

BL21 Competent Cells

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|----------------------|-----------------|--------------------|-----------|
| Shipping: On Dry Ice | Catalog numbers | | |
| Batch No.: See vial | 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 |



A Meridian Life Science® Company

Store at -80°C

Storage and stability:

BL21 Chemically Competent Cells are shipped on Dry/Blue Ice and stored at -80°C.

Expiry:

When stored under the recommended conditions and handled correctly, full activity of the cells is retained until the expiry date on the outer box label.

Product Specifications:

| Product Efficiency | Pack Size | Control Vector |
|--------------------|---------------------------------|-------------------------------------|
| Single Packs | 10 ⁷ cfu/µg of pUC19 | 1ml (10 x 100µl) pUC19 (100pg/µl) |
| Combo Pack | 10 ⁷ cfu/µg of pUC19 | 1.5ml (15 x 100µl) pUC19 (100pg/µl) |

Genotype:

BL21 F⁻ ompT hsdS_B(r_B m_B) gal dcm
BL21(DE3) F⁻ ompT hsdS_B(r_B m_B) gal dcm (DE3)
BL21(DE3)pLysS F⁻ ompT hsdS_B(r_B m_B) gal dcm (DE3) pLysS (Cam^R)
BL21(DE3)pLysE F⁻ ompT hsdS_B(r_B m_B) gal dcm (DE3) pLysE (Cam^R)

Safety precautions:

This product is for R&D use only, not for human use, or any other use. Please refer to the material safety data sheet for information regarding hazards and safe handling practice.

Notes:

Research Use Only.

Features

- High-level protein expression
- Protease deficient
- Transformation efficiency: ≥1 x 10⁷ 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 λDE3 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.

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 Control Vector (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.
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.

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

Transformation Efficiency Calculation for Control Vector

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:

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

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 |

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