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🌐 Protein Expression Using BL21(DE3) (C2527) V.2

[New England Biolabs](#)¹¹New England Biolabs

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dx.doi.org/10.17504/protocols.io.bdd4i28w**New England Biolabs (NEB)**Tech. support phone: **+1(800)632-7799** email: **info@neb.com****New England Biolabs**
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This protocol explains methods for Protein Expression Using BL21(DE3) Competent E. coli (C2527).

You can find the protocol for a large-scale Protein Expression [here](#).

DOI

dx.doi.org/10.17504/protocols.io.bdd4i28w<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), BL21, C2527

 protocol ,

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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



Biolabs Catalog #C2527H



Biolabs Catalog #C2527I

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

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1 Transform expression plasmid into BL21(DE3).

2 Plate on antibiotic selection plates.

3 

Incubate  **Overnight** at  **37 °C** .

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 µL** or **40 µL** of **100 Millimolar (mM) IPTG stock** (final concentration of 40 or 400 µM).

7 

Induce for 3-5 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.

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](#)).

9 For large scale, inoculate **1 L** of liquid medium (with antibiotic) with a freshly grown colony or **10 mL** of freshly grown culture.

10 

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

11 

Add **40 Micromolar (µM)** or **400 Micromolar (µM)** IPTG and express protein using optimal time/temperature determined in a small scale trial.