Catalog # BL21-201

TRANSFORMATION PROTOCOL

Product details:

BL21(DE3) chemically competent cells are suitable for transformation, high-level protein expression and production of recombinant proteins in bacterial systems. The strain lacks the *lon* and *ompT* proteases thus promoting stability of recombinant proteins. BL21(DE3) strain carries a copy of the T7 RNA polymerase under control of the IPTG inducible lacUV5 promoter, and as a result, is ideal for controlled expression of both E. coli and T7 promoter driven constructs. The transformation efficiency is ≥ $1x10^6$ cfu/µg pBR322 plasmid DNA.

***E. coli* genotype**:
fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS.
[λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5]

Suggested Transformation Procedure for Optimal Results:

1. Remove cells from - 80°C and let thaw on ice.
2. Gently mix cells by lightly flicking tube. Aliquot ~50-100 µl of cells into chilled, 17 x 100 mm polypropylene tube(s). Unused cells may be refrozen, but a 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 (≤5 µl per 50 µl cells) to cell suspension and gently swirl tube(s) for a few seconds to mix.
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 µl aliquots, 30 seconds is recommended for maximum efficiency.
6. Place tube(s) on ice for ~2 minutes.
7. Dilute transformation reaction(s) to 1ml by addition of 900-950 µl SOC. SOC medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$ & 10 mM $MgSO_4$. Other media can be used to grow transformed cells, including standard LB or TB broths. However, SOC is the optimal choice for recovery of the cells and for obtaining maximum transformation efficiencies.
8. Shake tube(s) ~200 rpm for 1 hour at 37°C.
9. Plate by spreading 50-200 µl of cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

Storage is recommended at - 80°C.

Usage: This product is intended for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.