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Feb 11, 2022

Protein Expression Using BL21(DE3), Large Scale (C2527) V.2

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dx.doi.org/10.17504/protocols.io.bhk2j4ye

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This is the protocol for a large-scale Protein Expression Using BL21(DE3) Competent E. coli cells (C2527).

DOI

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https://www.neb.com/protocols/0001/01/01/protein-expression-using-bl21de3-c2527

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T7 Expression Strain, expression plasmid, transform expression plasmid into BL21(DE3)

protocol ,

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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 - 20x0.05 ml **New England Biolabs Catalog #C2527H**

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

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

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

Determine the optimal time/temperature for the protein expression in a small scale trial.

1 Transform expression plasmid into BL21(DE3).

2 Plate on antibiotic selection plates.

3 

Incubate **Overnight** at **37 °C** .

4 

Inoculate **1 L liquid medium (with antibiotic)** with a freshly grown colony or with **10 mL freshly grown culture (from a single colony)** .

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Incubate at **37 °C** until OD_{600} reaches 0.4–0.8.

6

Induce with **4 µL** or **40 µL** of **100 Milimolar (mM) IPTG stock** (final concentration of **40 Micromolar (µM)** or **400 Micromolar (µM)**).

7

Express protein using optimal time/temperature determined in a small scale trial (3 to **05:00:00** 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.

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).

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