the time of manufacture.
2. Research Use Only.

Suggested Transformation Procedure for Optimal Results:

- 1) Remove cells from -80°C and let thaw on wet ice.
- 2) Gently mix cells by lightly flicking tube. Aliquot ~50-100 μ l of cells into chilled, 17 x 100mm polypropylene tube(s), e.g. Falcon 2059. Unused cells may be refrozen, but a small drop in efficiency may result. For optimal recovery, refreeze cells in a dry ice/ ethanol bath prior to storage at -80°C.
- 3) Add DNA solution ($\leq 5\mu$ l per 50 μ l cells) to cell suspension and gently swirl tube(s) for a few seconds to mix. If a control is desired, repeat this step with 2 μ l of the provided pUC19 in a separate tube.
- 4) Incubate on ice for 30 minutes.
- 5) Place tube(s) in 42°C water bath for ~45 seconds without shaking.
- 6) Replace tube(s) on ice for ~2 minutes.
- 7) Dilute transformation reaction(s) to 1ml by addition of 900-950 μ l SOC (see associated products). SOC Medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10mM NaCl, 2.5mM KCl, 10mM MgCl₂ & 10mM MgSO₄.
- 8) Shake tube(s) ~200 rpm for 60 minutes at 37°C.
- 9) Plate by spreading 5-200 μ l of cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

- When performing the pUC19 control transformation, plate 5 μ l of the transformation mixture on a LB agar plate containing 100 μ g/ml ampicillin. To facilitate cell spreading, place a pool of SOC (100 μ l) onto surface of plate prior to addition of transformation mixture.

Transformation Efficiency Calculation for Control DNA

$$\text{Transformation Efficiency (cfu/\mu g pUC19 DNA)} = \frac{\# \text{ colonies}}{\text{pg pUC19 transformed}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{Final volume (\mu l) of transformation mix}}{\text{Volume plated (\mu l)}}$$

For example:

If 40 colonies were obtained after transforming 20pg of pUC19 and plating 5 μ l of the final 1ml transformation mixture, the calculated transformation efficiency would be:

$$\frac{40 \text{ cfu}}{20 \text{ pg pUC19}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{1000 \mu\text{l}}{5 \mu\text{l}} = 4 \times 10^8 \text{ cfu/\mu g pUC19}$$

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