



Product Insert

BL21 Competent Cells

Catalogue Numbers:

BL21	1ml (10 x 100µl)	BIO-85031
BL21(DE3)	1ml (10 x 100µl)	BIO-85032
BL21(DE3)pLysS	1ml (10 x 100µl)	BIO-85033
BL21(DE3)pLysE	1ml (10 x 100µl)	BIO-85034
BL21 Combo Pack	1.5ml (15 x 100µl)	BIO-85035

Features

- High-level protein expression
- Protease deficient
- Transformation efficiency: $\geq 1 \times 10^7$ cfu/µg of pUC19

Applications

- Non-T7 promotor protein expression (BL21)
- T7 promotor expression (BL21 (DE3))
- Regulation of basal T7 promotor expression (BL21), (DE3) pLysS, BL21 (DE3) pLysE

Description

BL21 and its λ DE3 lysogenic are all-purpose *E. coli* host strains for high-level expression of a variety of recombinant proteins. All strains are deficient in both *lon* and *ompT* proteases, resulting in a higher level of intact recombinant proteins.

BL21 Competent Cells are an ideal host for optimal expression of proteins from vectors utilizing *E. coli* promoters (this strain lacks a T7 RNA polymerase).

The BL21(DE3) Competent Cells are designed for high-level protein expression and easy induction using T7 promotor constructs. These strains are lysogens of bacteriophage DE3, a lambda derivative containing the gene for T7 RNA polymerase under control of the *lacUV5* promotor. Induction with IPTG allows production of T7 RNA Polymerase, which then directs the expression of the target gene located downstream of the T7 promotor in the expression vector. Each BL21 (DE3) strain provides varying degrees of regulation and expression control.

The tightest regulation and expression control is achieved in the BL21(DE3)pLysE and BL21(DE3)pLysS strains, making them ideal for the expression of proteins that are toxic to *E. coli*. Recombinant proteins that are non-toxic to *E. coli* are generally expressed at higher levels in BL21 (DE3) cells than in BL21(DE3)pLysS or BL21(DE3)pLysE.

Product Specifications:

Control DNA:
pUC19 (100pg/µl)

Genotype:

BL21	F ⁻ <i>ompT</i> hsdS _B (r _B m _B ⁻) <i>gal dcm</i>
BL21(DE3)	F ⁻ <i>ompT</i> hsdS _B (r _B m _B ⁻) <i>gal dcm</i> (DE3)
BL21(DE3)pLysS	F ⁻ <i>ompT</i> hsdS _B (r _B m _B ⁻) <i>gal dcm</i> (DE3) pLysS (Cam ^R)
BL21(DE3)pLysE	F ⁻ <i>ompT</i> hsdS _B (r _B m _B ⁻) <i>gal dcm</i> (DE3) pLysE (Cam ^R)

Storage Conditions:

BL21 Competent Cells should be stored 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

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 storage at -80°C.
3. Add DNA solution ($\leq 5\mu\text{l}$ 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 ~45 seconds without shaking.
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 MgCl₂ & 10mM MgSO₄.
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

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

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:

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

Notes

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

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