

Protein Expression Using BL21(DE3), Large Scale (C2527)

New England Biolabs

Abstract

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

Citation: New England Biolabs Protein Expression Using BL21(DE3), Large Scale (C2527). **protocols.io**

dx.doi.org/10.17504/protocols.io.cjrum5

Published: 07 Nov 2014

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.

Before start

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

Materials

 BL21(DE3) Competent E.coli - 6x0.2 ml [C2527](#) 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.

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

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.

Express protein using optimal time/temperature determined in a small scale trial.

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.

NOTES

New England Biolabs 07 Nov 2014

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](#)).