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Version 1

Protein Expression Using BL21(DE3) (C2527) V.1

DOI

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Abstract

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

Guidelines

BL21(DE3) Genotype:

fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS

λ DE3 = λ sBamHI 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

MATERIALS

 BL21(DE3) Competent E.coli - 6×0.2 ml **New England Biolabs Catalog #C2527I**



- 1 Transform expression plasmid into BL21(DE3).
- 2 Plate on antibiotic selection plates.
- 3 Incubate overnight at 37°C.
 15:00:00
- 4 Resuspend a single colony in 10 ml liquid culture with antibiotic.
- 5 Incubate at 37°C until OD600 reaches 0.4–0.8.
- 6 Induce with 4 or 40 μ l of a 100 mM stock of IPTG (final concentration of 40 or 400 μ M)
- 7 Induce for 3 to 5 hours at 37°C.
- 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.