



Product Insert

CH3-Blue Competent Cells

Catalogue Numbers:

BIO-85039 $\geq 10^8$ cfu/ μ g of pUC19
 BIO-85040 $\geq 10^9$ cfu/ μ g of pUC19

Features

- Lacks *mcrA*, *mcrBC*, *mrr* and *hsdRMS* restriction systems
- Available in two efficiencies: $\geq 10^8$ or $\geq 10^9$ cfu/ μ g of pUC19

Applications

- Cloning of methylated DNA
- Ideal for subcloning and generating cDNA libraries
- Blue/White color screening

Description

CH3-Blue Chemically Competent Cells are a highly efficient derivative of *E. coli* K12, ideal for the construction of cDNA libraries using plasmid derived vectors. To facilitate the cloning of DNA that contains methylcytosine or 5-hydroxymethylcytosine, CH3-Blue lacks the *E. coli* restriction systems *mcrA*, *mcrBC*, *mrr* and *hsdRMS*. The *lacZ* mutation allows blue/white color screening and α -complementation of recombinants. The *recA1* and *endA1* markers minimize recombination events and improve the quality and yield of plasmid DNA.

Product Specifications

Efficiency	Pack Size	Control DNA
$\geq 10^8$ cfu/ μ g of pUC19	1ml (10 x 100 μ l)	pUC19 (10pg/ μ l)
$\geq 10^9$ cfu/ μ g of pUC19	1ml (20 x 50 μ l)	pUC19 (10pg/ μ l)

Genotype:

F Δ *mcrA* Δ (*mrr*-*hsdRMS*-*mcrBC*) Φ 80*lacZ* Δ M15 Δ *lacX74* *recA1* *endA1* *ara* Δ 139 Δ (*ara*, *leu*)7697 *galJ* *galK* λ -*rpsL* (Str^R)

Storage Conditions:

CH3-Blue 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
T4 DNA Ligase	500 Units	BIO-27026
Quick-Stick Ligase	50 Reactions	BIO-27027
IPTG	5g	BIO-37036
X-GAL	1g	BIO-37035
T4 DNA Polymerase	500 Units	BIO-27035

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 -80°C storage.
 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 ~30 to 45 seconds without shaking. For 50 μ l aliquots in Falcon 2059 tubes, 30 seconds is recommended for maximum efficiency.
 6. Replace tube(s) on ice for ~2 minutes.
 7. Dilute transformation reaction(s) to 1ml by addition of 900-950 μ l SOC. 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

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

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:

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

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