PRODUCT INFORMATION

Product Name : Zip Competent Cell BL21(DE3)
Code No. : DS255
Size : 100 µl × 10
Competency : > 1 × 10^6 cfu/µg (pBR322)

This product is research use only

Description :

The Zip Competent Cell BL21(DE3) is designed for a rapid transformation procedure. It requires neither heat shock nor culture after heat shock. Transformation of the Zip Competent Cell BL21(DE3) is completed within about 5 minutes. The time-saving procedure is a great benefit for researchers and experimenters.

The strain, BL21(DE3), is one of the popular strains for the protein expression. BL21(DE3) contains the λDE3 lysogen, which expresses T7 RNA polymerase under the control of the *lac*UV5 promoter[1)]. Upon addition of isopropyl-1-thio-β-D-galactopyranoside (IPTG), T7 RNA polymerase is expressed and it induces a high-level protein expression from T7 promoter driven expression vectors (e.g., pET). *E. coli* BL21(DE3) strain is a derivative of *E. coli* B strain and lacks both the *lon* protease and the *ompT* membrane protease which may degrade expressed proteins.

Genotype of *E coli* strain BL21(DE3) : F^- *ompT hsdS*(r_B^- m_B^-) *gal dcm* λ(DE3)
(λ(DE3): *lac*I, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)

Storage condition :

Stable at -80℃ with little or no loss in transformation efficiency for 12 months from the date of receipt. Competent cells are sensitive to variation in temperature. Must be stored at – 80 °C. Upon receipt, store the Zip Competent Cell BL21(DE3) in a freezer at - 80 °C directly from the dry ice shipping box.

Handling of competent cells :

• Competent cells are sensitive to mechanical shock. Excessive mixing should be avoided.
• After thawing competent cells on ice, cells tend to lose transformation efficiency gradually. Transformation should be started immediately after the cells have thawed on ice.
• Use of refrozen competent cells is not recommended.

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Quality Control :

Transformation was carried out according to the method described in this Product Information using supercoiled pBR322 plasmid. Transformants were plated on LB plates containing 50 µg/ml ampicillin. The efficiency was confirmed to be greater than 1×10^6 cfu/µg.

Comparative study of BL21 strains :

BioDynamics Laboratory Inc. offers three kinds of BL strains. These strains exhibit different features and are used depending on the experiments.

Strains	Induction Method	Superior Feature	Points to Note
BL21	Require phage CE6 infection	Tight regulation of basal level expression	Complex induction process
BL21(DE3)	IPTG	High-level expression	Frequent uninduced expression.
BL21(DE3)pLysS	IPTG	Reduction of basal level expression	Slightly lower expression than BL21(DE3)

Transformation Procedure :

● Materials to be supplied by user

- LB plates containing an appropriate antibiotic
- Ice bucket with ice
- Sterile spreader

● Transformation

1. Thaw the competent cells on ice (100 µl in a tube for each transformation).
2. Add DNA sample[*1] directly into the competent cells and mix by flicking the tube.
   [*1] The volume of the DNA sample should not exceed 5 % of that of the competent cells (i.e., 5 µl).
   [*2] If it is predicted that you will have too many colonies on the LB plate, dilute the plasmid solution with autoclaved water.
3. Leave the tube on ice for 5 minutes.
4. Spread the 100 µl of the cells to a 37ºC pre-warmed LB agar plate containing an appropriate antibiotic.
5. Incubate the plates at 37°C overnight.

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● Notes for transformation

1. As T7 expression method is a high-level protein expression system, some basal level expression of the target protein will occur in uninduced cells. This is likely problematic in cases which the target protein is toxic to *E. coli.* cells. In this case, it may be necessary to decrease the basal level expression as follows:

a) Use BL21(DE3)pLysS strain but not BL(DE3) strain.

The T7 Lysozyme encoded in a pLysS plasmid reduces the basal level of T7 RNA polymerase expression[2]. This leads to suppress the basal level expression of the target protein.

b) Use a low-copy, T7 driven expression vector.

c) Use liquid medium and agar plates supplemented with glucose (0.5 -1 %).

Glucose is known to decrease a basal expression from *lac*UV5 promoter[3].

2. Expression may vary among transformants. If large and small colonies are observed in the same plate, the expressed protein may affect the growth of the *E. coli* cells.

3. If the expressed protein is toxic to *E. coli* cells, transformants may not be obtained.

Protein Expression Procedure :

The following protocol is a general guide for the protein expression by use of T7 promoter driven expression vectors and BL21(DE3) cells or BL21(DE3)pLysS cells.

