AMID BIOSCIENCES

Protein Engineering Company

Product Manual

Product name: BL21(*λ*DE3)pBirA Chemically Competent *E. coli* Cells.
Catalog# BLB-201, **Size**: 10 X 0.1 ml/tube
Storage: at -80°C
Usage: This product is intended for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.

Product details:
The BL21(*λ*DE3)pBirA strain is recommended for expression and *in vivo* biotinylation of proteins fused with AviTag™ peptide or other peptide sequences recognized by *E. coli* BirA biotin ligase. This strain contains an IPTG-inducible BirA expression plasmid with the resistance gene for streptomycin/spectinomycin and allows high-efficiency protein expression of any gene that is under the control of a *T7* or *E. coli* promoter and has a ribosome binding site. The BirA plasmid is compatible for co-expression with many *E. coli* and *T7* promoter driven vectors (pET, pTrc, and etc).

Transformation efficiency: >1 x 10^4 cfu/µg pBR322

E. coli genotype: fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS pBirA(Str^R)
[λDE3 = 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 plasmid 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. Add 150 µl SOC to transformation reaction(s).
   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 100-200 µl of cell transformation mixture on LB agar plates containing streptomycin/spectinomycin and appropriate antibiotic for expression vector and incubate overnight at 37°C.

Suggested Induction Procedure
The following induction protocol is a general guide for *in vivo* biotinylation and co-expression of genes under the control of IPTG-inducible promoters.

1. Inoculate 5 ml aliquots of LB broth containing streptomycin at a final concentration of 50 µg/ml plus the antibiotic required to maintain the expression plasmid with single colonies from the transformation. Streptomycin serves to maintain BirA-expressing plasmid. Shake at 220–250 rpm at 37°C overnight.
2. Use this overnight culture to inoculate 1 L LB medium supplemented with streptomycin and the appropriate antibiotic and grow cells at 37 °C with shaking at 250 rpm.
3. When the culture OD600 reaches 0.6, remove 1ml as the non-induced control sample.
4. To induce protein expression, add IPTG to a final concentration of 1 mM. Add d-biotin at this point to a final concentration of 50 µM. Incubate the induced culture and the non-induced control sample with shaking at 220–250 rpm at 30°C for additional 4-6 hours.
   Note: These values for IPTG, biotin concentrations and induction time may require optimization depending on the gene expressed.
5. Remove 20 µl of the induced and the non-induced cultures after 2, 4, and 6 hours into clean microcentrifuge tubes. Add 20 µl of 2× SDS gel sample buffer to each microcentrifuge tube for analysis by SDS-PAGE.
6. After finishing the induction period, harvest cells by centrifugation at ~6,000×*g* for 10 min. The cells can be frozen or immediately processed for protein purification.