日本語データシート

PRODUCT INFORMATION

Product Name : BL21(DE3) expression competent cell pack
Code No. : DS265
Kit Component :

Component	Code No.	Contents
DynaCompetent Cells BL21(DE3)	DS250	5 tubes (100 µL/tube) *Yellow tube transfromation efficiency: 5 × 10^7 cfu/µg (pUC19)
DynaCompetent Cells BL21(DE3)plysS	DS260	5 tubes (100 µL/tube) *Clear tube transfromation efficiency: 5 × 10^7 cfu/µg (pUC19)
SOC medium		10 tubes (1 mL/tube)

Storage : Store at -80ºC

Product Description:

1) DynaCompetent Cells BL21(DE3)

DynaCompetent Cells BL21(DE3) is a chemically competent *E. coli* BL21(DE3) cell. The BL21(DE3) strain 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).

2) DynaCompetent Cells BL21(DE3) pLysS

DynaCompetent Cells BL21(DE3) pLysS is a chemically competent *E. coli* BL21(DE3)pLysS cell, suitable for high-level protein expression using the T7 expression system as well as DynaCompetent Cells BL21(DE3). The BL21(DE3)pLysS strain has a feature of containing T7 lysozyme gene in the pLysS plasmid. The T7 lysozyme suppresses the activity of T7 RNA polymerase[2], which reduces the basal level protein expression from the gene of interest. It is important if the protein is toxic to the E. coli cells. The presence of T7 lysozyme increases the tolerance of the *E. coli* cells against the toxicity. The pLysS plasmid contains a chloramphenicol resistant gene and a p15A replication origin which is compatible with those found in pUC19 and pUC derived plasmids.

E. coli BL21(DE3) and *E. coli* BL21(DE3)pLysS strain are a derivative of *E. coli* B strain and lacks both the *lon* protease and the *ompT* membrane protease which may degrade expressed proteins.

Genotype :

strain	Genotype
BL2(DE3)	F⁻ *ompT hsdS*(rB⁻ mB⁻) *gal dcm* λ(DE3) (λ(DE3): *lacI*, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)
BL21(DE3)pLysS	F⁻ *ompT hsdS*(rB⁻ mB⁻) *gal dcm* λ(DE3) pLysS (Cam^r) (λ(DE3): *lacI*, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)

Ver. 2.0

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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(DE3)	IPTG	High-level expression	Frequent uninduced expression.
BL21(DE3)pLysS	IPTG	Reduction of basal level expression	Slightly lower expression than BL21(DE3)

● **Notes**

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.

Composition of SOC medium supplied :

20 g/L	tryptone
5 g/L	yeast extract
0.5 g/L	NaCl
0.186 g/L	KCl
2.4 g/L	$MgSO_4$, anhydrous
4 g/L	glucose

Storage condition :

Stable at -80ºC with little or no loss in transformation efficiency for 12 months from the date of receipt. DynaCompetent Cells s are sensitive to variation in temperature. Must be stored at -80ºC. Upon receipt, store the DynaCompetent Cells BL21(DE3) and the DynaCompetent Cells BL21(DE3)pLysS in a freezer at -80ºC directly from the dry ice shipping box and store SOC medium at room temperature or -80ºC.

Quality Control :

Transformation was carried out according to the method described in this Product Information using supercoiled pUC19 plasmid. Transformants were plated on LB plates containing appropriate antibiotics.

- BL21(DE3) : containing 50 μg/mL of ampicillin
- BL21(DE3)pLysS: containing 50 μg/mL of ampicillin and 34 μg/mL of chloramphenicol

The efficiency was confirmed to be greater than 5×10^7 cfu/μg.

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Handling of Competent cells :

• Competent Cells are sensitive to mechanical shock. Excessive mixing should be avoided.

• After thawing DynaCompetent 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 DynaCompetent Cells is not recommended.

Transformation Procedure :

● Materials to be supplied by user

- LB plates containing antibiotic
  (Antibiotic* to select transformants and 34 μg/mL of chloramphenicol to keep pLysS)
- Ice bucket with ice
- 15 mL sterilized-polypropylene culture tubes
- 42°C water bath
- 37°C shaker
- Sterile spreaders

● Transformation

1. Thaw one tube of competent cells on ice. One tube contains 100 μL of cells for each transformation.
2. Add DNA sample* directly into the competent cells and mix by flicking the tube.
   * The volume of DNA sample should not exceed 5 % of that of competent cells (i.e. for 100 μL of competent cells, use ≦ 5 μL).
3. Leave the tube on ice for 20 minutes.
4. Heat Shock the cells by placing the tube in a 42°C water bath for 45 seconds. Do not mix or shake during this time.
5. Remove the tube from the water bath and place them on ice for 2 min.
6. Transfer the cells to a 15 mL sterilized culture tube containing 0.9 mL of SOC medium (pre-warmed at room temperature). Culture the cells at 37°C for 1 hr in a shaker.
7. Spread an aliquot of the cells* to a LB agar plate containing the appropriate antibiotic.
   * If plating <100 μL of the cells, mix SOC medium to the cells up to 100 μL then spread them.
8. Incubate the plates at 37°C overnight.

● 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.

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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 DynaCompetent Cells BL21(DE3) or DynaCompetent Cells 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.

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

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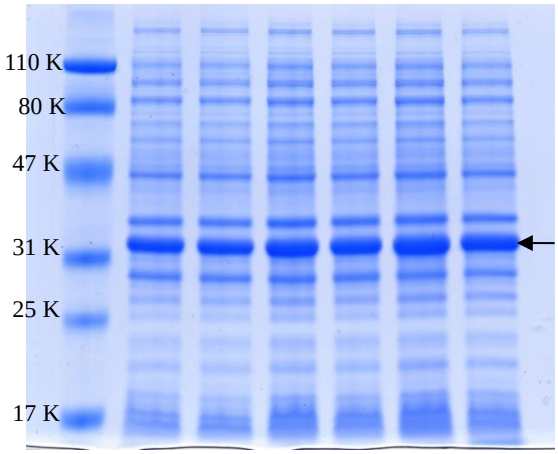

Fig. 1. Expression of a recombinant protein from BL21(DE3) cells
A gene of 32 KDa protein was cloned into a T7 promoter driven expression vectors (p32K). DynaCompetent Cells BL21(DE3) was transformed with the p32K, six colonies were picked and followed the "Protein Expression Procedure" as above. After 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.

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:

DS255	DynaCompetent Cells Zip BL21(DE3)
DS258	DynaCompetent Cells BL21(DE3) for Electroporation
DS230	DynaCompetent Cells JetGiga DH5α

● 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 may be 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.

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