CH3-Blue Competent Cells

Shipping: On Dry Ice

Exp. Date: See vial

Batch No.: See vial

Catalog numbers	
BIO-85039	≥10^8 cfu/µg of pUC19
BIO-85040	≥10^9 cfu/µg of pUC19

BIOLINE
A Meridian Life Science® Company

Store at –80°C

Storage and stability:
CH3-Blue Competent Cells are shipped on Dry Ice and can be stored for up to 6 months at –80°C.

Product Specifications:

Efficiency	Pack Size	Control DNA
≥10^8 cfu/µg of pUC19	1ml (10 x 100µl)	pUC19 (10pg/µl)
≥10^9 cfu/µg of pUC19	1ml (20 x 50µl)	pUC19 (10pg/µl)

Genotype:
F$^-$ Δ*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ* ΔM15 Δ*lacX*74 *rec*A1 *end*A1 *ara* Δ139 Δ(*ara*, *leu*)7697 *gal*U *gal*K λ- *rps*L (Str^R)

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

Features

- Lacks *mcr*A, *mcr*BC, *mrr* and *hsd*RMS restriction systems
- Available in two efficiencies: ≥10^8 or ≥10^9 cfu/µg 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 *mcr*A, *mcr*BC, *mrr* and *hsd*RMS. The *lac*Z mutation allows blue/white color screening and α-complementation of recombinants. The *rec*A1 and *end*A1 markers minimize recombination events and improve the quality and yield of plasmid DNA.

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 ~50-100µl 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 (≤5µl per 50µl cells) to cell suspension and gently swirl tube(s) for a few seconds to mix. If a control is desired, repeat this step with 2µl 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 50µl 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 900-950µl SOC. SOC Medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10mM NaCl, 2.5mM KCl, 10mM $MgCl_2$ & 10mM $MgSO_4$.
8. Shake tube(s) ~200 rpm for 60 minutes at 37°C.
9. Plate by spreading 5-200µl of cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

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

Transformation Efficiency Calculation for Control DNA

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

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

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

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

Product Citations:

1. Thompson, K. M. *et al. FEMS Micro. Lett.* **305(2)**, 143-7 (2010).

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