Protein Expression Using BL21(DE3) (C2527)

New England Biolabs

Abstract

This is the protocol for Protein Expression Using BL21(DE3) Competent E. coli (C2527). For large scale, please follow this protocol variant.

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Guidelines

BL21(DE3) Genotype:
fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS
λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5

NOTES:
1. **Caution:** This product contains DMSO, a hazardous material. Review the MSDS before handling.

2. **Storage and Handling**: Competent cells should be stored at -80°C. Storage at -20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above -80°C, even if they do not thaw.

Materials

BL21(DE3) Competent E.coli - 6x0.2 ml C2527I by New England Biolabs

Protocol

Step 1.
Transform expression plasmid into BL21(DE3).

Step 2.
Plate on antibiotic selection plates.

Step 3.
Incubate overnight at 37°C.

DURATION
15:00:00

Step 4.
Resuspend a single colony in 10 ml liquid culture with antibiotic.

Step 5.

Incubate at 37°C until OD600 reaches 0.4–0.8.

Step 6.
Induce with 4 or 40 µl of a 100 mM stock of IPTG (final concentration of 40 or 400 µM)

Step 7.
Induce for 3 to 5 hours at 37°C.

Step 8.
Check for expression either by Coomassie stained protein gel, Western Blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction only.

ANNOTATIONS

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If a fraction of the target protein is insoluble, repeat expression at a lower temperature (15 to 30°C) or test expression in Lemo21(DE3) (NEB #C2528).