Before starting:

1. Construct T7 promoter driven expression plasmid harboring a gene of interest using non-expression hosts.

2. Transform Competent Cell BL21(DE3) or Competent Cell BL21(DE3)pLysS with the expression plasmid.

Expression:

1. Following transformation, pick a colony and inoculate it into 3 ml of LB medium containing the appropriate antibiotic with shaking at 37°C, overnight. For the BL21(DE3)pLysS strain, it is preferable to add chloramphenicol at a final concentration of 34 μg/ml in the overnight culture to maintain pLysS.

2. The next morning, transfer 0.5 ml of the overnight culture to a new 10 ml of LB medium containing the appropriate antibiotic to select the expression plasmid. Grow the culture with shaking at 37°C until the OD_{600} reaches 0.5 (approximately 2 hrs but this depended on the expression plasmids).

When using BL21(DE3)pLys, chloramphenicol is not usually required in the short-period culture.

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3. When the OD_{600} reaches 0.5, transfer an aliquot (e.g., 1 ml) of the culture to a new centrifuge tube and centrifuge it to harvest cells. Store the cells at -80°C until analysis.

Add IPTG to a final concentration of 1 mM to the rest of the culture and grow the culture with shaking at 37°C for 3 hours.

The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.

4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 ml) and centrifuge it to precipitate the cells.

Analysis

1. Suspend the precipitated cells (from the 1 ml culture) in 200 µl of 1× PBS buffer.
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.
3. Heat the mixture at 85°C for 5 min, then centrifuge at 10,000 g for 10 min. Subject the supernatant (e.g., 5-25 µl) to SDS-PAGE. Western blot will help analyzing the expression of the target protein.

- 2 × SDS sample buffer : 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 20 % glycerol, 0.02% BPB, 62.5 mM Tris-HCl, pH6.8
- 1× PBS buffer.: 20 mM sodium phosphate, 150 mM sodium chloride, pH7.4

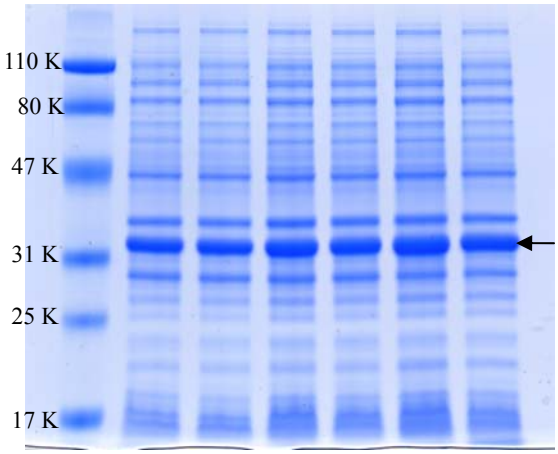

Fig. 1. Expression of a recombinant protein from BL21(DE3) cells
A gene of 32 KDa protein was cloned into a T7 promoter driven expression vectors (p32K). Competent Cell BL21(DE3) was transformed with the p32K, six colonies were picked and followed the "Protein Expression Procedure" as above. After induction, aliquot of the cells from each colony was subjected to 12.5 % polyacrylamide gel SDS electrophoresis. The gel was stained with Quick Blue Protein Staining Solution (BioDynamics Laboratory Inc. #DS500).
Lane 1: Molecular weight marker
Lane 2-7 : BL21(DE3) cells, clones 1-6
An arrow shows the expressed proteins.

● Notes for expression:

1. When expressing proteins in BL21(DE3) cells, if it takes longer time (5 hrs or more) to reach 0.5 at OD_{600} after inoculating the overnight culture (0.5 ml) to a new LB medium (10 ml), the expressed protein is likely toxic to *E. coli* cells. See a, b, and c in the section "Notes for transformation 1".
2. When BL21(DE3) cells lyse after induction with IPTG, the expressed protein is likely toxic to *E. coli* cells.

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Reference:

1) Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.

2) Moffatt, B.A. and Studier, F.W., Cell 49 (1987) 221-227

3) Pan, S. and Malcom, B.A., *BioTechniques* 29 (2000), 1234–1238

General reference in this Product Information

Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Related Products:

DS110	DNA Ligation Kit ver. 2	DS210	Competent Cell JM109
DS220	Competent Cell DH5α	DS225	Jet Competent Cell (DH5α)
DS240	Competent Cell BL21	DS250	Competent Cell BL21(DE3)
DS260	Competent Cell BL21(DE3)pLysS	DS500	Quick Blue Protein Staining Solution

● Purchaser Notification

This product is manufactured based on the T7 expression system which is the subject of US patent applications assigned to Brookhaven Science Associates, LLC (BSA). The product must be used only outside the United Stated and its territories. Neither this product nor materials prepared used by the T7 expression system are allowed to be distributed in the US and its territories without license of BSA. Information about license regarding the T7 expression system maybe obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 185, P.O. Box 500, Upton, New York 11973-5000, USA.