



Product Insert
BL21 Combo Pack

Catalogue Number:
BIO-85035

Features

- Contains, BL21(DE3), BL21 (DE3) pLysS and BL21 (DE3) pLysE competent cells
- High-level protein expression
- Protease deficient
- Provided in convenient 100µl aliquots
- Transformation efficiency: $\geq 1 \times 10^7$ cfu/µg of pUC19
- IPTG inducibility helps to minimize toxic effects of some recombinant proteins

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 derivatives 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 source of T7 RNA polymerase).

BL21 Combo Pack contains five aliquots of each of the following:
 BL21 (DE3) Green
 BL21 (DE3) pLysS Purple
 BL21 (DE3) pLysE Orange
 and sufficient control plasmid

Product Specifications:

Pack Size:
1.5ml (15 x 100µl)

Efficiency:
 $\geq 10^7$ cfu/µg of pUC19

Control DNA:
pUC19 (100pg/µl)

Genotype:
 BL21(DE3) $F^- ompT hsdS_B(r_g m_B^-) gal dcm$ (DE3)
 BL21(DE3)pLysS $F^- ompT hsdS_B(r_g m_B^-) gal dcm$ (DE3) pLysS (Cam^R)
 BL21(DE3)pLysE $F^- ompT hsdS_B(r_g m_B^-) gal dcm$ (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 ($\leq 5\mu$ 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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