Product Manual

Product name: BL21(λDE3) ΔserC ΔycdX Chemically Competent Cells for In Vivo Incorporation of Phosphothreonine

Catalog# BLPCY-201**, Size: 10 X 0.1 ml/tube**

Product details:
The BL21(*λ*DE3) ΔserC ΔycdX is an engineered strain for efficient co-translational incorporation of phosphothreonine (pThr) into recombinant proteins by an *Escherichia coli*. In this strain, deletion of serC gene which is responsible for the intracellular levels of phosphoserine along with deletion of a putative phosphatase, ycdX, enables homogeneous pThr incorporation at different sites in different proteins (Zhang et al.,2017).

BL21(*λ*DE3)ΔserC ΔycdX chemically competent cells are supplied as a pack of 10 convenient 100 µl/tube aliquots. The transformation efficiency is ≥ $1x10^6$ cfu/µg pBR322 plasmid DNA.

E. coli genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS ΔserC ΔycdX*

[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 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 200-250 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 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.

Important Note: Purchase of BL21(*λ*DE3) ΔserC ΔycdX cells includes research use limited license for use only at site of purchase. This license prohibits the purchaser from selling, assigning or transferring this product to any third party without the express written consent of Amid Biosciences, LLC. Please review this license before purchasing this product.

References

Zhang MS,et al. *Biosynthesis and genetic encoding of phosphothreonine through parallel selection and deep sequencing.* Nat Methods. 2017 Jul;14(7):729-736. doi: 10.1038/nmeth.4302. Epub 2017 May 29.